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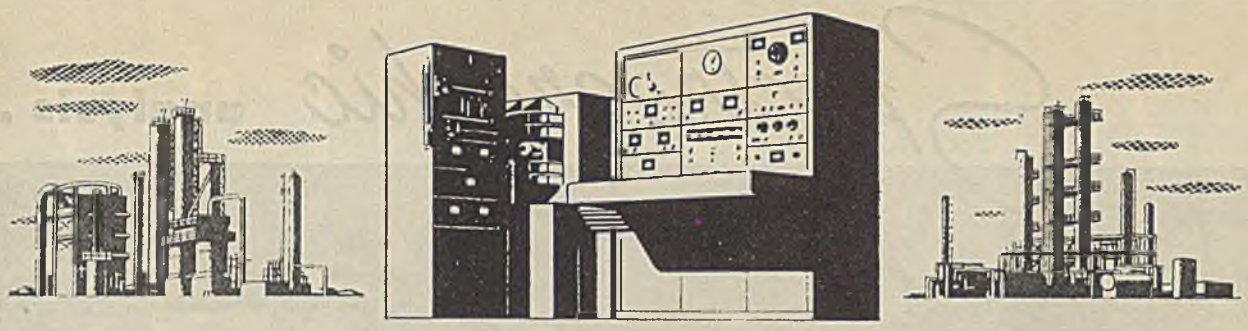

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Systematic Procedure for Identification of Synthetic Resins and Plastics

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The procedure given will identify most resins of commercial importance at the present time. Once the resin has been isolated in as pure a condition as possible it is necessary to determine to which of eight groups it belongs, then to proceed systematically to its tentative identification. Confirmatory tests are applied to confirm or disprove this. In the latter event, or if short cuts are possible because of the history of the resin, use is made of a classification of the properties for the various general types of resins.

EVERY chemist employing resinous substances is confronted at some time with the task of identifying a resin. No detailed procedure has so far appeared which would enable a systematic attack on this problem to be made, although a number of methods suitable for a few common resins are known (*1, 10, 13*). The method given in this paper has proved very serviceable in these laboratories for the past two years.

This method of analysis depends upon using a single resin for the group and systematic procedures. The general order of procedure to be followed on an unknown sample is:

1. Separation of the resin or resins from the solvents, plasticizers, fillers, pigments, and dyes
2. Separation of mixtures into individual resins
3. Classification of the separated resin according to the group tests
4. Identification by following the scheme for the group into which the resin falls, so as to arrive at the probable identity of the resin
5. Confirmation by specific tests

Cases will occur where the confirmatory tests do not yield a clear-cut identification. This may be due to imperfect separation of a mixture or the presence of a resin which is not covered in this paper. Some general reactions are given below which will help to identify the type of resin in such cases.

The scheme will function with the particular resins used and with allied resins; but it will not distinguish between different degrees of polymerization of the same monomer. It has not been possible to cover all the resins for each type; therefore, it is necessary to confirm the identification. Where the same resin is listed in different places in the tables it is because of similarity in properties of related resins which could not be distinguished without use of the trade name, or a few borderline cases which might fall in both places.

PREPARATION OF SAMPLE

In most fields there are published methods (*2, 8, 14, 16*) covering the separation of vehicles, pigments, or plasticizers from the resinous constituents that may be encountered. It is beyond the scope of this paper to do more than briefly indicate the methods employed.

REMOVAL OF VEHICLES AND PLASTICIZER. In handling a solution, addition of a nonsolvent such as ligroin or water to precipitate the resins is useful. The plasticizer will usually be left

Table I. Separation of Groups

(Sample previously separated from solvents, plasticizers, fillers, pigments, dyes, and other resins)

Halogens
- Strongly positive: Test according to Group A.
- Negative: Test for nitrogen and sulfur.

Nitrogen and sulfur
- Nitrogen positive, sulfur negative or very weakly positive: Test according to Group B.
- Nitrogen and sulfur positive: Test according to Group C.
- Nitrogen negative, sulfur positive: Test according to Group D.
- Nitrogen and sulfur negative: Test for saponification number.

Saponification No.
- Over 325: Test according to Group E.
- 120 to 325: Test according to Group F.
- Less than 120: Test for acetyl number.

Acetyl No.
- Over 40: Test according to Group G.
- Less than 40: Test according to Group H.

Table II. Separation of Group A

(Halogens present)

A. Test solubility in ligroin
- Soluble: Chlorinated diphenyls. Confirm by Test VI. Melt with very little decomposition. High refractive index
- Insoluble: Test solubility in hot acetone
  - Soluble: Polyvinyl chloride - acetate copolymers. Medium acetate type. Confirm by Test II
  - Insoluble: Test solubility in ethyl acetate
    - Soluble: Chlorinated rubber. Confirm by Test III
    - Insoluble: Test solubility in ethylene dichloride
      - Soluble: Polyvinyl chloride or low acetate polyvinyl chloride - acetate copolymers. Confirm by Test II
      - Insoluble: Test solubility in pyridine (B)

B. Solubility in pyridine
- Soluble: Test solubility in tetrachloroethane
  - Soluble: Polyvinyl chloride-acrylate copolymer. Hard resin. Low refractive index
  - Insoluble: Cashew nut oil polymer. Confirm by Tests II and VI. Soft sticky black resin
- Insoluble: Test solubility in tetrachloroethane
  - Soluble: Rubber hydrochloride. Confirm by Test VI
  - Insoluble: Test solubility in morpholine
    - Solvent and resin turn black. Polyvinylidene chloride resins. Confirm by Test II
    - Insoluble: Chloroprene rubbers. E type soluble in dioxane, other type insoluble. Confirm by Test II. Sulfur usually present

Table III. Properties of Resins in Group A

Test No.	Property Sought	Polyvinyl Resins: Chloride-Acetate: Chloride	Chloride-Acetate: Low acetate type	Chloride-Acetate: Medium acetate type	Chloride-acrylate	Polyvinylidene chloride	Chlorinated Diphenyls	Rubber Substitutes: Chloroprene	Rubber Substitutes: Chlorinated rubber	Rubber Substitutes: Rubber hydrochloride	Rubber Substitutes: Cashew nut oil polymer
I	Solubility										
	Acetone	I	IG	S	I	I	S	I	IG	I	I, Sl.G
	Ethyl acetate	I	I	S	I	I	S	I-IG	S	I	I, Sl.G
	Dioxane	S	S	S	I	I	S	I-S	S	I	IG
	Ligroin	I	S	S	I	I	S	I	S	I	I
	Benzene	I	I	I	I	I	S	I-IG	I	I	I
	Ethylene dichloride	S	S	S	IG	I	S	I-IG	S	I	IG
	Pyridine	IG	....	S	S	I	S	I-IG	S	I	S
	Acetic acid	I	....	I	I	I	S	I	I	I	I
	Morpholine	Sl.S.; G	....	....	IG	I liquid, resin turn black	....	I-IG	....	I	IG
	Tetrachloroethane	IG	....	S	S	I	S	I-IG	S	S	IG
II	Liebermann-Storch	Resin slowly turns blue	Resin slowly turns green to blue to brown	Resin slowly turns green to blue to brown	Neg.	Resin slowly turns yellow	Neg.	Red-brown	Neg.	Neg.	Red brown to dark brown
III	Carbonate fusion										
	Odor	(Resembles that of smoke from wet wood)			Gunpowder	Aromatic	None	Wet wood	Hypochlorite	Sl. rubbery	Oily
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	None	Pos.	Pos.	Pos.	Pos.
	Fume	V.Sl.	V.Sl.	V.Sl.	Heavy	V.Sl.	V.Sl.	Sl.	V.Sl.	Sl. white	Heavy
V	Carboxylic esters	Neg.	Neg.	Neg	Neg.	Neg.	Neg.	.....	Neg.	....	.....
VI	Odor on ignition	Pungent	Pungent	Pungent	Pungent	Pungent	Sl. acrid	Pungent and tarry	Pungent	Pungent	Oily
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	Pos.	Sl.	Pos.	Pos.
	Fume	Heavy yel.	Cons. yel.	Cons. yel.	V. heavy yel.	Nil	Nil	Heavy brown	White	Heavy yel.	Heavy yel.
	Distillate	Nil	Nil	Nil	Nil	Nil	Much	Sl.	Nil	Sl.	Sl.
XIII	Acetates	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	.....	Neg.	....	Neg.
	Refractive Index 20° C.	1.56	1.53	1.53	....	1.61	1.61–1.71	1.56	1.56	....	.....
	Specific Gravity $\frac{20°\ C.}{20°\ C.}$	1.2–1.6	1.35	1.35	1.38	1.6–1.75	1.34–1.95	1.14–1.24	1.64	....	.....

Key to Abbreviations

Cons., considerable or considerably
G, gel
I, insoluble
Neg., negative
Pos., positive
P.S.G., partly soluble gelled
S, soluble
Sl., slight
V., very
Yel., yellow

in solution. If test-tube experiments show this method is not feasible, the solution may be poured into boiling water or dried by evaporation. Both the latter methods leave the pigments and plasticizers in the resins and they will require further treatment.

Oily vehicles may usually be removed by precipitation of the resin by a nonsolvent.

Pigments and Fillers. Finely divided pigments sometimes offer considerable difficulty. If the pigment is held by Alundum or paper thimbles a Soxhlet extraction of the vehicle-free resins by a solvent will do. At times repeated filtration in the presence of a filter aid such as Filter-Cel on a suction or pressure filter, using a very dilute solution in a low-viscosity solvent, is required. Centrifuging will serve in some cases.

Dyes. Dyes are even worse to handle but precipitation by a nonsolvent, a procedure which must be repeated several times for removal of vehicles and plasticizers, usually leaves most of the dye in solution. Preliminary tests often indicate a solvent for the dye which has sufficient swelling action on the resin for a Soxhlet extraction of the dye to be made.

MIXED RESINS

Where a mixture of resins is suspected, these must be separated into as pure fractions as possible by suitable extraction or precipitation procedures designed to meet the particular case in hand. Fractions so separated, dried, and free from solvent are treated independently by the methods given below.

METHODS FOR SEPARATION OF GROUPS

The following tests are applied in the order given (Table I):

Halogens. Beilstein's copper wire test is convenient (*9*, *11*, *12*). All substances in Group A give a strong test. A faint test may be ignored as due to volatile impurities or salts. It is necessary to make sure that some of the resin actually enters the flame with the wire.

Halogens may also be detected in a portion of the filtrate from the sodium fusion by acidifying and boiling with nitric acid, then adding silver nitrate.

Nitrogen. The usual sodium fusion (*9*, *11*, *12*) with the development of Prussian blue in the presence of ferric salts is used.

Sulfur. A drop of the filtrate from the sodium fusion applied to a silver coin quickly develops a dark stain in the presence of sulfur.

Acid Number. While not required for group separation, this figure is conveniently obtained at this time and is useful as a confirmatory figure later.

Accurately weigh about 1 gram of resin and place it in 100 cc. of neutral dioxane, alcohol, or other suitable solvent. Then warm gently under an air condenser for about 1 hour. Titrate the free acid with aqueous 0.1 *N* sodium hydroxide, using phenolphthalein indicator.

$$\text{Acid number} = \frac{56.1 \times \text{normality of NaOH} \times \text{cc. of NaOH used}}{\text{weight of sample}}$$

Saponification Number. To the neutral solution above, in a soft-glass flask, add 25 cc.

Table IV. Separation of Group B

(Halogens absent, nitrogen present, sulfur absent)

- Apply Test IV
  - Blue color: Nitrocellulose or nitrocellulose acetate. Distinguish by rate of burning; acetate may be identified by method of Simonds and Ellis (*16*).
- No blue color: Apply Test XI
  - Negative: Apply Test III
    - Odor of NH_3 and burning hair. Polyamide resin. Confirm by Test VI. *Note.* Glue, gelatin, or albumenoids might appear here. Physical form, positive biuret reaction, solubility in hot water can be expected to indicate such substances.
    - Odor like hot aniline, butadiene-acrylonitrile copolymer. Confirm by action of acetone
  - Positive: Violet color. Apply Test XII
    - Negative: Test solubility in pyridine
      - Soluble: Melamine-formaldehyde resin. Confirm by Tests III and VI
      - Insoluble: Urea-formaldehyde resin. Confirm by Tests III and VI
    - Positive: Casein-formaldehyde resin. Confirm by weak sulfur test and Test VI

Table V. Properties of Resins in Group B

Test No.	Property Sought	Nitrocellulose	Urea-Formaldehyde Resin	Melamine-Formaldehyde Resin	Polyamide Resin	Casein-Formaldehyde Resin	Butadiene-Acrylonitrile Copolymer	Gelatin Glue Coagulated Protein
I	Solubility							
	95% ethanol	P.S.G.	I	I	I	I	I	I
	Acetone	S	I	I	I	I	P.S.G.	I
	Ether	I	I	I	I	I	I	I
	Ethyl acetate	S	I	I	I	I	IG	I
	Dioxane	S	I	I	I	I	IG	I
	Pyridine	S	I	S	I	I	IG	I-P.S
	Acetic acid	S	I	S	I	I	I	I-S
	Carbon tetrachloride	I	I	I	I	I	I	I
	Tetrachloroethane	I	I	P.S	I	I	IG	I
	Benzene	I	I	I	I	I	IG	I
	Hot water	..	..	..	..	..	..	S-I
II	Liebermann-Storch	NO_2 evolved	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
III	Carbonate fusion							
	Odor	Burning paper	NH_3	Formaldehyde	NH_3 + burning hair	NH_3 + burning hair	Like hot aniline	Burning hair and NH_3
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Nil	Pos.
	Fume	Sl.	Nil	Nil	Sl.	Sl.	Cons. white	V.Sl.
IV	Nitrates	Blue	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
VI	Odor on ignition	.....	NH_3 + burning hair	Formaldehyde	NH_3	Burning hair	Like hot aniline	Burning hair
	Char	.....	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
	Fume	.....	V.Sl.	Sl. white	Sl.	Heavy yel.	Heavy yel.	Heavy
	Distillate	.....	Nil	Nil	Sl.	Sl.	Cons.	Nil
XI	Formaldehyde	Red	Purple	Strong red violet	Neg.	Pale violet	Neg.	Neg.
XII	Xanthoproteic	....	Neg.	Neg.	Neg.	Pos.	.....	Weakly pos.
	Refractive index 20° C.	1.50	1.55	1.55	1.54	.....	1.52	.....
	Specific gravity $\frac{20° C.}{20° C.}$	1.4	1.16	1.16	1.1	1.35	0.96–1.01	.....

of aqueous 0.5 *N* sodium hydroxide and warm under reflux until the resin is all in solution but not less than 1 hour. If the resin does not dissolve, allow it to reflux overnight. Titrate excess sodium hydroxide with 0.5 *N* hydrochloric acid, using phenolphthalein or other indicator. For very dark solutions "Universal indicator" has proved useful. Where the resin is undissolved, vigorous shaking during titration is required to neutralize alkali absorbed by the swollen resin.

Table VI. Separation of Group C

(Halogens absent, nitrogen and sulfur present)

- Test solubility in hot water
  - Soluble: Gelatin. Confirm by Tests III and XI
  - Insoluble: Apply Test III
    - Odor of burning hair. Casein-formaldehyde resin. Confirm by Tests VI, XI, and XII
    - Odor of formaldehyde. Sulfonamide resins. Resins vary from viscous fluid to low-melting solids. Confirm by Acid No., Saponification No., and Test XI
    - Odor like hot aniline. See butadiene-acrylonitrile copolymer in Group B

$$\text{Saponification number} = \frac{56.1 \times \text{normality of NaOH} \times \text{cc. of NaOH used by sample}}{\text{weight of sample}}$$

Acetyl Number. Weigh about 2 grams of the sample accurately and place it in a Pyrex Erlenmeyer flask, add 20 cc. of pyridine-anhydride reagent, and place on a steam bath under an air condenser. Treat a blank on the reagents similarly. When all is in solution or after several hours if the resin only swells, add 25 cc. of neutral ethylene dichloride or benzene, stopper the flask, and shake vigorously. The resin should be dissolved or well broken up by this treatment. Add 100 to 150 cc. of distilled water and titrate with 0.5 *N* sodium hydroxide, using about twice the usual amount of phenolphthalein indicator. The titration requires vigorous shaking to remove the acid from the solvent layer and the red color should be permanent for at least one minute.

Reagent. 880 cc. of pyridine (Barrett's 2A grade), 120 cc. of 95% or better acetic anhydride.

$$\text{Acetyl number} = \frac{(\text{cc. of NaOH blank} - \text{cc. of NaOH sample}) \times 56.1 \times \text{normality of NaOH}}{\text{weight of sample}}$$

TESTS USED IN SCHEMATIC PROCEDURE AND FOR CONFIRMATION

Test I. Solubility. Place about 1 gram of sample in a test tube with 10 cc. of solvent, shake at room temperature several hours, then note carefully whether the resin is soluble, partly soluble, or insoluble, or whether there is any color in the solvent or resin.

Test II. Liebermann-Storch Reaction. Place a small fragment of resin on a spot plate and cover with a few drops of acetic anhydride. Now add 1 drop of concentrated sulfuric acid, so that it enters the liquid. Note the color reactions in the liquid and on the resin surface. Observe over a period of half an hour. List the colors in the order of their formation.

Test III. Odor on Carbonate Fusion. This test suppresses the acid constituents in the volatile decomposition products and allows some odors to be more readily recognized.

Fuse a piece of resin with 1.25 cm. (0.5 inch) of anhydrous sodium or potassium carbonate in a test tube. Note odors, fumes, and tendency to char.

Test IV. Nitrate. Dissolve a few crystals of diphenylamine in about 0.5 cc. of 90% sulfuric acid and place a drop of this reagent on a piece of the resin on a spot plate. An immediate intense blue color indicates nitrocellulose or esters such as cellulose nitroacetate. Even a few per cent of nitrocellulose in another resin will yield this test, but the color will develop more slowly.

Table VII. Properties of Resins in Group C

Test No.	Property Sought	Sulfonamide Resins[a] M.S.	M.H.P.	K	Gelatin, Glue, Albumenoids	Casein-Formaldehyde Resin
I	Solubility					
	95% ethanol	S	S	S	I	See under Group B. Sulfur test weakly positive
	Acetone	S	S	S	I	
	Ether	P.S	S	Sl.S.G.	I	
	Ethyl acetate	S	S	P.S.G.	I	
	Dioxane	S	S	S	I	
	Pyridine	S	S	S	I	
	Acetic acid	S	S	S	IG	
	Carbon tetrachloride	I	I	IG	I	
	Tetrachloroethane	S	S	S	I	
	Benzene	S	S	S	I	
	10% NaOH	P.S	S	I	P.S	
	Hot water	I	I	I	S	
II	Liebermann-Storch	Neg.	Neg.	Neg.	Neg.	
III	Carbonate fusion					
	Odor	Formaldehyde	Formaldehyde + aromatic	Formaldehyde + aromatic	Burning hair + NH_3	
	Char	Pos.	Pos.	Pos.	Pos.	
	Fume	Sl.	Sl.	Sl.	V.Sl.	
VI	Odor on ignition	Formaldehyde	Formaldehyde + aromatic	Formaldehyde	Burning hair	
	Char	Pos.	Pos.	Pos.	Pos.	
	Fume	Sl.	Sl.	V.Sl.	Heavy	
	Distillate	Sl.	Sl.	Sl.	Nil	
XI	Formaldehyde	Deep violet	Deep violet	Violet	Neg.	
	Refractive index 20° C.	1.59	1.596	1.56	.....	
	Specific gravity $\frac{20° C.}{20° C.}$	1.36	1.35	1.31	.....	

[a] Monsanto designations. M.S., soft, low melting point solid. M.H.P., solid, softening about 62° C. K, viscous fluid.

Table VIII. Separation of Group D[a]

(Halogens and nitrogen absent, sulfur present)

Test solubility in pyridine

Soluble: Organic polysulfides. Confirm by Test VI	Insoluble: Test solubility in carbon tetrachloride	
	Soluble: Isobutylene copolymer with diolefins. Confirm by weak sulfur test, Test VI, and solubility in tetrachloroethane and benzene	Insoluble: Vulcanized rubber. Confirm by Test VI. Beware of compounded butadiene-styrene copolymer. Odor of Test VI not strong with this resin

[a] Some crude dark cumarone resins and possibly others will show strong test for sulfur. If solubilities show substance definitely foreign to this group, ignore sulfur test and proceed to test for other groups.

Table IX. Properties of Resins in Group D

Test No.	Property Sought	Organic Polysulfide Rubber	Vulcanized Rubber	Isobutylene Copolymer with Diolefins
I	Solubility			
	95% ethanol	I	I	I
	Acetone	I	I	I
	Ether	I	I	I
	Ethyl acetate	I	I	I
	Dioxane	S	I	I
	Pyridine	S	I	I
	Acetic acid	I	I	I
	Carbon tetrachloride	I	I	S
	Tetrachloroethane	S	I	S
	Benzene	I	I	S
II	Liebermann-Storch	Pale red violet to brown to dark red brown	Neg.	Neg.
VI	Odor on ignition	Mercaptan + pungent	Burning rubber	Sl. aromatic
	Char	Pos.	Pos.	Sl.
	Fume	Heavy yel.	Heavy yel.	Cons. white
	Distillate	Cons.	Nil	Sl.
	Refractive index 20° C.	.....	1.52	1.52
	Specific gravity $\frac{20° C.}{20° C.}$	1.34	1.1–1.18	0.92

TEST V. CARBOXYLIC ESTERS (*5*). Place a small piece of resin in a clean test tube and add 1 cc. of 6% alcoholic (water-white) sodium or potassium hydroxide, then 1 drop of a saturated alcoholic solution of hydroxylamine hydrochloride. Shake and let stand 5 minutes. Heat for about 30 seconds while boiling, add 1 drop of 1% aqueous ferric chloride solution, and add carefully just sufficient 10% aqueous hydrochloric acid to dissolve the ferric hydroxide precipitate; then cautiously add a few drops in excess. A strong violet color indicates carboxylic acid esters. The color may be so strong that dilution with water is necessary in order to note it.

Too much hydrochloric acid will destroy the color and is to be avoided, but in negative tests a sufficient excess should be added (1 cc.) drop by drop to remove all doubt of the possibility that it is insufficient.

TEST VI. ODOR ON IGNITION. Heat a piece of resin strongly in a test tube, and note odor, fumes, charring, and presence of distillate.

TEST VII. TEST FOR PHTHALATES. Heat about 1 gram of resin with about 2 to 3 grams of pure phenol plus 5 drops of concentrated sulfuric acid until the melt turns orange or brown. Cool, dilute with water, and render alkaline with 10% aqueous sodium hydroxide. Characteristic red color of phenolphthalein indicates the presence of phthalates.

This test is preferred to the fluorescein test for phthalates and is less subject to error due to inexperience.

TEST VIII. TEST FOR PHENOLIC RESINS. This is the reverse of the test for phthalates. Heat about 1 gram of resin with about 1 gram of phthalic anhydride and 3 drops

Table X. Separation of Group E

(Halogens, nitrogen, and sulfur absent. Saponification Nos. over 325)

Apply Test II						
Resin slowly turns green			Resin slowly turns slightly brown.			Negative. Apply Test XI
Saponification Nos. 325–500, polyvinyl alcohol - acetate, with high polyvinyl acetate. Acetyl No. high	Saponification Nos. 500–700, polyvinyl esters. Apply Test XI		Apply Test V		Color of liquid changes to deep orange, polybasic acid from rosin. Confirm by Tests VI and XI. Solution in hot phenol and 3 drops of H_2SO_4 gives brilliant red which disappears on adding NaOH	Strong red, cellulose acetate. Confirm by Tests XIII and VI
	Red color, polyvinyl acetate. Confirm by characteristic odor. Test VI	Negative or pale violet or orange, vinyl ester copolymers	Red violet color, cellulose acetate. Confirm by Tests VI and XI. Insoluble in benzene	Negative, polymethyl acrylate. Confirm by Test VI. Soluble in benzene		Pale yellow or negative, cellulose aceto - propionate or butyrate. Confirm by Test VI and solubility

Table XI. Properties of Resins of Group E

Test No.	Property Sought	Polyvinyl Resins: Polyvinyl acetate	Polyvinyl Resins: Copolymers of vinyl acetate and fumarates or maleates	Polyvinyl Resins: High acetate polyvinyl alcohols	Cellulose Esters: High acetyl cellulose acetate	Cellulose Esters: Cellulose acetopropionate	Cellulose Esters: Cellulose acetobutyrate	Poly-methyl Acrylate	Rosin Polybasic Acid
I	Solubility								
	95% ethanol	S	S-IG	S-P.S.G.	I	I	I	IG	S
	Acetone	S	S-Sl.S.G.	IG	S-IG	S	S	S	S
	Ether	IG	IG-I	IG	I	I	I	IG	S
	Ethyl acetate	S	S-I	IG	S-I	S	S	S	S
	Dioxane	S	S-Sl.S.G.	IG	S	S	S	S	S
	Pyridine	S	S-Sl.S.G.	S	S-P.S.G.	S	S	S	S
	Acetic acid	S	S-IG	S-P.S.G.	P.S.G.	S	S	S	S
	Carbon tetrachloride	S	S-IG	I	P.S.G.-I	I	IG	IG	IG
	Tetrachloroethane	S	S-Sl.S.G.	S-I	S	S	S	S	P.S.G.
	Benzene	S	S-IG	IG	IG	I	IG	S	IG
II	Liebermann-Storch	Resin slowly turns green	Resin slowly turns green	Resin slowly turns green	Neg. or resin slightly brown	Neg.	Neg.	Resin slowly turns light brown	Dark orange
V	Carboxylic esters	Strong red violet	Strong red violet	Red-violet	Strong red violet	Strong red violet	Strong red violet	Neg.	Neg.
VI	Odor on ignition	Characteristic pungent	Ethereal + pungent	Pungent	Burning paper	Burning paper	Burning paper	Acrylate	Pine
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	V.Sl.
	Fume	Heavy yel.	Heavy yel.	Heavy	Sl.	Cons.	Heavy white	Heavy white	Nil
	Distillate	V.Sl.	Sl.	V.Sl.	Nil	Sl.	V.Sl.	Much	Complete
XI	Formaldehyde	Red	Neg. or red to orange	Neg. to V. pale red	Red	Pale Yel.	Neg.	Neg.	Orange
XIII	Acetates	Purple to black	Blue to black	Purple	Red orange	Neg.	Neg.	Red orange	Neg.
	Acid No.	4.6	4.8	4	5	2	3	3	29
	Saponification No.	600	400–600	325–540	550	500	500	375	354
	Refractive index 20° C.	1.47	.....	1.47–1.51	1.48	1.47	1.49	1.49	...
	Specific gravity $\frac{20° C.}{20° C.}$	1.19	.....	1.2–1.26	1.27	1.29	1.20	1.2	1.15

Table XII. Separation of Group F

(Halogens, nitrogen, and sulfur absent. Saponification Nos. 120 to 325)

Test solubility in ether

Insoluble: Test solubility in acetone

Insoluble: Do Test VI

Odor: Oily and acrolein, phenol and oil-modified alkyd. Confirm by Tests II and VII

Odor: Characteristic pungent, polyvinyl alcohol, medium acetate type. Confirm: Soluble in water. Tests XI and XIII

Soluble: Test solubility in 95% ethanol

Partly soluble gel. Do Test VI

Odor: Formaldehyde, regular alkyd. Confirm by Test VII

Odor: Acrolein and oily, oil-modified alkyd. Confirm by Test VII

Soluble: Apply Test VI

Odor: Butaldehyde, polyvinyl butylal or coacetal. Confirm by Tests III, XI, and XIV

Odor: Characteristic pungent, polyvinyl acetal of low hydrolysis. Confirm by Tests III, XI, and XIV

Soluble: Apply Test VII

Negative: Butyl phenol formaldehyde. Confirm by Tests I, II, VI, and XIV

Positive: Oil-modified alkyd. Confirm by Tests II, VII, and XII

Table XIII. Properties of Resins in Group F

Test No.	Property Sought	Polyvinyl Acetals, 70% Hydrolysis	Polyvinyl Coacetal, BuH-AcH, 20% Ac, 12% OH	Polyvinyl Alcohols, Medium Acetate Type	Butyl Phenol Formaldehyde	Alkyds: Regular	Alkyds: Oil-modified	Alkyds: Phenol and oil-modified
I	Solubility							
	95% ethanol	S	S	I	P.S.G.	P.S.G.	P.S.G.	IG
	Acetone	S	S	I	S	S	S	I
	Ether	IG	IG	I	S	Sl.-P.S.G.	S-I	I
	Ethyl acetate	S	S	I	S	S	..	..
	Dioxane	S	S	I	S	S	..	..
	Pyridine	S	S	I	S	S	..	..
	Acetic acid	S	S	I	S	S	..	..
	Carbon tetrachloride	IG	IG	I	S	IG	I	I
	Tetrachloroethane	S	S	I	S	S	..	..
	Benzene	S	IG	I	S	IG	..	..
II	Liebermann-Storch	Resin orange to dark brown	Resin red to red brown to brown	Resin light brown to red brown	Violet to brown to red brown to muddy green	Usually neg. rarely brown	Brown to dark brown	Red to brown red to dark brown
III	Carbonate fusion							
	Odor	Pungent AcH	Pungent BuH	Pungent	Balsam + formaldehyde	Formaldehyde + musty	..	..
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	..	..
	Fume	Heavy	Heavy	Heavy	Heavy	V.Sl.	..	..
V	Carboxylic esters	Red violet	Red violet	Red violet	Neg.	Violet	Violet	Red violet
VI	Odor on ignition	Pungent characteristic	Pungent BuH	Pungent characteristic	Phenol or cresol	Formaldehyde	Oily + acrolein	Oily + acrolein
	Char	Pos.	Pos.	Pos.	Pos.	Sl.	Nil	Pos.
	Fume	Heavy	Heavy	Heavy	Heavy brown	V.Sl.	Sl.	Sl.
	Distillate	Cons.	Nil	Nil	Cons.	Much	Complete	Cons.
VII	Phthalates	..	..	..	Neg. or weak pos.	Strong pos.	Pos.	Pos.
IX	Phenols	..	..	..	Red	Neg.	Neg.	Neg.
XI	Formaldehyde	Red	Orange	Red	V.Sl. violet	Neg.	Neg.	Neg.
XII	Xanthoproteic reaction	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.
XIII	Acetates	Red to green to blue	Slowly green	Violet to blue	Neg.	Neg.	Neg.	Neg.
XIV	Aldehydes	Red violet	Red violet	Neg.	Red violet	Neg.	Weakly pos.	Neg.
	Acid No.	6	8	4	2	20–50	25–50	10–30
	Saponification No.	255	140	120–325	125	150–250	140–225	150–250
	Refractive index 20° C.	1.46	....	1.51–1.55	1.66	1.57	....	....
	Specific gravity $\frac{20° C.}{20° C.}$	1.16	....	1.28–1.31	1.099	1.32	....	....

Table XIV. Separation of Group G

(Halogens, nitrogen, and sulfur absent. Saponification Nos. less than 120. Acetyl Nos. over 40)

A. Test solubility in hot water

- Soluble: Test solubility in benzene
  - Soluble: Polyethylene glycol waxes. Confirm: Soluble in dioxane, insoluble in ethyl acetate
  - Insoluble: Apply Test V
    - Positive: Polyvinyl alcohols, less than low acetate type. Confirm by Tests II and XIII
    - Negative: Methyl cellulose. Confirm by Tests II, VI, and XIII
- Insoluble: Test solubility in carbon tetrachloride (B)

B. Solubility in carbon tetrachloride

- Soluble: Test solubility in ether
  - Insoluble: Phenylphenol formaldehyde resin. Confirm by Tests VI and XII
  - Soluble: Do Test IX
    - Negative: Do Test XII
      - Negative: Ethyl cellulose. Confirm by Tests II and VI
      - Positive: Phenol indene cumarone. Confirm by Tests II and VI
- Insoluble: Test solubility in ethyl acetate (C)
  - Positive: Test solubility in 95% ethanol
    - Soluble: Substituted phenol formaldehyde. Confirm by Tests II, XII, and XIV
    - Insoluble: Butyl phenol formaldehyde. Confirm by Tests VI and XII

C. Solubility in ethyl acetate

- Soluble: Do Test VI
  - Odor of burning paper, ethyl cellulose. Confirm by Tests II and XIV
  - Any other odor: Test solubility in ether
    - Soluble
      - Odor of Test VI cresol. Shows cresol acetaldehyde. Confirm by Tests II, IX, and XII
      - Odor of Test VI slight. Shows modified rosin. Confirm by Test II. Resin is dark red color
- Insoluble: Test solubility in acetic acid (D)
  - Soluble: Do Test XI
    - Violet, polyvinyl formal. Saponification No. 20. Confirm by Tests II, V, and VI
    - Red, polyvinyl butylal. Acetyl No. about 250. Confirm by Tests II, V, and VI
  - Insoluble: Phenolic resin. Confirm by Tests VI, XI, XII, and XIV

D. Solubility in acetic acid

- Soluble: Test solubility in benzene
  - Soluble: Test solubility in 95% alcohol
    - Soluble: Do Test XI
      - Brown: Polyvinyl acetal. Saponification No. 30. Confirm by Tests II, V, VI, and XIV
      - Red: Polyvinyl butylal. Acetyl No. about 150. Confirm by Tests II, V, VI, and XIV
    - Insoluble: Polyvinyl butylal. Acetyl No. about 100. Confirm by Tests II, V, VI, XI, and XIV
  - Insoluble: Polyvinyl formal. Saponification No. about 100. Confirm by Tests II, V, VI, and XI
- Insoluble: Test solubility in 95% alcohol
  - Soluble: Rosin modified alkyd. Confirm by Tests II and VI
  - Insoluble: Benzyl cellulose. Confirm by Tests II, VI, and XIV

of concentrated sulfuric acid until a rich brown melt develops, cool, dilute with water, and render alkaline with 10% aqueous sodium hydroxide. Characteristic red color of phenolphthalein indicates presence of phenols. In cases where tarry matter obscures the color, dilute with water and confirm by discharging the color by acid. All phenolics tested, with the exception of an oil-modified one, gave a positive reaction with this test.

Test IX. Millon's Reagent for Phenolic Resins. Prepare the reagent by dissolving 10 grams of mercury in 10 grams of fuming nitric acid without heating, then dilute with twice its volume of water, and filter off any precipitate, or allow it to settle.

Heat a small piece of resin with 1 cc. of clear reagent and boil about 2 minutes. A red color indicates phenols.

As the test is characteristic of the phenol group it is also given by some proteins. The absence of nitrogen will, however, direct the test to phenolic resins. A few phenolic resins fail to yield a positive test.

Test X. Cumarone-Indene Resins. This is a modified form of Ellis test (*2*, p. 1261 footnote; *3*). With the latter it was found very difficult to decide whether the color was due to bromine or the resin. The modification gives a positive test with the usual cumarone resins but is negative with the low molecular weight polymers.

Dissolve 0.1 to 0.5 gram of resin in 10 cc. of chloroform, add 1 cc. of glacial acetic acid and 1 cc. of 10% bromine solution in chloroform, and let stand overnight. A red color indicates cumarone resins.

Do a blank at the same time.

Using 1 cc. of the highly colored solution, add about 1 to 2 cc. of 0.1 *N* sodium thiosulfate and shake vigorously. The blank will discharge to a light yellow color in the chloroform layer. A red color in the chloroform layer is evidence of the presence of high or medium molecular weight cumarone resins.

Test XI. Formaldehyde (*6*). Mix a small piece of resin and 2 cc. of 72% sulfuric acid (100 cc. of water and 150 cc. of concentrated sulfuric acid) plus a few crystals of chromotropic acid and heat by standing the test tube in a beaker of water at 60° to 70° C. for 10 minutes. Run a blank at the same time to avoid chance contamination from the laboratory air. A bright violet color indicates formaldehyde. Note the color after standing 1 hour at room temperature.

Test XII. Xanthoproteic Reaction. This test depends upon the presence of a phenyl group and is usually used to identify certain proteins which contain it. It is also shown by some oils and phenolic resins. It is sometimes useful as a confirmatory reaction.

Warm a small piece of resin with concentrated nitric acid for several minutes, cool, and add an excess of ammonium hydroxide. In the presence of a phenyl group the nitric acid is yellow, changing to an orange on addition of the ammonum hydroxide.

Test XIII. Acetates (*7*). Add a 5% aqueous solution of lanthanum nitrate and 1 drop of 0.1 *N* iodine solution, followed by a drop of concentrated ammonium hydroxide, to a piece of the resin on a spot plate.

In the presence of acetates or propionates a brown or blue coloration quickly develops in the resin. This may occur before the ammonium hydroxide is added and indicates addition of iodine to the resin.

When in doubt, warm a piece of resin with a few drops of concentrated hydrochloric acid in 1 cc. of water for about 10 minutes and apply the test to about 0.5 cc. of the water, making sure sufficient ammonium hydroxide is added to render it ammoniacal.

Test XIV. Aldehydes in Acetals (*4*). Heat a small piece of resin plus 1 cc. of reagent and 0.4 cc. of concentrated sulfuric acid on a steam bath for 2 to 3 minutes, then cool. Add a few drops of pure methanol and a layer of chloroform, then 0.5 cc. of concentrated hydrochloric acid, and shake the tube well. In the presence of aldehydes a red to purple color appears in the chloroform.

Reagent: 0.01 gram of azobenzene phenylhydrazine sulfonic acid in 100 cc. of distilled water.

CLASSIFICATION ACCORDING TO TYPES AND GENERAL REACTIONS

Where the substance does not appear to give the comfirmatory tests following its systematic separation by the above scheme, or where, because of its history, distinction between only a few substances is required, this classification according to types, with the reactions generally shown by them, will be found useful:

Acrylate Resins. Light-colored resins. n_D^{20} about 1.49. Specific gravity 1.2. Usually without filler. Soluble in acetone, esters, benzene; insoluble in CCl_4, 95% ethanol, ether. Test VI, sickly sweet odor of monomer, with practically complete distillation.

Alkyd Resins. Usually light color. n_D^{20} 1.54–1.59. Specific gravity 1.1–1.4. Test II, usually brown. Test V, usually positive. Test VI, formaldehyde, oily or acrolein odor, considerable distillation. Test VII, usually positive. Usually insoluble in 95% ethanol, ether, CCl_4.

Amino and Protein Resins. Usually light-colored. n_D^{20} 1.55. Specific gravity 1.1–1.35. All are insoluble in the solvents listed except melamine-formaldehyde, which is soluble in pyridine or acetic acid, and gelatin which is water-soluble.

Table XV. Properties of Resins in Group G

Test No.	Property Sought	Polyvinyl Acetals: Formaldehyde 75% hydrolyzed	Formaldehyde 95% hydrolyzed	Acetal 90% hydrolyzed	Butylal 95% Hydrolyzed: 8% OH	12% OH	20% OH	Polyvinyl Alcohol, Low Acetate Type	Polyethylene Glycol Waxes	Modified Rosin (Vinsol)
I	Solubility									
	95% ethanol	IG	I	S	I.Sl.G.	S	S	I	S	S
	Acetone	P.S.G.S	I	S	S	S	IG	I	S	S
	Ether	I	I	IG	IG	IG	I	I	I	S
	Ethyl acetate	S	I	S	S	S	IG	I	I	S
	Dioxane	S	S	S	S	S	S	I	S	S
	Pyridine	S	S	S	S	S	S	I	S	S
	Acetic acid	S	S	S	S	S	S	I	S	S
	Carbon tetrachloride	I	I	IG	IG	IG	IG	I	I	IG
	Tetrachloroethane	S	S	S	S	S	S	I	S	S
	Benzene	IG	I	S	S	S	IG	I	S	I
	Xylene	...	...	I	S	I	I	...	...	....
	Hot water	I	I	I	I	I	I	S	S	I
II	Liebermann-Storch	Resin slowly turns sl. green	Resin slowly turns bistre	Resin turns orange to red brown to dark brown	Resin turns orange to red brown to dark brown	Resin turns yel. to dark brown	Resin turns yel. to dark brown	Resin turns brown to sl. green	Neg.	Violet to brown to black
V	Carboxylic esters	Red violet	Red violet	Red violet	Red violet	Red violet	Pale violet	Red violet	Neg.	Neg.
VI	Odor on ignition	Formaldehyde	Formaldehyde	Characteristic pungent	BuH	BuH	BuH	Pungent	Pungent	V.Sl.
	Char	Pos.	Sl.	Sl.	Sl.	Sl.	V.Sl.	Sl.	Sl.	Pos.
	Fume	Cons.	Heavy white	Sl. white	Sl.	Sl.	Heavy	Cons.	Nil	Heavy yel.
	Distillate	Nil	Sl.	Sl.	Sl.	Nil	Nil	Nil	Complete	Much
VII	Phthalates	...	...	....	....	....	....	...	...	....
IX	Phenolic	...	...	....	....	....	....	...	...	....
XI	Formaldehyde	Deep violet	Deep purple	Brown	Red	Red	Red	Neg.	Neg.	Orange
XII	Xanthoproteic reaction	...	...	....	....	....	....	...	...	....
XIII	Acetates	Blue	Green	Red orange	Neg.	Neg.	Neg.	Blue	...	....
XIV	Aldehydes	Neg.	Neg.	Red violet	Red violet	Red violet	Red violet	Neg.	...	....
	Acid No.	4	4	5	5	4	4	4	3	117
	Saponification No.	100	20	30	10	10	10	119–0	0	0
	Acetyl No.	65	70	80	100	150	250	1080–1270	40–215	160
	Refractive index 20° C.	...	1.50	1.46	....	....	1.489	1.545–1.555	...	1.61
	Specific gravity $\frac{20° C.}{20° C.}$	...	1.23	1.16	....	1.18	1.11	1.31–1.33	1.15–1.20	1.20

Test No.	Property Sought	Rosin Modified Alkyd	Cellulose Ethers: Methyl	Ethyl	Benzyl	Phenolic Resins: Cresol-AcH	Butyl phenol formaldehyde	Phenyl phenol formaldehyde	Substituted phenol formaldehyde	Phenolic resin	Phenol Indene Cumarone
I	Solubility										
	95% ethanol	S	I	P.S.G.S	I	S	P.S.G.	I	S	IG	P.S.G.-S
	Acetone	S	I	I-S	Sl.S.G.	S	S	S	S	IG	S
	Ether	P.S.G.	I	I-S	I	S	S	Sl.S.G.	S	IG	S
	Ethyl acetate	S	I	S	S	S	S	S + G	S	IG	S
	Dioxane	S	IG	S	S	S	S	S	S	P.S.	S
	Pyridine	S	IG	S	S	S	S	S + G	S	S	S
	Acetic acid	IG	IG	S	I	S	S	Sl.S.G.	S	IG	S
	Carbon tetrachloride	IG	I	I-S	I-G	I	S	S	S	I	S
	Tetrachloroethane	S	P.S.G.	S	S	S	S	S + G	S + G	S	S
	Benzene	S	IG	I-S	I	IG	S	S	S	I	
	Xylene	.....	.....	I-S	.....	...	.....	.....	....	I	....
	Hot water	I	S	I	I	I	.....	I	I	I	....
II	Liebermann-Storch	Red violet to brown	Resin slowly turns yel. to olive	Orange to brown to black	Resin slowly turns orange to light brown	Red brown to orange	Violet to brown to red brown to muddy green	Neg	Yellow to red to red brown	Sl. pink	Scarlet to red brown to brown
V	Carboxylic esters	V. pale violet	Neg.	Neg.	Neg.	Neg.	.....	Neg.	....	....	....
VI	Odor on ignition	Formaldehyde	Burning paper and pungent	Burning paper	Benzald. pungent	Cresol	Phenol or cresol	Pungent formaldehyde	Coal gas	Phenolic + formaldehyde	Coal tar or indenelike
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Nil	Much	Nil
	Fume	Cons. yel.	Cons.	Heavy white	Heavy yellow	Cons. brown	Heavy brown	Nil	Nil	Heavy brown	V.Sl.
	Distillate	Cons.	Nil	Sl.	Sl.	Cons.	Cons.	Cons.	Complete	Sl.	Complete
VII	Phthalates	Weakly pos.	.....	.....	.....	Neg.	Neg. or weak pos.	Neg.	....	....	....
IX	Phenolic	Neg.	.....	.....	.....	Red	Red	Neg.	Red	Red weak	Neg.
XI	Formaldehyde	Neg.	Neg.	Pale violet	Neg.	Neg.	V. Sl. violet	Neg.	Neg.	Violet	Yellow
XII	Xanthoproteic reaction	.....	.....	.....	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
XIII	Acetates	.....	Red	Neg.	Neg.	...	Neg.	.....	....	....	....
XIV	Aldehydes	.....	V. weakly pos.	Red violet	Purple	...	Red violet	.....	Red violet	Red	....
	Acid No.	7	3	6	3	49	2	50	8	....	6
	Saponification No.	0	0	0	0	0	120	0	0	54	0
	Acetyl No.	90	120	65	100	370	295	270	127	450	150
	Refractive index 20° C.	1.54	.....	1.47	1.47	...	1.66	1.47–1.7	....	....	1.6–1.66
	Specific gravity $\frac{20° C.}{20° C.}$	1.14	.....	1.14	1.22	1.2	1.099	1.21–1.27	1.23	....	1.116

Table XVI. Separation of Group H

(Halogens, nitrogen, and sulfur absent. Saponification Nos. less than 120. Acetyl Nos. less than 40)

A. Test solubility in ether

- Soluble: Test solubility in 95% ethanol
  - Soluble: Test solubility in acetic acid
    - Soluble: Test for acid No.
      - Acid No. over 100, hydrogenated rosin. Confirm by Tests II and VI. Resin is brittle. Solid
      - Acid No. low, ethyl abietate. Confirm by Tests II and IV. Resin is viscous fluid
  - Insoluble: Do Test XII
    - Positive: Dihydromethyl abietate. Confirm by Test VI. Only partly soluble in pyridine
    - Negative: Low-polymer cumarone oil. Confirm by Test VI. Soluble in pyridine
- Insoluble: Test solubility in acetic acid (B)
  - Insoluble: Test solubility in dioxane
    - Insoluble: Terpene resin. Confirm: Soluble in ligroin, insoluble in pyridine or acetone
    - Soluble: Do Test X
      - Positive: Cumarone or polyindene resins. Confirm by Tests II and VI
      - Negative: Do Test II
        - Negative or pale orange, hydrogenated cumarone indene resin. Confirm by Tests VI and XIV. Light-colored resin, insoluble in acetone and ligroin
        - Positive: Red or purplish red, may change to green or brown, cumarone resin. Low m.p. semifluid resins are "soft" type. Harder solids "medium" type. Confirm by Tests VI and X. Mostly soluble in ligroin

B. Solubility in acetic acid

- Soluble: Polymethyl methacrylate. Confirm by Test VI. Soluble in CCl_4
- Insoluble: Apply Test X
  - Positive: High m.p. cumarone resin. Confirm by Tests II and VI
  - Negative: Test solubility in dioxane
    - Soluble: Polystyrene. Confirm by Tests II and VI. Insoluble in acetone
    - Insoluble: Test solubility in benzene
      - Soluble
        - Resin usually dark and may contain some sulfur, isobutylene copolymer with diolefins. Confirm by Test VI. Soluble in CCl_4
        - Resin light colored, polyisobutylene. Confirm by Test VI. Soluble in CCl_4
      - Insoluble: Butadiene-styrene copolymer. Confirm by Tests II and VI. Insoluble in CCl_4

Test III, NH_3 with burning hair odor characteristic of all except melamine-formaldehyde which gives a formaldehyde odor. Test VI, similar to Test III. Test XI, positive with urea-formaldehyde, melamine-formaldehyde, casein-formaldehyde. Negative with polyamide resin and gelatin. Test XII, positive with gelatin and casein-formaldehyde resins only.

Cellulose Esters. Usually light color. n_D^{20} 1.47–1.51. Specific gravity 1.2–1.4. Nitrocellulose burns rapidly, other esters burn slowly; former yields Text IV. Test V, positive except for nitrocellulose. Test VI, odor of burning paper. Test XI, negative to red. Saponification No., 500 to 550.

Cellulose Ethers. Usually light color. n_D^{20} 1.47. Specific gravity 1.10–1.25. Solubilities vary according to ethoxyl content, higher ethoxyl being more soluble. Methylcellulose soluble in hot water and insoluble in most other solvents. Test VI, odor of burning paper accompanied by benzaldehyde odor in case of benzyl cellulose. Test XIV, usually positive.

Chlorinated Diphenyls. Usually light color, vary from thin liquids to hard solids. n_D^{20} 1.61–1.71. Specific gravity 1.34–1.95. Yield strong halogen test. Soluble in all solvents listed in Table II. Test VI, slight acrid odor, much distillation.

Cumarone-Indene Resins. Vary from light- to dark-colored liquid or solid resins. n_D^{20} 1.6–1.66. Specific gravity 1.01–1.15. Insoluble in 95% ethanol and acetic acid. Usually soluble in ether, acetone, esters, dioxane, or pyridine.

Test II, red color characteristic which may change to brown or violet, rarely to green. Test VI, indenelike odor and almost complete distillation. Test X, positive with higher molecular weight polymers. Hydrogenated cumarone resin insoluble in acetone and Test II weak orange; otherwise similar.

Phenolic Resins. Vary from light to dark resins, usually solids. n_D^{20} 1.47–1.7. Specific gravity 1.1–1.27. Mostly soluble in pyridine, acetone, ether, tetrachloroethane, dioxane, and ethyl acetate, but usually insoluble in 95% ethanol. Heat-reacted forms may be insoluble in all solvents.

Test II, may be negative but browns predominate. Test VI, odor of phenol or formaldehyde with considerable distillation. Test IX, frequently positive. Test XII, positive. Test XIV, frequently positive.

Resin Products. Straw to highly colored liquids and solids. n_D^{20} 1.52–1.61. Specific gravity 1.03–1.22.

Soluble in most solvents used in solubility test with the following exceptions: Polybasic acid insoluble in CCl_4, benzene. Dihydromethyl abietate, partially soluble in pyridine or acetic acid. Red colored modified resin (Vinsol) insoluble in CCl_4 or benzene.

Table XVII. Properties of Resins of Group H

Test No.	Property Sought	Polymethyl Methacrylate	Terpene Resin	Polystyrene	Cumarone Resins: Low polymer oil	Cumarone Resins: Polyindene	Cumarone Resins: Hydrogenated cumarone indene resin	Cumarone Resins: High m.p. cumarone
I	Solubility							
	95 % ethanol	I	I	I	S	I	I	I
	Acetone	S	I	IG	S	S	I	S
	Ether	I	S	IG	S	S	S	S-I
	Ethyl acetate	S	Sl.S.G.	P.S.G.	S	S	S	S
	Dioxane	S	Sl.S.G.	S	S	S	S	S
	Pyridine	S	Sl.S.G.	S	S	S	S	S
	Acetic acid	S	I	I	I	I	I	I
	Carbon tetrachloride	I	S	S	S	S	S + G	S-I
	Tetrachloroethane	S	S	S	P.S	S	S	S
	Benzene	S	S	S	S	S	S	P.S-S
	Ligroin	I	S	I	....	....	I	....
II	Liebermann-Storch	Neg.	Neg.	Neg.	Scarlet to red violet	Bright red	Light orange	Red to purplish red to brown
V	Carboxylic esters	Neg.	Neg.	Neg.	Neg.	....	Neg.	....
VI	Odor on ignition	Sickly sweet odor of monomer	Like coal gas	Styrene	Indene	Indene	Indene	Indene
	Char	V.Sl.	Nil	Nil	Nil	Pos.	Nil	Sl.
	Fume	Sl. white	V.Sl.	Cons. white	Nil	Heavy yel.	Nil	Sl.
	Distillate	Complete	Complete	Complete	Complete	Much	Complete	Complete
X	Cumarone resins	Neg.	Neg.	....	Neg.	Pos.	Neg.	Pos.
XI	Formaldehyde	Neg.	Neg.	Neg.	Orange	Neg.	Neg.	Neg.
XII	Xanthoproteic reaction	...	...	....	Neg.	Neg.	Neg.	Neg.
XIII	Acetates	Neg.	Neg.	Neg.	....	....	Neg.	....
XIV	Aldehyde	...	...	....	....	....	Red	....
	Acid No.	4	2	2	2	7	6	2
	Saponification No.	20	0	0	0	0	0	0
	Acetyl No.	0	0	0	0	0	0	0
	Refractive index 20° C.	1.49	...	1.59	1.60	1.6–1.66	...	1.6–1.66
	Specific gravity $\frac{20° C.}{20° C.}$	1.19	...	1.05	1.01	1.10	...	1.10

Test II, red to violet color. Test V. If any odor it is pine or balsamlike, resin distills without residue. Saponification number low except for hydrogenated rosins and the polybasic acid. Acid number over 100 only in case of the Vinsol resin. Acetyl number zero except for Vinsol resin.

Rubber and Rubber Substitutes. *Halogen Containing.* Chloroprene is black rubbery resin, characteristic odor, insoluble in all solvents. Rubber hydrochloride and chlorinated rubber light colored. Former insoluble in all except tetrachloroethane; latter soluble in ethyl acetate, dioxane, and pyridine. Cashew nut oil polymer is sticky black resin soluble in pyridine only.

Butadiene Copolymers. Black rubbery solids. Butadiene-styrene copolymer insoluble in all solvents. Butadiene acrylonitrile copolymer insoluble in all solvents, contains nitrogen.

Polyisobutylenes and Copolymers with Diolefins. Light-colored fluids to rubbery solids. Copolymers usually dark colored.

Polysulfide Rubbers. Contain much sulfur. Specific gravity 1.34. Soluble in dioxane, pyridine, tetrachloroethane. Test II, red violet changing to brown. Test VI, mercaptan odor, considerable distillate.

Vulcanized Natural Rubber. Contains sulfur. n_D^{20} 1.52. Specific gravity 1.1–1.18. Insoluble in all solvents. Test VI, characteristic odor of burning rubber, no distillate.

Sulfonamide Resins. Light colored varying from soft viscous fluid to hard resins. n_D^{20} 1.56–1.60. Specific gravity 1.31–1.36. Soluble in most solvents shown in Table VI, insoluble in CCl_4. Test XI, strong violet.

Terpene Resin. Light-colored solid. Soluble in ether, CCl_4, tetrachloroethane, benzene, ligroin. Insoluble in 95% ethanol, acetone, dioxane, ethyl acetate, acetic acid. Test VI, odor like coal gas, distills completely.

Vinyl Resins. *Halogen-containing.* Strong test for halogen. Very light-colored solids. n_D^{20} 1.53–1.61. Specific gravity 1.2–1.75. Insoluble in ligroin and benzene. Test II, blue or green color slowly develops in resin.

Polyvinyl Esters. Colorless solids. n_D^{20} 1.47. Specific gravity 1.19. Polyvinyl acetate soluble in all the solvents except ether and ligroin. Copolymers insoluble in ether and ligroin but may also be insoluble in the other solvents.

Test II, resin turns green; this is characteristic. Test V, red violet. Test VI, characteristic odor, very slight distillate containing the acid usually acetic. Test XI, red to negative. Test XII, blue or purple to black. Saponification No. 400 to 600.

Polyvinyl Alcohol-Acetate. Light-colored resin. n_D^{20} 1.47–1.55. Specific gravity, 1.2–1.33. Low and medium acetates insoluble in everything except water. High acetates soluble in pyridine. Test II, green to brown color. Test V, red violet. Test VI, pungent acidic odor, no distillate. Test XIII, violet to blue or purple. Acetyl number, low acetate type, 1080 to 1270, Saponification No.: low acetate, 0 to 119; medium acetate, 120 to 325; high acetate, 325 to 540.

Polyvinyl Acetals. Light-colored resins. n_D^{20} 1.46–1.50. Specific gravity 1.11–1.23. All insoluble in ether, CCl_4, ligroin. All soluble in dioxane, pyridine, tetrachloroethane, or acetic acid.

Test II, usually brown. Test V, red violet. Test VI, characteristic odor of aldehyde indicates type; little distillate. Test XI, violet with formals, red or brown with acetals, red with butyrals. Test XIV, usually red violet, formals may be negative.

Polystyrene. Light-colored resin. n_D^{20} 1.59. Specific gravity 1.05. Soluble in dioxane, pyridine, CCl_4, tetrachloroethane, benzene. Insoluble in 95% ethanol, acetone, ether, acetic acid, ligroin. Test VI, odor of styrene, complete distillation. Saponification No. zero.

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Table XVII. Properties of Resins of Group H (*Continued*)

Cumarone Resins (*Cont'd*)		Rosin Products			Synthetic Rubbers		
Medium m.p. cumarone	Soft cumarone	Ethyl abietate	Dihydromethyl abietate	Hydrogenated rosin	Butadiene-styrene copolymer	Isobutylene copolymer with diolefins	Polyisobutylene
I	I	S	S	S	I	I	I
I-S	S	S	S	S	I	I	I
S	S	S	S	S	IG	I	Sl.S.G.
S	S	S	S	S	I	I	I
S	S	S	S	S	IG	I	I
S	S	S	P.S	S	IG	I	I
I	I	S	P.S	S	I	I	I
S	S	S	S	S	IG	S	S
....	....	S	S	S	IG	S	S
S	S	S	S	S	IG	S	S
P.S-S	S	....	....	....	IG	P.S	.....
Orange to brick red	Red to purplish red to green or brown	Red to violet to blue to black	Red to violet to purple to green	Red to violet to green to blue	Pale blue to gray green	Neg.	Neg.
....	....	Neg.	Neg.	Neg.	....	Neg.	.....
Indene	Indene	Pine	Pine	Faint balsam	Slight styrene	V.Sl. aromatic	Like coal gas
Nil	Nil	Nil	Nil	Nil	Pos.	Sl.	Nil
V.Sl.	V.Sl.	Nil	Nil	Nil	Cons. white	Cons. white	V.Sl.
Complete	Complete	Complete	Complete	Complete	Considerable	Sl.	Nil
Pos. to neg.	Pos. to neg.	....	....	....	....	Neg.	.....
Neg.	Neg. to weak violet	Red	Neg.	Neg.	....	Neg.	Pale violet
....	....	Pos.	Pos.	....	....	.....	.....
....	....	....	Neg.	Neg.	....	.....	.....
....	....	....	....	....	....	.....	.....
0	0	4	6	165	0	3	5
0	0	25	25	6	0	0	0
0	0	0	0	....	0	0	0
1.6–1.66	1.6–1.66	1.53	1.52	1.53	....	.....	1.51
1.10	1.10	1.03	1.03	1.06	0.94	0.92	0.912

Determination of Hydrocyanic Acid, Especially in Coke-Oven Gas

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The following method for rapid and accurate determination of hydrocyanic acid in coke-oven gas by a cyanogen bromide procedure, with a few changes should be of fairly general applicability in cyanide analysis. After absorption in potassium hydroxide in a special type of flask the sample is treated with an ammonium polysulfide solution to convert the cyanides to thiocyanates, thereby reducing the partial pressure of the free acid radical. The solution is then acidified at room temperature and carbon dioxide eliminated without appreciable loss of thiocyanate. Most of the gas is removed from the flask by at least partial evacuation, and, in the order named, the following reagents are added in excess: potassium bromide-bromate solution to convert thiocyanic acid to cyanogen bromide, phenol solution to eliminate excess bromine, and potassium iodide to reduce cyanogen bromide and substitute iodine. As this treatment is accomplished in an evacuated chamber, vapor pressure losses are zero. The liberated iodine is titrated with sodium thiosulfate solution. Studies were made which established conditions under which the action of the above reagents is substantially immediate. Laboratory time required for the analysis is about 15 minutes.

THE following procedure has been tried by the authors on many laboratory solutions and found satisfactory in contrast to other methods recommended for the determination of hydrocyanic acid by bromination followed by iodometric titration. It has been used successfully on coke-oven gas, where it is of particular value because of its wide range of applicability with respect to hydrocyanic acid concentrations. It also has a distinct advantage in that it integrates the analysis over a period of time as compared with the procedure of Seil (*11*) which employs an enlarged Tutwiler apparatus. This superiority is particularly desirable as the concentration of hydrocyanic acid in coke-oven gas often varies as much as perhaps 50% during a 30-minute interval. Incidentally, the Seil (*11*) method used on synthetic solutions of pure potassium cyanide standardized against silver nitrate gave results that were 16 to 20% high for quantities of hydrocyanic acid equivalent to a gas carrying 20 to 40 grains of hydrocyanic acid per 100 cu. feet, and a single test on a 2-grain gas showed 185% of the amount present. This appears to be due to the blank exhibited by dilute iodine solutions. (The Tutwiler iodine used in this test is approximately 0.013 *N*.) The Tutwiler procedure permits of no satisfactory compensation for this blank, nor can gas volume corrections be made.

The proposed method depends upon conversion of the cyanide or certain of its derivatives to cyanogen bromide, reduction of cyanogen bromide with potassium iodide, and titration of the liberated iodine with sodium thiosulfate solution. Several such procedures have been described in the literature. In the experience of the author and his associates, none of these have proved satisfactory, except perhaps under very restricted conditions. As the reactions involved have inherently great analytical advantages, a study of them was made which has resulted in the elimination of several sources of error, a widening of the scope of their application, and a considerable decrease in the time required for analysis. The laboratory time required for this analysis is about 10 to 15 minutes. Reproducibility obtained appears, in general, to be comparable to that involved in mechanical measurement of the standard solutions. This method was used to titrate a potassium thiocyanate solution carefully standardized by the Volhard procedure. The results varied from the Volhard by 1.5 parts per thousand. For the quantities (20-ml. titrations) the probable mechanical error was 2 parts per thousand.

SPECIAL APPARATUS AND SOLUTIONS REQUIRED

2 Shaw sulfur flasks (*13*).

Potassium hydroxide solution, 20%.

Ammonium polysulfide solution, which is prepared by taking 25 to 50 ml. of aqua ammonia, passing hydrogen sulfide through it at the rate of 2 to 3 bubbles per second, and adding an excess of micro sulfur to the solution. After about 15 minutes, the solution will be substantially saturated with sulfur and may be bottled for use. The ordinary analytical grade of yellow ammonium sulfide is not effective for this purpose.

Hydrochloric acid, concentrated reagent solution (1.18 sp. gr.). Bromide-bromate solution, 125 grams of potassium bromide and 25 grams of potassium bromate diluted to 1 liter with water, approximately normal with respect to potassium bromate. Phenol solution, approximately 5% phenol in water. Potassium iodide solution, about 50 grams per 100 ml. of solution. Thiosulfate solution, 0.1 *N* [0.01 *N* for a concentration of hydrocyanic acid, below 1 grain per 100 cu. feet of gas (2.832 cu. meters) about 0.002% hydrocyanic acid]. Starch indicator solution.

PROCEDURE I

(For concentrations of hydrocyanic acid above 5 grains per 100 cu. feet, 3.25 grams per 1000 cu. feet, about 0.01% hydrocyanic acid.)

Place 20 ml. of 20% potassium hydroxide solution in each of two Shaw sulfur flasks, connect in series for gas scrubbing, and scrub the gas at a rate of not more than 2.0 cu. feet per hour, with a meter at the end of the train. A 20 × 2.5 cm. (8 × 1 inch) test-tube trap may be used instead of the second sulfur flask. If the gas contains 10 grains of hydrocyanic acid per 100 cu. feet, a 1.0-cu. foot sample will give a final titration of about 5 ml. of 0.1 *N* thiosulfate solution. It is suggested that no more than 2.5 cu. feet of gas be taken as a sample because there is danger that a relatively large amount of carbon dioxide in the gas will destroy the caustic alkalinity and cause hydrocyanic acid to escape. After reading the meter, remove the train and combine the scrubbing solutions, employing as little wash water as possible. Add 10 to 15 drops of the ammonium polysulfide solution, shake to mix, and let stand 2 minutes. In special instances where the volume of acid gases present is small, the polysulfide treatment may be omitted, in which case the flask containing the caustic solution is evacuated before subsequent acidification.

Remove the stopper and make the solution in the flask just acid by slowly adding concentrated hydrochloric acid, meanwhile swirling the flask to mix. If no extra alkali (such as ammonia) is present, this treatment will require about 10 ml. of acid. The end point is indicated by the disappearance of the yellow color of ammonium sulfide. Use a sufficient excess of hydrochloric acid to yield approximately normal acidity at the time the potassium iodide solution is subsequently added (8 ml. of concentrated hydrochloric acid per 100 ml. of solution are generally satisfactory). Avoid high local concentrations of acid in the solution. Cool the flask to room temperature if necessary, and evacuate. If the solution is saturated with carbon dioxide, this operation must be done carefully at first. The suggested procedure is to turn the channeled stopper in the flask to an open position and to attach a light suction to the outlet stopcock, so that a gentle bubbling takes place which can be maintained by progressively cutting down the size of the opening in the stopper plug.

Finally put on full suction, remove most of the air, and disconnect from the suction line. Through the adjustable vent in the funnel top of flask add increments of potassium bromide-bromate solution with shaking until an excess of about 2.0 ml. of the reagent is present after 5 minutes' standing. Usually 5 to 10 ml. are required. After a little experience the depth of bromine color in the sample will be a sufficient guide. A large excess of bromine should be avoided. Wash the funnel top with water to remove excess potassium bromide-bromate and pass a few

drops into the flask. Wash the tubes of the flask also with a little water after the addition of each reagent has been completed. Shake to mix and let the flask stand 2 to 3 minutes. Add about 1 ml. of the 5% phenol solution, wash into a flask with a few milliliters of water, and shake to mix, so as to eliminate all traces of yellow bromine color in the solution. Then add 4 ml. of potassium iodide solution, shake, and let stand 2 minutes. The iodine produced is a measure of the hydrocyanic acid in the sample. Vent the flask to the air, place a little distilled water in the funnel top, and remove the stopper. Titrate the iodine with 0.1 *N* thiosulfate solution, adding starch indicator solution near the end. The titration, which is made in the "sulfur flask", is accomplished in two operations: initially swirling the flask to mix the thiosulfate and iodine, and then finishing after most of the iodine has disappeared by attaching a rubber tube to the inlet tube of the flask and blowing with the breath to mix during the addition of the last few milliliters of the thiosulfate.

CALCULATIONS. The reaction is based on the following equation:

$$CNBr + 2HI = HCN + HBr + I_2$$

Then

$$\frac{\text{Ml. of 0.1 } N \text{ thiosulfate} \times 2.084}{\text{cu. ft. of gas in sample (corrected)}} = \text{grains of HCN per 100 cu. ft. of gas (corrected)}$$

PROCEDURE II

(For concentrations of hydrocyanic acid below 5 grains per 100 cu. feet, 3.25 grams per 1000 cu. feet, 0.01% hydrocyanic acid.)

Under the preceding procedure the size of gas sample, because of carbon dioxide present, is roughly limited to 2.5 cu. feet, which would require on titration only 6 ml. of 0.1 *N* solution on a gas containing 5 grains of hydrocyanic acid per 100 cu. feet. Much smaller titrations are undesirable and the use of 0.01 *N* solutions introduces other well-known but not always realized complications. It has been found that, where ammonia is present in the gas in sufficient concentration, water can be used in place of potassium hydroxide solution as a scrubbing medium to yield sufficiently accurate results. For such a purpose the ammonia concentration of the gas is not permitted to drop much below 1000 grains per 100 cu. feet throughout the test. The problem of conveniently adding such an amount of ammonia has required some consideration, as the volume of pure ammonia gas desired (0.03 cu. feet per hour), is too small to be measured conveniently by a flowmeter; and aqua ammonia exhibits too high an initial partial pressure of ammonia to be used directly where it is necessary to scrub a large sample of gas, while with small gas samples an undesirably large amount of ammonia is left in the scrubbing solution. For all these reasons, procedure II, described below, is recommended for use where gas of very low hydrocyanic acid concentration is to be analyzed.

Set up two sulfur flasks and a 15 × 2.5 cm. (6 × 1 inch) test tube, each charged with 25 ml. of distilled water, as in procedure I, except that a glass tee is placed in the gas sampling line just before the first sulfur flask and a sulfuric acid trap for ammonia is inserted before the meter. Connect three 15 × 2.5 cm. (6 × 1 inch) test tubes charged with 25 ml. each of aqua ammonia solution (28% reagent grade) in series, and, from a compressed source, pass air or other inert gas through a flow meter, through the aqua ammonia, and through the tee into the gas stream before the hydrocyanic acid scrubbers. Adjust the gas rate through the ammonia solution to approximately 10% of that of the gas passing through the hydrocyanic acid scrubbers, and at the conclusion of the test deduct the volume of the inert gas from the total gas reading to obtain the true volume of sample. At a total gas rate of 2 cu. feet per hour this combination will maintain the desired ammonia concentration in the gas throughout the passage of 10 cu. feet of sample without introducing excessive amounts of ammonia into the scrubbing solution. A 10 cu. feet sample will yield a titration of about 5 ml. of 0.1 *N* thiosulfate if the gas has a hydrocyanic acid concentration of 1 grain per 100 cu. feet. For larger samples (or room temperatures much above 30° C.) a greater volume of aqua ammonia should be used. For the analysis of the sample add the contents of the test-tube scrubber to the second sulfur flask, add ammonium polysulfide, and follow the directions in procedure I.

It is suggested that the contents of the two sulfur flasks be titrated separately with 0.1 *N* thiosulfate and the two titrations added together for the hydrocyanic acid calculation, though, if desired, the iodine solutions can be joined and titrated as one. The separate titration permits a comparison that throws light on the scrubbing efficiency and consequently on the ammonia enrichment during scrubbing. The amount of thiosulfate required for titrating the solution in the first flask should be about four times that for the second flask. Under these conditions in this laboratory less than 2.5% error was indicated for analyses of a 1-grain gas. An additional water scrubbing unit will lower this error but will make the procedure more cumbersome. If it it desired to analyze gases having a hydrocyanic acid concentration below 1 grain per 100 cu. feet, it is suggested that 0.01 *N* thiosulfate solution be used, in which case the thiosulfate titration should be made at a temperature below 15° C. to avoid unreasonable blanks.

FURTHER APPLICATIONS OF METHOD

It seemed that if this method is satisfactory for determining hydrocyanic acid in gas, it would be of utility in analyzing a wide variety of miscellaneous solutions containing hydrocyanic acid derivatives. This has indeed proved to be the case. The procedure has given reliable results in the standardization of potassium cyanide and potassium thiocyanate solutions, in the analysis of two types of wet-process gas purification liquors where it is a great time saver (as thiosulfate and chloride do not interfere), and in analyses of pure samples of cyanogen bromide.

The ammonium polysulfide treatment may be omitted in all cases where the gas liberated by acidification is insufficient to destroy the vacuum. In the analysis of alkaline cyanides, such as potassium cyanide, a small excess of fixed alkali should be added to the sample before evacuating the flask to prevent vapor loss.

Although the recorded reactions of cyanogen bromide offer several possibilities for its determination (*1, 3, 5, 8, 9, 12*), an iodometric method seemed most practical for routine laboratory use. Difficulties were encountered, however, with previously recommended procedures (*2, 16, 17*). The authors therefore decided to investigate the following factors: time required for oxidation and reduction, effects of pH during oxidation and reduction, influence of nature of acid present, and action of certain alkaline salts prior to reduction.

In studying the reduction phenomena, pure cyanogen bromide was weighed and transferred quickly to water in a volumetric flask. This stock solution was kept at 0° C. and fresh solutions were frequently prepared. Aliquot samples were taken for individual analyses. The pipets were filled by pressure instead of suction and the contents delivered below the surface of a fixed volume of water in an ordinary glass-stoppered Erlenmeyer flask. Known quantities of acid and potassium iodide were added, and the flask was quickly stoppered and let stand for a definite period of time. Finally the liberated iodine was determined with sodium thiosulfate.

Table I. Influence of Sodium Carbonate and Trisodium Phosphate on Cyanogen Bromide Analysis

Concentration of cyanogen bromide stock solution, 15.58 grams per liter (purity 97.5%). Aliquot for each determination, 10 ml. Concentration of sodium carbonate and trisodium phosphate solutions, 1.34 *N*. Volume of solution after acidification, 100 ml.; acidity, 1 *N*. Potassium iodide added, 4 ml. (0.5 gram per ml.). Concentration of sodium thiosulfate, 0.0822 *N*.

Alkaline Solution Used	Quantity	Reaction Time with Alkaline Solution	pH	Sodium Thiosulfate
	Ml.			*Ml.*
				34.85 (Control)
Na_2CO_3	0.03	30 sec.	7.57	34.68
Na_2CO_3	0.03	30 min.	7.57[a]	34.49
Na_2CO_3	10	30 sec.	9.96	29.81
Na_2CO_3	10	30 min.	9.96	7.67
Na_2CO_3	25	30 sec.	10.04	29.40
Na_2CO_3	25	30 sec.	10.04	29.04
Na_3PO_4	0.03	30 sec.	7.5[b]	34.85
Na_3PO_4	0.03	30 min.	7.5[b]	34.73
Na_3PO_4	10	30 sec.	10.90	24.43
Na_3PO_4	10	30 sec.	10.90	24.51
Na_3PO_4	10	30 min.	10.90	0.03
Na_3PO_4	25	30 sec.	...	18.77
Na_3PO_4	25	30 sec.	...	17.93
Na_3PO_4	25	30 min.	...	0.00

[a] During time interval solution became slightly acid.
[b] During time interval pH dropped to 6.1.
Inclusion of only one figure indicates reproducible result.

A study of the influence of time on the reduction of cyanogen bromide showed that while Schulek (*10*) specifies a 30-minute delay, actually the reduction is complete in less than 0.5 minute under the conditions employed. In order to learn the effect of acid concentration, nine determinations were made, in concentrations varying from 0.1 to 6.0 *N*. The results agreed within the experimental error. An investigation of the effect of the nature of the acid present was made by using several concentrations of acetic and phosphoric acids, including the concentrations used by Chattaway and Wadmore (*3*), Møller (*6*), and Schulek (*10*). The success of the reduction was found to be independent of the source of hydrogen ion, excluding, of course, oxidizing and reducing acids.

Buchanan (*2*), in accordance with Stevens and Blackett (*15*), suggests the addition of sodium carbonate to the sample, followed by slight acidification before conducting the analysis. Nardin (*7*), who originated the use of sodium carbonate, claims that "by previously neutralizing sulfuric acid present, more reliable and slightly higher results are obtained". This suggestion was investigated, and also, for purposes of comparison, the action of trisodium phosphate, with the results shown in Table I.

These findings indicate that, if the solution is made just alkaline with sodium phosphate no observable destruction occurs in 30 seconds, but with sodium carbonate a small, though measurable, quantity of cyanogen bromide is lost. In both cases increased amounts of alkaline solutions and longer reaction periods cause very marked decomposition. With carbonate there is observed an additional loss due to expulsion of cyanogen bromide with the carbon dioxide liberated upon acidification.

RECOMMENDED PROCEDURE FOR PURE CYANOGEN BROMIDE

Although, as the foregoing data indicate, the analysis of cyanogen bromide may be conducted under widely varying conditions, in this laboratory the following procedure has been adopted:

When a solution is to be analyzed, a suitable quantity of hydrochloric acid is placed in a glass-stoppered Erlenmeyer flask together with 4 ml. of the potassium iodide solution. An aliquot of the sample containing 0.1 to 0.2 gram of cyanogen bromide is then delivered just under the surface of the acidified solution and the flask is quickly stoppered. The solution is of such volume and concentration that after the addition of the sample there will be present 100 ml. of 1 *N* acid.

If a solid product is to be analyzed, individual samples of 0.1 to 0.2 gram are weighed in small glass-stoppered bottles, and dropped into 100 ml. of 1 *N* hydrochloric acid containing 4 ml. of potassium iodide solution.

In either case, after at least 30 seconds the liberated iodine is titrated in the customary manner with 0.1 *N* thiosulfate solution.

DISCUSSION OF METHOD

Several procedures appear in the literature for using this reaction as a measurement of hydrocyanic acid in gas. The authors and several associates have tried these methods with inexact results. As cyanogen bromide hydrolyzes in neutral or alkaline solution, previous analysts have added the potassium hydroxide gas-scrubbing solution slowly to an excess of strongly acidified bromine water. At least in fuel-gas analysis, large amounts of carbon dioxide are frequently present in the sample. Losses of cyanogen bromide in the released carbon dioxide inevitably occur, causing low results by the procedure mentioned. In the method here recommended, the hydrocyanic acid is converted to relatively nonvolatile thiocyanic acid by ammonium polysulfide, permitting release of carbon dioxide to the air upon acidification. The reaction to form potassium thiocyanate does not take place unless the polysulfide molecule contains more than 2 atoms of sulfur. If hydrogen sulfide is absent from the gas, 5 drops of the polysulfide solution are sufficient. If hydrogen sulfide is present, the sulfide formed appears to react with the polysulfide added, tending to reduce the sulfur ratio below the 3 to 1 limitation. In dealing with unknown conditions, it is well to establish by trial and error the amount of polysulfide solution required. A very large excess is undesirable, but could not be readily avoided if the gas were actually scrubbed with polysulfide.

There is danger of hydrolysis of cyanogen bromide upon pouring an alkaline cyanide solution into acidified bromine water. This hydrolysis is extremely rapid in alkaline solution and there is the risk of a side reaction taking place at the interface of the two streams. A somewhat analogous condition exists if alkaline sulfide is run into acidified iodine, where very high results are occasioned by increased oxidation at the interface. The authors, therefore, recommend that in all cases the sample be acidified before addition of bromine. If, for any reason, too high a concentration of acid is present after bromination during the analysis of a sample, the concentration should be diminished by dilution and not by addition of alkalies.

Potassium bromide-bromate solution is used instead of bromine water because it is more convenient to handle and the bromine value of the solution is constant.

The acidity of the solution is fixed at approximately *N* hydrochloric acid, a convenient strength to complete bromine and iodine oxidation reactions. Hydrochloric acid is preferred over sulfuric acid for this purpose. Very large excesses of acid are to be avoided.

If too large an excess of bromine is used, a large amount of tribromophenol will be thrown down as a precipitate, which has a tendency to adsorb the iodine subsequently formed in the solution and thus to give low results. A small amount of the precipitate does no harm, but preferably there should be very little. On the other hand, too small an excess of bromine, as indicated by only a faint yellow color in the solution, affords low results.

Eymann (*4*) says that excess phenol causes low results. The authors cannot confirm this statement. It seems probable that he attributed the action of tribromophenol precipitate to phenol itself. Skirrow (*14*) has pointed out that halogenated phenols tend to adsorb iodine, thereby giving rise to low results in such a titration.

In this reaction one hydrocyanic acid molecule is equivalent to two atoms of iodine, whereas in the titration of thiocyanate with silver and ferric alum the relation is 1 to 1 and in the titration of sodium cyanide with silver in alkaline solution, the ratio is 1 to 0.5. These ratios greatly favor the cyanogen bromide method, especially where low concentrations of cyanides exist in the sample.

In sampling, especially where the gas is water-saturated or contains ammonia, it is very important that the scrubbers be attached as close to the gas main as possible, for a very small amount of water condensate in the sampling line will remove an appreciable amount of hydrocyanic acid, especially in the presence of much ammonia.

Bromine converts both hydrocyanic acid and thiocyanic acid to cyanogen bromide. Cyanic acid is not converted to cyanogen bromide. Ferrocyanide is not converted to cyanogen bromide but is oxidized to ferricyanide, which in turn releases iodine, giving high results for hydrocyanic acid and thiocyanic acid present. This effect is eliminated if zinc sulfate is added in excess before the bromine. Heavy metal salts capable of oxidizing hydriodic acid must be removed as they will interfere with the analysis. These findings are important in analyzing miscellaneous solutions, but only the first two factors are likely to be of significance in gas analysis.

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Photometric Determination of Phosphorus in Limestone

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A photometric method is described for the determination of phosphorus in limestone when present in amounts ranging from 0.002 to 0.4% P_2O_5. The sample is ignited to destroy organic matter, silica is removed by dehydration with perchloric acid, and phosphorus is determined in the filtrate by the phosphovanadomolybdate method. When applied to National Bureau of Standards samples of argillaceous limestone 1 and 1-a, containing 0.18 and 0.14% P_2O_5, respectively, results within 0.01% of the Bureau of Standards values were found. The effect of interfering elements and the use of a filter photometer are discussed.

THE accurate and rapid determination of phosphorus content of limestone used in the process is an important factor in carbide manufacture. Since the limestone usually contains 0.00 to 0.04% of phosphorus, great care must be exercised with gravimetric and volumetric methods of analysis if a high degree of accuracy and reproducibility is to be obtained. These methods are, however, too tedious and time-consuming for use in routine control.

Murray and Ashley (*6*) and Kitson and Mellon (*2*) used the colorimetric method suggested by Mission (*5*) to determine phosphorus in steel by converting the phosphorus to the yellow phosphovanadomolybdate complex. Willard and Center (*1, 7*) improved this colorimetric procedure by using perchloric acid instead of nitric acid to remove the silica, thus precluding high results from the formation of silicomolybdic acid. Koenig and Johnson (*3*) adapted the latter method to the determination of phosphorus in plants and in food materials.

The colorimetric determination of phosphorus as the phosphovanadomolybdate was found to be rapid and accurate when applied to the analysis of iron ores and plant ashes and offered promising possibilities for the analysis of limestones. If found applicable to this purpose, it was planned to adapt the method for use with a rugged, inexpensive photometer.

In this method the sample is calcined to destroy organic matter which, if not completely removed, imparts a slight color to the resultant solution; the calcium oxide is dissolved in perchloric acid and the resultant solution is fumed; and the yellow color of ammonium phosphovanadomolybdate develops upon the addition of ammonium vanadate and ammonium molybdate.

APPARATUS

A Beckman Model D quartz spectrophotometer fitted with matched rectangular cells 1 cm. square.

Fisher AC Electrophotometer fitted with 425-mμ blue filter and 3-ml. cylindrical absorption cells.

PRELIMINARY INVESTIGATION

Optimum conditions for the formation of the yellow phosphovanadomolybdate color have been investigated, and the amounts and composition of the necessary reagents have been established (*2, 3, 6, 7*). Temperature was not regarded as critical so long as the color was formed at room temperature—that is, 20° to 30° C. (*3, 6*). Different investigators found various periods necessary for the development of the color. Willard and Center (*7*) stated that 4 minutes was sufficient, while others mentioned periods ranging up to 30 minutes (*3, 6*). Murray and Ashley (*6*) quoted Mission (*5*) as stating that after the color was developed it was stable for 14 days; whereas Koenig and Johnson (*3*) found slightly lower transmittance after 12 to 24 hours. Willard and Center (*7*) found that less than 13 ml. of perchloric acid per 100 ml. allowed the formation of a precipitate upon the addition of ammonium molybdate and that more than 13 ml. retarded full development of the color. Willard and Center (*7*) also investigated the effect of iron and found that a 30-mμ spectral band centered at 450 mμ obviated interference from ferric perchlorate.

Since it was desired to use a filter-type monochromator for photometric measurements, it was necessary to establish the relationship between the absorption characteristics of the filter, the possible iron salts present, and the ammonium phosphovanadomolybdate.

The absorption spectrum of the filter was determined and transmittance curves were made for solutions of ferric nitrate and ferric perchlorate, both of which may be considered present when the reagents specified (*7*) are used. Solutions were prepared by adding 100 mg. of Fe_2O_3 as the corresponding salt to solutions containing 5 ml. of nitric acid and 17 ml. of perchloric acid and diluting each to 100 ml. A transmittance curve also was determined for a solution of ammonium phosphovanadomolybdate containing 0.1 mg. of phosphorus, prepared in the same manner as were solutions used for the calibration curve.

From data plotted in Figure 1 it can be seen that, although the absorption peak of the ammonium phosphovanadomolybdate is probably below 320 mμ, considerable absorption occurs in the spectral region covered by the blue filter. Figure 1 also shows that ferric perchlorate, even when present in as high an amount as 100 mg. of Fe_2O_3, absorbs only slightly in this region and that ferric nitrate exhibits more interference. For this reason, it was decided to use perchloric acid instead of nitric acid in the preparation of the ammonium vanadate reagent. In the absence of nitrates it appeared that only minor difficulty would be experienced when working with solutions containing appreciable quantities of iron.

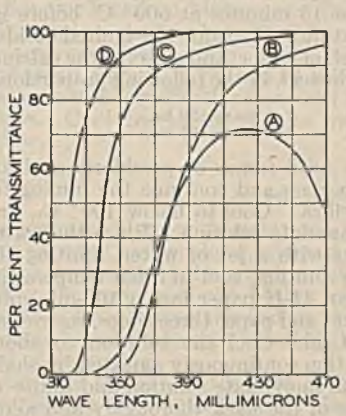

Figure 1. Transmittance Curves

A. Fisher 425 blue filter
B. Ammonium phosphovanadomolybdate, 0.1 mg. of phosphorus in 100 ml. of solution
C. Ferric nitrate, 100 mg. of Fe_2O_3 plus 5-ml. excess of nitric acid in 100 ml. of solution
D. Ferric perchlorate, 100 mg. of Fe_2O_3 plus 17-ml. excess of perchloric acid in 100 ml. of solution

REAGENTS

Ammonium Vanadate Solution. Dissolve 2.35 grams of ammonium metavanadate in approximately 400 ml. of hot water; add 14 ml. of 72% perchloric acid, cool, and dilute to 1 liter.

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Table I. Effect of Excess Perchloric Acid upon Phosphovanadomolybdate Color

	Per Cent Transmittance					
	$HClO_4$, 13-Ml. Excess		$HClO_4$, 15-Ml. Excess		$HClO_4$, 17-Ml. Excess	
Phosphorus, Mg.	After 30 min.	After 18 hours	After 30 min.	After 18 hours	After 30 min.	After 18 hours
0.0	94.5	94.5	94.0	94.0	94.5	94.5
0.2	72.8	72.4	72.9	72.3	73.0	72.6
0.4	58.2	57.7	58.5	57.8	59.0	58.6
0.6	48.0	48.0	48.6	48.5	49.2	48.8

Ammonium Molybdate Solution. Dissolve 100 grams of molybdic acid (85%) in a mixture of 300 ml. of water and 80 ml. of ammonium hydroxide. When dissolved, filter and boil filtrate 20 minutes; cool and dilute to 1 liter.

Standard Phosphorus Solution. Weigh out an amount of ammonium monohydrogen phosphate, the phosphorus content of which has been determined gravimetrically, equivalent to 0.1000 gram of phosphorus; dissolve in water and dilute to 1 liter. 1 ml. = 0.1 mg. of phosphorus. (Theoretical amount of ammonium phosphate required, 0.4263 gram.)

FACTORS AFFECTING COLOR DEVELOPMENT

Acid Concentration. Using procedures similar to those recommended by Willard and Center (7), the effect of excess perchloric acid was investigated (Table I).

Table I shows that although greater excesses of perchloric acid do not allow the formation of as intense a color as does the 13-ml. excess, the color progression is insignificant after solutions have stood for 30 minutes. A 17-ml. excess of perchloric acid was chosen because the larger excess aided in the rapid dehydration of silica whenever large samples were necessary.

Effects of Iron and Calcium. Iron in the concentration usually encountered in limestone causes little interference. Calcium salts have no effect on color development.

PROCEDURE

Calibration Curve. Transfer aliquots of the standard phosphorus solution to 100-ml. volumetric flasks containing 17 ml. of 72% perchloric acid. Add 10 ml. of ammonium vanadate solution, dilute to 75 ml., and cool to about 25° C. Add 7.5 ml. of ammonium molybdate solution, swirling the contents of the flask meanwhile to prevent precipitation. Dilute to the mark, mix thoroughly, and allow to stand for 30 minutes. Determine the percentage transmittance using a Fisher Electrophotometer with a 425-mμ blue filter. Plot the results on semilog paper.

Analytical Method. Ignite a sample of limestone (depending upon the phosphorus content) in a porcelain crucible for 30 minutes at 900° C. If a large amount of organic matter is present, ignite the sample for 15 minutes at 500° C. before igniting at the higher temperature. Transfer the ignited residue to a 150-ml. beaker, add 20 ml. of water, and dissolve the calcium hydroxide with 72% perchloric acid, in the following proportions:

0.5 gram:18 ml.	2.0 grams:20 ml.
1.0 gram:19 ml.	5.0 grams:25 ml.

Evaporate on a hot plate until fumes of perchloric acid are evolved; cover with a watch glass and continue the fuming for 5 minutes to dehydrate the silica. Cool to below 100° C., and add 10 ml. of ammonium vanadate solution. Rinse the watch glass and sides of the beaker with a jet of water, limiting the washings to 15 ml. Mix the solution, cool to room temperature and filter through a Whatman 41-H paper into a 100-ml. volumetric flask. Wash the beaker and paper three times, restricting the volume to less than 90 ml. Cool the solution to about 25° C., while keeping the solution continuously agitated by shaking; add 7.5 ml. of ammonium molybdate solution and dilute to the mark. Mix the contents of the flask thoroughly and allow to stand 30 minutes. Determine the percentage transmittance, using a Fisher electrophotometer with a 425-mμ glass filter. Calculate the percentage phosphorus from the number of milligrams of phosphorus found on the calibration curve.

APPLICATION TO STANDARD SAMPLES

Samples of Bureau of Standards argillaceous limestone and dolomite were analyzed by the photometric method. The values obtained by the Bureau of Standards, the mean of values by cooperating analysts, and the values by the photometric procedure are given in Table II. Up to this time the term "phosphorus" has been used, since it is customary to report the P_2O_5 present in limestone used for carbide manufacture in terms of the element. The Bureau of Standards certificates of analysis are on the P_2O_5 basis; therefore results on these samples are reported as the pentoxide.

PRECISION AND ACCURACY

Precision. The results reported in Table II are given as the nearest hundredth per cent P_2O_5. In Table III, the original values are given to show the precision of the method.

From the results in Table III the average deviation from the mean was found to be 0.0037% P_2O_5. The probable error of a single determination using the method of least squares (4) was found to be 0.0028% P_2O_5.

Accuracy. A comparison of the results in Table II shows that the results by the photometric method are within 0.01% P_2O_5 of the values reported by the Bureau of Standards when applied to samples containing 0.14 and 0.18% P_2O_5. A maximum deviation of 0.001% P_2O_5 from the Bureau of Standards value was obtained on a sample of dolomite reported to contain 0.002% of P_2O_5.

Table II. Analysis of Bureau of Standards Samples

Sample	Bureau of Standards Value P_2O_5	Average of Cooperating Analysts P_2O_5	Photometric P_2O_5	Deviation from Standard
	%	%	%	%
Argillaceous limestone, No. 1	0.18	0.18[a]	0.19[b]	+0.01
Argillaceous limestone, No. 1-a	0.14	0.15[c]	0.14[d]	0.00
Dolomite, No. 88	0.002	0.003[e]	0.002[f]	0.000

[a] Average of two values of 0.18 and 0.18.
[b] Average of three values ranging from 0.18 to 0.19.
[c] Average of ten values ranging from 0.108 to 0.18.
[d] Average of seven values, all 0.14.
[e] Average of two values of 0.002 and 0.004.
[f] Average of seven values ranging from 0.002 to 0.003.

Table III. Precision of Method

	P_2O_5 Found, %	Deviation from Mean, %
Sample 1-a	0.135	−0.005
	0.143	+0.003
	0.137	−0.003
	0.142	+0.002
	0.144	+0.004
	0.135	−0.005
	0.144	+0.004
Av.	0.140	

SUMMARY

A photometric method is described for the determination of phosphorus (0.002 to 0.4% P_2O_5) in limestone. The method is based upon the phosphovanadomolybdate color reaction, and may be used with a simple filter photometer. Calcium and iron salts, in the quantities encountered in limestone, cause no interference, and the organic matter is destroyed by a prior calcination.

Results obtained by the method have an accuracy and reproducibility adequate for the evaluation of limestone used for carbide manufacture. The procedure effects a great saving of time and reagents.

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Determination of Tin by a Modified Iodometric Method

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An accurate method for the determination of tin is proposed which avoids the usual sources of error encountered in precipitating tin as metastannic acid and those encountered in the reduction-oxidation procedure. The metal is dissolved in acid and, when necessary, collected with the use of ammonia and aluminum hydroxide, reduced with nickel, and titrated with standard iodine solution under a blanket of carbon dioxide.

IT IS generally accepted that the best method for determining tin is based on the reduction of the tin to its bivalent state with a metal, and subsequent oxidation with a standard iodine solution. Details of procedure vary widely and many difficulties are encountered that seriously affect the usefulness of the method.

Sources of error encountered are:

1. Incomplete precipitation of metastannic acid in making separations from copper
2. Loss of precipitated metastannic acid in filtering
3. Failure to obtain a satisfactory titration end point
4. Incomplete reduction of the material
5. Failure to prevent oxidation of stannous chloride by contact with air of the reduced solution

It is necessary to ensure the complete reduction of the tin to the bivalent state and to prevent its reoxidation by air. The former is not difficult and can be accomplished by the use of several metals. "Success in the latter depends on the maintenance of a nonoxidizing atmosphere during the entire operation, and is possible through the use of such expedients as the Bunsen valve or the use of a few grams of sodium carbonate" (*2*). The precipitation of tin by the usual nitric acid procedure is known to be incomplete at times (*4*) because metastannic acid often fails to coagulate sufficiently to be completely retained upon filtration. By the proposed method, precipitation is complete without filtration loss.

A series of determinations was made in order to select a metal to be employed as the reducing agent. The metals used were: iron (*3*), lead (*5*), antimony (*6*), and nickel (*1*). All these metals are capable of effecting the reduction of tin to the bivalent state. Nickel, alone, gave a very sharp end point. It is unnecessary to remove the undissolved nickel, thus eliminating from the analysis a step which is a troublesome source of error.

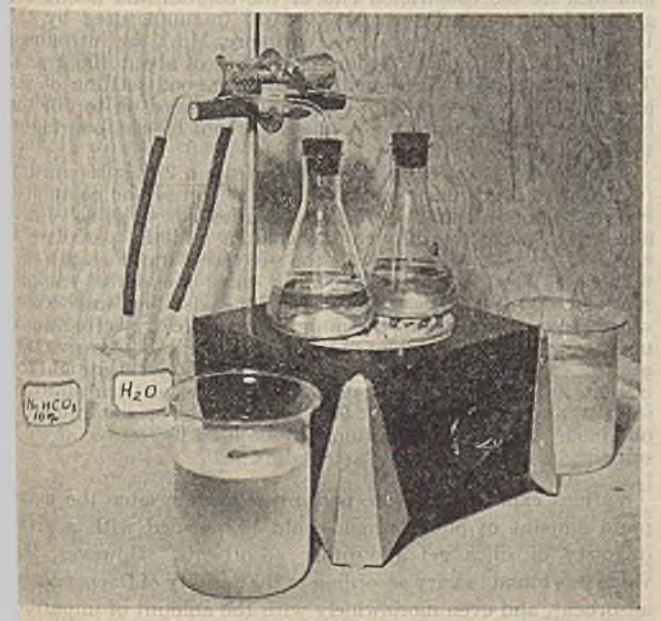

Figure 1. Apparatus

No claim to originality is made as regards the individual details of the procedure finally adopted. However, because the procedure outlined differs in some detail from any of those published and the results obtained are precise and accurate, it is believed that the following method is to be preferred.

REAGENTS REQUIRED

STANDARD TIN SOLUTION. Dissolve 5 grams of pure tin in 100 ml. of concentrated hydrochloric acid and dilute to 1 liter in a volumetric flask.

STANDARD IODINE SOLUTION. Dissolve approximately 11 grams of iodine in about 100 ml. of distilled water containing 20 grams of potassium iodide. Dilute to 1 liter in a volumetric flask and standardize against the standard tin solution after reduction of 25 ml. of the tin solution by the method given below.

STARCH SOLUTION. Add a thin paste made of 5 grams of soluble starch, 10 grams of sodium bicarbonate, and water to about 300 ml. of boiling water. Boil for 1 minute with stirring. Cool rapidly and dilute to 1 liter.

PURE NICKEL (shot or strip).

PREPARATION OF SAMPLES FOR REDUCTION

BRASS, BRONZE, AND COPPER BEARING MATERIALS (over 2% copper). Weigh 1 to 10 grams of material to give a tin content between 0.1 and 0.2 gram into 300-ml. Erlenmeyer flasks, add 10 to 30 ml. of nitric acid (1 to 1), and heat gently until the material is completely disintegrated. Boil until oxides of nitrogen are expelled, dilute to about 100 ml. with water, and add 3 to 5 ml. of 10% aluminum nitrate solution. Add ammonium hydroxide to the blue copper complex color, then 10 ml. in excess. Heat to boiling and filter through hard paper (Whatman No. 42 or comparable). Wash twice with 5% ammonium nitrate solution. Place the filter paper containing the precipitate in the original flask. Add 10 ml. of concentrated sulfuric acid, 5 ml. of concentrated perchloric acid, and a few drops of concentrated nitric acid. Heat gently, adding nitric acid dropwise as required to prevent darkening of the solution. Evaporate to sulfur trioxide fumes. Cool first in air, then in water, and proceed as directed below.

SOLDER BEARING METAL, etc. (less than 2% copper). Weigh the sample to contain between 0.1 and 0.2 gram of tin into 300-ml. Erlenmeyer flask. Add 10 ml. of concentrated sulfuric acid and about 5 grams of potassium sulfate, and heat until the material is completely dissolved or until lead sulfate, if present, turns white. Cool first in air, then in water, and proceed as directed below.

METHOD

Carefully dilute with water to about 100 ml., add 75 ml. of concentrated hydrochloric acid and 10 grams of nickel shot (10-mesh), and connect flask with a tube, one end of which extends below the surface of the beaker of water. Boil gently for 30 minutes, transferring the end of the outlet tube to a beaker of sodium bicarbonate solution (10%) several minutes before the end of the period.

Keeping the end of the tube below the surface of the sodium bicarbonate solution (Figure 1), place the flask in a suitable container of cold water. Allow to stand until cold. Remove the rubber stopper and, as rapidly as possible, introduce a small piece of dry ice (solid carbon dioxide). Then add more dry ice in sufficient quantity to keep the solution very cold (below 10° C.) and blanketed with carbon dioxide gas. (If solid carbon dioxide is not available, pellets of sodium bicarbonate may be substituted and will give satisfactory results if the solution is cooled by the use of ice.) Add 5 ml. of starch solution and titrate at once against the standardized iodine solution to a permanent blue end point. Calculate the percentage of tin in the sample.

Table I. Determination of Tin[a]

Bureau of Standards No.	Material	Tin Present %	Tin Found %	Deviation %
62	Manganese bronze	0.82	0.82	0.00
37	Sheet brass	1.013	1.012	0.001
53	Lead-base metal	10.91	10.91	0.00
127	Solder	34.88	34.87	0.01
52	Cast bronze	7.90	7.90	0.00
63	Phosphor bronze	9.91	9.91	0.00
54a	Tin-base metal	88.61	88.61	0.00

[a] Figures represent average of six determinations of each sample.

DESCRIPTION OF APPARATUS

The apparatus consists of Erlenmeyer flasks (300-ml.), stoppered with one-hole rubber stoppers. These are provided with bent glass and rubber tubing that extends to the bottom of the beakers containing, as they are used, water and bicarbonate of soda. The flask rests upon an electric heater that has a regulator to permit high, medium, or low heat adjustment. A ring stand, the base of which fits snugly under the heater, is provided with cross supports for the bent glass tubing. At the sides of the heater are two 80-ml. beakers partly filled with ice and water to facilitate rapid cooling.

Table I shows results obtained on National Bureau of Standard samples of varying tin content.

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The views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.

Purification of Solvents for Absorption Spectroscopy

An Adsorption Method

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A simple, rapid method for removing ultraviolet-absorbing impurities from hydrocarbon solvents by selective adsorption on silica gel columns is described. Solvents suitable for use in absorption spectrum measurements have been prepared by this method from commercial samples of cyclohexane, *n*-heptane, iso-octane, Skellysolve-B, and Skellysolve-F. In general, hydrocarbon solvents, both synthetic and commercial, which have been subjected to exhaustive chemical and physical purification have been noticeably improved by this adsorptive treatment. The advantages of the adsorption method over the usual methods are speed, simplicity of technique, and high yield of purified solvent.

IN RECENT years the advantages of liquid hydrocarbons over polar liquids as ultraviolet absorption solvents have gained recognition (*1*). The need for the use of pure hydrocarbons such as synthetic *n*-heptane, cyclohexane, and iso-octane instead of the commercially available petroleum fractions, which are hydrocarbon mixtures, has been demonstrated in specific examples (*2, 5, 11*). In this connection the purification of liquid hydrocarbons has been investigated.

The present methods for the preparation of hydrocarbons for use as solvents in absorption spectroscopy (*3, 8, 9, 11*) are as a rule long and cumbersome and result in poor yields. Usually the final products still contain significant amounts of impurities, probably aromatic and unsaturated compounds, which absorb radiations in the ultraviolet region.

The present paper describes a simple method for direct purification of commercial hydrocarbon solvents based on selective adsorption of the impurities by means of a suitable adsorbent. The adsorption procedure was suggested by the work of Mair, White, and others (*4, 7, 10*) who, in connection with an investigation of the composition of petroleum distillates at the National Bureau of Standards, showed that aromatic hydrocarbons can be separated from naphthenic and paraffin hydrocarbons by adsorption on silica gel.

The results which the authors obtained demonstrated the effectiveness of silica gel for purifying not only synthetic *n*-heptane, cyclohexane, and iso-octane for absorption spectroscopy, but also petroleum ether fractions (Skellysolves), if desired. In general, both synthetic and commercial hydrocarbons, even after they have been subjected to exhaustive chemical and physical purification, have been noticeably improved in ultraviolet transparency by adsorptive treatment.

APPARATUS AND PROCEDURE

The adsorption apparatus used consists of a glass tube, 120 cm. in length and 38 to 40 mm. in diameter, constricted at the lower end. A small plug of glass wool is placed on a perforated porcelain disk at the constricted end of the column and about 400 grams of silica gel, Davco 659528-2000 (manufactured by the Davison Chemical Corporation, Baltimore, Md.), are introduced with the aid of a powder funnel in batches of about 100 grams. The tube is tapped occasionally to ensure good settling of the adsorbent. Another plug of glass wool is placed on top of the column to prevent agitation of the adsorbent by the pouring of the solvent to be purified.

The solvent is added to the column from a 2-liter separatory funnel, care being taken not to allow the top of the column to run dry before all the solvent has been added. The solvent is allowed to percolate through the column and the percolate is collected in the same manner as the successive fractions of a distillation. The first fraction is in all cases the purest sample; successive fractions are acceptable until the adsorbent has become saturated with respect to the impurities. A test spectrogram is made to ascertain the extent of purification in the successive percolates. Usually a single passage of the liquid through the column suffices to produce a satisfactory ultraviolet-transmitting solvent, and a yield of about 90 to 95% of the original liquid is obtained. A simple distillation may be used to remove any adsorbent.

Various experiments were performed to determine the maximum amount of liquid which could be purified with a given quantity of silica gel and other adsorbents. However, this value was found to vary according to the activity of the particular adsorbent, and even more widely with the amount of impurities to be removed from different solvents or the same type of solvent

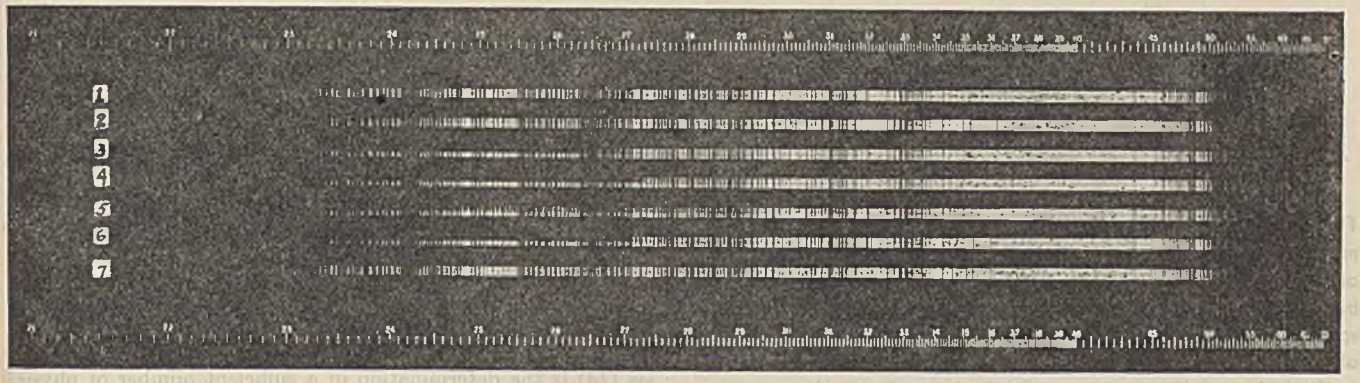

Figure 1. Comparative Spectrograms of Hydrocarbons

Upper portion, before purification; lower portion, after purification

1. Water, doubly distilled
2. Iso-octane
3. Skellysolve-F
4. Skellysolve-B
5. n-Heptane
6. Cyclohexane
7. Water, double distilled

Table I. Optical Density of Hydrocarbons before and after Purification by Silica Gel Adsorption Method (4-Cm. Cell)

Hydrocarbon	Purification	Wave Length 2300 Å.	2400 Å.	2500 Å.	2600 A.	2700 A.
Cyclohexane, Allied Chemical & Dye Corp.	Before	∞[a]	∞	∞	∞	∞
	After	0.10	0.00	0.00	0.00	0.00
Cyclohexane, Dow Chemical Co.	Before	∞	∞	∞	∞	0.49
	After	0.24	0.01	0.00	0.00	0.00
Cyclohexane, Du Pont Co.	Before	∞	∞	∞	∞	∞
	After	0.12	0.00	0.00	0.00	0.00
Cyclohexane, Eastman Kodak Co.	Before	∞	∞	∞	..	..
	After	0.52	0.10	0.00	0.00	0.00
Heptane Practical, Eastman Kodak Co.	Before	∞	0.61	0.84	0.72	0.50
	After	0.11	0.08	0.00	0.05	0.00
Iso-octane[b], Eastman Kodak Co.	Before	∞	∞	1.34	0.13	0.16
	After	0.05	0.02	0.00	0.06	0.00
Iso-octane[c], Rohm & Haas	Before	0.29	0.08	0.10	0.17	0.28
	After	0.00	0.00	0.00	0.00	0.16
Skellysolve-B[d], Skelly Oil Co.	Before	∞	∞	∞	∞	∞
	After	0.07	0.06	0.06	0.02	0.00
Skellysolve-F[e], Skelly Oil Co.	Before	0.30	0.98	1.05	0.98	0.05
	After	0.00	0.00	0.00	0.04	0.00

[a] ∞ represents insufficient transmission through 4-cm. layer to blacken photographic plate.
[b] 2,2,4-Trimethylpentane, b.p. 98–99° C.
[c] 2,2,4-Trimethylpentane, Bureau of Standards certified grade.
[d] Petroleum ether, b.p. 60–71° C. (A.S.T.M.).
[e] Petroleum ether, b.p. 30–60° C. (A.S.T.M.).

rom different commercial sources. Experience has shown that 100 grams of silica gel will purify 1 to 1.5 liters of cyclohexane, 2 liters of *n*-heptane, at least 4 liters of iso-octane, 0.5 to 1 liter of Skellysolve-B, and 2 to 2.5 liters of Skellysolve-F.

Other adsorbents such as alumina, magnesium oxide, various active clays, and carbons were tried. Effective improvement of the solvent was accomplished by the use of decolorizing carbons (Norit and Darco, Eastman), and activated charcoal, U.S.P. (Merck).

Activated silica gel of the type described is an efficient and readily available adsorbent; it was chosen especially because columns of this material are easily prepared and allow the solvent to pass through rapidly. This adsorbent can be readily reactivated for most purposes by washing thoroughly with water and heating in a stream of air at 325° C. for 24 hours (*4*).

Attempts were made to purify the hydrocarbons by adding the adsorbent directly to the solvent to form a slurry, and subsequently removing the adsorbent by filtration. The concentration of aromatics, as shown by spectrograms, was noticeably decreased. However, continuation of the slurry procedure showed that complete removal of the ultraviolet-absorbing impurities could be achieved, if at all, only after preparing a large number of such slurries with recharges of fresh adsorbent, thus requiring a high ratio of adsorbent to solvent purified.

Figure 1 shows absorption spectra of a series of different hydrocarbons before and after a single passage through a silica gel adsorbing column. It illustrates the effectiveness with which the ultraviolet-absorbing impurities are removed from various hydrocarbons.

The spectrograms were obtained by use of a Bausch & Lomb medium quartz spectrograph and a Hilger Spekker photometer. The absorption cell which usually contains the solvent was filled with the hydrocarbon as obtained commercially, and the cell which usually contains the solution was filled with the liquid obtained from a single passage through the silica gel. The photometer drum was set to provide equal apertures and consequently to permit equal amounts of light to strike each cell. The source of radiation was a high-voltage alternating current arc, using Therapeutic Carbon "B" rods, which provides a many-lined spectrum of iron superimposed on a continuous background of temperature radiation from the carbon (*6*). A corresponding quantitative representation of the improvement observed in the transmitting properties of these liquids throughout the spectral region 2300 to 2700 Å. is shown in Table I. The optical densities were obtained from measurements made with a Leeds & Northrup recording densitometer of spectrograms obtained by photographing each liquid, before and after purification, in 4-cm. cells, each against a matched empty cell representing 100% transmission.

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Sulfuric Acid Extraction in Hydrocarbon Type Analysis

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The extrapolation method based on acid extraction is suggested as a means of routine and control analysis of paraffin-naphthene-aromatic or paraffin-naphthene-aromatic-olefin mixtures low in olefin concentration, boiling in the kerosene-to-gas-oil range. The procedure described is suitable for hydrocarbon mixtures containing 20% or less of aromatics and olefins. Where applicable, the extrapolation method gives a direct measure of saturate content and properties. This is sufficient, in conjunction with a molecular weight determination, to establish the paraffin-naphthene ratio. At the same time a measure of olefin plus aromatic content is obtained and approximations as to the character of the unsaturates (olefins and aromatics) can be made.

TYPE analysis of hydrocarbons boiling above the gasoline range has recently assumed considerable importance, in keeping with the development of new petroleum cracking processes and high-solvency petroleum solvents (*10*). This paper suggests sulfuric acid extraction for routine type analysis of medium boiling range hydrocarbon solvents, refinery cracking charge stocks, and the like, which contain small to moderate proportions of aromatics and olefins, not exceeding 20% of the sample.

A comprehensive method of type analysis based on combustion for hydrogen content is available from the work of Deanesly and Carleton (*3, 4*). Organic combustion for hydrogen, however, is rather tedious for a refinery laboratory and there would appear to be considerable incentive to avoiding this step if possible without too great a sacrifice of accuracy (*12*). With the object of substituting some simpler procedure for routine examinations, the well-known use of sulfuric acid as an extracting agent has been considered in this laboratory.

Sulfuric acid extraction has been advocated from time to time by a number of investigators (*6, 7, 9, 11, 15*) as a means of separating unsaturated from saturated hydrocarbons in type analysis of mixtures. Use of the acid as an analytical tool has been objected to (*6, 13*), however, on the generally valid grounds of inaccuracy due to incomplete removal of unsaturates, solubility of saturates in the acid extract, or chemical attack by the acid (*18*).

A stepwise extraction procedure and graphical interpretation of the extraction data have been investigated by Fisher and Eisner (*6*) for hydrocarbon type analysis. In Fisher and Eisner's method, acid ranging in concentration from 75 to 98% by weight was employed in a series of about ten successive treatments, at a constant ratio of 3 volumes of acid to 1 volume of oil. Graphs relating the physical constants and volume of unsulfonated oil were obtained which showed density and refractive index maxima in the earlier extraction stages, followed by progressive decreases to approximately constant values in the later stages. The maxima were interpreted as corresponding to complete olefin removal and incipient aromatic extraction, and the approximately constant later values of density and refractive index were interpreted as representing complete aromatic extraction and incipient removal of saturates.

Earlier work indicated that a two- or three-step extraction procedure, utilizing strong acid and varying the volume of acid, might yield linear volume–physical constant data and thereby simplify analysis.

In the experiments described below, it was found that acid sufficiently strong to remove aromatic compounds completely from a kersoene cut also reacted with the saturates present. Nevertheless, the action of the acid on the saturates could be allowed for by using several different ratios of acid to oil and extrapolating to a hypothetical zero acid to oil ratio.

PRINCIPLE OF TYPE ANALYSIS BY SULFURIC ACID

The principle underlying the use of sulfuric acid in type analysis (*15*) is the determination of a sufficient number of physical properties of a mixture before and after reaction with the acid to furnish calculation of the proximate composition, by appropriate combination of the data.

Complete structural type analysis of hydrocarbon mixtures involves complex ramifications (*3*) which are beyond the scope of absorption or extraction methods. For the purposes of routine analysis, however, any procedure which will give the per cent of paraffins plus naphthenes, the paraffin-naphthene ratio, the percent of olefins plus aromatics, and an independent determination of olefins, may be considered as yielding a type analysis.

Specifically, if accurate values of the per cent of saturates present and density and refractive index of the saturates can be obtained by the use of acid on a hydrocarbon mixture, then combined with molecular weight determination and bromine number determination on the original mixture, the composition can be expressed (*16*) in terms of per cent of paraffins, olefins, napthenes, and aromatics by established methods of interpreting specific refractivity (*3, 5, 10*) and bromine number (*5, 15*).

The primary object of the experimental work reported here has been to ascertain if the necessary data on volume per cent, density, and refractive index of the saturates present can be obtained by sulfuric acid extraction with sufficient accuracy, using acid strong enough to react completely with the olefins and aromatics. A secondary object has been to observe the effect of acid extraction on the aniline point, as a knowledge of this property is frequently of value (*8*).

Table I. Properties of Saturated Hydrocarbons Solvent 160-S

Gravity, ° A.P.I.	47.5	Density, $^{20}_{4}$	0.7864
Initial boiling point, ° F.	385	Dispersion $(N_F - N_C) \times$	
5%	393	10^4, 20° C.	77.4
10%	396	Specific dispersion	98.4
50%	407	Aniline point, ° F.	168.3
90%	425	Carbon, %	85.2
95%	433	Hydrogen, %	14.8
D.P.	437	Molecular weight	190
Flash point, ° F., T.C.C.	160	Bromine No.	0.0
Refractive index, $^{20}_{D}$	1.4368	Naphthenic rings per molecule, calculated	0.54

MATERIALS AND APPARATUS

After a number of preliminary tests on known hydrocarbon mixtures, 101% sulfuric acid—i.e., anhydrous sulfuric acid containing 4.4 weight % of free sulfur trioxide—was selected as extracting agent.

A single saturated hydrocarbon fraction, a light kerosene fraction described in Table I, was employed in all the tests. Synthetic mixtures (refer to Table II) were made up using this fraction with various olefins and aromatics. The xylene used was Baker's C.P. grade, diisobutylene and cyclohexane were Eastman purified materials, and the aromatic amyl derivatives were samples obtained through the courtesy of Sharples Chemicals, Inc. All these unsaturated substances were used in their state of purity as received.

The extractions were carried out in graduated bottles similar to Stoddard solvent bottles (*1*), but specially made to contain 10-ml. additional volume.

The optical measurements were made with an Abbe refractom-

Table II. Extraction of Solutions of Unsaturated Hydrocarbons in Solvent 160-S by 101% Sulfuric Acid

Blend No.	Unsaturated Component	Vol. % Unsaturated Component	Ratio by Volume Acid:Oil	Absorption, Vol. %	Raffinate Properties: Refractive index, $^{20}_{D}$	Raffinate Properties: Density, $^{20}_{4}$	Raffinate Properties: Aniline point, °F.	Original Solution Properties: Refractive index, $^{20}_{D}$	Original Solution Properties: Density, $^{20}_{4}$	Original Solution Properties: Aniline point, °F.	Solute Properties: Refractive index, $^{20}_{D}$	Solute Properties: Density, $^{20}_{4}$
0	None	0.00	4:1	27.5	1.4330	0.7743	174.5	..	..	..	..	..
			3:1	20.5	1.4340	0.7772	173.0					
			2:1	13.5	1.4350	0.7803	171.5					
			Ext. 0:1	−0.5	1.4368	0.786	168.5					
1	Xylene	20.0	4:1	46.0	1.4328	0.7722	173.5	1.4478	0.8033	134.0	1.4953	0.8612
			3:1	39.5	1.4340	0.7760	172.0					
			2:1	33.0	1.4345	0.7800	170.5					
			Ext. 0:1	20.0	1.4368	0.7878	167.5					
2	Monoamyl benzene	20.0	4:1	34.0	1.4343	0.7780	172.0	1.4465	0.7999	139.5	1.4869	0.8577
			3:1	30.0	1.4350	0.7800	171.0					
			2:1	26.0	1.4358	0.7822	170.0					
			Ext. 0:1	18.0	1.4372	0.7864	167.8					
3	Diamyl benzene	20.0	4:1	32.0	1.4347	0.7789	172.0	1.4463	0.7996	150.5	1.4850	0.8537
			3:1	28.5	1.4351	0.7806	171.0					
			2:1	25.0	1.4358	0.7819	170.0					
			Ext. 0:1	18.0	1.4368	0.7849	167.8					
	Monoamyl naphthalene	20.0	4:1	36.0	1.4340	0.7769	173.5	1.4638	0.8220	135.5	1.5731	0.9641
			3:1	32.0	1.4347	0.7786	172.0					
			2:1	27.5	1.4354	0.7819	170.5					
			Ext. 0:1	19.2	1.4370	0.7879	167.5					
5	Diamyl naphthalene	20.0	4:1	37.5	1.4337	0.7758	173.5	1.4597	0.8162	142.5	1.5527	0.9340
			3:1	33.0	1.4346	0.7784	172.0					
			2:1	28.5	1.4354	0.7807	171.0					
			Ext. 0:1	19.5	1.4371	0.7858	168.6					
6	Cyclohexene plus xylene	12.5	4:1	35.5	1.4350	0.7777	172.0	1.4447	0.7976	130.5	1.4470	0.8105
			3:1	32.0	1.4355	0.7806	170.0					
		12.5	2:1	28.5	1.4361	0.7830	168.5				1.4953	0.8612
			Ext. 0:1	21.5	1.4374	0.791	164.4					
7	Diisobutylene	20	4:1	34.5	1.4327	0.7719	174.0	1.4330	0.7727	157.0	1.4106	0.7157
			3:1	30.0	1.4331	0.7750	172.5					
			2:1	25.5	1.4338	0.7780	171.0					
			Ext. 0:1	16.5	1.4350	0.7840	168.0					

eter, density determinations were made in 2- or 3-ml. pycnometer bottles, and aniline points were determined (*2*) using microburets and 5-ml. samples.

PROCEDURE

The extractions were carried out following the method of Thomas, Bloch, and Hoekstra (*15*), at ice-water temperature. Three extractions were made in each case, employing acid-oil ratios of 4 to 1, 3 to 1, and 2 to 1.

After the volume of hydrocarbon absorbed was measured, the extracts were discarded and the raffinates washed with sodium carbonate solution and dried over sodium carbonate. The density, refractive index, and aniline point of the washed and dried raffinates were determined and extrapolated graphically to a fictitious 0 to 1 acid-oil ratio.

The data obtained in the extraction experiments are recorded in Table II, including the extrapolated values.

ACCURACY OF SATURATES DETERMINATIONS

From the limited amount of data presented, it can be estimated that in the case of paraffin-naphthene-benzene or paraffin-naphthene-naphthalene mixtures, where the concentration of aromatic is 20% or less, the accuracy of the extrapolation method is approximately as follows:

Property	Estimated Accuracy
Volume per cent of saturates	∓0.2%
Density of saturates	∓0.005
Refractive index of saturates	∓0.0005
Aniline point of saturates	∓0.8° F.

When the mixture contains olefinic derivatives at 12 to 20% concentration (see Table II), the inaccuracy is approximately doubled.

CALCULATION OF UNSATURATE PROPERTIES

Density and refractive index are approximately volume-additive properties in higher boiling hydrocarbon mixtures (*4, 11, 14*); hence the approximation equations below may be set up:

$$Fu^{(V)} = \frac{dm - ds}{du - ds} = \frac{Nm - Ns}{Nu - Ns}$$

Table III. Calculation of Properties of Unsaturated Compounds Mixed with Naphthenes and Paraffins, by Extrapolation Method

Unsaturated Compound	Data, Table II, Blend No.	Properties of Unsaturated Compound: Refractive Index, $^{20}_{D}$ Calculated	Refractive Index, $^{20}_{D}$ Observed	Density, $^{20}_{4}$ Calculated	Density, $^{20}_{4}$ Observed
Xylene	1	1.4918	1.4953	0.8653	0.8612
Monoamyl benzene	2	1.4888	1.4869	0.8614	0.8577
Diamyl benzene	3	1.4894	1.4850	0.8666	0.8537
Monoamyl naphthalene	4	1.5737	1.5731	0.9619	0.9641
Diamyl naphthalene	5	1.5530	1.5527	0.9417	0.9340
Diisobutylene	6	1.4230	1.4106	0.7160	0.7157

where

$Fu^{(V)}$ = volume fraction of unsaturates as determined by extrapolation method
dm = density of original mixture
ds = density of saturates, as extrapolated
du = density of unsaturates
Nm = refractive index of original mixture
Ns = refractive index of saturates, as extrapolated
Nu = refractive index of unsaturates

In Table III, the densities and refractive indices of the unsaturates used in the extraction tests have been calculated by the above formulas.

The agreement of calculated and observed values averages about ∓0.005 for the densities and ∓0.004 in the case of the refractive indices. Calculation of unsaturate aniline points was unsatisfactory.

DISCUSSION

It is evident from the experimental results in Table II that the accuracy of the extrapolation method is seriously reduced by olefins at high concentration. Other data on olefins at high concentration (above 20%) indicate that large proportions of olefinic compounds can make extrapolation indefinite.

For hydrocarbon mixtures containing 20% or less unsaturates, however, it appears to be a safe guide to consider that the extrapolation method fails when a linear continuation of the data to zero acid-oil ratio is not immediately apparent.

Application of the extrapolation method to hydrocarbon mixtures containing much more than 20% of olefins or aromatics is not practicable in any case with the present technique, inasmuch as the raffinate quantities would be too small for ordinary manipulation.

The extrapolation method of analysis appears at first sight to have the fundamental drawback of giving as unsaturated any substance which has at least one unsaturated group per molecule, regardless of the saturated character of the rest of the molecule. This is not actually a disadvantage in many cases, however, by virtue of the fact that the density and refractive index of the extracted unsaturated material can be approximated sufficiently to give a general idea of the nature of the unsaturates, especially if this information is supplemented by determinations of bromine number and optical dispersion (*5*, *16*, *17*).

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Determination of Large Amounts of Manganese

Modified Persulfate Method

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IN A STUDY of the reaction of ammonium persulfate with manganous salts in acid solution, it was discovered that by the addition of disodium hydrogen phosphate, the manganese can be oxidized and the excess persulfate decomposed by boiling; finally by the use of osmic acid, as recommended by Gleu (*4*), the permanganate may be stoichiometrically titrated with sodium arsenite solution. These modifications adapt the persulfate method to the accurate determination of manganese in larger amounts than has been possible heretofore and extend the usefulness of the procedure to the determination of manganese in such materials as manganese ores and ferromanganese. In this paper are given brief descriptions of the experimental work and a method of procedure for manganese in high-grade manganese ore.

Two major modifications in the usual persulfate method were adopted to permit accurate determination of moderately large amounts of manganese: (1) addition of disodium hydrogen phosphate to retard the decomposition of the permanganate, and (2) establishment of heating limits to decompose the excess persulfate, yet not affect the permanganate. Permanganate ion tends to decompose upon heating in an acid solution, the ionic equation for the reaction being

$$MnO_4^- - 4H^+ - 3e = MnO_2 - 2H_2O \qquad (1)$$

This equation shows that the reduction of the MnO_4^- involves the oxidation potential equation

$$E = E_0 - RT/3F \ln (MnO_4^-)(H^+)^4 \qquad (2)$$

Assuming the oxidation potential of sodium to be positive, the E_0 of this equation will be negative. Thus, upon examination, it is found that if the hydrogen-ion concentration, or the permanganate-ion concentration, is decreased, the oxidation potential, E, becomes negatively smaller or actually larger, making the reduction less likely. Since it was desired to increase the limit on the amount of manganese present, the hydrogen-ion concentration or acidity was reduced through addition of disodium hydrogen phosphate.

While the reduction in hydrogen-ion concentration should, according to Equations 1 and 2, greatly reduce the rate of permanganate decomposition, it should not affect the rate of decomposition of the excess persulfate, since the ionic equation for the reduction of persulfate is

$$S_2O_8^{--} - 2e = 2SO_4^{--} \qquad (3)$$

As can be observed from this equation, no hydrogen ions are involved in the process of reducing persulfate ion.

PROCEDURE

Although the maximum amount of manganese that can be present in a sample is still under investigation, it has been found that samples containing up to 0.05 gram of manganese can be effectively employed. Thus, an aliquot containing from 0.03 to 0.05 gram of manganese can be accurately analyzed by this procedure.

The sample first must be powdered, dried, and dissolved in an aqua regia solution. This solution is filtered and the residue is fused with c.p. sodium carbonate in a platinum crucible. If the carbonate turns green, manganese has been left in the precipitate and the fused mass must be dissolved in the filtrate. To the resulting solution is added enough concentrated sulfuric acid to give 100 cc. of 6 *N* sulfuric acid solution when diluted to 250 cc.—approximately 33 grams or 18 cc. The aqua regia is now evaporated off or the solution is evaporated to fumes of sulfur trioxide. The remaining solution is quantitatively washed into a 250-cc. volumetric flask and diluted to the mark. From this solution 25-cc. portions can be pipetted into flasks for analysis.

To each portion to be analyzed are added from 3 to 5 grams of disodium hydrogen phosphate, followed by approximately

Table I. Decomposition of Permanganate with and without Disodium Hydrogen Phosphate

Experiment No.	Time of Heating	Disodium Hydrogen Phosphate Added	Sodium Arsenite Required	Sodium Arsenite Required for Blank	Variation of Trial from Blank
	Min.	*Grams*	*Cc.*	*Cc.*	*Cc.*
1	16 (water bath)	5	23.37	23.37	0.00 (none)
2	16 (water bath)	3	23.35	23.37	−0.02
3	16 (water bath)	1	22.89	23.37	−0.48
4	16 (water bath)	0	22.85	23.37	−0.52
5	5 (open flame)	0	22.77	23.37	−0.60
6	5 (open flame)	5	23.36	23.37	−0.01
7	15 (open flame)	5	23.36	23.37	−0.01

10 cc. of sirupy phosphoric acid. After the phosphate has completely dissolved. 10 cc. of freshly prepared 20% ammonium persulfate are added. The oxidation is catalyzed by 5 cc. of 0.1 *N* silver nitrate. The solution is now placed in a boiling water bath for 14 to 20 minutes or boiled gently over an open oxidizing flame 3.5 to 5 cm. in height for as close to 2 minutes as possible, then cooled immediately to 20° or 25° C. in a cold water bath. Then the solution is made strongly acid by adding 25 cc. of 6 *N* sulfuric acid. A few drops of the osmium tetroxide catalyst are added and the solution is ready for titration.

This may proceed in two different ways, by direct titration or by back-titration. The first method consists of running sodium arsenite titer into the solution being analyzed until it becomes just colorless, or orange if the *o*-phenanthroline indicator is used. In the second method sodium arsenite is run into the test solution until it is colorless, and a few milliliters are added in excess. This excess is then back-titrated with standardized potassium permanganate to the first sign of pink, or, if sodium diphenylamine sulfate indicator is used, to a purple color. The amount of manganese present is calculated by a simple volumetric equation.

DISCUSSION

In performing the experimental work on this project, solutions of 0.1 *N* potassium permanganate, free from manganese dioxide, were made up, then reduced to manganous ions with hydrogen peroxide which was almost completely chloride-free. From this point the procedure described was followed.

Thus, the decomposition of the permanganate ion was determined by preparing the test solutions as above and heating for various times (Table I). Phosphate was absent or present in a varying amount, and in no case did the procedure necessitate addition of any oxidation or reduction agents, since it was the permanganate decomposition that was being determined. The blanks, which were used to compare these results, were prepared by pipetting a definite amount of the potassium permanganate solution into a flask, acidifying, and titrating to an end point with the sodium arsenite.

The second modification in the procedure was determined purely through experiment. After the permanganate was kept from decomposing by the phosphate and the persulfate was again used, the comparative results began to run low once more. It was reasonable to assume that in some manner the decomposition of the persulfate had affected the oxidation of the manganese. Although what actually happens is not known, it was found that by heating the test solution a so-called point of equilibrium could be established at which the persulfate is completely decomposed, but does not affect the permanganate formed by oxidation. This was accomplished by following the routine procedure up to the point which called for heating to decompose the persulfate. At this point, the solution was heated for various lengths of time both in a water bath and over an open flame until the desired points were found. The results and comparison with the blank are given in Table II.

Many other trials were made to determine the limits on the amounts of reagents that might be used. In all cases the trials were identical with those reported in Table II, except that the reagent in question was varied from one extreme to the other. It was found that the amount of sirupy phosphoric acid used can vary between 7 and 13 cc. and still maintain the accuracy of the procedure. The amount of silver nitrate catalyst required may vary up to about 10 cc. but must not be less than 3 cc. The primary addition of sulfuric acid, which is often made while making up the original sample, should be between 0.08 and 0.025 equivalent. The minimum limit is established for this reagent, but the maximum limit may be somewhat larger, since no experimental trials were made above this point. The secondary addition of sulfuric acid is indefinite, as long as the test solution is made definitely acid.

As an indication of the accuracy of this procedure, several trials, using the method described, were made on manganese ore No. 25a, obtained from the Bureau of Standards (Table III). Another trial was made on the same sample using the bismuthate method (*1*, *5*). The manganous ions were oxidized to permanganate by means of an excess of sodium bismuthate, the excess was filtered off, and the residue washed with dilute nitric acid. The test solution was titrated with sodium arsenite in a definitely acid solution.

REAGENTS

Osmium Tetroxide Catalyst. Break a 1-gram capsule of osmium tetroxide beneath the surface of 390 cc. of 0.1 *N* sulfuric acid and make sure all has dissolved before filtering glass off. Osmium tetroxide emits poisonous vapors when standing in air. Preserve catalyst in a glass-stoppered bottle.

Silver Nitrate Solution. Dissolve approximately 17 grams of the silver nitrate salt in 1 liter of distilled water.

Ammonium Persulfate Solution. Dissolve 20 grams of the persulfate in 100 cc. of distilled water at room temperature. This solution must be prepared fresh each time, since it decomposes upon standing.

Table II. Effect of Time of Heating Solution on Decomposition of Ammonium Persulfate

Experiment No.	Time of Heating	Sodium Arsenite Required	Sodium Arsenite Required for Blank	Variation of Trial from Blank
	Min.	*Cc.*	*Cc.*	*Cc.*
1	12 (water bath)	23.49 (no constant end point)	23.37	−0.12
2	14 (water bath)	23.35	23.37	−0.02
3	16 (water bath)	23.37	23.37	0.00
4	18 (water bath)	23.36	23.37	−0.01
5	20 (water bath)	23.35	23.37	−0.02
6	22 (water bath)	23.31	23.37	−0.06
7	24 (water bath)	23.26	23.37	−0.11
8	5 (open flame)	23.28	23.37	−0.09
9	4 (open flame)	23.31	23.37	−0.06
10	3 (open flame)	23.34	23.37	−0.03
11	2 (open flame)	23.36	23.37	−0.01
12	1 (open flame)	23.51 (no constant end point)	23.37	−0.14

Table III. Results of Actual Trials on Standard Samples

Experiment No.	Type of Procedure	Manganese Determined	Standard Value	Error
		%	%	%
1	Persulfate	56.67	56.62	−0.09
2	Persulfate	56.65	56.62	−0.05
3	Persulfate	56.57	56.62	−0.09
4	Persulfate	56.62	56.62	None
5	Bismuthate	56.26	56.62	−6.38

SODIUM ARSENITE SOLUTION. Dissolve approximately 7 grams of sodium hydroxide pellets in as little cold water as possible, then dissolve exactly 4.9456 grams of pure dry arsenious oxide in this solution. Finally, neutralize with 1 *N* sulfuric acid, transfer to a 1-liter volumetric flask, and dilute to the mark. This gives an exactly 0.1 *N* solution of sodium arsenite.

POTASSIUM PERMANGANATE SOLUTION. Filter through a Jena glass crucible an approximately 0.1 *N* solution which has aged several days, and standardize against pure dry sodium oxalate.

ACKNOWLEDGMENT

The author wishes to acknowledge his indedtedness to B. F. Brann, professor of physical chemistry at the University of Maine, whose excellent cooperation and progressive suggestions were largely responsible for this paper.

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Determination of Rate of Cure of GR-I and Natural Rubber

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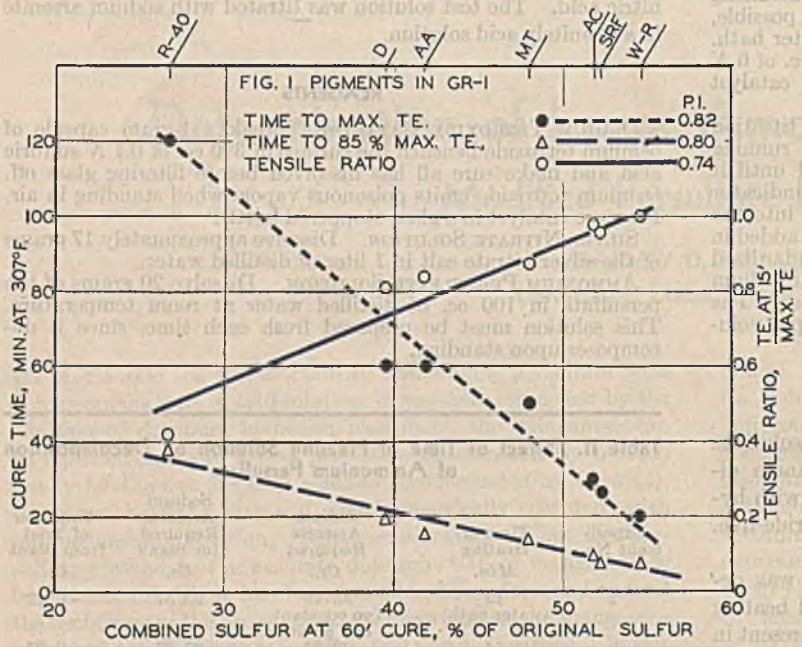
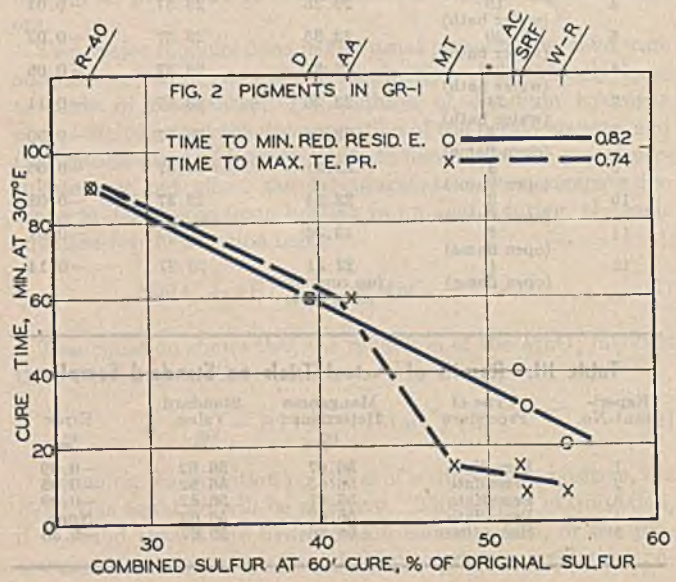




Indexes of rate of cure previously used in natural rubber and GR-S have been compared in GR-I (Butyl). Combined sulfur varies with cure time in a manner similar to that observed in natural rubber and GR-S. The T-50 temperature varies only slightly with cure time and does not appear to be satisfactory as an index of rate of cure in GR-I. Tensile ratio (tensile at an undercure/maximum tensile) appears to be a useful index of rate of cure in GR-I. For any series of stocks a convenient undercure is, as in the case of GR-S and natural rubber, a cure giving a tensile ratio of about 0.60 for the stocks in the series having intermediate cure rates.

IN A previous publication two of the authors studied various methods for determining the rate of cure of natural rubber and GR-S (*2*). Evidence was presented to show that the tensile ratio, the ratio of tensile strength at an undercure to maximum tensile strength, was a useful index of rate of cure.

TESTS IN NATURAL RUBBER ON PRODUCTION CARBON BLACKS

In order to examine further the applicability of the tensile ratio to practical rate of cure problems, tensile ratio, T-50 at 10 minutes, and T-50 at 45 minutes were determined for forty-four sam-

Table I. Tensile Ratio and T-50 for Forty-four Production Channel Black Samples

(Expressed as per cent of a standard control)

	Tensile Ratio, Tensile 10 Min./ Tensile 45 Min.	T-50, 10 Min.	T-50, 45 Min.
Average Grade AA (EPC)	112	117	115
Average Grade A (MPC)	106	103	119
Average Grade D (MPC)	103	99	111
Coefficient of correlation			
Tensile ratio *vs.* T-50 at 10-minute cure (all grades)			+0.30
Tensile ratio *vs.* T-50 at 45-minute cure (all grades)			−0.26
T-50 at 10-minute cure *vs.* T-50 at 45-minute cure (all grades)			−0.10

Table II. Pigments in GR-I

	Gum	Witcarb R	MT	Continex SRF	Continental AA	Continental D	Continental R-40	Acetylene Black
GR-I (Butyl)	100	100	100	100	100	100	100	100
Zinc oxide	5	5	5	5	5	5	5	5
Stearic acid	1	1	1	1	1	1	1	1
Sulfur	2	2	2	2	2	2	2	2
Tetramethylthiuram disulfide	1	1	1	1	1	1	1	1
Mercaptobenzothiazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Witcarb R (W-R)	...	78	...	...	...	...	...	...
Medium thermal (MT)	...	...	50	...	...	...	...	...
Continex SRF	...	...	...	50	...	...	...	...
Continental AA (EPC)	...	...	...	...	50	...	...	...
Continental D (MPC)	...	...	...	...	...	50	...	...
Continental R-40 (CC)	...	...	...	...	...	...	50	...
Acetylene black (AC)	...	...	...	...	...	...	...	50
Cure at 307° F., Min.								
				Modulus at 400% Elongation, Pounds per Square Inch				
8	...	...	...	250	200	200	...	350
15	...	135	150	315	300	300	175	500
30	100	175	200	500	475	435	200	725
60	150	200	250	700	650	650	250	925
90	175	200	285	735	800	850	350	975
180	200	200	325	735	925	880	400	1000
				Tensile at Break, Pounds per Square Inch				
8	280[a]	1760	1075	1675	1200	1380	390	1250
15	1100[a]	1875	1460	1820	1960	2160	740	1570
30	2530[a]	1875	1600	1900	2150	2475	1400	1600
60	2530[a]	1825	1675	1850	2335	2670	1675	1600
90	2000[a]	1800	1525	1800	2300	2630	1750	1600
180	1975[a]	1800	1525	1750	2300	2650	1700	1500
				Elongation at Break, %				
8	1300[a]	1000	960	935	1000	1110	1200	825
15	1200[a]	875	900	815	915	960	1100	750
30	1050[a]	835	815	725	850	925	950	650
60	875[a]	815	775	675	800	885	925	600
90	825[a]	800	735	650	715	790	900	590
180	825[a]	800	675	635	685	770	800	575
				Breaking Set, %				
8	...	30	25	30	60	68	150	50
15	...	25	25	25	50	50	75	38
30	...	25	25	25	43	45	58	35
60	...	23	20	25	35	35	55	35
90	...	20	15	20	27	35	50	30
180	...	18	15	20	25	30	50	19
				Tear, Pounds per Square Inch				
8	40	200	90	200	185	280	85	200
15	45	150	85	200	325	405	135	215
30	30	145	70	190	395	475	175	250
60	30	125	50	150	415	495	200	220
90	35	115	50	160	385	440	200	220
180	25	125	75	160	390	435	...	...
				Durometer, Instantaneous				
8	24	40	40	48	51	51	51	51
15	26	40	40	49	53	55	51	55
30	27	42	42	50	54	56	51	56
60	30	45	45	55	56	59	53	60
90	30	46	45	53	57	62	54	60
180	30	45	43	51	57	62	51	60
				Durometer, 30-Second Reading				
8	16	30	30	39	38	39	35	41
15	21	34	35	41	43	46	39	47
30	22	38	39	45	45	46	40	51
60	28	40	41	50	50	52	40	57
90	29	41	41	50	51	55	42	57
180	29	41	40	47	51	56	42	56
				Rebound at 100° C., %, Bashore				
8	37	30	34	28	21	19	18	22
15	38	31	36	29	22	20	18	23
30	43	32	37	30	23	21	20	23
60	46	32	39	31	24	23	21	25
90	48	33	41	33	26	25	22	25
180	46	32	40	31	26	26	23	24
				Abrasion Loss, Du Pont, Cc. per Hp.-Hour				
30	[b]	1320	1590	346	...	...	...	229
60	[b]	1020	760	265	401	424	...	160
90	...	...	...	...	...	...	534	...
180	[b]	937	663	249	329	361	481	157
				Heat Buildup, ° F.[c]				
90	[d]	195	[d]	201	...	...	...	253
150	...	...	...	...	257	268	[e]	...
				Williams Plasticity, Inch				
..	0.110	0.118	0.116	0.124	0.141	0.150	0.166	0.139
				Tubing Rate, Grams per Minute[f]				
..	15.0	26.0	24.0	27.0	22.2	20.8	19.2	29.7
				T-50, ° C.				
15	−44.0	−39.8	−37.5	−29.3	−18.9	−17.5	−10.8	−14.0
90	...	−43.0	...	...	−25.5	−23.8	−14.3	...
				Combined Sulfur, % of Original Sulfur				
60	49.8	54.6	47.9	52.3	41.8	39.5	26.6	51.9
				Tensile Ratio, 15-Minute/Maximum				
..	...	1.0	0.87	0.96	0.84	0.81	0.42	0.98

[a] Results questionable owing to tendency of gum stock to tear in tensile jaws.
[b] Test block crumbled when abraded.
[c] Heat rise of center of test plug over room temperature (70° ± 3° F.) on St. Joe flexometer after 20 minutes at 475 pounds' vertical load and 0.130-inch horizontal deflection. Face plate temperature of 100° ± 2° F. used at beginning of each test.
[d] Test plugs blew out in less than 5 minutes under conditions of test.
[e] Test plug could not be successfully cured.
[f] 0.5-inch Royle tuber, 3/16-inch die, 24 r.p.m. screw speed, and 170° F. barrel temperature.

ples of channel black collected weekly from several different operating units. The blacks were milled in the following test formulation which was cured at 280° F.:

Smoked sheet	100
Stearic acid	4.0
Pine tar	1.5
Sulfur	3.0
Phenyl-β-naphthylamine	1.0
Zinc oxide	3.0
Mercaptobenzothiazole	1.0
Carbon black	50.0

Under the conditions used all the channel blacks reached a tensile in 45 minutes sufficiently close to the maximum tensile so that the more readily determined ratio (tensile at 10 minutes/tensile at 45 minutes) could be used for tensile ratio in place of (tensile at 10 minutes/maximum tensile). All samples were tested alongside a standard control and the average values reported in Table I are expressed as per cent based on the control. Percentages are calculated so that higher values indicate faster cure rate.

Considering the average values of the three grades of black tested, the tensile ratio and T-50 at 10-minute cure indicate decreasing rate of cure in going from AA to D. This is in agreement with actual experience. T-50 at 45 minutes, however, does not follow the expected trend. Likewise, the coefficient of correlation between tensile ratio and T-50 at 10 minutes is +0.30, which for the number of stocks used shows some interdependence, there being less than one chance in twenty that this correlation could arise through random sampling errors. On the other hand, T-50 at 45 minutes shows no correlation with either tensile ratio or T-50 at 10 minutes. Just why the T-50 at 10 minutes and T-50 at 45 minutes should agree so poorly for these stocks is not clear; however, the evidence indicates that both tensile ratio and T-50 at 10 minutes

can be used as indexes of cure rate. The tensile ratio has the advantage of being very simply obtained from tensile data which are usually determined anyhow in evaluating a stock.

RATE OF CURE OF GR-I

The rate of cure of GR-I (Butyl) was studied, following the approach previously used for natural rubber and GR-S (*2*). Two series of stocks were used in this work. The first series, formula and tests on which appear in Table II, consists of one inorganic and various carbon pigments and covers a wide range of cure rates. The second series (Table III) includes only channel carbon blacks and covers a narrower cure range. A loading of 25.6 volumes per 100 volumes of GR-I was used for all pigments. Physical properties, T-50 tests, and combined sulfur data are included in the tables. A.S.T.M. standard test conditions were used except where otherwise specified. A different shipment of GR-I was used in each set. Although both sets are consistent within themselves, appreciable differences were found between the two sets. These differences may be due to variation in the polymer or to unintentional differences in processing the two sets.

Combined sulfur was calculated from the sulfur originally present by subtracting the free sulfur determined by the sodium sulfite extraction method (*1, 4*).

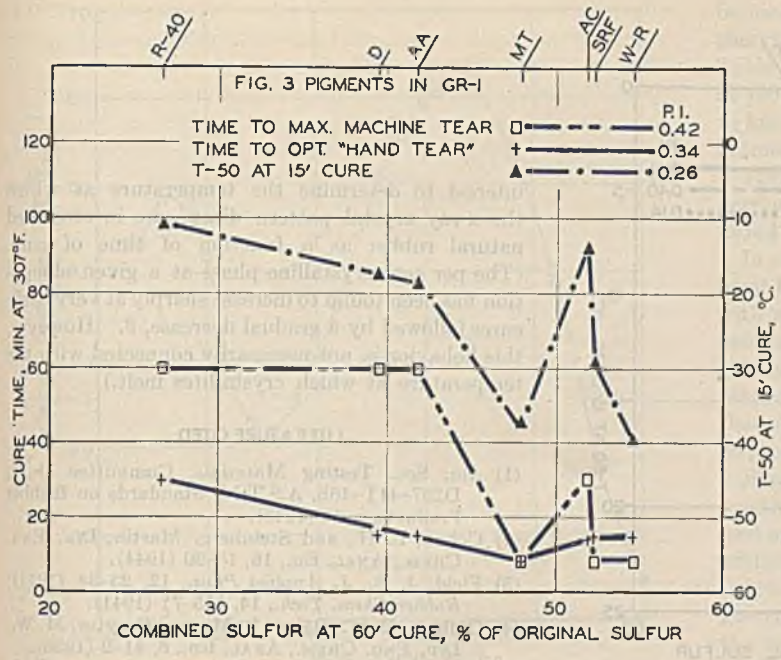
The sulfur determinations are subject to two principal errors: (1) the tetramethylthiuram disulfide in the formula may interfere, giving high results for free sulfur, and (2) slow diffusion through GR-I may lead to incomplete extraction of the free sulfur in the standard 2-hour extraction time. Determinations were therefore carried out at various extraction times with the results shown in Table IV. In the case of the uncured stock, a value equal to essentially all the free sulfur is obtained in 8 hours. Since for the cured stock the per cent of sulfur extracted also appears to be leveling off at about this time, the 8-hour extraction was adopted throughout. (Since this work was completed a new method for determination of sulfur in Butyl based on methyl ethyl ketone extraction has appeared, *5*.)

The physical test data in Tables II and III were used to determine time to reach maximum tensile, tensile product, tear, and rebound; time to reach minimum reduced residual elongation (breaking set divided by tensile); time to reach 85% and other percentages of maximum physical properties; tensile ratio (ten-

Table III. Channel Blacks in GR-I[a]

	Continental AAA	Continental AA	Continental A	Continental D	Continental F	Continental R-20	Continental R-30	Continental R-40
GR-I (Butyl)	100	100	100	100	100	100	100	100
Zinc oxide	5	5	5	5	5	5	5	5
Stearic acid	1	1	1	1	1	1	1	1
Sulfur	2	2	2	2	2	2	2	2
Tetramethylthiuram disulfide	1	1	1	1	1	1	1	1
Mercaptobenzothiazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Continental AAA (EPC)	50	...	...	...	...	...	...	...
Continental AA (EPC)	...	50	...	...	...	...	...	...
Continental A (MPC)	...	...	50	...	...	...	...	...
Continental D (MPC)	...	...	...	50	...	...	...	...
Continental F (HPC)	...	...	...	...	50	...	...	...
Continental R-20 (CC)	...	...	...	...	...	50	...	...
Continental R-30 (CC)	...	...	...	...	...	...	50	...
Continental R-40 (CC)	...	...	...	...	...	...	...	50
Cure at 307° F., Min.								
	Modulus at 400% Elongation, Pounds per Square Inch							
8	250	180	200	...	...	210	150	75
15	375	280	250	320	475	300	300	125
30	650	400	400	450	550	450	375	250
60	750	600	600	625	600	650	550	350
90	800	700	750	775	675	700	725	400
180	900	800	900	950	800	875	775	525
	Tensile at Break, Pounds per Square Inch							
8	1275	775	925	800	800	1075	525	335
15	1750	1400	1500	1600	1600	1725	1450	550
30	2010	1675	2100	2250	2225	2475	2300	1100
60	2325	2000	2400	2425	2500	2650	2525	1350
90	2300	1950	2400	2475	2475	2725	2600	1400
180	2250	1925	2325	2400	2400	2725	2575	1535
	Elongation at Break, %							
8	1120	1055	1200	1170	930	950	900	800
15	980	890	1025	1035	835	860	880	730
30	880	825	895	870	800	850	950	775
60	795	760	800	815	790	775	845	770
90	700	700	740	740	755	750	805	700
180	700	655	705	705	740	740	780	700
	Breaking Set, %							
8	53	53	68	75	75	40	110	165
15	40	38	53	50	53	50	92	75
30	32	35	45	45	50	50	70	70
60	30	30	38	38	50	45	70	60
90	25	33	35	33	38	38	65	60
180	25	25	30	30	35	37	58	60
	Tear, Pounds per Inch							
8	260	150	145	130	145	250	120	135
15	380	225	235	350	310	395	175	145
30	425	315	480	475	430	495	430	190
60	475	365	495	485	475	495	545	195
90	450	350	460	475	485	500	545	200
180	450	350	460	415	475	500	530	215
	Durometer, Instantaneous							
8	47	46	45	50	40	47	45	25
15	49	48	47	51	45	50	50	30
30	50	49	49	51	49	52	51	35
60	52	50	52	55	52	54	53	50
90	52	51	54	56	51	55	57	49
180	52	50	55	54	50	54	57	50
	Durometer, 30-Second Reading							
8	35	35	31	36	28	38	33	20
15	40	40	39	41	39	43	40	22
30	43	41	41	45	40	45	45	26
60	46	45	46	48	47	48	46	40
90	48	48	50	50	46	50	50	41
180	48	45	50	50	48	49	50	43
	Rebound at 100° C., %, Bashore							
8	24	25	23	23	25	22	19	19
15	25	27	24	25	25	23	21	21
30	31	33	30	29	27	26	23	26
60	32	34	30	30	28	25	22	24
90	33	33	31	30	29	27	25	25
180	61	32	29	28	28	27	24	24
	Heat Buildup, ° F.							
150	242	242	247	251	248	256	[b]	[c]
	Tubing Rate, Grams per Minute							
..	18.4	18.8	18.2	18.6	17.3	14.6	14.7	16.4
	T-50, ° C.							
15	−21.3	−21.0	−20.4	−20.5	−20.2	−19.6	−16.0	−10.8
90	...	...	−25.0	−24.4	−24.0	−25.5	...	...
	Combined Sulfur, % of Original Sulfur							
30	38.0	36.2	31.9	30.7	...	...	30.2	...
60	45.4	43.6	40.0	40.0	40.0	40.2	41.6	32.6
	Tensile Ratio (15-Minute/Maximum)							
..	0.75	0.70	0.63	0.65	0.64	0.63	0.56	0.36

[a] Same test conditions as listed in Table II.
[b] Blew out in 18.5 minutes under conditions of test.
[c] Test plug would not cure properly even after 4 hours at 307° F.



sile at 15 minutes/maximum tensile); and tensile product ratio (tensile product at 15 minutes/maximum tensile product). The 15-minute cure was selected, as this cure gave tensile ratios between 0.30 and 1.00 for all stocks and ratios of about 0.60 for stocks having intermediate cure rates. Time to reach optimum cure as judged by hand tear was also determined. No attempt was made to determine the break in the modulus versus cure time curve, as the change in slope of this curve is very gradual for the GR-I formulation used. The indexes of rate of cure which have been proposed in the literature and also those indexes which were found to correlate closely with combined sulfur are plotted against combined sulfur in Figures 1 to 5. Prediction indexes (P.I. = $1 - \sqrt{1 - r^2}$, where r is the coefficient of correlation) showing the extent of correlation of the various indexes of rate of cure with combined sulfur are tabulated on the figures.

In both the pigment and channel black series, tensile ratio shows a high degree of correlation with combined sulfur. Time to 85% maximum tensile, time to optimum tensile, time to optimum machine tear, and time to optimum tensile product also show a significant correlation within the 95% confidence limits for both sets. The minimum in the reduced residual elongation versus cure-time curve is not an absolute minimum but a mathematical minimum followed by a maximum and then a further drop to lower levels. For this reason, the minimum is difficult to determine unless a considerable number of cures are available in the minimum region. The minimum could be determined for only five of the pigment series and two of the channel black series.

The GR-I gum stock was not included in the graphs or calculations because of the questionable character of most of the stress-strain data. This stock tore so easily that it was extremely difficult to prevent test dumbbells from tearing in the jaws of the tensile machine. The values reported were obtained by using a narrow die (0.090 instead of 0.250 inch in width) and disregarding all samples which did not break near the center of the test dumbbell. Judging from the combined sulfur results, the T-50 value for the gum stock also appears too low compared with the T-50 values for the loaded stocks. In natural rubber, too, it has been found that the T-50 cannot be used in comparing the rate of cure of a gum stock with that of loaded stocks (*2*). In such a comparison, T-50 is out of line with practically all other indexes of rate of cure.

Of those indexes showing good correlation with combined sulfur, tensile ratio is for many purposes one of the most convenient to determine. It therefore appears that tensile ratio is a useful index of rate of cure in GR-I as well as in natural rubber and GR-S.

The T-50 temperature decreases slightly with increasing cure time. The decrease is greater than for GR-S but much less than for natural rubber. In view of the small difference between T-50 at 15 minutes and T-50 at 90 minutes, the rather large differences between stocks containing various pigments were surprising. Apparently this behavior may be due to the effect of some other property of the stock, such as set, on T-50, rather than to rate of cure. In any case it appears doubtful that the T-50 temperature can be used as a satisfactory index of rate of cure for GR-I.

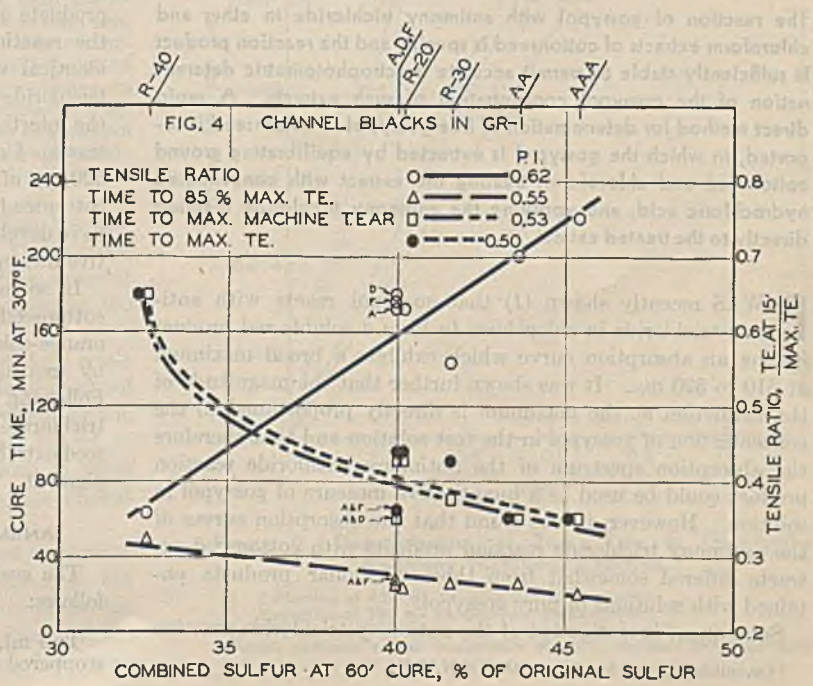
The T-50 temperature may be associated with the melting point of crystallites formed at low temperature in the stretched polymer. Since GR-S does not crystallize and GR-I crystallizes to only a limited extent at high elongation, we might expect them

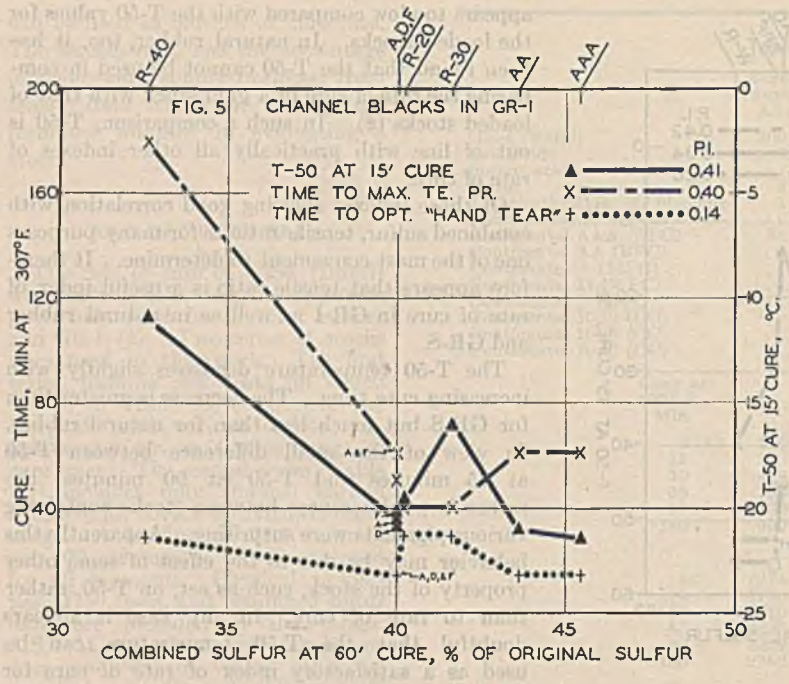
Table IV. Effect of Extraction Time on Amount of Sulfur Extracted

(Expressed as per cent of original sulfur)

Stock	Extraction Time 2 Hours	4 Hours	5.5 Hours	8 Hours	16 Hours
Continental AA in GR-I (uncured)	75.3	...	95.2	99.4	...
Continental D in GR-I[a] (60-min. cure)	55.5	57.9	...	57.9	58.9

[a] A different cure than reported in Table II.





to show, respectively, no and very little variation in T-50 temperature with time of cure. In this connection it might be of interest to determine the temperature at which the x-ray crystal pattern disappears in stretched natural rubber as a function of time of cure. (The per cent crystalline phase at a given elongation has been found to increase sharply at very early cures followed by a gradual decrease, *3*. However, this behavior is not necessarily connected with the temperature at which crystallites melt.)

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Quantitative Determination of Extractable Gossypol in Cottonseed and Cottonseed Meal

A Spectrophotometric Method

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The reaction of gossypol with antimony trichloride in ether and chloroform extracts of cottonseed is specific and the reaction product is sufficiently stable to permit accurate spectrophotometric determination of the gossypol concentration of such extracts. A rapid direct method for determination of free gossypol in cottonseed is reported, in which the gossypol is extracted by equilibrating ground cottonseed and chloroform, treating the extract with concentrated hydrochloric acid, and applying the antimony trichloride reaction directly to the treated extract.

IT WAS recently shown (*1*) that gossypol reacts with antimony trichloride in chloroform to form a soluble red product having an absorption curve which exhibits a broad maximum at 510 to 520 mμ. It was shown further that the magnitude of the extinction at the maximum is directly proportional to the concentration of gossypol in the test solution and that therefore the absorption spectrum of the antimony trichloride reaction product could be used as a quantitative measure of gossypol in solution. However, it was found that the absorption curves of the antimony trichloride reaction products with cottonseed extracts differed somewhat from those of similar products obtained with solutions of pure gossypol.

Subsequent investigation of the antimony trichloride reaction products with cottonseed extracts has shown that in most cases the reaction product exhibits, initially, an absorption curve identical with that obtained with pure gossypol. Antimony trichloride evidently reacts more rapidly with gossypol than with the interfering reactants which may be present in cottonseed extracts. Consequently, when the absorption maximum at 510 to 520 mμ of the reaction product of antimony trichloride with a cottonseed extract is determined before interfering reactions have developed, the height of this maximum serves as a quantitative measure of the concentration of gossypol in such an extract.

In some hydraulic press-cake meals, as well as in some raw cottonseed, much of the "free" gossypol occurs in the form of an orange-colored pigment (*2*) which can be converted into gossypol by treating the extracts with concentrated hydrochloric acid. Following such treatment, these extracts react with antimony trichloride in a manner completely analogous to that of cottonseed extracts containing negligible amounts of the orange-colored pigment.

ANTIMONY TRICHLORIDE REACTION WITH PURE GOSSYPOL

The gossypol–antimony trichloride reaction is carried out as follows:

To 1 ml. of a chloroform solution of purified gossypol in a glass-stoppered absorption cell there are added 1 drop of acetic anhy-

[1] On military leave for duty with U.S.N.W.R.

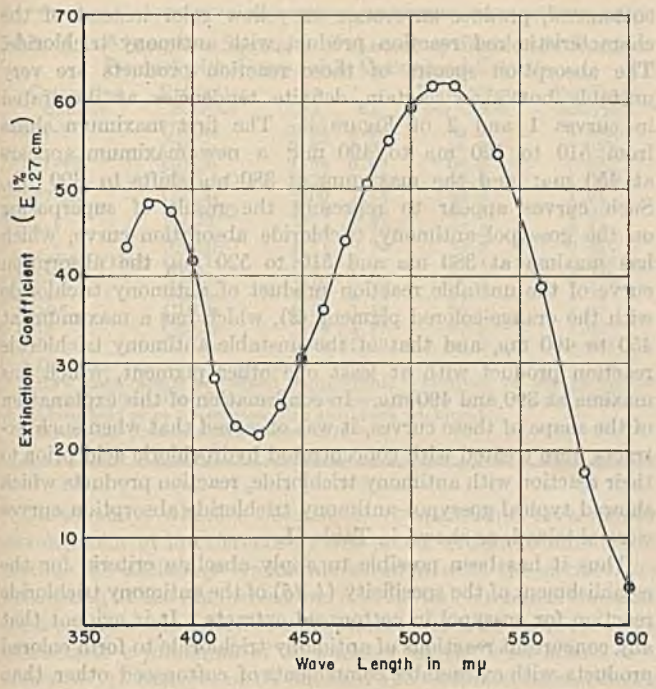

Figure 1. Absorption Curve of Antimony Trichloride Reaction Product with Pure Gossypol

dride and 5 ml. of a saturated chloroform solution of antimony trichloride. The acetic anhydride is added to prevent the development of the hazes produced by moisture in the extracts. For the same reason it is desirable to mix the reagents directly in the absorption cells and thus avoid unnecessary exposure to atmospheric moisture. The transmission of the solution is measured against that of a blank which consists of 1 ml. of chloroform, 1 drop of acetic anhydride, and 5 ml. of a saturated chloroform solution of antimony trichloride.

The volumes of reagents indicated were chosen to suit the capacity of the cells designed for the Coleman double monochromator spectrophotometer which was used for all absorption measurements reported here. The Coleman spectrophotometer is equipped with cells having an estimated optical depth of 1.27 cm. and all extinction coefficients were, therefore, expressed in terms of this optical depth rather than the customary 1.0-cm. depth. Extinction coefficients are defined by the equation $E = \frac{\log I_0/I}{cl}$ where $\log I_0/I$ is the extinction, I_0, the intensity of light transmitted by the blank, I the intensity of light transmitted by the solution, c the concentration of the solution, and l the length of the path of light through the liquid. The extinction coefficients were calculated in terms of the optical depth of the absorption cells used (1.27 cm.) and, for convenience in calculating the concentration of gossypol in test extracts, they were expressed in terms of the concentration in grams of gossypol per 100 ml. of the original solution to which the acetic anhydride and the antimony trichloride solution were added.

The absorption curve of the antimony trichloride reaction product with pure gossypol is shown in Figure 1. The curve is characterized by two maxima, a broad one in the visible range at 510 to 520 mμ, and a sharper one in the near ultraviolet at 380 mμ, and by a minimum at 430 mμ.

As shown in Figure 2, a straight-line relationship exists between the values of $\log_{10} I_0/I$ and the concentration of gossypol at 520, 430, and 380 mμ for concentration of gossypol in the original solution ranging from 0.004 to 0.016 gram per 100 ml. of solution. These values were obtained with solutions of three preparations of gossypol prepared according to two independent procedures (*1*, *2*).

The existence of two well-defined, widely separated absorption maxima permitted the mathematical characterization of the absorption spectrum (*4*, *15*) of the gossypol–antimony trichloride reaction product in terms of the ratios of the magnitudes of the maxima to each other and to the minimum. These ratios can be used for establishing the specificity (*4*, *15*) of the reaction for gossypol contained in extracts of cottonseed. As shown in Table I, the value of the ratio R_a, $\log_{10} I_0/I$ at 520 mμ to $\log_{10} I_0/I$ at 430 mμ, is 2.68 ± 0.23, and the ratio R_b, $\log_{10} I_0/I$ at 520 mμ to $\log_{10} I_0/I$ at 380 mμ, is 1.22 ± 0.07 for pure gossypol in the antimony trichloride reaction.

The absorption spectrum in the range 370 to 600 mμ reaches its maximum development within 10 minutes after the reagents are mixed and is stable for at least 24 hours.

In contrast to the reaction of gossypol, the orange-colored pigment from cottonseed (*2*) does not form a stable reaction product with antimony trichloride. When a chloroform solution of the orange-colored pigment is first treated with concentrated hydrochloric acid and then reacted with antimony trichloride, the reaction product exhibits an absorption spectrum identical with that of the gossypol–antimony trichloride reaction product with respect to both positions and ratios of maxima and minimum.

Because of its broadness and its position in the visible wavelength range, the absorption maximum at 510 to 520 mμ serves best as a standard for computing gossypol concentrations in test solutions. When dilutions ranging from 0.004 to 0.016% gossypol of nine different solutions of three preparations of pure gossypol obtained by two different procedures (*1*, *2*) were used, the values for $E^{1\%}_{1.27\,cm.}$ at 520 mμ shown in Table I were obtained. From these data it may be concluded that the value of $E^{1\%}_{1.27\,cm.}$ at 520 mμ is 65.5 ± 1.9 for gossypol in the antimony trichloride test where the concentration of gossypol is expressed in terms of grams per 100 ml. of the original solution.

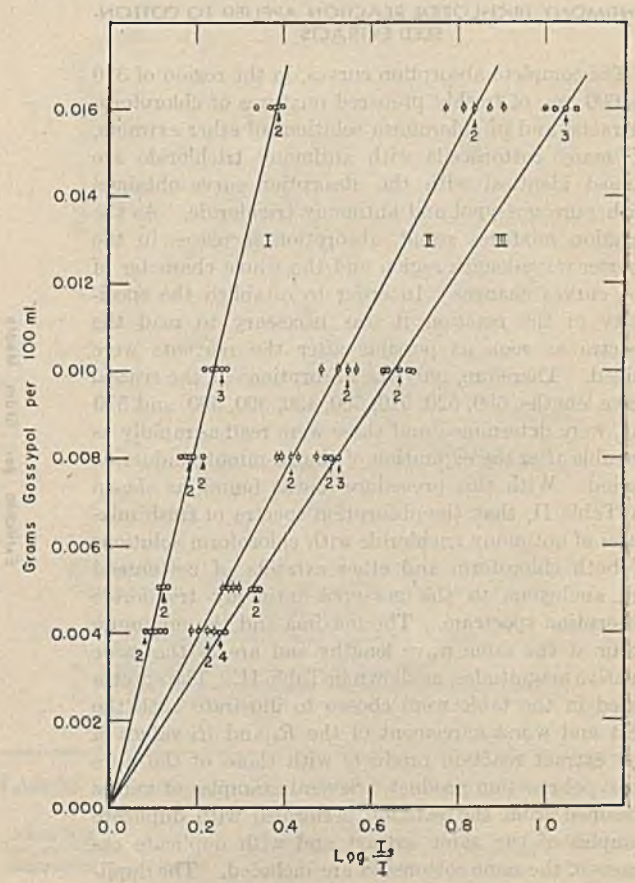

Figure 2. Demonstration of Beer's Law in Gossypol–Antimony Trichloride Reaction

I. Extinction at 430 mμ *vs.* gossypol concentration
II. Extinction at 380 mμ *vs.* gossypol concentration
III. Extinction at 520 mμ *vs.* gossypol concentration
Arabic numerals indicate multiplicity of points obtained in independent tests.

Table I. Absorption Spectrum of Antimony Trichloride Reaction Product with Pure Gossypol

Gossypol Preparation	Concentration, Gram/100 Ml.	R_a[a]	R_b[b]	$E^{1\%}_{1.27\ cm.}$ at 520 mμ
FSBP	0.010	2.49	1.17	63.8
	0.010	2.88	1.29	62.9
	0.016	2.72	1.15	62.5
	0.008	3.16	1.29	63.7
	0.008	2.84	1.28	61.9
	0.004	3.02	1.37	65.0
Y_{00}	0.010	2.69	1.22	69.9
	0.005	2.49	1.20	68.4
	0.010	2.69	1.26	65.8
	0.005	2.57	1.20	67.4
	0.005	2.79	1.24	64.6
	0.016	3.03	1.32	63.9
	0.008	2.82	1.24	64.5
	0.004	2.49	1.15	65.0
FSB[c]	0.016	2.80	1.27	66.9
	0.016	2.77	1.16	65.4
	0.016	2.66	1.29	65.4
	0.008	2.58	1.21	63.6
	0.008	2.53	1.15	66.3
	0.008	2.47	1.28	66.3
	0.004	2.11	1.11	63.0
	0.004	1.99	1.08	65.0
	0.004	2.68	1.34	65.0
	0.010	2.69	1.26	68.8
	0.010	2.61	1.19	66.8
	0.010	2.86	1.19	66.8
	0.005	2.56	1.10	65.6
	0.005	2.69	1.22	67.4
Y_{00}[c]	0.016	2.73	1.24	65.4
	0.008	2.83	1.22	66.3
	0.004	2.96	1.27	66.0
Average values		2.68	1.22	65.5
Deviation from mean		±0.23	±0.07	± 1.9

[a] $R_a = \log_{10} I_0/I$ at 520 mμ to $\log_{10} I_0/I$ at 430 mμ.
[b] $R_b = \log_{10} I_0/I$ at 520 mμ to $\log_{10} I_0/I$ at 380 mμ.
[c] Solutions treated with concentrated hydrochloric acid before reaction with antimony trichloride.

ANTIMONY TRICHLORIDE REACTION APPLIED TO COTTONSEED EXTRACTS

The complete absorption curves, in the region of 370 to 600 mμ, of freshly prepared mixtures of chloroform extracts, and of chloroform solutions of ether extracts, of many cottonseeds with antimony trichloride are almost identical with the absorption curve obtained with pure gossypol and antimony trichloride. As the reaction mixtures stand, absorption increases in the shorter wave-length region and the whole character of the curves changes. In order to establish the specificity of the reaction it was necessary to read the spectra as soon as possible after the reagents were mixed. Therefore, only the absorptions at the critical wave lengths, 530, 520, 510, 500, 430, 390, 380, and 370 mμ, were determined and these were read as rapidly as possible after the expiration of the 10-minute induction period. With this procedure it was found, as shown in Table II, that the absorption spectra of fresh mixtures of antimony trichloride with chloroform solutions of both chloroform and ether extracts of cottonseed are analogous to the gossypol–antimony trichloride absorption spectrum. The maxima and the minimum occur at the same wave lengths and are of the same relative magnitudes, as shown in Table II. The spectra listed in the table were chosen to illustrate both the best and worst agreement of the R_a and R_b values of the extract reaction products with those of the pure gossypol reaction product. Several examples of values obtained from the reaction performed with duplicate samples of the same extract and with duplicate extracts of the same cottonseed are included. The duplication of the values of E at 520 mμ, even though the values of the ratios are not duplicates, demonstrates that the value of E at 520 mμ is an accurate measure of the gossypol concentration.

Chloroform and ether extracts of some hydraulic-pressed cottonseed meals, as well as of some raw cottonseed, produce an orange or yellow color instead of the characteristic red reaction product with antimony trichloride. The absorption spectra of these reaction products are very unstable but show certain definite tendencies as illustrated in curves 1 and 2 of Figure 3. The first maximum shifts from 510 to 520 mμ to 490 mμ; a new maximum appears at 450 mμ; and the maximum at 380 mμ shifts to 390 mμ. Such curves appear to represent the result of superposing on the gossypol–antimony trichloride absorption curve, which has maxima at 380 mμ and 510 to 520 mμ, the absorption curve of the unstable reaction product of antimony trichloride with the orange-colored pigment (*2*), which has a maximum at 450 to 460 mμ, and that of the unstable antimony trichloride reaction product with at least one other pigment, which has maxima at 390 and 490 mμ. In confirmation of this explanation of the shape of these curves, it was observed that when such extracts were treated with concentrated hydrochloric acid prior to their reaction with antimony trichloride, reaction products which showed typical gossypol–antimony trichloride absorption curves were obtained, as shown in Table II.

Thus it has been possible to apply absolute criteria for the establishment of the specificity (*4, 15*) of the antimony trichloride reaction for gossypol in cottonseed extracts. It is evident that any concurrent reactions of antimony trichloride to form colored products with extractable components of cottonseed other than gossypol would alter the shape of the curve and thus change the values of the ratios. In the absence of such interfering reactions the absorption serves as a direct measure of the gossypol concentration in the extracts.

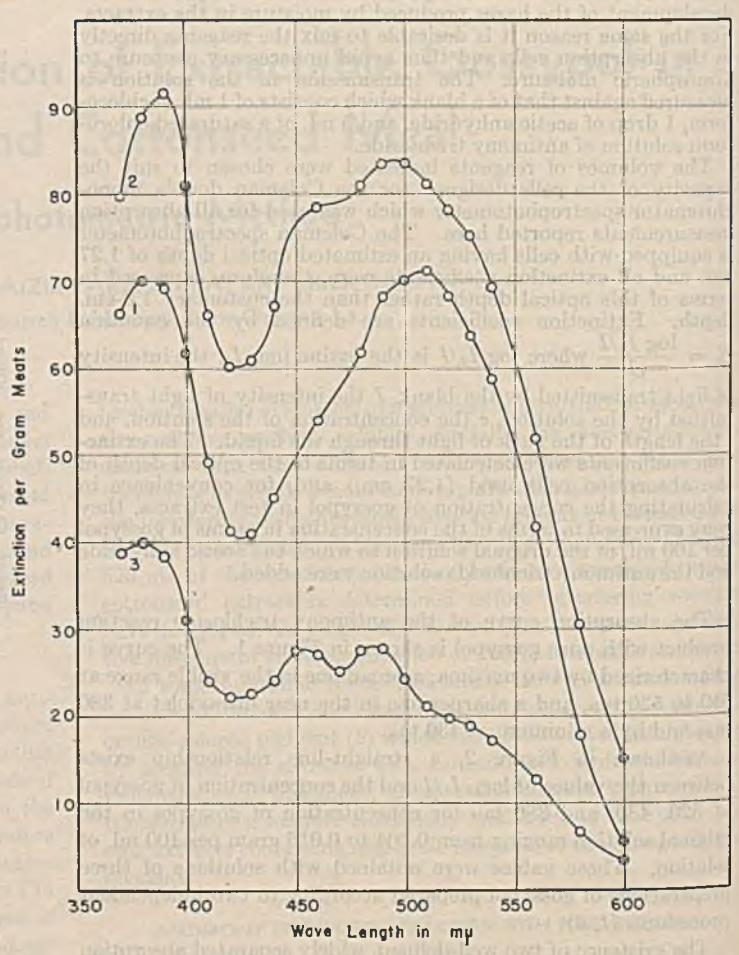

Figure 3. Absorption Curves of Antimony Trichloride Reaction Products with Cottonseed Extracts

1. Reaction product with chloroform extract 15 to 20 minutes after mixing reactants
2. Reaction product with chloroform extract 24 hours after mixing reactants
3. Reaction product with Skellysolve F extract 24 hours after mixing reactants

Table II. Absorption Spectrum of Antimony Trichloride Reaction Product with Gossypol in Cottonseed Extracts

Cottonseed Sample	Extraction Solvent	R_a	R_b	E at 520 mμ per Gram	Per Cent Gossypol
C-77-I	$CHCl_3$	1.93	1.06	56.2[a]	0.858
	$CHCl_3$	2.07	1.07	56.2[a]	0.858
C-77-VII	$CHCl_3$	1.59	0.90	64.8	0.991
	$(C_2H_5)_2O$	2.21	1.13	65.5	1.00
C-77-IX	$CHCl_3$	2.00	1.13	66.3	1.01
	$(C_2H_5)_2O$	2.14	1.18	66.3	1.01
205e[b]	$CHCl_3$	1.64	1.10	22.5	0.344
	$(C_2H_5)_2O$	1.71	0.95	22.5	0.344
PC-C-77-VIIa[c]	$CHCl_3$	1.23	0.92	15.1	0.235
PC-7[c]	$CHCl_3$	1.78	1.04	32.1	0.481
205a[b]	$CHCl_3$	1.98	1.15	84.2	1.29
PC-9[c]	$CHCl_3$	1.94	1.12	70.0	1.07
105b	$CHCl_3$	2.04	1.17	67.2	1.03
C-78	$CHCl_3$	2.29	1.19	45.2	0.681

[a] Duplicate tests of same extract.
[b] Raw cottonseeds contained considerable quantities of orange-colored pigment, so that chloroform solutions were treated with concentrated hydrochloric acid prior to reaction with antimony trichloride.
[c] Hydraulic-pressed meals treated as in [b].

Fortunately, whether because of compensation or a slower development of interference at this point, within 10 minutes of mixing the antimony trichloride solution with the extract the absorption at 520 mμ increases to a value which remains constant for a considerable period of time, ranging from 24 hours for some extracts to a minimum of 30 minutes for the least stable of a series of 117 extracts of 64 different raw cottonseeds and meals examined. Therefore, the value of the extinction at 520 mμ of the antimony trichloride reaction products with chloroform solutions of ether or chloroform extracts of cottonseed, or with acid-treated chloroform solutions of ether or chloroform extracts of hydraulic-pressed cottonseed meals determined within 10 to 40 minutes after the reagents are mixed, is a true measure of the gossypol content of the extract.

As is shown in Table III, the addition of measured amounts of pure gossypol to cottonseed extracts produces increases in the extinction at 520 mμ of the reaction product with antimony trichloride which are quantitatively proportional to the amount of pure gossypol added. The values shown in Table III establish the precision of the method. When conducted as described, the antimony trichloride reaction method permits the determination of the gossypol content of ether and chloroform extracts of cottonseed and of hydraulic-pressed cottonseed meals with a duplicability within ±1% of the total gossypol concentration of the extracts. This is the limit of precision of the spectrophotometer as it is used in the test.

Absorption spectra curves of antimony trichloride reaction products with cottonseed oils or Skellysolve F extracts differ markedly from that of the gossypol–antimony trichloride reaction product. As shown in curve 3 of Figure 3, when the antimony trichloride reaction product of such an extract has stood for some time, well-defined absorption maxima at 380, 450, and 480 to 490 mμ develop. Treatment of chloroform solutions of the oils or Skellysolve F extracts with concentrated hydrochloric acid prior to reaction with antimony trichloride causes the formation of pink reaction products. The absorption spectra curves no longer exhibit a maximum at 450 mμ and absorption at 510 to 520 mμ is increased, but absorption at 390 and 490 mμ remains high, so that the absorption curves only very slightly resemble the gossypol–antimony trichloride absorption curve. These observations indicate the presence of the orange-colored pigment in cottonseed oils and Skellysolve F extracts. They indicate further, however, that the orange-colored pigment occurs in such relatively small amounts that even when it has been converted to gossypol by the action of hydrochloric acid, the gossypol–antimony trichloride reaction is masked by the reaction of the preponderant interfering pigments. Chloroform and ether extracts of nondefatted cottonseed evidently contain some of this interfering substance, but in much lower relative concentrations, so that its only effect is to reduce the stability of the spectrophotometric test.

Since it has been possible to establish the specificity of the antimony trichloride reaction for gossypol in extracts prepared from 64 different samples of raw cottonseed and hydraulic-pressed cottonseed meal, the validity of the method can be considered to be established beyond reasonable doubt. Consequently, it appears superfluous to make a systematic comparison of the present method with other methods for the determination of gossypol.

The spectrophotometric method for the determination of gossypol proposed by Lyman, Holland, and Hale (*8*) suffers from the disadvantage that the dianilinogossypol absorption curve on which the method is based exhibits only one maximum in the visible region. Because of this limitation, the authors are able to state concerning the specificity of their method only that "there appears to be no source of error due to other pigments present in cottonseed meal", and "if there are other substances besides gossypol in cottonseed meal which give color with aniline, these substances must be closely related to gossypol". When various cottonseed extracts are treated with the carbonyl reagent, dinitrophenylhydrazine, the absorption spectra of the reaction mixtures indicate that carbonyl compounds other than

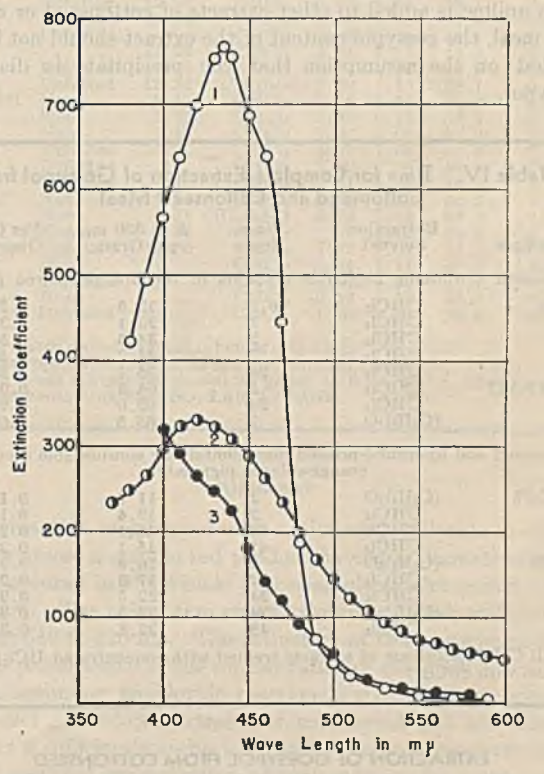

Figure 4. Absorption Curves of 2,4-Dinitrophenylhydrazine Reaction Products with Cottonseed Components

1. 2,4-Dinitrophenylhydrazone of orange-colored pigment of cottonseed
2. 2,4-Dinitrophenylhydrazone of gossypol
3. 2,4-Dinitrophenylhydrazine reaction product with chloroform extract of cottonseed

Table III. Determination of Gossypol Added to Cottonseed Extracts

Sample of Seed or Meal Extracted	Gossypol in Extract	Pure Gossypol Added to Extract	Total Gossypol in Final Mixture	Gossypol Found	Recovery of Gossypol
	G./100 ml.	*G./100 ml.*	*G./100 ml.*	*G./100 ml.*	%
202a	0.00208	0.00416	0.00624	0.00631	101.0
PC-1	0.00516	0.00074	0.00590	0.00599	101.5
	0.00387	0.00147	0.00534	0.00542	101.4
	0.00184	0.00323	0.00507	0.00508	100.2
C-101	0.00184	0.00586	0.00770	0.00760	98.7
	0.00368	0.00439	0.00807	0.00764	94.7
	0.00460	0.00366	0.00826	0.00812	98.3

gossypol and the orange-colored pigment occur in the cottonseed extracts. If these carbonyl compounds also react with aniline, as predicted by Lyman, Holland, and Hale (*8*), they will interfere in the aniline-spectrophotometric method for gossypol whenever they occur in cottonseed extracts. Consequently, no direct comparison of the two spectrophotometric methods was made.

Experience with the aniline precipitation method as modified by Halverson and Smith (*6*) and Smith (*13*) was similar to that recently reported by Lyman, Holland, and Hale (*8*) in that duplicate analyses were obtained with difficulty. No attempt was made to confirm their observation (*8*) that the dianilinogossypol precipitates were frequently impure. On the other hand, the antimony trichloride method nearly always indicated a higher gossypol concentration in a given extract than the aniline precipitation method. This occurred even when the extracts contained no detectable amounts of the orange-colored pigment. In such cases it seems most logical to conclude that the observed discrepancies are due to incomplete precipitation of dianilinogossypol from the extracts. According to a recent report (*10*), the addition of an ether solution of aniline to an ether solution of gossypol results in the precipitation of a mixture of dianilino- and tetraanilinogossypol. If a similar reaction should occur when aniline is added to ether extracts of cottonseed or cottonseed meal, the gossypol content of the extract should not be calculated on the assumption that the precipitate is dianilinogossypol.

Table IV. Time for Complete Extraction of Gossypol from Cottonseed and Cottonseed Meal

Sample	Extraction Solvent	Time, Hours	*E* at 520 mμ per Gram	Per Cent Gossypol
Cottonseed containing negligible amounts of the orange-colored pigment				
17a	$CHCl_3$	0.5	13.5	0.206
	$CHCl_3$	1	23.1	0.352
	$CHCl_3$	2	34.6	0.528
	$CHCl_3$	2	34.2	0.523
	$CHCl_3$	24	36.1	0.552
C-77-VII	$CHCl_3$	2	64.5	0.975
	$CHCl_3$	24	63.0	0.963
	$(C_2H_5)_2O$	2	65.5	1.00
Cottonseed and hydraulic-pressed meal containing considerable amounts of orange-colored pigment[a]				
PC-77	$(C_2H_5)_2O$	2	11.7	0.179
	$CHCl_3$	2	12.4	0.189
	$CHCl_3$	24	15.1	0.235
	$CHCl_3$	48	15.1	0.235
205c	$(C_2H_5)_2O$	2	16.8	0.257
	$CHCl_3$	2	17.3	0.264
	$CHCl_3$	24	22.5	0.344
	$(C_2H_5)_2O$	24	22.5	0.344
	$CHCl_3$	48	22.8	0.348

[a] All $CHCl_3$ solutions of extracts treated with concentrated HCl prior to reaction with $SbCl_3$.

EXTRACTION OF GOSSYPOL FROM COTTONSEED

With a rapid and accurate method available for the determination of gossypol in cottonseed extracts, the accurate determination of gossypol in cottonseed requires only a reliable and convenient method for extracting gossypol from the seed.

It has been reported that both the duration of extraction and type of extraction apparatus, as well as the moisture content of the seed or hydraulic-pressed meal, affect the amount of gossypol extracted. Most investigators (*5–8, 11–13*) recommend exhaustive extraction, usually in an apparatus of the reflux type which is designed for thorough rinsing of the substance from which soluble material is extracted. Apparatus of the reflux type must be used when the seed or meal is to be freed entirely from gossypol, or when, as is the case in the use of gravimetric methods, a large amount of gossypol is required for the determination. On the other hand, a simple equilibration can be used effectively when the concentration of extractable gossypol in the seed or meal is to be determined by means of the sensitive spectrophotometric method. The conditions for adequate equilibration can be ascertained by determining what volume of solvent and what contact time must be employed, so that an increase in the proportion of solvent or in the time of contact produces no increase in the concentration of gossypol in solution. An aliquot portion of an extract obtained under these conditions can be used directly in the spectrophotometric method for determining the concentration of extractable gossypol in the seed.

Table V. Equivalence of Equilibration and Butt Extraction with Ether

Cottonseed Sample[a]	Kind of Extraction	Time of Extraction, Hours	R_a	R_b	*E* at 520 mμ per Gram Seed	Per Cent Gossypol
C-77-VIII	Equilibration	2	1.97	1.03	61.9	0.945
	Equilibration	24	2.15	1.16	64.6	0.986
	Equilibration	72	1.90	1.04	62.8	0.960
	Butt	24	2.05	1.23	68.8	1.05
	Butt	72	1.95	1.03	68.8	1.05
C-77-IX	Equilibration	24	2.03	1.16	60.3	0.922
	Butt	24	2.03	1.19	62.9	0.960
	Equilibration[b]	2	2.00	1.13	66.3	1.012
	Equilibration	2	2.14	1.18	66.3	1.012

[a] Both series of cottonseed samples contained negligible amounts of orange-colored pigment.
[b] Solvent of equilibration was chloroform.

As is shown in Table IV, complete extraction of gossypol from ground cottonseed meats or hydraulic-pressed meal by equilibration with chloroform requires 2 hours when the seed contains negligible amounts of the orange-colored pigment, and 24 hours when the seed or meal contains a considerable concentration of this pigment.

That diethyl ether and chloroform extract equal amounts of the pigments is demonstrated in Tables II, IV, VI, and VIII. Murty, Murty, and Seshadri (*9*) also have reported the equivalence of ether and chloroform for the extraction of gossypol.

In order to confirm the equivalence of simple equilibration and exhaustive extraction, direct comparisons of extracts of replicate samples of the same seeds were made (Table V).

The results of experiments to determine the effect of moisture on the extraction of free gossypol by chloroform and ether are shown in Table VI. These data show, in agreement with the recent report of Murty, Murty, and Seshadri (*9*), that moisture does not play an important role unless it is very low, as in the case of desiccated seed, or so high as to interfere with the "wetting" of the seed by chloroform. Halverson and Smith (*7*) and Lyman, Holland, and Hale (*8*) observed that continued extraction of wet hydraulic-pressed cottonseed meal by wet ether at elevated temperatures gives increased amounts of gossypol. They attributed their results to the absence of a sharp boundary between "free" and "bound" gossypol in cottonseed meal. In view of the results obtained with raw cottonseed, it is more probable that the extraction methods employed by these investigators cause the liberation of some of the bound gossypol. Consequently, for the extraction of free gossypol from cottonseed meal the present authors have employed only the mild conditions of extraction found adequate for the extraction of free gossypol from raw cottonseed.

It is apparent from Table VII that proportions of chloroform to seed varying from 25 to 125 ml. per gram afford complete extraction of gossypol. Consequently, the spectrophotometric method can be used for accurate determination of the gossypol content of seeds of widely varying gossypol content.

In contrast with the consistent results obtained by the equilibration of ground cottonseed kernels and of hydraulic-pressed meal with chloroform and ether shown in the preceding tables and in Table VIII, very erratic results were obtained when the seed was first defatted by extraction with Skellysolve F. As is shown in Table VIII, extraction of gossypol from defatted seed was erratic regardless of the nature of the solvent or of the moisture content of the seed. Moreover, despite published reports that no gossypol can be detected in Skellysolve F extracts

(*3, 7, 14*), it was found, as is shown in Table VIII, that the indicated gossypol content of Skellysolve F–defatted seed is almost invariably less than that of the nondefatted seed. In view of these facts, it is apparent that accurate gossypol determinations can be obtained only by the direct extraction of nondefatted seed with ether or chloroform.

PROCEDURE FOR DETERMINATION OF EXTRACTABLE GOSSYPOL OF COTTONSEED AND COTTONSEED MEAL

REAGENTS. The chloroform and acetic anhydride should be C.P. grade and the antimony trichloride should be anhydrous, C.P. grade.

The saturated chloroform solution of antimony trichloride is prepared as follows: Wash about 30 grams of finely ground antimony trichloride with a small volume of chloroform. Add 100 ml. of chloroform to the washed crystals, warm the suspension, shake it vigorously, and allow it to cool to room temperature.

The gossypol used for the standardization should be purified as previously described (*1, 2*).

PROCEDURE. A measured volume of chloroform is added to a weighed sample of ground cottonseed kernels or meal. (For the extraction of raw cottonseed of the usual range of gossypol content, 25 ml. of chloroform to 0.25 gram of ground kernels produce an extract which can be used directly in the antimony trichloride test without dilution. For most hydraulic-pressed meals a larger proportion of meal to chloroform must be used.) The flask is stoppered and the mixture is allowed to stand, with occasional shaking, for 24 hours. A sample of the extract is withdrawn from the equilibration mixture in a manner which prevents evaporation and filters the extract—e.g., by covering the tip of a pipet with cotton or by attaching an inverted sintered-glass suction funnel to the end of a pipet. The sample is then shaken vigorously with concentrated hydrochloric acid, about 10 drops per 5 ml. of extract, and the mixture is allowed to stand for 24 hours.

One milliliter of the extract is transferred to a glass-stoppered absorption cell by means of a pipet. One drop of acetic anhydride and 5 ml. of a saturated chloroform solution of antimony trichloride are introduced into the absorption cell and the mixture is thoroughly agitated.

Within 10 to 40 minutes after the reagents are mixed, the transmission of the test solution at 520 mμ is read against that of a blank consisting of 1 ml. of chloroform, 1 drop of acetic anhydride, and 5 ml. of a saturated chloroform solution of antimony trichloride.

The concentration of gossypol in the original seed or meal is calculated by means of the following equation:

$$\%\ \text{gossypol} = \frac{\log I_0/I \times V/W}{E^{1\%}\ \text{gossypol}}$$

Log I_0/I is the extinction at 520 mμ of the gossypol–antimony trichloride test solution. I_0 is the transmission of the antimony trichloride reagent blank at 520 mμ. I is the transmission of the gossypol–antimony trichloride test solution at 520 mμ. V is the volume of solvent used in the extraction of the seed. W is the weight of cottonseed extracted. $E^{1\%}$ gossypol is the extinction coefficient at 510 to 520 mμ, as previously defined, calculated for 1% gossypol in the original solution before reaction with antimony trichloride. With the absorption cells used in the Coleman double monochromator spectrophotometer $E^{1\%}_{1.27\ \text{cm.}} = 65.5 \pm 1.9$.

Table VI. Effect of Moisture on Extraction of Gossypol from Cottonseed

Treatment of Ground Meats[a]	Solvent	E at 520 mμ per Gram	Per Cent Gossypol
None	$CHCl_3$	66.3	1.013
H_2O added	$CHCl_3$	61.0	0.932
None	$(C_2H_5)_2O$	66.3	1.013
H_2O added	$(C_2H_5)_2O$	70.4[b]	1.073
None	$CHCl_3$	64.8	0.990
Dried[c]	$CHCl_3$	11.3	0.107
Dried[c]	Wet $(C_2H_5)_2O$[d]	69.5	1.061
Dried[c]	Wet $(C_2H_5)_2O$[d]	65.3	0.998
Dried[c]	Wet $(C_2H_5)_2O$[d]	67.0	1.022
Dried[e]	$(C_2H_5)_2O$	62.9	0.961
Dried[e]	$CHCl_3$	62.9	0.961
Wet[f]	$(C_2H_5)_2O$	65.2	0.996

[a] Original meats contained 8.51% moisture.
[b] Sediment observed in filtered extract.
[c] Dried in desiccator to 3.12% moisture.
[d] Ether contained 1% H_2O and 2.5% C_2H_5OH.
[e] Dried as in [c], then exposed to moist air for 24 hours. Final meats contained 7.57% moisture.
[f] Dried as in [c], then moistened with 2 drops of H_2O per 0.25 gram of ground meats.

Table VII. Effect of Variation in Ratio of Volume of Extraction Solvent to Weight of Cottonseed

Cottonseed Sample	Ml. of $CHCl_3$ per Gram of Meats	E at 520 mμ per Gram	Per Cent Gossypol
C-77-VIII	25	62.0	0.947
	50	61.0	0.932
	125	62.0	0.947
C-77-VII	25	63.6	0.971
	50	63.0	0.962
C-77-IX	25	66.3	1.01
	100	64.8	0.990
205e	50	22.8	0.348
	100	22.9	0.350

Table VIII. Extraction of Gossypol from Defatted and Nondefatted Cottonseed Meats

Cottonseed Sample	Treatment of Ground Meats Prior to Equilibration	Per Cent Moisture	Equilibration Solvent	R_a	R_b	E at 520 mμ per Gram[a]	Per Cent Gossypol
C-77-VII	None	8.70	$(C_2H_5)_2O$	2.21	1.13	65.5	1.00
	None	8.70	$CHCl_3$	1.59	0.90	64.8	0.991
	None	8.70	$CHCl_3$	1.90	1.01	64.5	0.985
	None	8.70	$CHCl_3$	1.92	1.10	64.5	0.985
	Defatted	12.29	$CHCl_3$	1.57	1.01	30.6	0.467
	Defatted	12.29	$CHCl_3$	1.70	0.97	58.5	0.893
	Defatted	12.29	$CHCl_3$	1.83	0.98	39.1	0.597
	Defatted	12.29	$CHCl_3$	2.14	1.19	39.2	0.598
	Defatted	[b]	$CHCl_3$	1.86	1.19	36.1	0.552
	[c]	12.29	$CHCl_3$	1.81	1.13	43.2	0.660
	Defatted	12.29	$(C_2H_5)_2O$	2.10	1.11	39.2	0.598
	Defatted	12.29	$(C_2H_5)_2O$[d]	2.30	1.12	38.1	0.582
C-77-VIII	None	7.17	$CHCl_3$	2.04	1.10	61.0	0.932
	None	7.17	$CHCl_3$	1.85	0.99	62.0	0.947
	None	7.17	$CHCl_3$	1.94	1.03	61.0	0.932
	Defatted	.....	$CHCl_3$	1.97	1.03	61.9	0.945
	Defatted	.....	$CHCl_3$	1.89	1.09	46.8	0.716
	Defatted	.....	$CHCl_3$	1.81	1.07	46.2	0.710
C-77-IX	None	8.51	$CHCl_3$	2.00	1.13	66.3	1.01
	None	8.51	$(C_2H_5)_2O$	2.14	1.18	66.3	1.01
	None	8.51	$CHCl_3$	2.07	1.10	64.8	0.991
	Defatted	12.50	$CHCl_3$	1.79	1.07	42.5	0.648
C-105b	None	.....	$CHCl_3$	2.04	1.17	67.2	1.027
	Defatted	.....	$CHCl_3$	1.82	1.09	41.7	0.637
C-78	None	.....	$CHCl_3$	2.29	1.19	45.2	0.690
	Defatted	.....	$CHCl_3$	1.80	1.13	23.4	0.357

[a] Calculated on basis of meats before defatting.
[b] Five drops H_2O added to 0.5 gram of defatted meats.
[c] Five drops refined cottonseed oil added to 0.5 gram of defatted meats
[d] Ether containing 1% H_2O, 2.5% C_2H_5OH.

SUMMARY

The reaction of gossypol with antimony trichloride in chloroform produces a soluble red product having a characteristic absorption curve in the visible and near ultraviolet region of the spectrum. The absorption curve exhibits a broad, stable maximum at 510 to 520 mμ. The extinction at this absorption maximum is proportional to the concentration of gossypol.

The antimony trichloride reaction is specific for gossypol in ether and chloroform extracts of cottonseed and the reaction product is sufficiently stable to permit the accurate determination of the gossypol concentration of such extracts by means of the spectrophotometer.

The gossypol–antimony trichloride reaction has been used as a means for determining the optimum conditions for the extraction of free gossypol from cottonseed. Chloroform and ether extract equal amounts of gossypol. Equilibration of meats and solvent for 24 hours is adequate for complete solution of extractable gossypol. Moisture is an important factor in the extraction of gossypol only in the case of very dry seeds. The proportions of solvent to meats may be varied within wide limits.

A rapid, direct method for the determination of "free" gossypol in cottonseed has been reported in which the gossypol is extracted by equilibrating ground cottonseed and chloroform. The extract is treated with concentrated hydrochloric acid and the antimony trichloride reaction is applied directly to the treated extract.

The method has been shown to be applicable to the determination of the free gossypol content of hydraulic-pressed meal in which gossypol occurs to a large extent in the form of an orange-

colored pigment which is not precipitated by aniline but is readily converted to gossypol.

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Determination of Thiamine by the Thiochrome Method

Effects of Temperature and Dissolved Oxygen on Fluorescence of Quinine Standard and of Thiochrome

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The effect of dissolved oxygen and changes in temperature upon the quinine standard used in the thiochrome reaction and upon thiochrome solutions is large enough to warrant an attempt to control these variables. Temperature effects can be minimized by the use of a water bath to keep the quinine at a standard temperature. The effect probably does not alter the thiochrome fluorescence very much if the room temperature does not vary greatly. Oxygen effects can be minimized by controlling the temperature of the quinine so that no dissolved air is lost, or by the use of glass standards. Because of the shaking operation the oxygen content of thiochrome solutions is probably a constant factor. Since several types of instruments used to measure fluorescence will gradually heat up the cuvette chamber, the quinine standard should not be left in the instruments.

IT HAS long been apparent to many analysts that the thiochrome method of assaying the thiamine content of biological and other products is occasionally subject to unexplained sources or error that appear and disappear in an erratic manner. Usually these errors limit the accuracy of the method to from ±5% to ±10% (*5*), but they may be considerably larger, as has occasionally been observed in this laboratory. While engaged in an attempt to run down some of these sources of error the authors became suspicious of the accuracy of their quinine standard. The order in which samples of thiamine were oxidized and read against the quinine standards appeared to affect the results. If a sample of material was assayed twice on any given day, and if several hours elapsed between the two oxidations, the last result was the higher if the same quinine standard was used for both oxidations. This phenomenon made it appear as if the quinine exhibited less fluorescence the longer it was used on any given day. The authors had been using fresh daily aliquots of the standard, kept at about 6° C. when not in use.

According to Vavilov's equations (*6*) the fluorescence of a substance in solution is a function of the absolute temperature, other variables being constant. Vavilov also demonstrates the quenching of fluorescence by foreign molecules in the fluorescing solution. He divides quenching into two types (*13*): quenching by redistribution of the absorbed radiant energy among the degrees of freedom of the fluorescence molecule itself and collisions of the second type, which may or may not involve a chemical reaction. A consideration of Vavilov's work, together with the observations mentioned above, led to the conclusion that the possible effects of temperature and quenchers on the fluorescence of quinine and thiochrome should be investigated.

Quenching by redistribution of energy among the molecules of the fluorophor, if it occurs in the quinine standards and in concentrations of thiochrome usually used in the thiochrome method, would appear to be a constant factor, at least for quinine. The only possibilities for quenching by foreign molecules lie in the presence of dissolved atmospheric gases in both quinine and thiochrome solutions, of potassium chloride, sodium sulfate, water alkali, or ferricyanide in the thiochrome solution, and of sulfuric acid in the quinine solution. The quenching effect of dissolved oxygen was noted by Weil-Malherbe and Weiss (*14*), who found that oxygen at one atmosphere of pressure quenched thiochrome fluorescence in isobutyl alcohol 27.5% and quinine sulfate fluorescence in 0.1 *N* sulfuric acid 17.5% when both substances were examined in a concentration of 10 mg. per liter. The quenching ability of electrolytes has been intensively studied by Stoughton and Rollefson (*9*, *10*, *11*), who found the chloride ion to be a strong quencher for quinine.

The fluorescence of quinine is profoundly affected by the presence of acid. It changes in color from blue to violet from pH 3.8 to 4.5 (*4*) and decreases to zero at pH 9 (*3*). At pH 2 the fluorescence is proportional to the concentration of quinine and at pH 3 is a logarithmic function of concentration (*2*). Changes in pH change the fluorescence spectrum of quinine sulfate (*7*). It has also been reported (*1*) that the spectral line of fluorophors is changed by changes in temperature.

PROCEDURE

Three aliquots of the quinine standard (0.3 microgram per ml. of 0.1 *N* sulfuric acid) and three aliquots of thiamine oxidized to thiochrome (1.0 microgram thiamine aliquots oxidized to thiochrome and dissolved in 18-ml. aliquots of isobutanol) were placed in glass cuvettes. The cuvettes were stoppered and the thiochrome cuvettes were covered with metal covers to exclude light. Two sets of each series were connected to manifolds. Oxygen was blown through one set for 15 minutes and nitrogen was blown through the other set for the same length of time. Volume changes from the blowing were prevented by first saturating the gases with water vapor (quinine samples) or isobutyl alcohol (thiochrome samples). The third set of each series was left intact

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(no added gas). All the cuvettes were placed in a water bath at about 6° C. Another quinine standard was heated to 35° C. in a water bath and bubbles of expelled air were removed. It was maintained at this temperature by the water bath and used as a reference standard. The series of quinine and thiochrome solutions were read at various temperatures from 6° to 50° C. and the galvanometer readings they produced in a fluorometer were recorded.

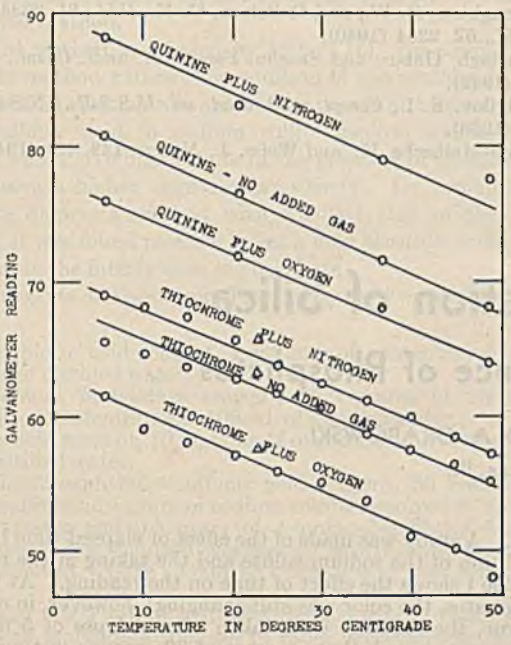

Figure 1. Quenching Effect of Temperature upon Quinine Sulfate and Thiochrome

0.3 μg. of quinine sulfate per ml. of 0.1 *N* H_2SO_4. 1.0 μg. of thiamine oxidized to thiochrome and dissolved in 18 ml. of isobutanol

The results are shown in Figure 1. Clearly, within the temperature range studied, the fluorescence as measured by galvanometer deflection is a linear function of temperature, and oxygen is a strong quenching agent for both thiochrome and quinine. As the solutions warmed up from 6° C. to a room temperature of 25° C. the galvanometer deflection varied about 5 units. Since it is customary to keep quinine solutions under refrigeration, it is clear that such solutions should be warmed to room temperature before use.

To make sure that the effects noticed were not caused by decomposition, particularly by decomposition of thiochrome, the solutions were cooled from 50° to 22° C. and readings were again taken on the thiochrome solutions. These readings, shown by the triangle points in Figure 1, indicate that no decomposition of thiochrome took place. A fresh sample of quinine was taken and read against the standard held at 35° C., then heated to 50° C., cooled, and read again. Both readings were 90 galvanometer units. Then the fresh sample was placed in the fluorometer and allowed to stand in the ultraviolet beam for 45 minutes. At the end of that time it read 87.5 galvanometer units. Apparently quinine is stable to heat up to 50° C. and to the light from the authors' fluorometer.

In this laboratory it has been customary to test the fluorometer with the quinine standard both before and after each reading. During the interval between the readings of successive samples (during the oxidations) the quinine is allowed to stay in the cuvette chamber, as the instrument (a Colemen Model 12 photofluorometer) is provided with a shutter, so that the light can be shut off from the cuvette chamber. It was decided to find out if the quinine standard was warmed by the instrument during the 2-hour period it takes to oxidize and read the daily run.

A sample of quinine was taken from the refrigerator at about 6° C. and placed in the instrument. The instrument was turned on and temperature readings were taken at short intervals for 2 hours. The resulting curve is shown in Figure 2. In 2 hours the solution warmed up to 35° C. A similar curve was run on another type of fluorometer in which the cuvette chamber is separate from the light source. This sample was taken at room temperature and the curve is also shown in Figure 2.

A comparison of Figure 2 with Figure 1 makes it clear that the quinine standard should not be left in either type of instrument. One instrument in a 2-hour period can raise the temperature of the quinine standard to 35° C.

If the quinine standard is taken from a refrigerator at 6° C. and used immediately, setting the instrument at a galvanometer reading of 80 with it, and if it is allowed to warm up to 35° C. in the machine while in use, the variable resistances in the fluorometer will have to be changed in an amount corresponding to a galvanometer deflection of 8.75 units to keep the quinine reading at 80 units. This is obvious from Figure 1 (curve for quinine, no added gas), since a temperature rise of 6° to 35° C. will cause the galvanometer deflection of the quinine to drop from 80.75 to 72.0 units, a difference of 8.75 units. This can cause errors of 10.9% in the determination of an unknown. Since the more probable variations in temperature of the quinine solution would perhaps involve only a 10° variation, the more common errors caused by temperature changes may well lie in the neighborhood of 3 galvanometer units if the galvanometer readings are made in the neighborhood of 80 units. This would introduce errors of the order of 4%. No errors will be introduced from temperature variations, provided both quinine and thiochrome solutions are read at the same temperature. It is apparent that for best results both the quinine standard and thiochrome solutions should be maintained at the same constant temperature within ±2° or 3° C.

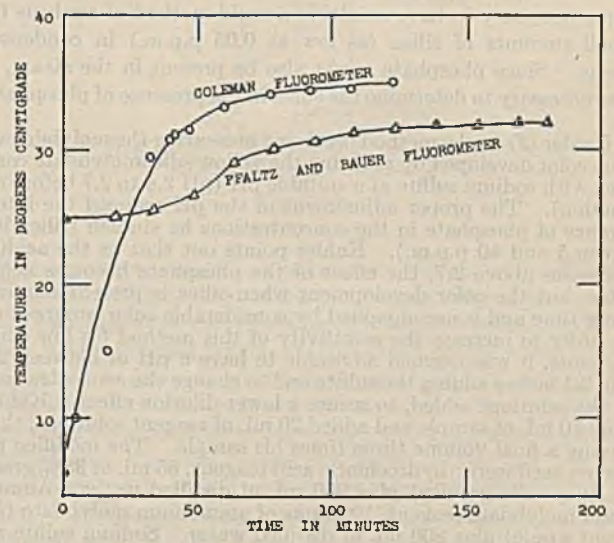

Figure 2. Heating of Quinine Sulfate Solution in Cuvette by Fluorometers

The effect of dissolved oxygen also depends upon the temperature. This effect is probably more or less constant in the case of the thiochrome because of the shaking operation when the thiochrome is extracted with isobutanol, and nonexistent in the quinine standard in cases where the temperature is controlled in such a way that bubbles of dissolved gases are not expelled. It could be eliminated by the use of glass standards such as those described by Vastagh and Szegho (*12*) and Lowenstein (*8*). Elimination of temperature changes caused by placing of solutions on or near open windows, radiators, or steam pipes, and cooling of isobutanol to room temperature after distillation are among the considerations suggested by the data presented.

To test the effect of potassium chloride on the thiochrome fluorescence, samples were run both with and without the addition of potassium chloride. The resulting temperature-

fluorescence curves were identical with those in Figure 1 for thiochrome with no added gas. Identical curves were also obtained when a sample of thiamine was oxidized with twice the usual amount of alkaline ferricyanide, indicating that reagent has no appreciable quenching effect beyond the amounts necessary for oxidation.

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Photometric Determination of Silica

In Condensed Steam in Presence of Phosphates

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IN RECENT years much difficulty has been experienced in steam power plants because of silica deposition on turbine blades. As part of the study of the cause of this difficulty, it became necessary to have available a rapid method of analysis for small amounts of silica (as low as 0.05 p.p.m.) in condensed steam. Since phosphate might also be present in the steam, it was necessary to determine the silica in the presence of phosphate.

Kahler (*2*) used a method involving measuring the molybdenum blue color developed by reducing the yellow silicomolybdate complex with sodium sulfite at a suitable pH (pH 2.4 to 2.7 before reduction). The proper adjustment of the pH reduced the interference of phosphate in the concentrations he studied (silica between 5 and 40 p.p.m.). Kahler points out that as the acidity decreases above 2.7, the effect of the phosphate becomes negligible, but the color development when silica is present requires more time and is accompanied by considerable color progression. In order to increase the sensitivity of this method for low silica contents, it was deemed advisable to have a pH of between 2.2 and 2.4 before adding the sulfite and to change the concentrations of the solutions added, to secure a lower dilution effect. Kahler used 10 ml. of sample and added 20 ml. of reagent solutions, thus having a final volume three times his sample. The modified reagents used were: hydrochloric acid reagent, 55 ml. of 38% grade (1.19 specific gravity) plus 900 ml. of distilled water. Ammonium molybdate reagent, 10 grams of ammonium molybdate (reagent grade) plus 800 ml. of distilled water. Sodium sulfite reagent, 135 grams of sodium sulfite (anhydrous reagent grade) plus 800 ml. of distilled water. Sodium silicate solution, 10.0 mg. as silica per liter. Fifty milliliters of sample were used for analysis, and 5 ml. of hydrochloric reagent, 10 ml. of ammonium molybdate reagent, and 10 ml. of sodium sulfite were added, giving a final volume of 75 ml. or 1.5 times the sample.

It was realized that this procedure might not eliminate the effect of phosphate entirely, but it was thought best to try it, since the increased sensitivity was desirable and most of the samples being tested were free from phosphate.

APPARATUS

A Coleman spectrophotometer Model 11 was used for colorimetric comparison of the solutions. It was noticed that the time interval elapsing between addition of the ammonium molybdate reagent and of the sodium sulfite reagent, as well as the time elapsing between addition of the sodium sulfite reagent and taking of the reading, had a marked effect on the final reading. The time interval between addition of the ammonium molybdate reagent and the sodium sulfite reagent was standardized at 1 minute. A study was made of the effect of elapsed time between the addition of the sodium sulfite and the taking of the readings and Table I shows the effect of time on the reading. At the end of 10 minutes, the color was still changing; however, in order to save time, the readings were taken after a lapse of 5 minutes. The change was such that an error of 30 seconds in time would produce an error of 0.5%, which was considered well within the range of accuracy desired. The results shown in Figure 1 were obtained under these conditions of testing. A 40-mm. cell was used for testing and comparison was made at a wave length of 700 millimicrons.

The results obtained showed the method to be fairly sensitive for silica, but it was thought that a more sensitive method might be developed.

Schwartz (*3*) described a method for determining silica colorimetrically in the presence of phosphate, in which he made use of the yellow color developed by the yellow complex silicomolybdate and destroyed the phosphomolybdic acid complex by adding oxalic acid. Schwartz reagents and test procedure were as follows:

Hydrochloric acid reagent, 1 volume of concentrated acid to 1 volume of distilled water, 1 to 1.

Ammonium molybdate reagent, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Oxalic acid reagent, 10.0 grams of oxalic acid dihydrate per 100 ml. of distilled water.

Add and mix 1 ml. of hydrochloric acid solution and 2 ml. of ammonium molybdate solution in rapid succession to 50 ml. of sample. Wait 5 to 10 minutes for full color development, then add and mix 1.5 ml. of oxalic acid solution and determine color intensity.

Table I. Effect of Time on Color Development in Modified Kahler Method

(2 p.p.m. of SiO_2 in sample tested)

Elapsed Time after Addition of Sulfite *Min.*	Transmittance %
2	44.3
3	43.3
4	43.0
5	42.8
6	42.5
7	42.3
8	42.1
9	42.1
10	42.0

Figure 1 gives per cent transmittance at 410 millimicrons for various concentrations of silica using 19-mm. test tubes. A 5-minute interval was used after addition of the ammonium molybdate solution before addition of the oxalic acid solution. There was no change in transmittance reading in a period from 2 to 15 minutes after the addition of the oxalate solution.

This method eliminates the effect of time in taking the reading of transmittance; however, its sensitivity is about the same as the Kahler method.

A third method, suggested by Imhoff (*1*), was similar to the Schwartz method without the addition of the oxalic acid; however, the final solution was reduced by means of 1-amino-2-naphthol-4-sulfonic acid in sodium sulfite-bisulfite solution. This method would give high results in the presence of phosphate but would have a higher degree of sensitivity. By combining the complete Schwartz method with the last step in the Imhoff method, it was found possible to get a very sensitive method and to eliminate the interference of phosphate.

The reagents and test procedure finally used were as follows:

Hydrochloric acid reagent, 1 volume of concentrated acid to 1 volume of distilled water, 1 to 1.

Ammonium molybdate reagent, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Oxalic acid reagent, 10.0 grams of oxalic acid dihydrate per 100 ml. of distilled water.

1-Amino-2-naphthol-4-sulfonic acid reagent, 30 grams of sodium bisulfite and 1 gram of sodium sulfite dissolved in 200 ml. of distilled water and 0.5 gram of 1-amino-2-naphthol-4-sulfonic acid added. The solution was heated slowly until the last reagent dissolved. Care should be taken not to heat solution too hot.

Add and mix 1 ml. of hydrochloric acid solution and 2 ml. of ammonium molybdate solution in rapid succession to 50 ml. of sample. Wait 5 minutes, then add and mix 1.5 ml. of oxalic acid solution, followed by 2 ml. of the 1-amino-2-naphthol-4-sulfonic acid. Determine silica color intensity with suitable instrument at a wave length of 700 millimicrons after 1 minute.

Table II. Effect of Phosphate on Transmittance

(Using Schwartz method with amino acid)

SiO_2	PO_4	Transmittance
P.p.m.	*P.p.m.*	%
0.1	0	94.0
0.1	0	95.0
0.1	0	94.0
0.1	0	94.5
0.1	0	94.0
0.1	50	93.8
0.2	0	89.5
0.2	0	90.3
0.2	0	90.0
0.2	0	89.8
0.2	0	90.0
0.2	50	89.0
0.5	0	76.5
0.5	0	76.8
0.5	0	76.3
0.5	50	76.3
1.0	0	60.0
1.0	0	60.0
1.0	0	60.0
1.0	50	59.8
2.0	0	37.0
2.0	0	37.0
2.0	0	36.5
2.0	50	36.5

Tests conducted using standard silica solutions showed no effect on per cent transmittance with a time interval from 2 to 15 minutes after addition of the final reducing agent. With a time interval of only 3 minutes after addition of the ammonium molybdate, a final color of less intensity was developed; however, as no change was determined when a time interval of 5 and 10 minutes was used, this time was set at 5 minutes.

Figure 1 shows the per cent transmittance with a 40-mm. cell and 19-mm. test tubes. When phosphate in amounts equal to 50 p.p.m. was added to silica solutions no appreciable deviation from the curve on phosphate-free silica solutions was detected (Table II).

When 50 p.p.m. of phosphate was present and the oxalic acid was not added (Imhoff method), the transmittance of a blank without silica present was 1%; however, when a similar test was run with oxalic acid present, the transmittance was 100%. This shows that the phosphate had a marked effect on the Imhoff method, but that oxalic acid entirely eliminates the phosphate effect.

Table II shows that the accuracy of the method is about 0.02 p.p.m. of silica.

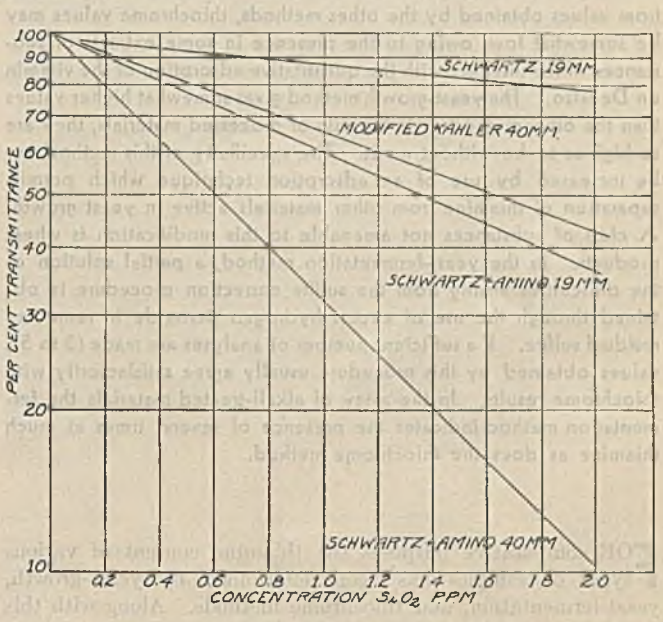

Figure 1

Since reagents used add a small amount of color to the test solution and this might vary, owing to the possibility of dissolving silica from the glass containers used, a blank solution was prepared, using the same volume of silica-free distilled water as the test sample, to which the regular amount of reagents were added at the time the test sample was being tested. This blank was then put in the spectrophotometer, the reading adjusted to 100% transmittance, and the comparison made on the test sample. This eliminated the intereference of the reagents or silica in the reagents. The blank usually read about 97% transmittance when compared with distilled water to which no reagents had been added.

This modification of the Schwartz method has a degree of accuracy of 0.01 p.p.m. in determining silica in amounts from 0.02 to 2.0 p.p.m., when a 50-ml. sample is used. If the silica is above 2 p.p.m., the Schwartz method would work better, since it would require less dilution. These tests have all been conducted on distilled water or condensed steam free from color or organic material. The effect of organic matter in the water has not been studied.

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Thiamine Determination

Comparative Study of Yeast-Growth, Yeast-Fermentation, and Thiochrome Methods

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The yeast-growth, yeast-fermentation, and thiochrome methods for thiamine determination have been studied, with an attempted evaluation of certain modifications in the yeast methods. The thiochrome method appears to be a satisfactory means of determining the thiamine content of various types of natural and processed materials. Judging from values obtained by the other methods, thiochrome values may be somewhat low, owing to the presence in some extracts of substances which interfere with the quantitative adsorption of the vitamin on Decalso. The yeast-growth method gives somewhat higher values than the other methods. In the case of processed materials, they are so high as to be without merit. The specificity of this method can be increased by use of an adsorption technique which permits separation of thiamine from other materials active in yeast growth. A class of substances not amenable to this modification is wheat products. In the yeast-fermentation method, a partial solution of the difficulties arising from the sulfite correction procedure is obtained through the use of excess hydrogen peroxide in removing residual sulfite. If a sufficient number of analyses are made (3 to 5), values obtained by this procedure usually agree satisfactorily with thiochrome results. In the assay of alkali-treated materials the fermentation method indicates the presence of several times as much thiamine as does the thiochrome method.

FOR comparative purposes the thiamine content of various types of samples has been determined by yeast-growth, yeast-fermentation, and thiochrome methods. Along with this comparative study, some work has been done on possible modifications of the methods involving yeast. Among the samples assayed were two (15 and 16, Table I) used for checking purposes by the American Association of Cereal Chemists and three samples (9, 11, and 13, Table I) used in collaborative studies sponsored jointly by the Research Corporation of New York and the American Association of Cereal Chemists. Included also were samples which had been subjected to heat treatment in alkaline solution; the thiamine content of these, in which deliberate destruction of the vitamin had been effected, was estimated by the methods under consideration.

The thiochrome and yeast-fermentation methods have been subjected to comparative study and standardization by Frey and Hennessy (*7*) and collaborators. The mean values obtained by the two methods for cereal samples and dry yeast agree remarkably well and are in agreement with mean values obtained using animal methods (rat-growth and rat-curative); but the range of deviation of individual values from a given mean is considerable, in the fermentation method being as high as 59%, in the chemical one as high as 122%. These studies were made on cereal products with one exception, and values for each sample obtained by a single method vary considerably.

Cheldelin and Williams (*2*) report that thiamine values for food samples determined by the yeast-growth method of Williams, McMahan, and Eakin (*20*) are in good agreement in most cases with the values obtained by the thiochrome method as reported by Lane, Johnson, and Williams (*13*), Nordgren and Andrews (*15*), and Conner and Straub (*3*); these comparisons, however, were not made on the same sample preparations. Only in the case of foods which have been subjected to processing involving heat treatment did Cheldelin and Williams observe the yeast-growth method to show striking disagreement (too high values).

No previous comparisons have been made using the yeast-growth and yeast-fermentation methods.

In a comparative study such as this, it is desirable to prepare extracts of samples which are suitable for assay by each method employed. Since thiamine often occurs in yeast and animal tissues as cocarboxylase, and since the pyrophosphoric ester is inactive in the yeast growth test (*20*) and thiochrome pyrophosphate is not extractable with isobutanol (*12*), a hydrolyzing agent must be used to convert cocarboxylase to thiamine if the yeast-growth or thiochrome methods are to be used.

Lohmann and Schuster (*14*) have shown that the above conversion can be accomplished enzymatically with suitable enzyme preparations. Pyke (*17*) and Dawson and Martin (*4*) have used digestion with pepsin followed by digestion with takadiastase, and Harris and Wang (*8*) have used incubation with takadiastase and papain following a preliminary heating in the presence of acid. Cheldelin *et al.* (*1*) used digestion with takadiastase and papain, and in this study their procedure was adopted; the phosphatase preparation used is sold under the trade name Clarase.

ASSAY METHODS

YEAST GROWTH. The method of Williams, McMahan, and Eakin (*20*), which is based on the stimulatory effect of thiamine on the growth of *Saccharomyces cerevisiae*, Old Process strain, was used. Yeast growth was measured turbidimetrically.

Though the basic procedure of the growth method was retained throughout this work, the preparation and treatment of extracts to be assayed were varied. Thiamine content of the following types of extracts was estimated: (a) extracts of enzyme-digested samples, (b) extracts of alkali-digested samples, and (c) eluates of extracts (a) and (b) prepared by adsorption of sample aliquots on Decalso followed by elution with acidified potassium chloride.

YEAST FERMENTATION. The procedure recommended by Schultz, Atkin, and Frey (*18*) was followed in determining the thiamine content of the following types of sample extracts: (a) extracts of enzyme-digested samples, and (b) extracts of alkali-digested samples.

A commercial fermentometer was used.

In some cases this method was modified to the extent that, instead of using sulfite treatment as a means of correcting for non-thiamine activity, preliminary adsorption and elution using Decalso were performed before the fermentation test was applied.

THIOCHROME. The procedure of Hennessy (*9*) was followed, especial care being taken to standardize the timing of all operations beginning with the oxidation of the sample. The thiamine content of eluates of the following types of extracts was estimated: (a) extracts of enzyme-digested samples, and (b) extracts of alkali-digested samples.

Fluorescence was determined with a Pfaltz-Bauer fluorophotometer.

PREPARATION OF SAMPLE EXTRACTS AND ELUATES

ENZYME-DIGESTED SAMPLES (1a). In order to obtain certain materials to be assayed in a finely divided condition, they were homogenized in a Waring Blendor. Two per cent Clarase and 2% papain were added to each sample, together with 0.5% sodium acetate–acetic acid buffer (pH 4.5), and after the addition of 0.5 ml. of benzene the mixture was incubated for 24 hours. At the end of the incubation period the samples were steamed 30 minutes, made to volume, filtered, steamed 10 minutes for sterilization, and then stored in the refrigerator until assayed. The amount of buffer used in preparing the respective extracts depended on the approximate thiamine content of the sample, since Hennessy (*9*) has found preferred volumes from which adsorption should be effected, as well as preferred amounts of thiamine to be adsorbed per column of Decalso.

This procedure was varied somewhat for samples of high starch content; for these, in addition to an extract prepared in the manner above, an extract was prepared by diluting the incubated mixture to its final volume and filtering prior to the 30-minute steaming period. The extract was refiltered if solid material separated out on steaming. This type of extraction is designated as 1a′ in Table I.

Alkali-Digested Samples (2a). The samples listed in Table II were put in solution, the pH was adjusted to 9 by addition of sodium hydroxide, and the solution autoclaved 1 hour at 7 kg. (15 pounds) pressure. After cooling, the pH of each solution was adjusted to 4.5 by the addition of sulfuric acid and the volume brought to its final value.

Eluates (1b, 1b′, 2b). An aliquot of each of the sample extracts containing 0.5 to 10 micrograms of thiamine was adsorbed on a column of Decalso and eluted with acidified potassium chloride, in accordance with the method described by Hennessy (*9*). The treatment of the zeolite prior to adsorption and the entire base-exchange procedure used were those recommended by Hennessy.

DISCUSSION OF METHODS

Yeast Growth. Williams and co-workers (*16, 21, 23, 24*) have used the yeast-growth method in investigations of small amounts of tissues, in which all other methods were of necessity excluded because of the relatively large samples required. It was found to be highly sensitive and to give reproducible and seemingly consistent results of the right order of magnitude; recovery tests indicated that it was sufficiently specific to be of value when applied to fresh tissue extracts. It requires inexpensive equipment and many tests per day can be run by one individual. However, the finding of Cheldelin and Williams (*2*) that yeast-growth values for materials which have been heated during processing are much higher than corresponding thiochrome values has made it evident that the yeast-growth method cannot be applied in its original form to certain types of materials.

A modification has proved useful in overcoming this discrepancy. The thiamine in sample extracts was separated from other materials known to stimulate yeast growth by taking advantage of the selective adsorption of Decalso for the vitamin, and the contents of the eluates from adsorption were measured. Neither 5-(2-hydroxyethyl)-4-methylthiazole, which was found in this investigation to be 68% as active as thiamine on an equimolecular basis, nor 4-amino-5-ethoxymethyl-2-methylpyrimidine which is 14% active, is adsorbed on Decalso from solutions in sodium acetate buffer (pH 4.5) in the routine adsorption procedure. [Deutsch (*5*) reports that "more than 95% of the pyrimidine is also removed by zeolite"; the pyrimidine referred to is presumably "(III) the pyrimidine portion, 4-amino-2-methyl-5-ethoxymethylpyrimidine". He fails to indicate, however, whether or not the adsorbent used was activated Decalso and at what pH adsorption was effected.] Indirect evidence points to an analogous adsorption behavior of the 5-hydroxymethylpyrimidine derivative which is formed by the hydrolytic cleavage of thiamine, since eluates from the Decalso adsorption of alkali-treated samples (Table II) measured in the yeast-growth method proved to have only a small percentage of the activity determined for the corresponding whole extracts.

Yeast Fermentation. In preliminary investigations made in connection with work done at this institution (*22*) in determining the thiamine content of food samples, considerable difficulty has been encountered in obtaining satisfactory replicate values for the sulfite-treated samples. This difficulty was removed in part by the use of excess hydrogen peroxide in destroying residual sodium sulfite, a precautionary measure recommended by Josephson and Harris (*11*) for the microfermentation method. But for some samples this precaution did not alleviate the difficulty. It has been reported recently that the thiazole and pyrimidine sulfonic acid resulting from the sulfite cleavage of thiamine are slightly stimulatory in the microfermentation method (*5*), and it is not unlikely that they are similarly active in the macromethod.

A modification involving adsorption was attempted in order to eliminate the sulfite treatment of samples, but it failed to eliminate entirely the interfering substances. It was established that filtrates with combined washings from Decalso adsorption of

Table I. Comparison of Values from Thiochrome, Yeast-Growth, and Yeast-Fermentation Determinations

Sample	I Thiochrome (1b, 1b′)	II Yeast Growth (1b, 1b′)	III Yeast Growth (1a, 1a′)	IV Yeast Fermentation (1a, 1a′)	V Yeast Fermentation (1b, 1b′)	Deviation: II from I	II from IV	III from I	III from IV	IV from I	V from I
	Micrograms per gram					%	%	%	%	%	%
Plant and animal tissues											
1 Peanuts, fresh (1a)	7.1	7.0	14.7	7.1	..	− 1.4	− 1.4	+107	+107	0	...
2 Oatmeal (1a)	6.8	7.9	8.1	8.0	7.2	+15.6	− 1.2	+ 18	+ 1.2	+ 16.8	+ 5.9
3 Oatmeal (1a′)	7.6	9.5	10	8.4	6.9	+25	+ 13	+ 32	+ 19	+ 10.5	− 9.2
4 Beef muscle (1a)	0.59	0.63	1.3	0.94	0.76	+ 6.8	− 33	+114	+ 34	+ 59.4	+ 29
5 Potatoes, white (1a)	1.13	1.18	1.82	1.41	1.34	+ 4.4	− 16	+ 61	+ 29	+ 25	+ 19
6 Potatoes, white (1a′)	1.11	1	1.65	1.21	1.22	− 9.9	− 8.2	+ 49	+ 36	+ 9	+ 9.9
7 Potatoes, white (dehydrated) (1a)	2.97	5	4.25	3.3	..	+68	+ 51	+ 43	+ 29	+ 11	...
8 Green peas (dehydrated) (1a)	5.77	6.9	8.5	7.5	..	+20	− 8	+ 47	+ 13	+ 30	...
9 Dry yeast (1a)[a]	598	658	735	654	625	+10	+ 0.6	+ 23	+ 13	+ 9.4	− 5.4
10 Wheat germ (1a)	18.4	24.5	20.6	16.7	..	+33	+ 47	+ 12	+ 23	− 9.2	...
11 Whole wheat (1a)[a]	4	6.65	..	5.2	5.1	+66	+ 28	...	...	+ 29	+ 27
12 Whole wheat (1a′)	4.2	5.2	5.2	4.3	5.0	+24	+ 21	+ 24	+ 21	+ 24	+ 19
13 White flour (1a)[a]	0.69	1.24	..	0.81	1.34	+79.5	+ 53	...	...	+ 17	+ 94
14 White flour (1a′)	0.68	0.93	0.98	0.57	1.01	+37	+ 63	+ 44	+ 72	− 16	+ 49
15 Cereal product (1a)[b]	24.9	30.3	31.9	25.9	26.8	+22	+ 19	+ 28	+ 23	+ 4	+ 7.6
16 Rice product (1a)[b]	2.8	3.4	4.6	3	4.9	+21	+ 13	+ 64	+ 53	+ 7	+ 75
Processed materials[c]											
17 Peanuts, roasted (1a)	2.8	3.3	15.3	2.8	..	+17.8	+ 17.8	+446	+446	0	...
18 Yeast extract (1a)	17.1	19.4	63.6	23.7	..	+13.4	− 18.2	+272	+168	+ 38.6	...
19 Rice bran concentrate (1a)	50.2	51.2	170	75.8	..	+ 2	− 32.4	+240	+124	+ 51	...
20 Whole wheat bread (1a)	1.2	2.2	2.4	1.03	..	+83	+114	+100	+133	− 13	...
Urine	*Micrograms per ml.*										
21 Sample 1 (1a)	0.061	0.081	..	0.23	0.5	+33	− 65	...	...	+277	+719
22 Sample 2 (1a)	0.15	0.18	1.23	0.54	..	+20	− 67	+720	+128	+260	.

[a] Received from R. R. Williams. These samples, designated as Nos. 6, 4 and 3 by the Research Corporation Committee, have respective thiamine contents of 702, 5.1, and 0.92 micrograms per gram. Indicated mean values were derived from results of nineteen collaborators who used thiochrome method (*7*). Many individual values deviated widely from the mean.

[b] Obtained from J. S. Andrews, of General Mills, Inc. Thiamine content, 25 and 3.0 micrograms per gram, respectively.

[c] Subjected to more or less cooking, may contain thiamine fragments.

samples contain considerable quantities of material stimulative to fermentation, and further that 4-amino-5-ethoxymethyl-2-methylpyrimidine, and probably the corresponding 5-hydroxymethylpyrimidine derivative as well, each of which stimulates fermentation (*18, 19*), are not adsorbed on Decalso under the conditions employed. Hence it appeared that eluates from Decalso adsorption might serve as suitable test materials. The salt concentration in the eluate depresses fermentation somewhat, so that it was necessary to add to each standard bottle the amount of potassium chloride in the aliquot of eluate being tested. In no case was more than 5 ml. of an eluate used.

In checking the fermentometer it was found that some of the bottles, regardless of placement in the shaker, permitted settling of the yeast during the 3-hour shaking period, and as a consequence smaller volumes of carbon dioxide were evolved than from those bottles in which no sedimentation occurred. This source of error was easily eliminated by replacing the imperfect bottles which had irregular seals between the walls and bottoms.

Thiochrome. In this method based on the oxidative conversion of thiamine to thiochrome, a possible source of error arises from the fact that thiamine may be incompletely adsorbed on the zeolite. Egaña and Meiklejohn (*6*) have found by means of recovery experiments that urine samples which contain blood or bile, as well as those from individuals whose thiamine intake is low, contain some material which prevents complete adsorption of the vitamin on Decalso, while normal urine appears free from this inhibitory material. Such inhibitory substances may be of more general occurrence than is appreciated, in which case the effects would be particularly apparent in the case of extracts containing a low concentration of thiamine. That urine contains an unidentified material which interferes with the adsorption of pantothenic acid on charcoal was found by Hogg (*10*).

The presence of interfering fluorescent materials in the eluates can also serve as a source of error, especially if such materials are labile to oxidation, so that their contribution to fluorescence in the oxidized sample differs from that in the unoxidized blank.

Values obtained by the chemical method have been chosen as reference values for this comparative study.

Table II. Effect of Alkali Treatment on Thiamine Content

Samples	Thiochrome (2b)	Yeast Growth (2b)	Yeast Growth (2a)	Yeast Fermentation (2a)
	Micrograms per gram			
Yeast extract (2a)	0.02	1	65.2	5.8
Yeast extract (2a)	0.33	2.57	46	3.9
Rice bran concentrate (2a)	3.6	9.3	187	18.7
Thiamine, crystalline (2a)	0	4[a]	4.5[a]	13[a]

[a] Values in terms of % thiamine activity intact after alkali treatment.

DISCUSSION OF RESULTS

The comparative results obtained for enzyme-digested samples, together with the percentage deviation of values from corresponding thiochrome values, are listed in Table I. In the case of values from yeast-growth determinations, percentage deviations from yeast-fermentation results are also indicated. In Table II, values for the thiamine content of the alkali-treated samples are presented.

Of the samples assayed in this study which have been used previously in two sets of collaborative determinations, Nos. 15 and 16 were found to give thiochrome and fermentation values in close agreement with reported contents, but values lower than the reported means were obtained for Nos. 9, 11, and 13. Since the authors' values for the last three were obtained at least a year and a half later, it is not unlikely that in these samples some loss of thiamine occurred during storage.

Comparison of Values from Yeast-Growth and Thiochrome Determinations. Without exception, values obtained for whole extracts (1a, 1a′) by the yeast-growth method are higher than corresponding values from thiochrome determinations. In the case of heat-processed materials and urine, the deviations are of far greater magnitude than for the unprocessed materials assayed. This is probably due to the presence of active fragments of the thiamine molecule in these samples.

For most samples, values for eluates (1b, 1b′) are significantly lower than those for the corresponding whole extracts in the yeast-growth determination, and are in better agreement with values from the chemical method. Fifteen of the 22 eluates listed in Table I gave values within ±25% of the thiochrome values, 6 of them being within ±10%. For wheat products, use of the adsorption technique accomplishes no improved agreement of values from yeast-growth assay with those from thiochrome determinations. Evidently wheat products contain some growth-promoting substance other than thiamine which is not separated from the vitamin by the base-exchange procedure.

Comparison of Values from Yeast Fermentation and Thiochrome Determinations. Values listed in Table I obtained by thiochrome and yeast-fermentation assay are in fair agreement, 15 of the 22 fermentation values agreeing within ±25% with thiochrome figures. In the case of rice bran concentrate, beef muscle, and urine, fermentation values are more than 50% higher than thiochrome values. For rice bran concentrate, the nonthiamine material active in the fermentation test (determined by sulfite treatment) amounts to approximately 50% of the total stimulatory material present; for urine, it represents approximately 70% of the total. These correction terms are certainly high, but such corrections do not of themselves invalidate results because in other samples assayed—viz., roasted peanuts, yeast extract, whole wheat bread, and white flour—the nonthiamine active material amounts to 50% or more of the total stimulatory material present, yet agreeing values are obtained by the two methods. It may be that the rice bran concentrate, beef muscle, and urine samples contain some material which interferes with the adsorption of thiamine on Decalso, so that values from the fluorometric method are lower than the actual thiamine content; that urine may contain such material has been pointed out by other workers (*6*). An alternate interpretation is that these samples contain some active material other than thiamine which is inactivated by sulfite treatment, so that it appears as thiamine in the fermentation assay.

For those eluates assayed in the yeast-fermentation method, values were obtained which for the most part agree as well or better with thiochrome values than do the corresponding values obtained using sulfite treatment. For urine, white flour, and the rice product this was not the case; for these, values for the eluates were considerably higher. Since the assay of eluates does not give consistently improved values, it cannot be recommended as an adequate substitute for the usual procedure involving sulfite correction.

Comparison of Values from Yeast Growth and Yeast-Fermentation Determinations. Yeast-growth values for whole extracts (1a, 1a′) of unprocessed materials are from 1 to 100% higher than corresponding fermentation values; values for processed materials are entirely out of line, as observed in the previous comparison. Values for eluates (1b, 1b′) assayed by yeast growth are in better agreement with fermentation results, and for most samples the agreement is fairly good; 12 of the 22 eluate values in Table I agree with the corresponding fermentation values within ±25%. Dehydrated potatoes, white flour, wheat germ, and whole wheat bread gave discordant results by the two methods.

Comparison of Values for Alkali-Treated Samples. The results listed in Table II show that pure thiamine subjected to heat treatment at an alkaline pH is, according to thiochrome measurement, completely destroyed. In the yeast-growth method it retains 4 to 4.5% of its activity, and in the yeast-fermentation, 13%.

For alkali-treated samples tested, thiochrome values are far

lower than are corresponding values from either of the yeast methods. Eluate yeast-growth values are in better agreement with thiochrome values than are any others, but they too are significantly higher.

In each extract there appears to be some material which is (1) relatively stable to alkali and heat treatment, (2) adsorbed on Decalso and eluted with acidified potassium chloride, (3) labile to sulfite treatment, and (4) stimulatory to yeast growth and fermentation.

ACKNOWLEDGMENT

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Device for Rapid Closing of Weighing Bottles

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WHEN large numbers of vacuum-oven moisture determinations are made, it is convenient to use a device designed to facilitate closing a number of weighing bottles at the same time. Closing each bottle by hand is not only tedious but slower, and this delay between opening the oven and closing the bottles may give some samples a chance to absorb moisture from the atmosphere.

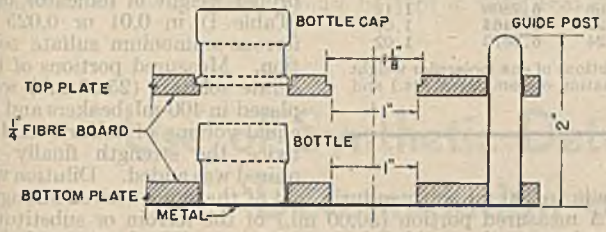

Figure 1. Type A. Cross Section Showing Openings for Bottles and Guide Post

Two devices, fundamentally alike but adapted to different forms of bottle closure, have been used in this laboratory. Each consists of two plates. The top plate supports the lids or stoppers and serves both to open and to close the bottles; the bottom plate, similar in both types, keeps the bottles in fixed positions. Both plates are placed in the oven where they rest on the brackets that ordinarily support the shelves.

Plates of type A (Figure 1) were designed to fit a vacuum oven in the form of a cylinder 22.5 cm. (9 inches) in diameter by 45 cm. (18 inches) long, and the pair accommodates 40 Parr weighing bottles, 25 mm. wide and 20 mm. high, having outside-fitting ground-glass lids. The bottom plate consists of a continuous metal sheet, 0.16 cm. ($^1/_{16}$ inch) thick, riveted to thicker (0.6-cm., 0.25-inch) nonmetallic sheet material (such as fiberboard) having round holes, uniformly arranged in parallel rows, to fit the bottoms of the bottles. Metal is used for the continuous base to ensure good heat conductivity to the bottles. The top plate, which holds the lids, is made of nonmetallic sheet material and has holes corresponding to those in the bottom plate and large enough to let the uncovered weighing bottles go through. These holes have a slightly larger diameter from the upper surface to about halfway through the plate, in order to hold the lids in place. One end of the top plate has a hole corresponding in position and size to a guide post at one end of the bottom plate.

To load this device, the top plate is placed on the bottom one, the closed bottles are placed in the holes, their lids are loosened, and the top plate is slowly raised. The bottoms of the bottles will remain on the lower plate, while the lids will be lifted with the top plate. After the drying period, the bottles are closed by moving the upper plate, loaded with the caps, downward along the guide post. The closed bottles may then be lifted out of the holes.

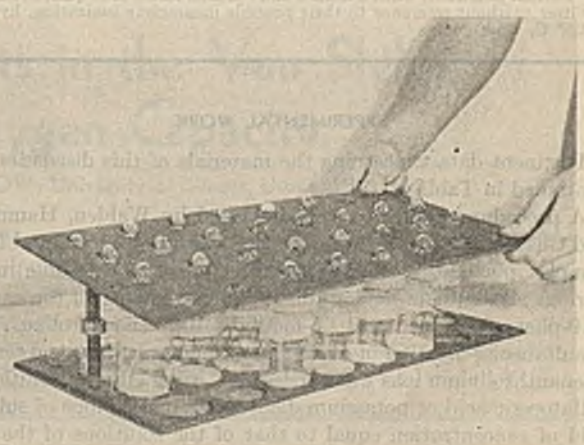

Figure 2. Type B. Stoppers Being Lowered onto Bottles

A similar device, type B (Figure 2), accommodates 32 bottles, 40 mm. wide by 50 mm. high, having inside-fitting ground-glass stoppers. The top plate has slits and grooves at right angles to each other. All the slits are parallel. The bottles are placed in the bottom plate with the flattened tops of the stoppers parallel to the direction of the slits in the top plate, which is lowered until it rests on the weighing bottles; the tops are turned through a 90° angle to fit into the grooves; and the top plate is slowly lifted, removing the stoppers which are suspended from it. After drying, the stoppers may be lowered onto the bottles, the tops turned in the direction of the slits, and the top plate lifted off.

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Substituted 1,10-Phenanthroline Ferrous Complex Oxidation-Reduction Indicators

Potential Determinations as a Function of Acid Concentration

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This paper deals with determination of the variation in the potential at which the phenanthrolinium ion is oxidized from the ferrous to the ferric form as a function of acidity. The data include those for the substituted complex ions in which the 5- or 6-position hydrogen is replaced by methyl, nitro, chloro, or bromo radicals, and for the complex ions in which the 5- and 6-position hydrogens have been replaced by both the methyl and nitro groups.

THE first application of the phenanthrolinium complex ion as an indicator in oxidation-reduction reactions was described by Walden, Hammett, and Chapman (*8*). The nitro substitution product was first studied by Hammett, Walden, and Edmonds (*1*), and its first practical application was in the determination of oxalic acid, described by Smith and Getz (*6*). The synthesis of the materials is described by Smith and Getz (*5*) and Richter and Smith (*4*). The spectrophotometric properties of these products were studied by Moss, Mellon, and Smith (*3*).

Table I. Physical Constants of Phenanthrolines and Their Substitution Products

Compound	M. P. (Anhydrous) ° C.	Common Name of Ferrous Sulfate Complex	Mol. Wt.	Amount Required for 1000 Ml. of 0.01 *M* Fe^{++} Complex *Grams*	Formal Oxidation E.M.F. *Volts*[a]
5-Nitro-1,10-phenanthroline	202	Nitro-ferroin	225.20	6.7560	1.25
5-Nitro-6-methyl-1,10-phenanthroline	269	Nitromethyl-ferroin	239.23	7.1768	1.22
5-Bromo-1,10-phenanthroline monohydrate	119	Bromo-ferroin	277.11	8.3134	1.12
5-Chloro-1,10-phenanthroline monohydrate	123	Chloro-ferroin	232.66	6.9800	1.12
1,10-Phenanthroline monohydrate	117	Ferroin	198.22	5.9464	1.06
5-Methyl-1,10-phenanthroline monohydrate	114	Methyl-ferroin	212.24	6.3673	1.02

[a] Formal potential, when oxidized and reduced forms are equal, in sulfuric acid solutions of one molecular weight per liter (without reference to their possible incomplete ionization, hydrolysis, formation of complexes, etc.) and at 25° C. (*7*).

EXPERIMENTAL WORK

Pertinent data concerning the materials of this discussion are contained in Table I.

A procedure similar to that described by Walden, Hammett, and Chapman (*8*) was employed when both the ferrous and ferric complex phenanthrolinium ions were found to be stable in the various strengths of acid studied. This was true in the case of 1,10-phenanthroline and 5-methyl-1,10-phenanthroline. The simultaneous titration of a mixture of ferrous sulfate and ferrous phenanthrolinium ions was carried out, using either a solution of sulfatoceric acid or potassium dichromate in solutions of sulfuric acid of concentration equal to that of the solutions of the ions being titrated. The range of acidity employed was from 1 to 8 moles per liter. The ceric-cerous and ferrous and ferric potentials were separately determined under the same conditions and the values of these two reference points reconfirmed.

A typical sample titration graph is shown in Figure 1. Titration conditions used were:

10 ml. of an approximately 0.1 molar ferrous sulfate solution in sulfuric acid (4 gram molecules of sulfuric acid per liter)

15 ml. of an approximately 0.1 molar solution of methyl-ferroin added to an equal volume of sulfuric acid (8 gram molecules of sulfuric acid per liter)

Dilution to 200 ml. by addition of sulfuric acid (4 gram molecules of sulfuric acid per liter)

Titration using 0.1 *N* potassium dichromate dissolved in sulfuric acid (4 gram molecules of sulfuric acid per liter)

Frequently employed reference point potentials of various systems as used at different acid concentrations are given in Table II.

GENERAL PROCEDURE FOR DETERMINATION OF FORMAL ELECTRODE POTENTIALS

For some of the phenanthrolinium ions the potential of the higher reference system is not sufficient to oxidize the ferrous to the ferric ion complex. To use a perchloric acid solution of perchloratoceric acid because of its higher potential in some cases was unsatisfactory because of the formation of insoluble ferrous perchlorate complex phenanthrolinium ions. In other cases equally insoluble sulfuric acid complex ions resulted at the higher acidities.

The ferric complex phenanthrolinium ions (with the exception of oxidized ferroin and methyl-ferroin) were not stable for more than a short interval, especially at higher acid concentrations. In these cases solutions of 0.01 or 0.025 *N* sulfatocerate ion in solutions containing 1 to 8 gram molecules of sulfuric acid per liter were prepared by dissolving pure ammonium nitratocerate in concentrated sulfuric acid, followed by gradual dilution to the proper amount. The substituted ferroin indicator solutions of 0.01 or 0.025 *M* concentrations were prepared by solution of the proper weight of indicator base (Table I) in 0.01 or 0.025 *N* ferrous ammonium sulfate solution. Measured portions of the cerate solution (25.00 ml.) were placed in 400-ml. beakers and an equal volume of sulfuric acid of twice the strength finally required was added. Dilution was made to 200 ml. with sulfuric acid of the same desired strength.

A measured portion (50.00 ml.) of the ferroin or substituted ferroin was then added in one portion with vigorous stirring. The potential of the resulting solution was read at once, using a saturated calomel electrode and a bright platinum electrode as references. Any condition such as results from instability of the ferric phenanthrolinium ions was indicated by a gradual fall of potential which could be observed without difficulty. Such instability was more pronounced at higher acid concentrations and

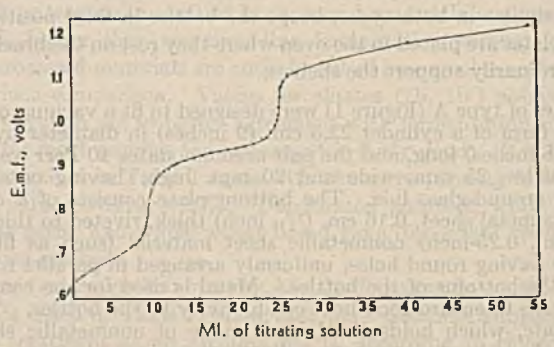

Figure 1. Simultaneous Titration of Ferrous and Phenanthrolinium Ferrous Ions

In 4 *M* sulfuric acid solution, using potassium dichromate in 4 *M* sulfuric acid throughout

Table II. Formal Electrode Potentials of Various Systems in Sulfuric and Hydrochloric Acid Solutions[a]

Potential Determined	Sulfuric Acid Concentrations (Moles per Liter) 1	2	4	6	8
	E.M.F., Volts				
$Fe^{+++} \longleftrightarrow Fe^{++}$	0.68	0.68	0.68	0.68	0.68
$Cr_2O_7 \rightarrow 2Cr^{+++}$	...	1.11	1.15	1.30	1.35
$Ce(SO_4)_3^{--} \rightarrow Ce^{+++}$	1.44	1.43	1.42	...	1.40
	Hydrochloric Acid Concentrations (Moles per Liter) 1	2	3	4	
	E.M.F., Volts				
$Fe^{+++} \longleftrightarrow Fe^{++}$	0.69	0.68	0.67	0.66	
$Cr_2O_7^{--} \rightarrow 2Cr^{+++}$	1.09	1.11	1.19	1.15	

[a] Determinations of present study are taken from Smith and Getz (*6*).

Table III. Formal Oxidation Potential of Ferroin and Substituted Ferroin Indicators at Various Strengths of Sulfuric Acid

Indicator	Sulfuric Acid Strength 0.5 *M*	1 *M*	2 *M*	3 *M*	4 *M*	6 *M*	8 *M*
	Oxidation Potential, Volts						
Nitro-ferroin	1.26	1.25	1.22	...	1.12	1.12	1.11
Nitromethyl-ferroin	...	1.23	...	...	...	...	...
Bromo-ferroin	1.13	...	...	...	...	...	...
Chloro-ferroin	...	1.11	1.10	...	1.04	0.97	...
Ferroin	...	1.06	1.03	1.00	0.96	0.89	0.76
Methyl-ferroin	...	1.02	1.00	0.96	0.93	0.86	0.70
2,2′-Dipyridyl-ferroin	...	0.97	...	...	0.92	...	...

with ferric phenanthrolinium ions of highest electrode potential. The systems showed no appreciable change in potential during the time required for reading the potential of the first mixing.

The data obtained are found in Table III. By determination of potentials in many cases by both procedures, the values were shown to be reliable within 0.02 volt. The values obtained by either procedure duplicated those of Walden, Hammett, and their co-workers as corrected by Hume and Kolthoff (*2*). As previously assumed (*2*), the oxidation potential of the bipyridylinium ferrous complex ion is not so high as that of ferroin.

In using the data of Tables II and III as a guide to titrations employing visual equivalence point determinations rather than potentiometric observations, it must be kept in mind that the color change from red in reduced solutions to faint blue in oxidized solutions requires approximately 90% oxidation of the indicator ion before the red hue is eliminated. The oxidation potential is thus effectively approximately 60 millivolts higher than the values give in Table III. The values given in Table III are claimed to be valid to within ±20 millivolts and in most cases better.

SUMMARY

Formal oxidation potentials of the ferric-ferrous and the dichromate-chromic systems have been determined in 1 to 8 *M* sulfuric and hydrochloric acid solutions. The use of such data in the selection of the proper indicator systems for determination of reaction and points is suggested.

A general procedure for use in determination of the formal electrode potentials of reversible oxidation-reduction indicators of the ferroin and substituted ferroin group is described.

The oxidation potential of the phenanthrolinium ion and nitro, bromo, chloro, methyl, and nitromethyl phenanthrolinium ions is given in various sulfuric acid strengths from 1 to 8 *M*.

For the system of indicators studied the range of oxidation potentials found varies from 0.7 to 1.26 volts, with all gradations between represented.

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Abstract of a portion of a thesis presented in partial fulfillment of the requirements for the Ph.D. degree in the Graduate School, University of Illinois.

Use of Synthetic Detergents in the Van Slyke Determination of Oxygen Capacity

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MODIFICATIONS of the original Van Slyke procedure (*5*, *7*) for the determination of blood oxygen capacity have been concerned chiefly with mechanical and manipulative improvements (*1*, *2*, *3*, *6*, *8*, *9*). It occurred to the authors to test several synthetic detergents, of different types, as possible substitutes for the saponin prescribed by Van Slyke as the hemolytic agent.

The results below indicate that several common detergents may conveniently be used in place of the less readily available, more expensive, and mildly irritating saponin. Sendroy's procedure (*2*) has been used in these determinations on rabbit and horse blood. It is reasonable to assume that the modified method can be extended to the blood of other species.

A saturated solution of each of the detergents, with the exception of the RO-C, was prepared in a freshly made potassium ferricyanide solution containing 23 grams per 100 cc. The saturated solutions were prepared by adding one volume of potassium ferricyanide of twice the desired concentration to an equal volume of detergent solution containing 16 grams per 100 cc. and filtering. The source of each of the detergents used can be ascertained by reference to the 1943 list (*4*). In the case of the RO-C (a cationic detergent of the alkyldimethylbenzyl ammonium chloride type, Winthrop Chemical Company), 8 grams per 100 cc. were used, an amount equal to that of the saponin prescribed by Van Slyke. The RO-C reacted slowly with potassium ferricyanide and is not considered suitable for use with it as the oxidizing agent.

Table I. Oxygen Capacity Determinations on Fresh Oxalated Rabbit Blood Diluted with 1% NaCl Solution

Detergent	Type	Volume % O_2
Saponin	Natural polycyclic glucoside, Merck	11.99
Duponol WA	Long-chain alcohol sulfate	12.06
Aerosol O.T.	Sodium dioctyl sulfosuccinate	11.72

The results in Table I suggest that Duponol WA may be readily employed in place of saponin, but that the use of Aerosol O.T. yields slightly low values. A favorable check in this analysis is ±0.2 volume % (*8*). All determinations, including blanks, were carried out in duplicate.

Table II indicates that each of the three detergents tested will give satisfactory results on fresh oxalated rabbit blood diluted with 1% sodium chloride. The use of Nacconol FSNO, an alkyl aryl sulfonate type, led to similar values. A freshly prepared

Table II. Oxygen Capacity Determinations

Detergent	Type	Volume % O_2
Saponin	Merck product	10.40
Duponol W-20	Long-chain alcohol sulfate	10.39
Aerosol O.S.	Isopropyl naphthalene sodium sulfate	10.49
Arctic Syntex M.	Sulfate of glycerol monolaurate	10.52

and used RO-C–potassium ferricyanide combination led to slightly low results.

Additional experiments also indicated that either potassium dichromate or iodine in 10% potassium iodide may be substituted in equimolecular amounts for potassium ferricyanide and used with saponin. This aspect of the problem was not pursued further.

Accordingly, in view of the experiments described in this report, it is suggested that synthetic detergents of the long-chain alcohol sulfate type, the alkyl aryl sulfonate type, or the monoglyceride sulfate type be used as hemolytic agents in the determination of blood oxygen capacity with potassium ferricyanide as the oxidizing agent. It is, of course, possible that other readily available detergents may be equally effective.

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From the senior thesis of M. A. Swerdlow, February, 1944.

Quantitative Method for Determination of Maltose in the Presence of Glucose

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AN ACCURATE and rapid method is needed for analysis of mixtures of sugars, and especially for mixtures of maltose and glucose, but this method cannot be applied as outlined below to mixtures of these two sugars and other carbohydrates, such as "malt sirup" and "corn sirup".

Two methods cited by Browne and Zerban (*1*) are representative of the usual procedures that have been advocated: that of Morris, which combines copper reduction, polarization, and selective fermentation, and the shorter one of Steinhoff, which makes use of two copper solutions—i.e., a Soxhlet and a modified Barfoed. A more recent method is that of Schultz, Fisher, Atkin, and Frey (*3*), which is based on three fermentations, in which the evolved gas volumes represent the sugars acted upon, and the maltose and "β-amylase attackable substances" are computed by difference. This method is similar to that developed by the author at about the same time (*2*) for the "maltose fraction" in flour. Aside from the question of accuracy, these methods are involved and cumbersome.

Tomoda and Taguchi (*4*) have reported a polarimetric procedure for analysis of mixtures of glucose and maltose and of glucose and fructose similar to the one described herein but differing in detail. Their method has been condemned, apparently on the basis of misquotation of their statements regarding the accuracy of their maltose determinations. However, they claim that in four determinations of maltose in a maltose-glucose mixture the error was −1.10% in one and 0.0% in the other three.

The difference in the ability of various sugars to combine with bisulfites was noted by the author in the course of work on fermentations wherein bisulfites were present and this difference was made use of in the analyses of sugar mixtures for maltose. Although in the work of Tomoda and Taguchi the same principle was employed, it is believed desirable, because of the simplicity and accuracy obtainable, to describe a somewhat different method of application of this principle.

This polarimetric method is based on the fact that the optical rotation of glucose may be reduced to zero by addition of a sufficient quantity of soluble bisulfite, but the rotation of maltose and dextrins is affected only very slightly. Incidentally, the rotation of lactose and other reducing sugars is also lowered by the presence of bisulfites and the rotation of the sugar alcohols is unaffected. The speed and accuracy of this method are comparable with those of polarimetric determinations in general, but the sensitiveness is somewhat less.

The method of evaluation is based on Biot's additive rule of optical rotations—namely, $[\alpha]_x = X[\alpha]_1 + (1 - x)[\alpha]_2$ when $[\alpha]_x$ is the specific rotation of the mixture, $[\alpha]_1$ and $[\alpha]_2$ are the specific rotations of the individual components, and x is the fraction of one of them. Browne and Zerban (*1*) point out that the specific rotations used must take into account the solvent concentration. Since the concentration of the total sugars is constant, that of the water is approximately so, and an empirical relationship is adequate for this method, using observed values rather than specific rotations.

Table I. Optical Rotation of Maltose (Hydrate)–Dextrose (Anhydrous) Mixtures and Corresponding Percentages of Maltose

(In 30% bisulfite solution at 20° C. in 200-mm. tube)

Maltose, %	0	20	40	50	60	80	100
Dextrose, %	100	80	60	50	40	20	0
° S.[a]	0	11.3	22.9	28.8	34.3	46.0	57.8
Maltose (calcd.), %	0	19.72	39.96	50.26	59.85	80.27	100.86

[a] ° S., degrees on International Sugar Scale.

METHOD

The first requirement in the use of this method is a set of standard values for the optical rotation of maltose and glucose and mixtures of known proportions of these sugars in the presence of sodium bisulfite.

Because of the difficulty of dissolving relatively large quantities of bisulfite in sugar solutions of 10% or greater concentration, the writer prefers the following procedure in their preparation:

A series of seven solutions is prepared, each solution containing 10 grams of total sugar and not less than 75 ml. of water. The proportion of glucose to maltose should be 10 grams to 0, 8 to 2, 6 to 4, 5 to 5, 4 to 6, 2 to 8, and 0 to 10. To each of seven sugar flasks graduated to 110 ml. are added 30 grams of sodium metabisulfite or its equivalent of sodium bisulfite, and one of the sugar solutions is transferred to each. The flasks are shaken to dissolve the metabisulfite, cooled to 20° C., the contents made up to a volume of 110 ml. with distilled water, mixed, and polarized at 20° C. The length of the polariscope tube need not be specified but should be the same for all determinations.

The observed rotations are then plotted against the percentage of maltose and will lie on practically a straight line defined by these points. The percentage of maltose present may be determined by referring the readings to the graph or by multiplying (° S.) by the tangent of the line, which in the present work was 1.745.

In Table I are given the optical rotations of solutions of maltose hydrate, *d*-glucose (anhydrous) and sodium metabisulfite by polarization in a 200-mm. tube at 20°, and the corresponding computed percentages of maltose.

The accuracy of the method is indicated by the values obtained. The maximum deviation from the actual values in terms of per cent maltose was 0.28 and the minimum 0.04, with an average deviation of 0.20% for the five mixtures.

To determine the amount of maltose in a mixture of glucose and maltose, determine first the amount of total sugars by some accepted method. To each sugar flask used add 10 grams of the unknown mixture or the amount of its solution which contains 10 grams of total sugars. Dilute to approximately 75 ml. and proceed as described for the solutions of known sugar content. From the observed rotation the percentage of the total amount of sugar present as maltose can be calculated as described or may be determined with the use of the graph prepared from the data obtained upon the "known" solutions.

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Estimation of Pyridine Content of Pyridine–Acetic Acid Mixture Used in Riboflavin Determination

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IN A PART of their procedure for the determination of riboflavin, Conner and Straub (*1*) used a pyridine–acetic acid mixture to elute the riboflavin from the Florisil adsorbent. After treatment with oxidizing agents, the fluorescence of the riboflavin in the eluate was determined. When a number of riboflavin determinations are made by their procedure, a considerable quantity of the used pyridine–acetic acid mixture is collected. By distillation, the pyridine together with water and acetic acid may readily be recovered as a mixture, but unless some simple method is available for determining the concentration of these components, the mixture is worthless. The acetic acid may readily be determined by titration with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator, and its concentration calculated in the customary manner, but a convenient standard method is not available for determination of the pyridine concentration. However, after some experimenting it was found that pyridine could be separated from the mixture by means of a strong solution of caustic soda. Using this principle, a method was devised for determining the approximate pyridine concentration of such a mixture.

Table I. Apparent Recovery of Pyridine from Mixtures

Pyridine in Original Solution	20.0 *N* Sodium Hydroxide: Water and pyridine mixture	20.0 *N* Sodium Hydroxide: Acetic acid, water, and pyridine mixture	15.3 *N* Sodium Hydroxide: Water and pyridine mixture	15.3 *N* Sodium Hydroxide: Acetic acid, water, and pyridine mixture
Per Cent by Volume				
16.1	19.0	19.0	19.0	19.0
18.1	21.5	21.3	21.5	21.5
20.1	23.8	23.8	24.5	24.5
22.1	26.5	26.5	27.0	26.5
24.1	29.0	29.3	29.5	29.5

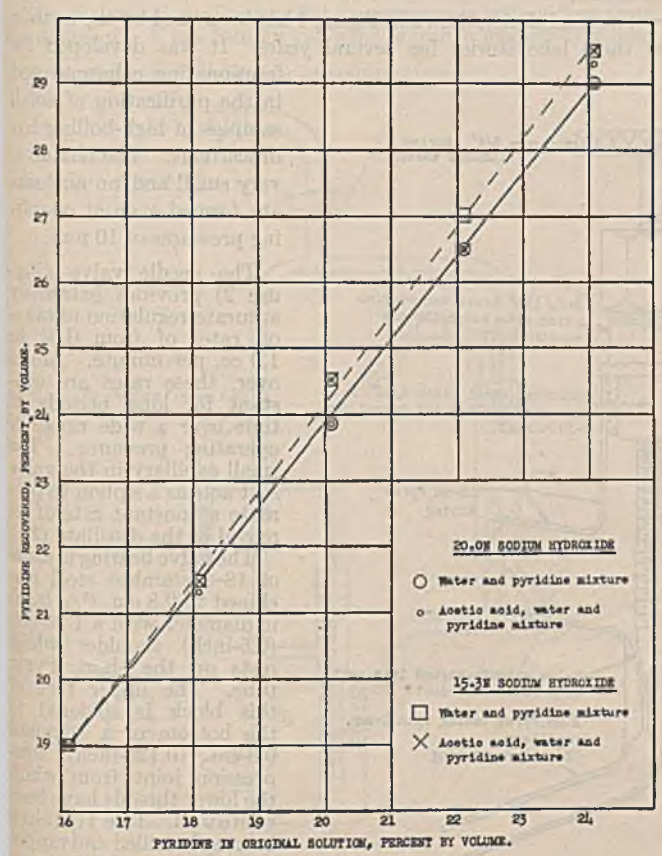

Figure 1

METHOD. After distillation of the pyridine–acetic acid mixture, a 20-ml. portion of the distillate is poured into a graduated 25-ml. glass-stoppered cylinder. To this there are added 5 ml. of 20.0 *N* sodium hydroxide. After shaking vigorously, the cylinder is set aside for 15 minutes, during which time the liquid separates into two layers. The volume of the top layer is noted.

When this method was used it was found that the pyridine was not recovered in a pure state. Hence, it was necessary to find the relationship between amount of crude pyridine recovered—i.e., the top layer of liquid—and the amount of pyridine that was originally present in the mixture. Furthermore, the amount of crude pyridine recovered might be influenced either by the amount of acetic acid present or by the concentration of the sodium hydroxide used.

In order to determine this relationship and the effect of sodium hydroxide and acetic acid concentration, a quantity of reagent grade pyridine of such purity that it distilled between 114° and 116° C. was selected and two series of mixtures were prepared. The first series consisted of several mixtures of water and pyri-

dine, each of which differed from the other, in its pyridine concentration. The second series consisted of mixtures of water, pyridine, and acetic acid. In this series the acid concentration of each mixture was 0.3 *N* but the mixtures differed from each other in pyridine concentration. The pyridine concentration of each mixture was then determined by the above method. The mixtures were again analyzed in the same manner, except that 15.3 *N* instead of 20.0 *N* sodium hydroxide was used. The results obtained are shown in Table I and Figure 1.

DISCUSSION

The results in Table I clearly show that when the mixtures were 0.3 *N* with acetic acid, the amount of crude pyridine obtained was for all practical purposes the same as when no acid was present in the mixtures. Hence, it may be assumed that acetic acid in concentrations of 0.3 *N* or less will not affect the amount of crude pyridine recovered. When the concentration of the sodium hydroxide solution was 15.3 *N* instead of 20.0 *N*, a measurable difference was obtained in the amount of crude pyridine recovered. Hence, the strength of this solution should be maintained at approximately 20 *N* as specified in the method. When this concentration is used, the volume figure for the top layer of liquid may be directly converted to per cent pyridine by means of the approximate curve in Figure 1.

SUMMARY

To determine the concentration of pyridine in distillates consisting of pyridine, water, and acetic acid, the pyridine, in an impure state, is separated by treating the distillate with strong sodium hydroxide. The relationship between this crude pyridine and the percentage of pure pyridine present in the distillate is then determined by means of a curve.

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A Precision Head for Small Fractionating Columns

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THE most satisfactory head design for laboratory columns of all kinds is the total condensation partial take-off type originally reported by Loveless (*1*) and modified by Whitmore and Lux (*5*) and others. This design is not subject to the mechanical difficulties inherent in heads of the partial condensation total take-off variety (*3*, *5*) and permits accurate regulation of the distillation rate. It is particularly adaptable for use on fractionating columns which utilize small samples, since the holdup is negligible if the head is properly constructed.

[1] Present address, General Aniline & Film Corp., Easton, Pa.

When such columns are operated under reduced pressure, there is a tendency for the distillate to dissolve the lubricant in the conventional stopcock, allowing air to leak in around the barrel and rise through the tiny pool of reflux liquid above the take-off tube. This situation prevents accurate adjustment of the distillation rate and at very low operating pressures (10 to 25 mm.) may cause air-locks which entirely prevent the removal of the distillate.

The head design shown in Figure 1 has been used by the author in these laboratories for several years. It was developed for fractionating columns used in the purification of small samples of high-boiling hydrocarbons. The holdup is very small and no air-locks are formed even at operating pressures of 10 mm.

The needle valve (Figure 2) provides extremely accurate regulation of take-off rates of from 0.01 to 1.0 cc. per minute. Moreover, these rates are constant for long periods of time over a wide range of operating pressures. The small capillary in the valve seat acts as a siphon to promote a constant rate of removal of the distillate (*2*).

The valve bearing is made of 18–8 stainless steel machined to 0.8 cm. ($^5/_{16}$ inch) in diameter with a 1.25-cm. (0.5-inch) shoulder which rests on the short Pyrex tube. The upper face of this block is soldered to the bottom of a standard 0.3-cm. (0.125-inch) compression joint from which the lower threads have been removed. The finished bearing is drilled and tapped to receive the 8–32 thread on the valve spindle.

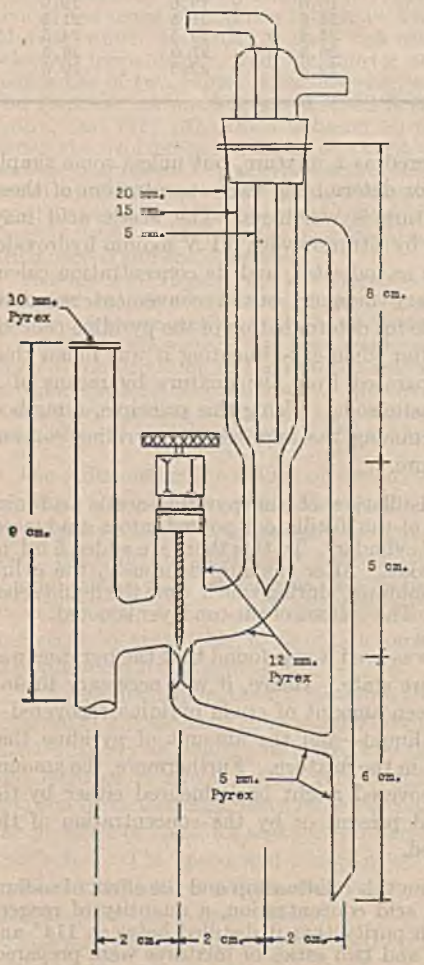

Figure 1. Head Detail

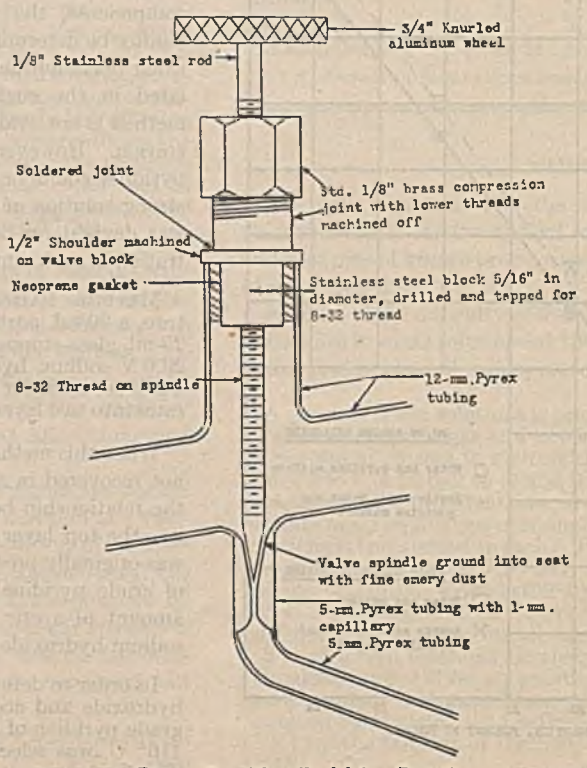

Figure 2. Needle Valve Detail

The needle-valve spindle is of 0.3-cm. (0.125-inch) stainless steel rod threaded to fit the valve bearing. A small knurled wheel is threaded to the top of the spindle. A fine point is ground on the end of the valve spindle with a Carborundum wheel and the valve seat produced by grinding this point into the capillary tubing with a fine grade of emery dust.

Strands of absorbent cotton impregnated with a stiff grease are stuffed around the spindle in the depression of the bearing and compressed to an air-tight seal by the hexagonal nut. A small piece is cut from 0.3-cm. (0.125-inch) Neoprene tubing and slipped over the end of the valve bearing, so that the assembled unit fits tightly to the inside of the Pyrex tube. When properly packed and assembled, such a bearing will easily retain vacuums of 3 to 5 mm.

An efficient column for use with this head consists of a Pyrex tube, 8 mm. in inside diameter, providing 30 cm. of packed section. This inner tube is surrounded by a 35-mm. tube wound with Nichrome wire over asbestos spacer cords. The inner tube and heating jacket is covered by a piece of 45-mm. Pyrex tubing which acts as as an insulator. The inner tube was packed with Wilson helices (*6*) 0.24 cm. ($^3/_{32}$ inch) in diameter. The column was operated at reduced pressures in conjunction with the fraction receiver described by Towne, Eby, and Young (*4*). This column proved to be very efficient in the purification of small samples (10 to 25 cc.). It had 14.5 theoretical plates at total reflux and an H.E.T.P. of 2.06 cm.

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A Circulating Device for Use with a Hydrogen Electrode

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THE device described here has been found useful in connection with a hydrogen electrode in two circumstances: first, when the solution, whose hydrogen-ion concentration is being measured, contains a very soluble gas which would be carried away if the hydrogen were allowed merely to bubble through it. A presaturator may be used, but under certain conditions this is rather impractical. The second situation is when a deuterium electrode is desired. With the device described here macroquantities of the gas may be used, but an excessive amount is not required. These two conditions were present in measurements made by the authors on the second ionization constant of deutero-carbonic acid. The results of these measurements have been reported elsewhere (*1*) but the circulating devices has not been adequately described.

The construction of most of the apparatus is self-evident from the diagram. *V* is a Bunsen valve made from a medicine dropper bulb. The proper size of slit can be found with a few trials and it may be inserted through the end of the wide tubing when the rubber stopper is removed. The mercury in the side arm is raised and lowered about 10 cm. by means of a motor and eccentric, raising and lowering a leveling bulb at a rate of about 15 times per minute. When the mercury rises the pressure in the adjacent part of the apparatus increases. Hence, the hydrogen bubbles through the solution around the platinum electrode until the pressure in the entire apparatus becomes uniform. When the mercury is lowered the hydrogen escapes through the Bunsen value. Thus the hydrogen tends to circulate through the apparatus. During this period the stopcock of the cell, S_2, should be kept closed. When an e.m.f. measurement is made the circulation may be stopped and the stopcock of the cell opened if desired.

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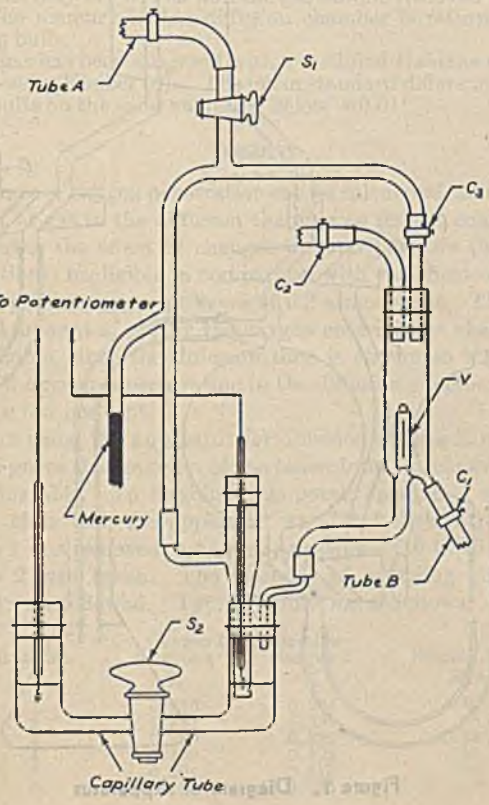

The apparatus is filled and flushed out by proper manipulation of clamps C_1, C_2, C_3, stopcocks S_1 and S_2, and the mercury column, hydrogen being admitted through tubes *A* and *B*. Admittance of dry hydrogen would alter the concentration of the solution slightly. This is ordinarily a very small error but may be avoided to a large extent by allowing the hydrogen to bubble through water, or better yet, through a sample of the solution before it is admitted to the apparatus through *A* and *B*. As a precautionary measure all rubber connections should be kept coated with collodion. In the authors' apparatus the volume, including the space in the cell above the solution, was 35 cc. For one determination about 100 cc. of hydrogen (deuterium) were used, the excess gas being used for flushing. It is usually most convenient to fill the device with hydrogen, so that when the mercury column is at its mean position the total pressure in the system is equal to that of the atmosphere. The corrections to apply in order to obtain the partial pressure of the hydrogen are obvious. In the authors' measurements, at equilibrium, the fluctuations in e.m.f. due to change in hydrogen pressure were less than 0.1 millivolt.

In order to avoid condensation it is necessary to keep the gas-phase portion of the apparatus at a temperature somewhat higher than that of the solution. This was accomplished by fastening the circulating device to a wooden block and suspending it in a small box in such a manner that the bottom of the box was slightly above the surface of the water in the thermostat in which the cell was immersed. When the thermostat was adjusted to 25° C. a current of air warmed to 30° C. was passed up through the bottom of the box through several holes. The hydrogen which circulates through the solution is also warmed to about 30° C. and this introduces a slight but unavoidable error.

This circulating device is obviously not limited to the particular type of cell depicted here. Harned and Scholes (*2*) have described in an extremely brief manner what is evidently an elaborate device for the circulation of hydrogen. Apparently their device is somewhat similar to the one described here.

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Apparatus for Measuring Rate of Gas Penetration through Food-Packaging Materials

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An apparatus is described for measuring the rate of gas penetration through flexible materials. The apparatus is particularly suitable for determining the rate of oxygen penetration into pouches used for food packaging. The absolute accuracy of the measurements is determined mainly by the accuracy of gas analysis. The relative accuracy can be changed by varying the time of penetration and thus the difference between start and end concentration of oxygen in the gas inside the pouch.

A MAJOR factor in the spoilage of many dehydrated food products is the partial pressure of oxygen in the gas surrounding the food, particularly when the product contains fat. According to Holm, Schaffer, and Haller (*5*) butter oil containing a concentration of oxygen less than 0.5% by volume "will remain in good condition for a long storage period even under what would generally be considered severe conditions of storage". Some manufacturers, therefore, remove oxygen by evacuation; others replace air with an inert gas. Before the war, metal containers were used to package the product and ensure a low oxygen content over a long period. Because of the scarcity of metals these containers have been replaced by pouches constructed of cellophane or similar materials. No satisfactory method has so far been made available to determine how well such packages maintain the desired low partial oxygen pressure.

The rate of change of partial oxygen pressure in the gas inside a package is among the most important criteria for grading a container.

Elder (*2*), Shuman (*8*), and Todd (*9*) recently described manometers for measuring gas permeability of film materials without regard to changes in partial pressure. Shuman gives examples of measuring "air transmission". Air transmission thus determined, however, is no suitable criterion for judging packaging materials. An undesirable material with a high permeability for oxygen and a low permeability for nitrogen may show a lower rate of air transmission than a desirable material with low oxygen and high nitrogen permeability. The manometers might, of course, be used to measure separately the permeabilities of pure gases and thence to draw conclusions as to the probable transmission of these gases from mixtures such as air.

For testing packing materials and particularly pouches, however, the authors preferred to work out a method which determines the major criterion—namely, changes in partial oxygen pressure—directly, and is applicable especially in cases where no significant differences in total pressure are involved.

The rate of penetration by diffusion (dependent on differences of partial pressure only) can be determined by gas analysis or other means designed to measure oxygen concentration. Such studies have been made by several investigators. Krogh (*7*) designed a metal diffusion chamber to measure the diffusion rate of oxygen through animal membranes, from a gas to a liquid or from a liquid to a liquid. His equipment did not lend itself to the proposed studies. Harvey and Morrison (*3*) used cultures of luminous bacteria to determine oxygen concentration. According to them, the oxygen concentration just allowing perceptible luminescence is about 1 part by weight of oxygen dissolved in 3.7×10^6 cc. of sea water. To obtain relative data on the rate at which oxygen penetrates various materials, Hill (*4*) used the luminous bacteria as an indicator. Alexejev and Matalskiĭ (*1*) determined the diffusion rate of gaseous mixtures through rubber membranes; but the present writers have been unable to obtain data on the type of apparatus or the method of analysis used.

APPARATUS

The apparatus was designed mainly for measuring changes in the oxygen concentration of a gas separated from air by various packaging materials. These measurements are particularly valuable when mechanical flaws are absent.

Although different types of apparatus have been tested in this laboratory, only the one found satisfactory is described here (Figure 1). This consists of a glass diffusion chamber, *A*, having an outside diameter of 6 cm. and a length of about 18 cm. Gas may be introduced and sampled through a tube, *B*, the end of which is about 8 cm. below the top of the diffusion chamber. *B* connects to either a nitrogen tank or a gas-sampling bulb, through the three-way stopcock, *C*, and rubber tubing *D*. A glass receptacle, *E*, containing mercury is fastened to the diffusion chamber with a piece of bicycle inner tubing, *F*. The orifice, *G*, of chamber *A* may then be closed with a glass bell, *Q*, or with a sack of the packaging material in question. It has been desirable to hold the glass bell or packaging material in the mercury with

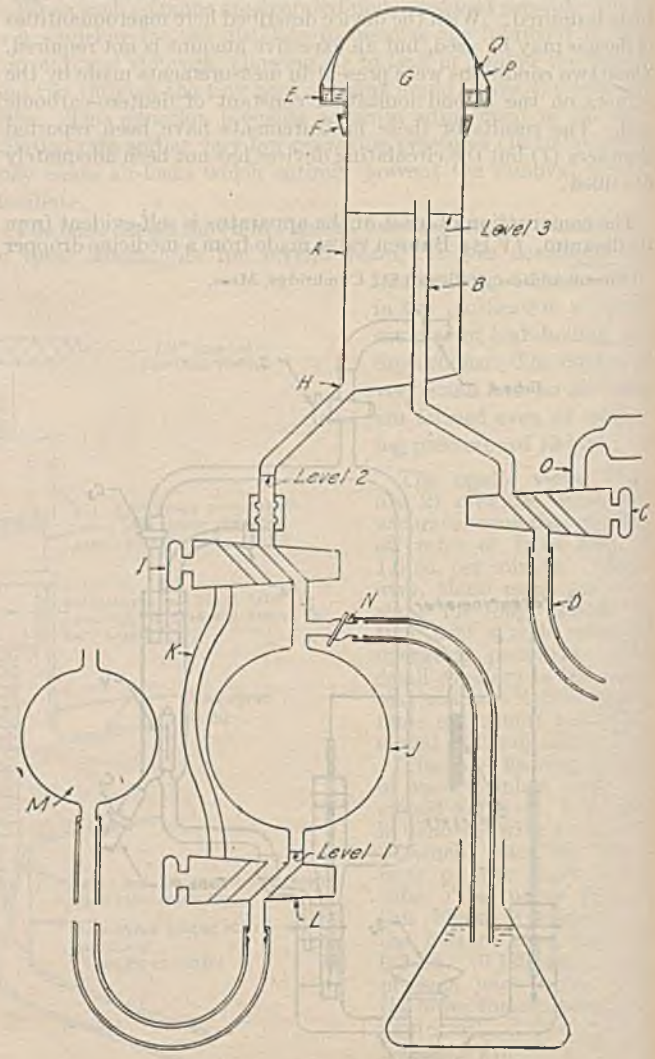

Figure 1. Diagram of Apparatus

Scotch tape, *P*, fastened over the top. Tube *H* is connected by a piece of rubber (pressure) tubing with a three-way stopcock, *I*. One opening of this stopcock is connected to the 250-ml. glass bulb, *J*, while the other connects with a glass tube, *K*. Thus by manipulating stopcocks *I* and *L*, connection to the diffusion chamber may be made either through bulb *J* or through tube *K*. Stopcock *L* connects with the mercury-leveling bulb by means of rubber tubing.

In operation, the mercury-leveling bulb, *M*, and stopcocks *I* and *L* are manipulated so that *K* is completely filled with mercury. This tube remains filled during measurements. The glass bell or packaging material is held solidly in the mercury seal by Scotch tape. *M* is adjusted so that the mercury stands at level 1. Stopcock *N* is opened, and stopcock *I* is turned to connect bulb *J* and chamber *A*. Stopcock *C* is opened into tube *B*, and gas is allowed to flow from a nitrogen tank through *A*. The excess gas escaping through *N* is allowed to bubble through water. Thus a constant positive pressure is maintained in the apparatus during replacement of the gas.

After the gas has flowed for a few seconds, *M* is raised so that the mercury rises from level 1 just to the top of *J*. The leveling bulb is then lowered, and the mercury allowed to fall to level 1. Meanwhile the nitrogen must be kept flowing fast enough to ensure constant escape of gas through *N* as measured by constant bubbling through the water seal. Two manipulations of the leveling bulb usually give satisfactory replacement of the air. Stopcocks *C* and *N* are then closed simultaneously, and the tube at the lower end of *C* is connected by rubber tubing to a gas-sampling bulb containing mercury. *C* is now opened into tube *O*, and the mercury in the gas-sampling bulb is forced up to flow into *O*. *C* is then opened into *B*, and *I* and *L* are adjusted to open the connection between *M* and *A* through *J*. *M* is raised, and simultaneously the mercury in the sampling bulb is lowered. This drives the gas from *A* into the sampling bulb.

When the mercury in the apparatus reaches level 2, stopcocks *I* and *C* are closed, and the sampling bulb is removed for analysis of the sample. *M* is then lowered; and *N* is opened, allowing all the mercury, except that above *I*, to flow back into the leveling bulb. This mercury may be used in other apparatus during the diffusion study.

At the expiration of the time allotted for diffusion, a second sample is taken. (Diffusion time must be determined according to the permeability of the material to be tested. As a rule, 48 hours is satisfactory.) For the second sample the gas-sampling bulb is connected and manipulated as before. *M* is raised to a point about level with the top of the diffusion chamber; *I* and *L* are adjusted so that the mercury flows into the diffusion chamber through *K*. As the mercury flows, the gas sample is collected in the sampling bulb. When the mercury reaches level 3 the stopcocks may be shut off and the gas sample removed for analysis. The mercury in the diffusion chamber is returned to the leveling bulb.

The gas has been analyzed with a modified Haldane apparatus described by Kleiber (*6*). The mean standard difference between two results on the same sample is below ±0.01%.

RESULTS

The rate of oxygen penetration can be calculated, assuming the amount of gas in the diffusion chamber to remain constant and considering the effect of changes in total pressure (barometric fluctuations) negligible in comparison with the effect of a difference of partial oxygen pressure of 0.2 atmosphere. The change in total amount of gas by the oxygen entering the chamber also is negligible, since the diffusion time is chosen so that the increase of oxygen concentration in the diffusion chamber does not exceed a few per cent.

Before using the apparatus for diffusion studies it was necessary to prove that samples of gas taken from the chamber would be comparable. To establish this point, opening *G* was closed with a glass bell, the apparatus was filled with nitrogen, and sample 1 was removed. As soon as possible (10 to 15 minutes), sample 2 was taken. The methods of sampling already described were followed. Typical results are as follows:

Run No.	Oxygen Concentration Sample 1	Oxygen Concentration Sample 2	Elapsed Time
	%	%	*Min.*
1	0.210	0.204	10
2	0.357	0.359	15
3	0.229	0.227	15
4	0.190	0.198	15
5	0.204	0.210	10
6	0.189	0.195	10

As a second check on the reliability of the diffusion equipment it was necessary to be sure there were no leaks. To test for leaks the glass bell was placed over opening *G*, and the apparatus was filled with nitrogen as before. A sample was secured, the equipment was allowed to stand, and then a second sample was taken:

Run No.	Oxygen Concentration Sample 1	Oxygen Concentration Sample 2	Elapsed Time
	%	%	*Hours*
1	0.173	0.178	48
2	0.210	0.209	48
3	0.220	0.223	22
4	0.187	0.186	48

Since the differences in samples 1 and 2 in these tabulations lie below the figure given by Kleiber as the standard error of the oxygen determination with the apparatus used, the diffusion chamber may be considered satisfactory, and the small discrepancies between samples may be ignored. Leaks have not been observed during the period of study (over 7 months), and thus routine checks before each diffusion study would not seem necessary.

To determine the rate of diffusion of oxygen one must know the volume into which the gas diffuses.

The diffusion chamber was filled with water, and the volume of water was then measured by draining the liquid to level 2 and allowing it to drain from tube *B* just to stopcock *C*. When diffusion rates were measured on pouches, the volume of the pouches was taken by calibrating with water. Since the open end of the sack is placed in mercury to a depth of 1.5 cm., the water level in calibrating is placed 1.5 cm. from the top of the sack. The total volume then is the sum of the diffusion chamber volume plus the volume of the sack.

Flexible sacks may be immersed in the mercury seal by attaching them to glass or metal rings with Scotch tape. Metal hooks on the top of rings permit the attachment of rubber bands, which submerge the juncture of sack and ring into the mercury. Flat pieces of flexible material have been crimped over the top of the diffusion chamber and held in place by a rubber band. This method may be criticized because it introduces folds and creases in the material. With the low rate of penetration observed in the authors' measurements, however, those irregularities in the surface areas can hardly affect the rate of exchange.

The following data, collected on a sack made of MSAT No. 480 laminated cellophane, will serve as an example of the results:

The total volume into which the oxygen diffused was 900 ml.; the area of sack exposed was 500 sq. cm.; the diffusion time was 555 hours; the temperature was 30° C. The oxygen content at 0 hours was 0.054%; at 555 hours, 0.175%. Thus the diffusion rate may be calculated as follows:

$$\frac{\text{Volume of gas in diffusion chamber and sack} \times \text{increase in } O_2 \text{ concentration}}{\text{Sack area exposed to air} \times \text{time}} = \text{rate/unit area/unit time}$$

or substituting

$$\frac{900 \times (0.175 - 0.054)0.01}{500 \times 555} = 0.0000039 \text{ ml. of } O_2\text{/sq. cm./hour}$$

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Use of an Alternating Current Solenoid in Freeze Tests

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NUMEROUS types of apparatus have been developed in the past two years to determine the relative flexibility and brittle point of synthetic materials at subzero temperatures. In employing any "freeze test" to judge the suitability of a material for low-temperature service, the mechanics of the test device used and the methods of conditioning the sample (time, temperature, etc., *2*) should be considered in addition to the actual test data obtained.

In order to reproduce test results the velocity of the member inducing the deformation must be constant, since brittleness is a function of the rate of bending. Inability to reproduce test results is frequently due to lack of control of velocity, and hence of the energy supplied by the test device.

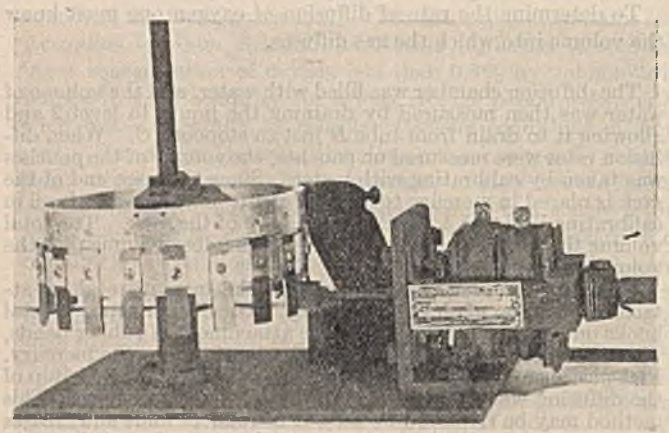

Figure 1

The bent loop test described by Martin (*6*) and tentatively adopted by A.S.T.M. (Designation D736–43T) is an excellent type of test for determining the relative flexibility of a series of test specimens, but the procedure for conducting the test fails to specify a specific rate at which the specimens are to be flexed. Most apparatus for performing this test employs either a crank and lever or a direct-push type of mechanism which is hand-operated, permitting some variation of the rate of flexing. The direct-push type is the simpler of the two devices and is the one more commonly used. The rate at which this push-type device can be operated depends upon the number and flexibility of the samples under test. Other flexibility tests, such as the bend test in which the specimen is bent through 90° over a 0.125-inch pin, or the bend test in which the mandrel size depends on the gage of the material being tested, are hand-operated. Thus the rate of flexing varies from test to test and from operator to operator, and variations observed in test results from laboratory to laboratory depend, to a large extent, on the human element involved in the test.

The weakness of most of the test devices which have been developed to determine the brittle point of synthetic materials is due also to the lack of controlled velocity. Bell Telephone Laboratories in presenting their hand-operated brittle point apparatus (*8*) stated that better test results were obtained if only one sample was under test at a time. Improved results were due to better control of velocity. This apparatus has been modified by Morris, James, and Werkenthin (*7*) to give better control of temperature and higher velocity at point of impact. Kemp, Malm, and Winspear (*5*) have fitted the original Bell apparatus with an electric motor and a set of gears in an attempt to control the rate at which the sample strikes the arm. Both groups of investigators have concluded that it is necessary to restrict an individual test to a single sample. The Bell Telephone Laboratories' apparatus which has been tentatively adopted by A.S.T.M. (D746–43T) operates with a quadrant having a peripheral speed of 6.5 ± 0.5 feet per second.

Chatten, Eller, and Werkenthin (*1*) have developed a test device in which a swinging pendulum imparts a hammer blow to the test specimen. Graves and Davis (*4*) have presented an apparatus in which a spring-actuated hammer imparts the blow to the test specimens. The motorized apparatus, the pendulum device, and the spring-actuated hammer are very good from the standpoint of control.

Another brittle point test in which the specimen is subjected to a hammer blow was developed in the Goodrich Laboratories by Garvey (*3*). Its outstanding feature is economy of sample, and its main disadvantage is lack of control in imparting the hammer blow.

ALTERNATING CURRENT SOLENOID AS A SOURCE OF POWER

An alternating current solenoid is a simple, efficient, easily controlled source of power which may readily be applied to many existing devices for measuring freeze resistance. It will supply energy at a reasonably constant rate, if it is operated at constant voltage. By varying the voltage, the speed at which the plunger of the solenoid travels may readily be controlled. A very simple way of controlling the desired voltage is by use of a Variac, an autotransformer which can easily be set to any desired output voltage.

The use of an alternating current solenoid as it has been applied to the Du Pont brittleness tester is shown in Figure 1. The brittleness tester consists of an aluminum drum from which the samples are suspended. The drum may be rotated from outside the working chamber in order to place the sample in front of the hammer. This hammer is made of linen impregnated with a phenolic resin and is actuated by the solenoid. The leading edge of the hammer has been machined to a 0.0625-inch radius. The drum has twenty numbered slots from which samples, 2 × 0.5 × 0.075 ± 0.010 inches, are suspended. It is counterbored above each slot and a spring-activated pin drops into this hole to ensure proper alignment of each sample with the hammer before testing. The lower edge of the slot, over which the samples must bend or break when struck by the hammer, has been machined to a $^1/_{32}$ inch radius. Each sample is held in place by a machine screw with a square washer. This apparatus is designed to operate in a cold, dry atmosphere (carbon dioxide).

The alternating current solenoid which is the source of power for the hammer is of the pull-type, having a 1-inch stroke, and is rated as having a 14-pound pull. This pull-type solenoid was converted to a push-type by drilling a 0.25-inch hole through the end of the frame and into the center of the cross section of the plunger. A brass rod on which the hammer is mounted was screwed into the end of the plunger. A second solenoid, much smaller in size, is used to return the hammer to its starting position. Both solenoids are individually controlled and are operated on a 110-volt 60-cycle circuit.

TEST PROCEDURE. The general test procedure when there are a large number of compounds to be tested is as follows:

Four test pieces, 2 × 0.5 × 0.075 ± 0.010 inches, of each of five compounds are fastened to the drum with the machine screws just tight enough to hold the samples in place, the drum is put on the test stand, and the unit is installed in the cold box. After the desired temperature has been maintained for 1 hour the samples in slots 1, 5, 9, 13, and 17 are tested. When any one of these samples fails the remaining three pieces of the same compound are tested as checks. After exposure at the next lower temperature, the same procedure is repeated, starting with samples in slots 2, 6, 10, 14, and 18. Thus, one sample of each compound is tested at each temperature and when failure occurs the remaining untested pieces of the compound are tested. If all four test pieces of a compound are tested without failure the procedure is repeated, starting with the first test piece of that group at the next lower temperature.

In general, the test is started at −30° F. and the temperature is lowered in 5° F. intervals with 1-hour exposure at each temperature to ensure temperature equilibrium. The test unit is adjusted so that the hammer travels approximately 0.25 inch (depending on thickness of sample) after contacting the test piece

Table I. Du Pont Brittleness Test Data

(Hammer striking sample 0.25 inch below bottom edge of drum and traveling 0.25 inch after making contact)

Compound	Temperature of Brittle Point, ° F.				
Buna S	−90°	−90°	−90°	−90°	−90°
Neoprene Type GN	−65°	−65°	−65°	−65°	−65°
Neoprene Type FR	−75°	−70°	−70°	−75°	−75°
Rubber	−65°	−65°	−60°	−65°	−65°

and strikes it 0.25 inch below the edge of the drum, thus producing a bend of approximately 45°, as is shown in Figure 2. The reproducibility of the brittle point for a given compound is excellent. When failure occurs the sample breaks off squarely at the lower edge of the drum.

Table I shows the results obtained with the Du Pont brittleness tester on four compounds which were tested five different times over a period of 3 weeks.

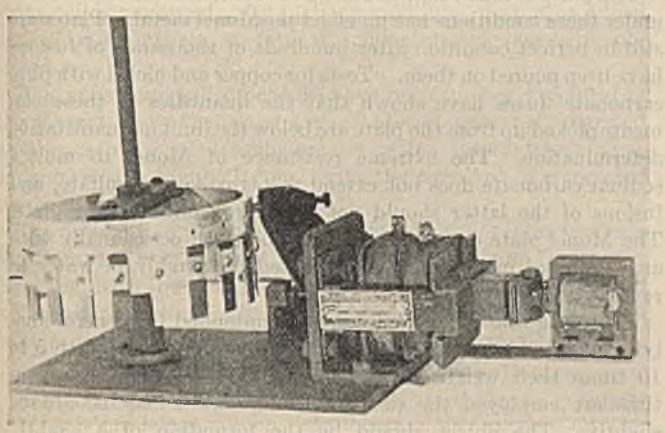

Figure 2

This apparatus may also be used to determine the low-temperature flexibility of coated fabrics. This test may be conducted by replacing the present drum with one that has a longer skirt on which loops of the material to be tested are mounted. After being conditioned at the desired temperature, each loop is compressed by the hammer against the drum until the distance between hammer and drum is equal to twice the gage of the fabric.

Figure 3 shows an isometric sketch of a proposed brittleness test apparatus employing an alternating current solenoid of the push-type.

The basic design of this apparatus is similar to the Bell Telephone Laboratories' (*4*) motorized apparatus, but the energy to bend the sample at a constant rate is supplied by an alternating current solenoid instead of a motor. This solenoid is rated as producing a 5-pound push at a maximum stroke of 1 inch. The inside dimensions of the tank containing the immersion medium are 15 × 4.5 × 6 inches, and sixteen specimens, 1.50 × 0.25 × 0.075 ± 0.010 inches, may be mounted on the sample holder. A movable bar, not shown in the figure, supports the specimens in a horizontal position during the conditioning period. It is moved to the front of the tank before testing the specimens. The edge of the hammer that strikes the sample has a 0.0625-inch radius, which is the same as the radius on the arm used in the Bell Telephone Laboratories' apparatus. The position of the sample holder on which the specimens are mounted may be varied by changing the size of the inserts at the supports.

As may be seen in Figure 3, the structure supporting the solenoid is hinged, in order that it may be swung back to simplify the operation of removing and replacing the specimen holder. The sample holder is equipped with small eye-bolts, so that hooks may be used in removing and replacing the holder. Alignment of the solenoid with the test specimen is assured by a latch which drops into the notches shown on the upper rod supporting the solenoid. The immersion medium, usually methyl alcohol, ethyl alcohol, or acetone, is cooled by circulation through a coil placed in an acetone and dry ice bath. The immersion medium inlet is at the

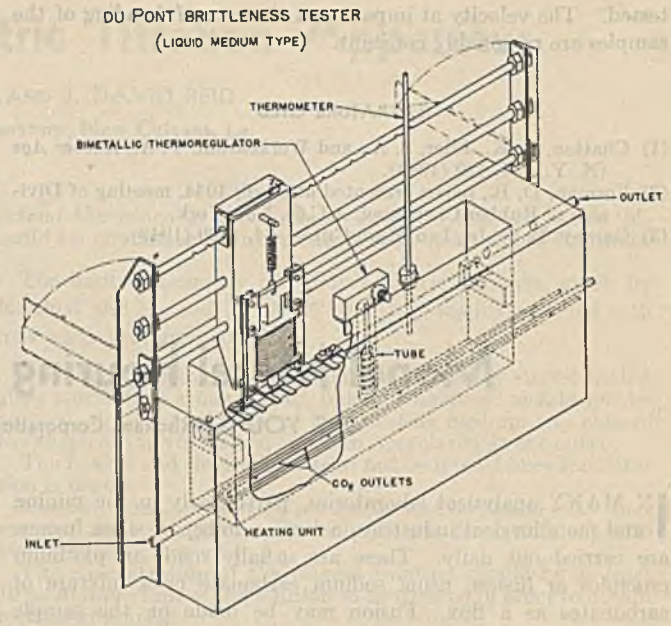

Figure 3

bottom of the tank on the left end and the outlet is at the top on the right end. The tube shown along the bottom of the tank is for the purpose of bubbling carbon dioxide through the medium in order to give mild agitation and to produce a carbon dioxide blanket over the medium to reduce fire hazard. In case fumes from the immersion medium constitute a health hazard, the apparatus should be operated under a hood.

The pump used to provide circulation of the immersion medium may be controlled by a bimetallic thermoregulator located in the tank as shown. The bimetallic thermoregulator is mounted in a tube in which a nonvolatile liquid, such as a low-temperature hydraulic oil, is used to prevent volatile vapors from entering the chamber which houses the contact points where arcing may occur. A condenser placed in the circuit with the bimetallic thermoregulator and the motor of the pump will reduce the arcing to a minimum and help retain the sensitivity of the thermoregulator. The temperature of the immersion medium is indicated by the thermometer which is mounted next to the bimetallic thermoregulator. The heating unit enables one to warm up the immersion medium rapidly.

The velocity at the instant of impact of the hammers for both the 14- and 5-pound solenoids was determined and found to vary linearly from 7 to 9 feet per second when the voltage was varied from 100 to 120 volts. Therefore, when the solenoids are operated at rated voltage (110 volts) the velocity of the hammers at the instant of impact is 8 feet per second.

The time of travel of the hammers from the point of zero velocity to the point of impact is very short; hence the velocity at impact is affected by the phase of the voltage cycle at which the solenoid becomes energized. This seems to indicate that a more scientific test unit might be obtained if a direct current solenoid were used. However, the use of a direct current solenoid is not only impracticable in many cases, but is believed to be unnecessary. It was found experimentally with the unit which is operated in air that the kinetic energy imparted by the solenoid at rated voltage was 3.5 times the kinetic energy necessary to break the samples. In view of this fact, the slight variation in the kinetic energy resulting from energizing the solenoid at various phases of the voltage cycle is negligible.

ADVANTAGES

Operation is simple and efficient. The human element has been eliminated as much as possible from the test.

A variable number of samples may be tested at one time. Stocks of any hardness or those swollen by solvents can be

tested. The velocity at impact and the rate of bending of the samples are reasonably constant.

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Presented before the Division of Rubber Chemistry, American Chemical Society, at its New York meeting.

Monel Metal Pouring Plate for Silica Fusions

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IN MANY analytical laboratories, particularly in the mining and metallurgical industries, a large number of silica fusions are carried out daily. These are usually made in platinum crucibles or dishes, using sodium carbonate or a mixture of carbonates as a flux. Fusion may be made on the sample directly or on the insoluble portion after prior acid treatment.

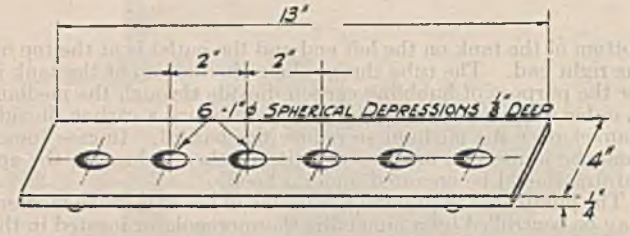

Figure 1. Pouring Plate

Since these determinations are generally used for control purposes, speed is essential. If the fusion is allowed to solidify in the crucible, a long time is required to dissolve the contents in the dilute hydrochloric acid usually employed. The practice of allowing the melt to cool on the sides of the crucible requires careful manipulation, and even then most of the sample solidifies at the bottom. When solution of the solidified melt is slow, more hydrochloric acid is often added to hasten the action. However, as the resulting solution usually must be evaporated to dryness to remove silica, the increase in bulk prolongs the evaporation period. When a fusion reacts slowly with acid, or adheres to the crucible, there is a temptation to hasten solution by scraping with a glass rod, which usually results in ultimately deforming or injuring the platinum. The time lost in waiting for cooled fusions in platinum crucibles to dissolve is particularly important to those laboratories where an abundant supply of platinum ware is not available.

This difficulty can be overcome by pouring the molten fusion into a depression on a Monel metal plate. The material quickly solidifies in the form of a flat button which is transferred with platinum-tipped tongs to a beaker or casserole, together with the platinum crucible. A few milliliters of dilute hydrochloric acid suffice to dissolve the thin film of solidified melt left in the platinum crucible or dish, which can be washed and removed for another fusion in a minute or two. Owing to its surface, the button rapidly goes into solution in dilute acid. The chilling effect produced by pouring on the Monel plate gives a product which dissolves more readily than a fusion allowed to cool in the crucible. If necessary, solution of the button may be aided by breaking up with a flat-tipped glass stirring rod.

This procedure was first brought to the writer's notice some time ago in the laboratories of the International Nickel Company of Canada at Copper Cliff, Ontario, where it had been in use for many years. It has since been applied successfully to a wide variety of products.

The plate is illustrated in Figure 1. Molten sodium carbonate under these conditions has no effect on Monel metal. Plates are still in perfect condition after hundreds of thousands of fusions have been poured on them. Tests for copper and nickel with pure carbonate fluxes have shown that the quantities of these elements picked up from the plate are below the limit of quantitative determination. The extreme resistance of Monel to molten sodium carbonate does not extend to peroxide or bisulfate, and fusions of the latter should not be poured onto such a plate. The Monel plate can be cleaned and polished occasionally with any standard metal cleaner and rinsed thoroughly in water to remove traces of polish.

The plate illustrated in Figure 1 is intended for general analytical work where 0.5- to 2.0-gram samples are fused with 5 to 10 times their weight of carbonate flux. Where other quantities are employed the size of the depression may be altered slightly. The object should be the formation of a slightly rounded button which will fill the depression but not overflow to give a thin layer on the plate. In the latter case the thin edge of the button may break in several pieces when picked up with the tongs. A slightly larger plate to contain two rows of depressions may be used where a large number of fusions are carried out as routine determinations.

Remove the crucible from gas burner or electric furnace when contents have fused, pour onto plate, place crucible on Transite or Alberene stone table top or on the plate itself, pour the next crucible, place the first solidified button in the corresponding crucible, and transfer to beaker or casserole. It is not good practice to put a red-hot platinum crucible on a Monel plate, but after the crucible is poured it is almost invariably cool enough to place directly on the plate beside the button if desired. Tip the button from the crucible into the beaker and add a little dilute hydrochloric acid to the crucible. Continue pouring in this manner, wash, and remove the crucibles. While the main portion of the fusion contained in the button is dissolving, the platinum crucibles or dishes may be used for further fusions.

Spectrophotometry

Two papers have been prepared by the Research Department of the Calco Chemical Division, American Cyanamid Co., and are available through the Advertising Department, Bound Brook, N. J.

"Spectrophotometry and the Colorist", Calco Technical Bulletin 756, prepared by E. I. Stearns, discusses interpretation of spectrophotometric data and suggests methods of application to mill production and research problems.

"Identification of Organic Pigments by Spectrophotometric Curve Shape", Calco Technical Bulletin 754, prepared by R. Abbott and E. I. Stearns, illustrates the general method of approach to the problem of identification of organic pigments by their characteristic absorption curve shape.

A Simplified Conductometric Titration Apparatus

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THE advantages and disadvantages of conductometric titrations are too well known to be detailed here. It is often advantageous to titrate mixtures or turbid solutions conductometrically, and this method has been used in this laboratory for the analysis of complex mixtures encountered in research on the fireproofing of cotton cloth. However, the complicated apparatus ordinarily used discourages more general application of the method. The apparatus described by Whittemore and his coworkers (*3*, *4*, *5*) is perhaps the simplest. In their method the voltage is adjusted to a constant value after each addition of titrating solution and the current passing through the conductivity cell is measured. By plotting milliliters of solution added against milliamperes, a typical conductometric curve is obtained.

This apparatus and procedure have been further simplified in this laboratory by the use of a constant-voltage transformer and an alternating current milliammeter of low internal resistance, as diagramed in Figure 1, considering the circuit at *M* completed through *A*.

The innovation, though simple, makes the conductometric titration very easy, since it is only necessary to read one meter, compared with the former procedure involving the adjustment of a fluctuating voltage with a potentiometer, reading this on a voltmeter, and simultaneously reading the milliammeter.

The constant-voltage transformer, T_1, is the type generally used for 8-volt lamps in such instruments as the Coleman spectrophotometer and thus is generally available. Its cost is less than that of the meter, transformer, and potentiometers it replaces. The more common 115-volt constant-voltage transformer with an auxiliary step-down transformer is equally suitable and this combination may be substituted for T_1. Along with the constant-voltage transformer it may be necessary to have an additional constant load to meet its minimum load requirement and avoid overheating.

Because of war conditions, the sale of meters is restricted, and, unfortunately, an alternating current milliammeter is more rarely used than other types in a chemical laboratory. A low-range alternating current voltmeter is nearly always available, however. In this laboratory, the 2.5-volt range of an alternating current meter of the rectifier type, 1000 ohms per volt, was used instead of a milliammeter as follows:

A radio-type transformer, T_2, commonly known as an audio-output transformer, with an impedance ratio of about 500 to 1 (turn ratio of 22 to 1), was used as a current transformer to convert the relatively high current at a small voltage drop to a much higher voltage, and impress this voltage on the meter. It was then possible to read the current directly from the meter in relative units. If actual values are desired, the factor may be obtained from a consideration of the meter and transformer constants, or by direct calibration. This circuit is shown in Figure 1, considering the circuit at *M* completed through *B*. The transformer to be used as T_2 should be selected to reflect the resistance of the meter as 5 ohms in series with the cell—for example, to use the components cited above, a transformer matching 2500 ohms to 5 ohms was used. In general, satisfactory results are obtained using a value within the limits of 4 to 8 ohms for the low-impedance winding.

Electrodes are conveniently made by welding platinum disks to platinum wire and sealing each into appropriately shaped glass tubes as shown in Figure 1. The size of the electrodes and the distance between them are chosen to give a convenient initial conductance. For example, in the titration of approximately 100 ml. of 0.001 *N* solution with 0.01 *N* reagents, disks 1 cm. in diameter and spaced about 2.5 cm. apart were used. In the titration of approximately 100 ml. of 0.01 *N* solutions with 0.1 *N* reagents, disks 0.3 cm. in diameter and spaced about 3 cm. apart were used. It was found that the apparatus described was generally suitable for cell-electrolyte combinations which resulted in resistances of the order of 400 to 10,000 ohms. It was not found necessary to extend the range in the direction of lower resistances, as this could be circumvented by titrating with more dilute solutions.

The limit of accuracy of conductometric methods, given by Kolthoff and Sandell (*2*) as 0.5 to 1%, is readily attained with reasonable precautions:

The solution being titrated should be uniformly stirred with a glass stirrer (not a metal one), but a "whirlpool" should not be allowed, since the addition of the titrating medium will change the shape of the vortex and cause an irregularity in the curve.

The beaker and electrodes should not be moved once the titration is begun.

The two transformers (if T_2 is used) must be placed with their cores at right angles or sufficiently distant from each other to avoid inducing current in T_2.

The concentration of the reagent solution should be at least 10 to 20 times that of the solution to be titrated in order to obtain rectilinear graphs.

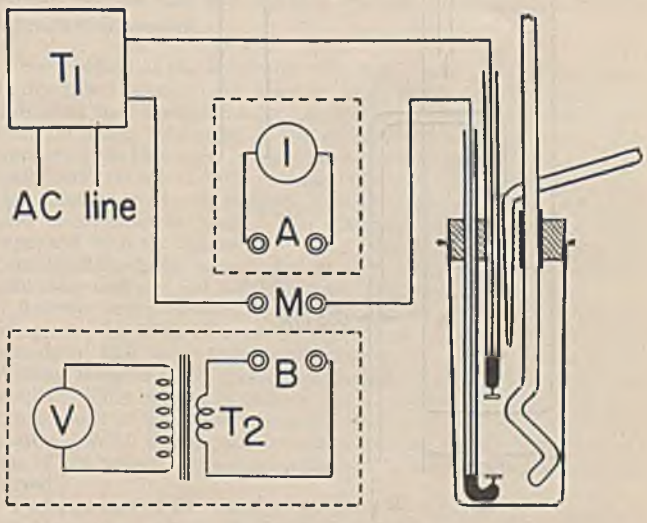

Figure 1. Diagrams of Simplified Conductometric Apparatus

T_1. 8-volt constant-voltage transformer (or combination of 115-volt constant-voltage transformer and 115- to 8-volt step-down transformer)

M. To be completed through either circuit *A* or *B*

Circuit *A*. *A*, metering circuit using milliammeter *I*
I, low-resistance 0- to 30-milliampere alternating current meter

Circuit *B*. *B*, metering circuit using T_2 and voltmeter *V*
T_2, audio-output transformer
V, 0- to 2.5-volt alternating current meter, 1000 ohms per volt

Graphs of conductometric titrations obtainable with this apparatus are of the same type and precision as shown by other authors (*1*, *3*, *4*, *5*). The text by Britton (*1*) is particularly useful for interpretation of the graphs obtained.

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An Improved Timing Siphon

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CYCLIC processes can best be controlled by the use of a clock motor or other similar device. However, a substitute for a mechanical timer is often needed. In this laboratory the common siphon proved unsatisfactory for such use because after the first delivery of water, the siphon tube remains full of bubbles. In this case, the siphon vessel may never again fill up, the water siphoning out as fast as it runs in, or the siphon may operate when the vessel is only one-fourth or one-half full.

This difficulty is overcome in the design given in Figure 1.

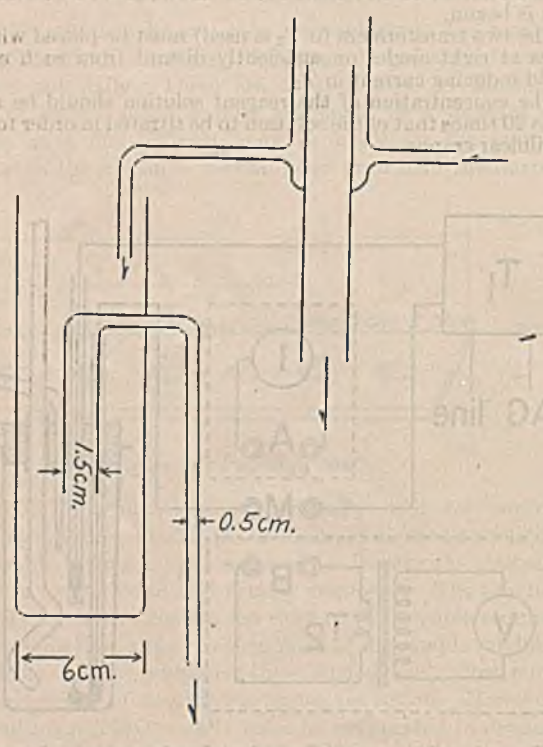

Figure 1

The central tube is 1.5 cm. in diameter and joins on to a 5-mm. tube. This large central tube completely breaks up the bubble string and gives cycles which are accurate to 2 or 3% if the water temperature does not vary too greatly. The size of the outer vessel can be varied to any size, while the central tube and the draining tube can be enlarged to any degree, provided the ratio of sizes is not made smaller and the draining tube does not become too large actually to function as a siphon. A conventional constant-head device furnished a constant flow of water to the vessel containing the siphon.

The conductivity of the water can be used to work the control mechanisms as follows: Two electrodes which are intermittently bathed by the water can be connected to a source of power and a suitable relay which in turn can actuate pumps, valves, lights, etc. A satisfactory relay circuit is described by Rudy and Fugassi (*1*).

Liquids other than water can be used with this device, with due regard for abnormal viscosity or for vapor pressure which might cause bubble formation due to the lower pressure at the top of the siphon tube.

In place of conductivity control, a photoelectric control may also be used.

By controlling the rate of influx of water, the rate of draining with the siphon, and the placing of the electrodes, almost any length of cycle can be obtained.

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Simple Automatic Pump for Collecting Gases at Low Pressures

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IN A particular piece of work it was found desirable to maintain a reaction mixture at low pressure and at the same time to collect evolved gases. Since the reaction was being followed for extended periods, a manually controlled pump such as a Toepler was inconvenient. The following simple design was found to be efficient and required virtually no attention during long periods of operation.

The details of the design are shown in the figure. It consists essentially of a mercury diffusion pump in which the compressed gas, instead of being removed by a mechanical pump in the usual way, is entrained by the condensed mercury, as in the ordinary Sprengle pump, carried down the capillary tube, *A*, and discharged into the gas holder, *C*. A second capillary, *B*, returns the mercury from the gas holder to the boiler.

The height of the diffusion pump above the mercury boiler must be sufficiently great to keep the hydrostatic pressure of the mercury and entrained gas in *A* always greater than the mercury alone in *B*. In the present design, which operated successfully on systems where the pressure was as high as 3 cm. of mercury, this height is about 40 cm. Pumping speeds should be greater as this head is increased. In order that the collected gas should not occupy too large a volume, the gas holder should be located about 40 cm. below the level of the mercury in the boiler.

Capillary tubing of 2-mm. diameter is satisfactory for the top half of *A*, while the use of 1-mm. tubing in the bottom half greatly reduces the tendency for compressed gas bubbles to move up the tube. A heating element of about 60-watt capacity wrapped about the tube delivering mercury vapor to the jets reduces bumping in the boiler and eliminates excessive refluxing of the mercury.

This pump is particularly useful for collecting gases at low pressures. Its limited capacity makes it rather slow at higher pressures. The design described reduced the pressure in a 350-cc. volume from 35 to less than 10^{-5} mm. in 3 hours. A lowering from 1 to 10^{-5} mm. was obtained in the same system in 15 minutes.

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Determining Volatile Bases in Fish

Comparison of Precision of Certain Methods

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Methods were investigated for determining total volatile base and tertiary volatile base in fish flesh as an index of spoilage. Sampling methods tested included use of press juice, protein-free press juice, 60% ethanol-leached samples, samples "liquidized" with 60% ethanol, and samples of ground fish suspended directly in solution. Volatile base was removed by microdiffusion, distillation, and aeration. Most precise results were obtained for total volatile base by extracting the fish flesh with 60% ethanol and removing the volatile base by distillation from the solution made alkaline with borax. Tertiary volatile base could best be determined by a slight modification of the microdiffusion method of Beatty and Gibbons (3) whereby a sample extracted with 60% ethanol was used in place of press juice.

THE determination of volatile bases in fish has been widely used as an index of the freshness of fish. A considerable number of procedures have been suggested, and since results obtained by the different methods are not always in agreement, the comparative results obtained by some of them have been studied. These were procedures which obtain the total volatile base by separation from alkaline solution into an excess of standard acid or which determine tertiary amines by separation in the presence of formaldehyde.

SAMPLING TECHNIQUE

An experimental lot of fish of varying degrees of freshness was prepared as follows: Fifty-five eviscerated silver salmon were procured when 4 hours out of the water. Twenty fish were stored at room temperature and 30 in crushed ice. Five were dressed immediately and the flesh, free of skin and bones, was ground twice through an electric meat grinder, mixed thoroughly, and packed in 0.2-kg. (0.5-pound) cans. The sealed cans were immediately frozen and stored at about −15° C. (5° F.). At suitable intervals the salmon stored at room temperature or in ice were sampled by removing five fish and treating as above. It was assumed that no increase in volatile base occurred after freezing, and the cans of frozen fish were used for subsequent tests. (All tests were completed within 6 months; additional samples stored 3.5 years showed an average increase in total volatile base of 18%. Greatest decomposition occurred in the fresher samples, where significant changes may have occurred during the 6 months' storage period.)

The method of bringing the volatile base into solution prior to distillation into standard acid is of importance in obtaining accurate results. If both volatile base and other nitrogenous substances are present during distillation, the latter may break down to give added volatile base. Low results may be obtained if an attempt is made to extract the volatile base from the protein or if the protein is removed from solution by precipitation. In the first case all the base may not be extracted and in the second case it may be lost by being adsorbed on the protein precipitate.

In obtaining press juice for the tests, the ground fish flesh was placed in a canvas cloth and pressed in a Carver laboratory press at a pressure of 8000 to 10,000 pounds per square inch (562 to 703 kg. per sq. cm.). Since the fish had been frozen, relatively large volumes of press juice were obtained (100 to 200 ml. of juice per kg. of flesh). The press juice ordinarily was clear, but became cloudy upon standing. When made alkaline for the distillation and particularly in the presence of formaldehyde, it frequently gelled, especially if from stale fish. With fresh fish the juice usually became very cloudy, and sometimes considerable sediment settled out, but as a rule it did not solidify.

A series of tests was made with press juice from which the protein had been precipitated by trichloroacetic acid. Twenty-five milliliters of the juice were treated with a few milliliters of a saturated trichloroacetic acid solution in a centrifuge tube. After centrifuging, the clear solution was decanted into a 100-ml. volumetric flask, and the voluminous precipitate was washed three times with 10-ml. portions of water. The combined solution and washings were made up to 100 ml. for the determination. The solution was clear and little or no sediment separated either upon standing or when made alkaline.

The exceedingly gummy nature of the precipitate formed when trichloroacetic acid was added to the press juice made washing difficult, and doubtlessly considerable of the volatile base was left adhering to the precipitate. In some instances the precipitate was more gummy than in others, making for irregular adsorption of volatile base. A more easily washed precipitate could be obtained by precipitating diluted press juice, but so high a dilution was required (ten times or more) that the resulting sample did not contain enough volatile base to give reproducible results.

Extractions of the fish both with water and with 60% ethanol as described for meat by Allen (1) were tried. Preliminary tests indicated that for fish, as for meat, much more consistent results were obtained by means of the ethanol extractions; so all tests were made in this way. Forty grams of fish were stirred mechanically with 60 ml. of 60% ethanol, in a 250-ml. centrifuge bottle, the solution was centrifuged, and the clear solution decanted into a volumetric flask. The stirring and centrifuging were repeated with two more 75-ml. portions of 60% ethanol and the combined leachings were made up to 250 ml. The final solution was clear and did not solidify upon being made alkaline.

Another series of tests was run in which the fish was finely disintegrated in 60% ethanol by means of a liquidizer. (Several brands of this type apparatus were tried. Of those used, the Waring Blendor with special aluminum container gave the best results. This special container had a screw-type lid which prevented loss of solution during operation and gave quantitative results. With equipment in which the lid merely sat upon the top of the vessel, an appreciable loss of solution sometimes occurred.)

Forty grams of fish and 100 ml. of 60% ethanol were liquidized for 5 minutes, after which time the fish was completely disintegrated and formed a stable suspension from which the solid could be removed completely only by centrifuging. The solids remaining in the centrifuge tube were washed with two 25-ml. portions of 60% ethanol and the combined solution and washings were made up to 250 ml. The resulting solution was clear immediately after preparation but a fine precipitate usually formed after a short time giving a cloudy appearance.

SEPARATION OF VOLATILE BASE

Determinations were run using the Conway and Byrne (4) microdiffusion technique as modified for fish by Beatty and Gibbons (3), and also using press juice freed of protein by trichloroacetic acid treatment. In applying the microdiffusion technique improvised glassware was used in place of the regular Conway dishes which were not readily available. A 5-ml. beaker for holding the standard acid was placed inside a Stender dish (60 mm. in diameter and 28 mm. deep). In order to assure that the lids of the Stender dishes in all cases fit tightly, it was necessary to regrind them with Carborundum and use a fairly heavy coating of stopcock grease. The ratio of surfaces of alkaline test sample and of standard acid exposed to total volumes is less in this improvised equipment than in standard Conway dishes but preliminary experiments showed that by increasing slightly the time of standing and temperature of incubation, recovery of ammonia from both pure ammonium chloride solution and standard ammonium chloride added to fish solutions was adequate (recovery consistently over 90%, usually better than 95% from solutions 0.001 N or stronger).

Samples prepared by extraction with alcohol were also dis-

tilled at atmospheric pressure. The presence of the alcohol during the distillation materially aided in reducing foaming. An excess of borax was used in place of magnesium oxide because it not only reduces hydrolysis of nitrogenous substances by virtue of its lower pH, but also, being denser than magnesium oxide, has less tendency to cause foaming. This distillation procedure is a considerable improvement over the ordinary aqueous distillations using magnesium oxide and a battery of six or more can easily be run with little or no attention.

Aeration was carried out in the apparatus exactly as described by the A.O.A.C. (*2*) procedure for meat, in which case finely ground fish flesh was suspended in water. In other cases fish flesh was liquidized with 60% ethanol and the resulting solution made alkaline and aerated without removal of fish particles. Aeration units were set up in batteries of six units in parallel and some difficulty was encountered in obtaining uniform aeration. This difficulty was overcome by use of capillary tubing of appropriate lengths as entrance tubes into each of the six tubes containing the fish samples. By adjusting the length of this tubing the pressure could be equalized, permitting uniform aeration in all samples. Four such batteries of six units were run at once.

An aeration period of 5 hours was used in all cases. Further aeration gave a slight increase in volatile base but this increase continued indefinitely as long as the aeration was carried out, and probably represented a breakdown of the protein or other nitrogenous substances. All determinations were run in triplicate to allow for discarding occasional samples which did not receive adequate aeration when the inlet tubes to the sample vessels became clogged with fish flesh. Little or no difficulty was encountered with tubes becoming clogged when samples prepared in the liquidizer were used. In these cases the fish, being of a much finer state of subdivision, did not settle out as long as the aeration continued.

Tertiary volatile base was determined in each case by addition of neutral formaldehyde to the final solution of fish. The difference between the amount of standard alkali used in the determination and the blank was taken as equivalent to the volatile base present.

DISCUSSION OF RESULTS

Fish stored at room temperature for 1 day were still reasonably fresh but were slightly stale after 2 days (see Tables I and II). Accordingly, if total volatile base or tertiary volatile base is to be used as an index of spoilage a large increase would be anticipated between fish stored for 1 and 2 days. With the fish

Table I. Individual Determinations and Averages for Total Volatile Base in Silver Salmon[a]

		Milligrams of Nitrogen per 100 Grams of Fish or per 100 Milliliters of Press Juice							
			Microdiffusion			Distillation		Aeration	
Sample No.	Storage Conditions of Fish	Press juice	Protein free press juice	Leached with 60% ethanol	Liquidized with 60% ethanol	Leached with 60% ethanol	Liquidized with 60% ethanol	Suspended in water (A.O.A.C.)	Liquidized
1	Fish 4 hours out of water. Fresh	11.2	9.5	4.0	14.3	7.2	11.8	7.3	17.3
		11.6	6.5	3.9	14.7	12.9	12.0	7.2	17.1
		13.8	..	..	..	..	..	..	15.4
	Av.	12.2	8.0	4.0	14.5	10.1	11.9	7.3	16.6
2	1 day at room temperature. Fresh	11.7	7.3	5.3	11.9	13.0	11.8	9.7	16.4
		13.1	11.8	5.1	12.6	13.3	12.1	9.4	16.0
		11.9	7.3	..	..	13.1	11.5	..	15.8
		..	..	..	..	12.2	11.9	..	..
	Av.	12.2	8.8	5.2	12.3	12.9	11.8	9.6	16.1
3	2 days at room temperature. Slightly stale	18.0	15.6	12.4	24.3	16.5	20.6	17.7	26.8
		18.8	14.3	12.3	23.6	20.8	21.2	11.0	27.2
		17.6	..	..	..	21.6	21.3	..	25.4
		18.7	..	..	..	..	21.6	..	..
	Av.	18.3	15.0	12.4	24.0	19.6	21.2	14.4	26.5
4	3 days at room temperature. Very stale	28.0	23.1	19.2	42.8	34.8	31.0	37.0	40.7
		24.2	19.0	21.0	47.3	32.2	31.4	26.3	25.5
		15.6	..	..	46.8	29.4	31.4	35.6	30.6
		24.7	..	..	45.4	..	31.7	..	..
	Av.	23.1	21.0	20.1	45.6	32.1	31.4	33.0	35.3
5	4 days at room temperature. Putrid	39.8	35.0	35.4	44.2	47.0	35.4	28.2	48.0
		38.0	32.0	34.2	47.5	44.5	36.2	28.0	48.0
		35.4	..	..	..	..	35.3	27.2	46.7
		35.7	..	..	..	..	36.4	..	..
	Av.	37.2	33.5	34.8	45.9	45.8	35.8	27.8	47.6
A	3 days in ice. Fresh	12.6	12.3	..	13.1	12.1	11.1	7.6	14.6
		13.0	10.8	..	13.3	12.9	12.1	10.4	12.6
		13.5	..	..	..	11.5	11.3	8.9	15.2
		12.0	..	..	..	14.1	12.1	..	..
		..	..	..	..	13.2	..	..	..
	Av.	12.8	11.6	..	13.2	12.8	11.7	9.0	14.1
B	6 days in ice. Fresh	13.3	7.6	12.7	13.3	12.6	13.3	4.7	15.3
		11.0	8.2	13.3	13.1	12.0	13.5	4.9	15.7
		14.2	..	..	..	13.6	..	10.1	..
		13.3	..	..	..	13.6	..	..	..
	Av.	12.9	7.9	13.0	13.2	13.0	13.4	6.6	15.5
C	10 days in ice. Slightly sweet odor	11.4	9.0	10.9	15.9	11.7	12.0	14.9	15.3
		12.6	11.3	10.5	16.8	11.6	12.1	12.2	13.7
		11.0	8.8	..	..	12.4	..	9.9	15.7
		12.5	11.1	..	..	12.1	..	..	..
		11.7	..	..	..	13.2	..	..	..
	Av.	11.8	10.1	10.7	16.4	12.2	12.1	12.3	14.9
D	13 days in ice. Slightly stale	15.4	13.2	12.8	19.9	19.1	17.4	22.4	21.1
		18.3	9.7	14.4	20.6	19.5	17.7	14.6	23.8
		18.7	..	..	..	19.5	..	16.7	22.7
		15.4	..	..	..	17.4	..	..	..
		18.1	..	..	..	22.8	..	..	..
		..	..	..	..	22.8	..	..	..
	Av.	17.2	11.5	13.6	20.3	20.2	17.6	17.9	22.5
E	15 days in ice. Stale	18.3	18.6	21.7	19.9	23.0	19.5	27.4	27.7
		20.3	16.9	19.7	21.1	22.7	19.8	22.4	27.5
		24.4	18.8	..	..	..	..	20.1	27.6
		20.0	..	..	..	..	..	..	..
		22.8	..	..	..	..	..	..	..
	Av.	21.2	18.1	20.7	20.5	22.9	19.7	23.3	27.6
F	17 days in ice. Very stale to slightly putrid	91.0	26.4	27.1	30.4	33.5	30.9	17.7	38.6
		34.0	17.6	29.0	30.8	33.3	30.8	17.2	39.0
		21.7	..	..	..	..	..	..	36.8
	Av.	48.9	22.0	28.1	30.6	33.4	30.9	17.5	38.1

[a] Each determination reported was started from beginning of sampling and was carried out separately, so that differences in results are due to combined sampling errors, errors in separations, and titration errors.

stored in ice a more gradual deterioration took place. After 6 days the fish were still fresh, but after 10 days, although not at all stale, they had developed a slightly sweet odor. After 13 days in ice the fish were slightly stale. Corresponding increases in volatile base content were found with both lots of fish, and larger or smaller increases were obtained by all the methods of analysis.

SAMPLING METHODS

Errors due to sampling methods would be most apparent as poor precision when several determinations were run on the same lot of fish. Of the five sampling methods used, outstandingly high reproducibility was obtained by use of the "liquidizer". Precision was uniformly high by this method, and there can be little doubt but that of the methods tried it gives by far the most homogeneous sample, which is also readily adaptable to subsequent steps of the determination.

Use of press juice, while simpler and somewhat less time-consuming than the use of the liquidized sample, gave very poor precision especially when working with stale samples. This is believed to be caused by the tendency of press juice from such samples partially to solidify when made alkaline. Leaching the fish by stirring the flesh (previously ground in a meat grinder) with several portions of 60% ethanol gave good precision; this method is very time-consuming, but can be used if a liquidizer is not available.

METHODS OF REMOVING VOLATILE BASE

In preliminary tests the three methods of separating volatile base gave good precision when used on pure solutions in absence of fish. Errors occurring in the presence of fish were due largely to decomposition of protein, or other nitrogenous compounds, such as trimethylamine oxide during the separation from the alkaline solution. The results obtained did not indicate any outstanding advantage for any one method in all cases.

MICRODIFFUSION. This procedure has two advantages. First, it is convenient, especially with respect to saving in time, since many tests can be run simultaneously and little attention is required during the separation. The second advantage lies in the low temperature which can be maintained during separa-

Table II. Individual Determinations and Averages for Tertiary Volatile Base in Silver Salmon[a]

		Milligrams of Nitrogen per 100 Grams of Fish or per 100 Milliliters of Press Juice							
		Microdiffusion				Distillation		Aeration	
Sample No.	Storage Conditions of Fish	Press juice	Protein free press juice	Leached with 60% ethanol	Liquidized with 60% ethanol	Leached with 60% ethanol	Liquidized with 60% ethanol	Suspended in water (A.O.A.C.)	Liquidized
1	Fish 4 hours out of water. Fresh	0.00	0.23	0.00	0.13	0.00	1.00	0.21	0.51
		0.00	0.00	0.04	0.10	0.00	1.10	0.21	0.51
		0.30	..	..	..	...	...	0.21	0.51
	Av.	0.10	0.12	0.02	0.12	0.00	1.05	0.21	0.51
2	1 day at room temperature. Fresh	0.27	0.00	1.13	0.17	1.21	0.70	0.39	0.22
		0.00	0.38	1.05	0.10	0.72	0.67	0.29	0.73
		0.00	..	..	..	0.70	...	0.34	0.44
		0.64	..	..	..	0.77	...	...	...
	Av.	0.23	0.19	1.09	0.14	0.85	0.69	0.34	0.46
3	2 days at room temperature. Slightly stale	3.6	1.75	4.3	2.3	8.2	8.1	5.2	9.4
		3.2	4.2	4.2	2.2	8.2	8.7	5.4	9.2
		2.7	..	..	2.3	8.2	8.5	4.4	9.7
		..	..	..	2.4	...	...	...	...
	Av.	3.17	2.98	4.25	2.3	8.2	8.4	5.0	9.4
4	3 days at room temperature. Very stale	9.5	4.1	6.6	3.5	13.4	13.3	5.1	11.9
		6.8	2.5	6.9	3.5	12.4	13.8	5.1	11.7
		0.73	..	..	3.7	12.7	13.3	...	12.1
		3.7	..	..	3.8	...	...	...	...
		6.5	..	..	..	...	...	...	...
	Av.	5.4	3.3	6.75	3.62	12.8	13.5	5.1	11.9
5	4 days at room temperature. Putrid	12.4	6.1	8.0	3.9	15.3	14.7	6.5	13.6
		10.9	4.1	8.5	3.8	15.4	14.2	8.2	13.4
		8.3	..	..	3.9	...	...	11.1	...
		8.0	..	..	4.0	...	...	...	...
	Av.	9.9	5.1	8.25	3.9	15.4	14.5	8.6	13.5
A	3 days in ice. Fresh	0.26	0.00	0.12	0.25	0.61	1.00	0.40	0.31
		0.32	0.78	0.00	0.28	0.61	1.30	0.49	0.31
		0.75	..	..	0.07	0.42	...	0.40	...
		..	..	..	0.05	0.30	...	...	...
	Av.	0.44	0.39	0.06	0.16	0.49	1.15	0.43	0.31
B	6 days in ice. Fresh	0.88	0.50	0.60	0.35	1.30	1.30	0.45	1.14
		0.20	0.45	0.60	0.38	1.30	1.05	0.45	1.14
		0.22	..	..	..	1.30	...	...	...
		..	..	..	..	1.11	...	...	...
	Av.	0.43	0.48	0.60	0.37	1.25	1.18	0.45	1.14
C	10 days in ice. Slightly sweet odor	0.90	0.28	0.80	0.80	2.00	2.3	1.29	1.87
		0.83	3.2	0.70	0.70	2.00	2.1	1.24	1.87
		0.25	1.38	..	..	1.97	...	...	...
		0.58	..	..	..	...	...	...	...
		1.55	..	..	..	...	...	...	...
	Av.	0.82	1.62	0.75	0.75	1.99	2.2	1.27	1.87
D	13 days in ice. Slightly stale	3.3	2.4	3.6	1.28	5.8	5.2	4.2	5.9
		1.5	3.3	3.0	1.25	5.0	5.3	4.0	5.7
		1.7	..	..	..	5.0	...	3.8	6.1
		3.9	..	..	..	4.8	...	...	...
		4.3	..	..	..	5.5	...	...	...
	Av.	2.94	2.85	3.3	1.27	5.2	5.25	4.0	5.9
E	15 days in ice. Stale	5.0	4.5	3.6	2.2	5.3	6.7	6.3	7.4
		1.49	4.5	5.0	1.67	6.0	7.0	6.6	7.5
		2.0	5.8	..	..	...	...	...	7.4
		5.8	..	..	..	...	...	...	...
		4.5	..	..	..	...	...	...	...
	Av.	3.76	4.93	4.3	1.93	5.7	6.85	6.45	7.4
F	17 days in ice. Very stale to slightly putrid	7.8	8.2	7.1	2.4	12.1	10.5	6.4	9.7
		6.4	7.8	7.0	2.5	12.0	10.9	7.5	9.9
		3.3	..	..	..	...	...	6.9	...
		6.0	..	..	..	...	...	...	...
	Av.	5.87	8.0	7.05	2.45	12.1	10.7	6.9	9.8

[a] Each determination reported was started from beginning of sampling and carried out separately, so that differences in results are due to combined sampling errors, errors in separations, and titration errors.

tion, which assures a minimum decomposition of nitrogenous compounds.

Disadvantages are a lack of high precision, necessity of special equipment, and the need for great care in cleansing glassware and in making titrations. The lack of precision is due to errors in titration which occurred even when using a microburet because of the very small sample size. A fairly high dilution of the fish is required if a liquidizer is employed, owing to the relatively large volume of liquid needed to operate this equipment. In practice it was found that a concentration of fish corresponding to only about 150 grams per liter could be prepared and only 0.3 gram of fish is present when 2 ml. of this solution are used. With fresh samples having low volatile base content, such a small sample impairs precision.

Distillation. Standard Kjeldahl distillation apparatus can be used, results can be obtained in a very short time, and very high precision is readily obtained, owing to the larger fish sample used (up to 40 grams per titration).

Disadvantages include the necessity of watching the distillations to prevent foaming, and the high results obtained for the tertiary volatile base determinations where values up to ten times as high as by the other procedure were found with fresh samples. The distillation procedure seems to be most suitable for determining total volatile base but it cannot be used for determining the tertiary bases unless allowance is made for the higher results obtained, especially with fresh fish.

Aeration Procedure. Aeration is carried out at room temperature, so that a minimum of decomposition of nitrogenous constituents takes place. However, since no attempt is made to remove such nitrogenous material as is done with the other methods, even a slight decomposition of the large concentration of these interfering substances may be more serious than in the other methods. This method has the advantage of requiring a minimum of time to prepare the sample, since the centrifuging and washing steps are eliminated. Disadvantages include use of special equipment, long aeration time, and need for constant attention during aeration to prevent clogging of the aeration tubes. This method is rather cumbersome and is not recommended, although reasonably precise results are obtained.

RECOMMENDED PROCEDURE

Forty grams of fish are placed in a liquidizer (preferably with a tight-fitting lid) with 100 ml. of 60% ethanol and mixed for 5 minutes. The contents of the liquidizer are transferred quantitatively to a 250-ml. centrifuge bottle, using 60% ethanol as wash solution, centrifuged for 10 minutes, and decanted into a 250-ml. volumetric flask. The solids in the centrifuge bottles are stirred with 25 ml. of 60% ethanol, centrifuged, and decanted into the volumetric flask, and the washing and centrifuging repeated with a second 25 ml. The volume is made up with 60% ethanol.

For the tertiary volatile base determination, a 2-ml. aliquot is pipetted into the outer section of a Conway dish, 2 ml. of neutral formalin solution are added, 1.00 ml. of 0.005 N hydrochloric acid is pipetted into the center dish, and then with the lid in place except for a small opening for the pipet, 1 ml. of saturated potassium carbonate solution is added from the quick-draining pipet. The lid, previously well greased at the ground-glass section, is quickly slid into place, the contents of the dish are mixed by a slight rotary motion, and the dish is incubated for 3 hours at 40° C. Blanks are run simultaneously in exactly the same way except for substituting 2 ml. of 60% ethanol for the fish solution. After incubation the excess acid is titrated, using a microburet and a mixed indicator, either methyl red–methylene blue or methyl red–bromo cresol green. The indicator solution should first be adjusted to the neutral point by the addition of dilute acid or alkali. Determinations should be carried out in triplicate.

The same procedure can be used for determining total volatile base except for the omission of added formalin, and use of 1.00 ml. of 0.020 N acid in the center of the Conway dish. However, the following distillation procedure is preferred by the authors because of the advantages previously mentioned:

The contents of the volumetric flask (after aliquots for tertiary volatile base have been withdrawn) are transferred to a 500-ml. Kjeldahl flask and 4 glass beads and 5 grams of powdered borax are added. The flask is quickly connected to the distillation equipment and 100 ml. of distillate are collected in 50 ml. of 0.05 N hydrochloric acid. If great difficulty should be encountered with foaming, as sometimes occurs with very stale samples, a few drops of caprylic alcohol may be added, but an excess should be avoided. A blank should be run simultaneously, using 60% ethanol in place of fish solution. Excess acid in the distillate is titrated with standard alkali, using methyl red as an indicator.

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Polarographic Use of Organic Reagents

Magnesium with 8-Hydroxyquinoline

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CARRUTHERS (2) has described a polarographic method for the determination of magnesium by reduction of the 8-hydroxyquinoline in a phosphate buffer solution of the precipitated magnesium inner complex salt. The present work is concerned with the determination of magnesium by polarographic measurement of the excess 8-hydroxyquinoline without removing the precipitate and avoids the difficulties which arise from the necessity for filtration.

APPARATUS AND MATERIALS

A Leeds & Northrup Electrochemograph equipped with the cell arrangement described by Furman, Bricker, and Whitesell (3) was used for the polarographic measurements. The work was done at room temperature (23° to 26° C.).

8-Hydroxyquinoline obtained from Paragon Testing Laboratories was recrystallized three times from ethanol-water mixtures. The standard solution contained 0.5 gram per liter in 5% ethanol.

The ammonia–ammonium chloride buffer (0.25 M in total ammonia, ammonium chloride approximately 0.036 M) was made from polarographically pure ammonium chloride and C.P. ammonium hydroxide and was adjusted to pH 10 with a Leeds & Northrup pH meter.

A standard magnesium solution (100 mg. per liter) was made by dissolving the appropriate amount of magnesium metal containing 0.1% maximum impurity in the smallest amount of 0.01 M hydrochloric acid and diluting the solution to the proper volume.

Other chemicals used were of analytical reagent grade tested for magnesium. In most cases it was absent.

The capillary had the following characteristics: m = 0.6695 mg. per second, t = 4.55 seconds at 1.0 volt against the satu-

A method for the determination of magnesium by the polarographic estimation of the excess 8-hydroxyquinoline left after precipitation of the magnesium salt is reported. The only major interfering cation which is common is titanium. The solubility of magnesium 8-hydroxyquinolate in ammonia–ammonium chloride buffer of pH 10 is 1.9 × 10^{-6} mole per liter. The method can be applied to estimation of magnesium in water and plant materials.

rated calomel electrode (S.C.E.) in the buffer solution; the head of mercury was 41.9 cm. Oxygen was removed by passing purified nitrogen through the solution for 15 minutes. All polarograms were taken at $^1/_{10}$ sensitivity unless otherwise indicated.

METHOD

The method is based on the decrease in wave height of a given concentration of 8-hydroxyquinoline by the precipitation of part of it by magnesium in a buffered solution at pH 10 without removal of the precipitate.

In all this work 25-ml. volumetric flasks were used, unless some other size is indicated. Five milliliters of the standard 8-hydroxyquinoline solution and 10 ml. of the buffer were placed in the flask. For the original concentration, the flask was filled to the mark, mixed well, and the polarogram taken. For the precipitation, a given amount of the standard magnesium solution or of the unknown was added to the buffer and 8-hydroxyquinoline in the flask. The flasks were filled to the mark, mixed well, and shaken at frequent intervals for 1 to 2 hours, depending on the amount of magnesium. The polarogram was then taken. Figure 1 shows the character of the wave when the amount of magnesium changes with the same original concentration of 8-hydroxyquinoline in each case.

The polarogram consisted of two waves (Figure 2). The first wave had an $E_{1/2}$ = 1.39 volt *vs.* S.C.E. which did not shift appreciably with concentration in the range under consideration. The second wave had an $E_{1/2}$ = 1.61 volts *vs.* S.C.E. This half-wave potential shifted slightly with concentration and also considerably with slight changes in pH and so was not investigated further. The height of the wave that had $E_{1/2}$ = 1.39 volt was proportional to the concentration, as Table I shows. The decrease in wave height due to precipitation by the magnesium was proportional to the amount of magnesium present, and gave a straight-line calibration curve between 5 and 200 micrograms of magnesium in the 25-ml. volumes used.

SOLUBILITY OF MAGNESIUM 8-HYDROXYQUINOLATE

A small amount of moist magnesium 8-hydroxyquinolinate prepared by precipitation in the usual way was washed with dilute ammonia and water until the washings were free of chloride and were colorless. Ten milliliters of the buffer were diluted to 25 ml. and saturated by intermittent shaking for 12 hours in contact with some of the moist preparation. A polarogram was taken at $^1/_5$ sensitivity. The wave height corresponded to a concentration of 1.9 × 10^{-6} mole per liter of magnesium 8-hydroxyquinolinate, which is equal to 46 micrograms of magnesium per liter. The maximum error due to the solubility of the precipitate is 1 microgram of magnesium in 25 ml. This error is in general smaller because of the decrease of the solubility of the precipitate due to the excess of 8-hydroxyquinoline.

INTERFERENCES: Lundell and Hoffman (*5*) list the cations precipitated by 8-hydroxyquinoline. Any cations that are precipitated under the conditions used will interfere and must be removed or converted into complexes which are not precipitated. Electrolysis with the Melaven cell (*5*, *6*) removes the common interfering ions except aluminum, titanium, and calcium. 25 to 50 mg. of ammonium tartrate will keep in solution 150 micrograms of aluminum in the 25-ml. volumes used. No reagent was found that would keep titanium in solution; this must be removed by precipitation as the hydroxide when present. Calcium can be tolerated in amounts up to 0.5 mg. in 25 ml. with no interference.

DETERMINATION OF MAGNESIUM IN TAP WATER

The tap water available is the type obtained from limestone and dolomite beds. The iron content is low (0.6 p.p.m.) and with the size of sample taken causes no interference.

PROCEDURE: Fifty milligrams of ammonium tartrate were dissolved in 10 ml. of the buffer and 5 ml. of the standard 8-hydroxyquinoline solution were added. A 5-ml. sample of the water was added and diluted to the mark. After 2 hours' shaking and standing, the polarogram was taken. The results are shown in Table II.

DETERMINATION OF MAGNESIUM IN PLANT MATERIALS

Samples of tobacco obtained from the Connecticut Agricultural Experimental Station and of dried pine seedling cuttings obtained from Ray Dawson of the Biology Department, Princeton University, were analyzed.

PROCEDURE: Samples were dried, ashed, and dissolved in dilute sulfuric acid. The resulting solution was electrolyzed with the Melaven cell and filtered to remove the small amount of black precipitate due to the manganese. The filtrate was made up to some standard volume and an aliquot taken such that about 100 micrograms of magnesium were present. The procedure as for water determination was followed (Table III).

DISCUSSION

The strength of the standard 8-hydroxyquinoline solution is not too critical, but 0.5 gram per liter fits the procedure best.

Table I. Constancy of I_d/c with C for 8-Hydroxyquinoline

C, Moles/Liter × 10^4	I_d, Microamperes, Corrected for I_r	I_d/c
0.276	0.28	1.01
0.552	0.56	1.01
0.690	0.70	1.01
1.379	1.38	1.00
2.070	2.08	1.01
2.758	2.76	1.00
	Av.	1.01

Table II. Analysis of Tap Water

Magnesium Present, P.P.M.		
Polarographic	Colorimetric (*4*)	Gravimetric[a] (*1*)
5.2	5	5

[a] Weighed as magnesium 8-hydroxyquinolinate.

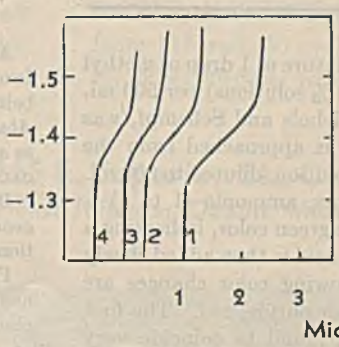

Figure 1. Effect of Magnesium on 8-Hydroxyquinoline

C = 1.379 × 10^{-4} mole per liter
Magnesium: (1) none, (2) 2 micrograms per ml., (3) 4 micrograms per ml., (4) 3.8 micrograms per ml.

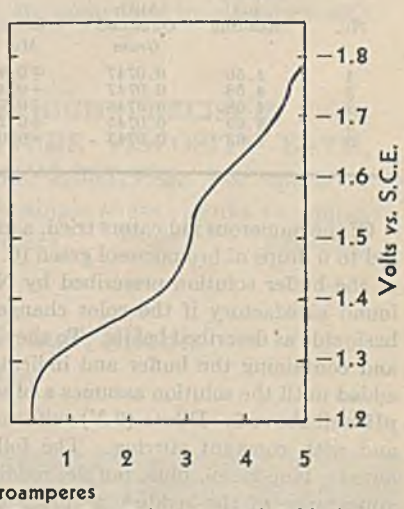

Figure 2. Polarogram of 8-Hydroxyquinoline

C = 2.758 × 10^{-4} mole per liter at pH 10

Table III. Determination of Magnesium in Plant Materials

Sample No.	Weight of Sample	MgO Found	MgO Reported[a]
	Gram	%	%
	Tobacco		
1	0.2020	1.15	1.24
1	0.1987	1.17	...
2	0.1959	1.45	1.54
2	0.2045	1.39	...
3	0.2318	1.58	1.59
4	0.1898	1.76	1.70
4	0.1956	1.70	...
	Pine Seedling Cuttings		
	0.3360	0.14	...
	0.3437	0.14	0.12[b]
	0.3769	0.12	...

[a] MgO determined by gravimetric separation and determination of Mg as pyrophosphate.
[b] Spectroscopic value. Sample CaIa (*7*).

The solution decomposes slightly with time and a blank has to be run each time it is used. The decomposition can be followed polarographically, but at the present time the decomposition products are not known.

The precipitation has to be carried out at room temperature because ammonium 8-hydroxyquinolate is too volatile even at 60° C. Ammonium tartrate has no effect on either I_r or I_d, and hence the calibration data are good for solutions containing small amounts of tartrate.

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NOTE ON ANALYTICAL PROCEDURE

Use of a Color Indicator in the Tannin Method for Determination of Beryllium and Aluminum

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THE accuracy of the tannin method, as modified by Nichols and Schempf (*2*), for the separation and determination of beryllium and aluminum depends on an accurate control of the pH during precipitation of the aluminum. The only means now available for obtaining this control is the pH meter, an instrument very difficult to obtain at the present time. Because of the strategic importance of these metals a search for a suitable color indicator seemed advisable.

Table I. Use of Color Indicator in Analysis

(Al_2O_3 taken, 0.0743 gram; BeO taken, 0.0753 gram)

No.	Beckman pH Reading	Al_2O_3 Obtained	Error	BeO Obtained	Error
		Gram	*Mg.*	*Gram*	*Mg.*
1	4.50	0.0747	+0.4	0.0750	−0.3
2	4.58	0.0747	+0.4	0.0752	−0.1
3	4.68	0.0748	+0.5	0.0748	−0.5
4	4.69	0.0745	+0.2	0.0758	+0.5
5	4.62	0.0743	±0.0	0.0750	−0.2

Of the numerous indicators tried, a mixture of 1 drop of methyl red to 6 drops of bromocresol green (0.1% solutions) per 500 ml. of the buffer solution prescribed by Nichols and Schempf, was found satisfactory if the color change is approached from the basic side as described below. To the solution, diluted to 500 ml. and containing the buffer and indicators, ammonia (1 to 1) is added until the solution assumes a blue-green color, indicating a pH well above 5. Dilute (6 *N*) sulfuric acid is then added slowly and with constant stirring. The following color changes are noted: blue-green, blue, purple, reddish purple, red. The first appearance of the reddish purple was found to coincide very closely with a pH of 4.6, the pH necessary for the complete separation of the two metals. The color change is definite and easily distinguished. If desired, however, it may be checked against the Clark and Lubs buffer mixture (*1*) having a pH of 4.6.

In order to determine the value of this mixed indicator in the analysis, a series of determinations was made on a solution mixture of aluminum and beryllium sulfates of known concentration, the results of which are shown in Table I.

Although in each analysis the Beckman pH reading was taken, no further adjustment of the acidity was made. The accuracy obtained compares favorably with that of Nichols and Schempf.

LITERATURE CITED

(1) Lange's Handbook of Chemistry, 4th ed., p. 943, Sandusky, Ohio, Handbook Publishers, 1941.
(2) Nichols, M. L., and Schempf, J. M., Ind. Eng. Chem., Anal. Ed., 11, 278 (1939).

Fifteen-Year Collective Index for Analytical Edition

A fifteen-year collective index of the Analytical Edition of Industrial and Engineering Chemistry, complete through 1943, is being prepared by Charles L. Bernier, associate editor of *Chemical Abstracts*, with the expectation of being able to issue it early in 1945, as a pamphlet of the same page size as regular issues of the Analytical Edition, if sufficient paper for printing can be obtained.

Both subject and author indexes are to be included, following in general the practice of *Chemical Abstracts*, but with certain variations suggested by the special nature of this analytical index.

Present plans contemplate furnishing copies of the index at a nominal price to any subscriber to the Analytical Edition who places his order before publication, and selling copies after that time at a somewhat higher price. At present no definite price can be quoted, as it will depend somewhat upon the number of copies printed. It will be very helpful if those wishing to receive the index will notify Walter J. Murphy, Editor, 1155 Sixteenth St., N. W., Washington 6, D. C., preferably prior to October 1.