PROCEDURES FOR THE CHEMICAL ANALYSIS OF WOOD AND WOOD PRODUCTS

(As used at the U. S. Forest Products Laboratory)

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The U. S. Forest Products Laboratory is maintained at Madison, Wis., in cooperation with the University of Wisconsin.

PREFACE

This manual is a compilation of procedures used at the U.S. Forest Products Laboratory for the chemical analysis of wood and wood products. It was assembled in order to bring together, in a single, readily available form, the analytical procedures in current use along with pertinent references and selected, representative, analytical data. Some of these procedures and many of the techniques and modifications included have not been available previously in the literature.

All of the methods required for complete chemical characterization of wood are not included, but rather, only methods which the Forest Products Laboratory has had considerable experience with and found useful, accurate, and reliable.

The manual is meant to be a working guide for the analytical chemist and technician involved in wood chemistry and is also, to a limited extent, meant to be a source book for information on the composition of wood and for supplementary information regarding the analytical techniques and apparatus used. Revisions of the manual will be issued as required by advances in technology.

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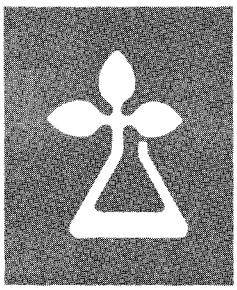

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DETERMINATION OF ACETYL AND C-METHYL GROUPS[1]

Part I--Acetyl Groups

Principle

The organic substance containing O-acetyl or N-acetyl groups is hydrolyzed in an acidic or alkaline medium. After hydrolysis, the volatile hydrolytic products are steam distilled out of the reaction mixture; the acetic acid in the distillate is determined by titration with standard sodium hydroxide solution. At the Forest Products Laboratory, the alkaline hydrolysis procedure is generally used (always for wood). The acidic hydrolysis is used for pure, stable compounds or when the sample is insoluble in alkali and can tolerate this more vigorous treatment (no components present that might be oxidized to acetic acid).

Equations

1. $CH_3CO\text{-}OR \xrightarrow{HOH(H^+ \text{ or } OH^-)} ROH + CH_3COOH \text{ (or } CH_3COO^-)$

2. $CH_3CO\text{-}N(R_1)(R_2) \xrightarrow{HOH(H^+ \text{ or } OH^-)} HN(R_1)(R_2) + CH_3COOH \text{ (or } CH_3COO^-)$

3. $CH_3COOH + NaOH \xrightarrow{\text{titration}} CH_3COONa + H_2O$

Apparatus

A Wiesenberger apparatus[1] (fig. 1) consisting of:

1. A heart-shaped, long-neck, ground-joint reaction flask, approximately 7 in. high.

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[1]Based on "The Microanalytical Determination of C-Methyl and Acetyl Groups," by E. Wiesenberger. Makrochem. 33: 51-69 (1947).

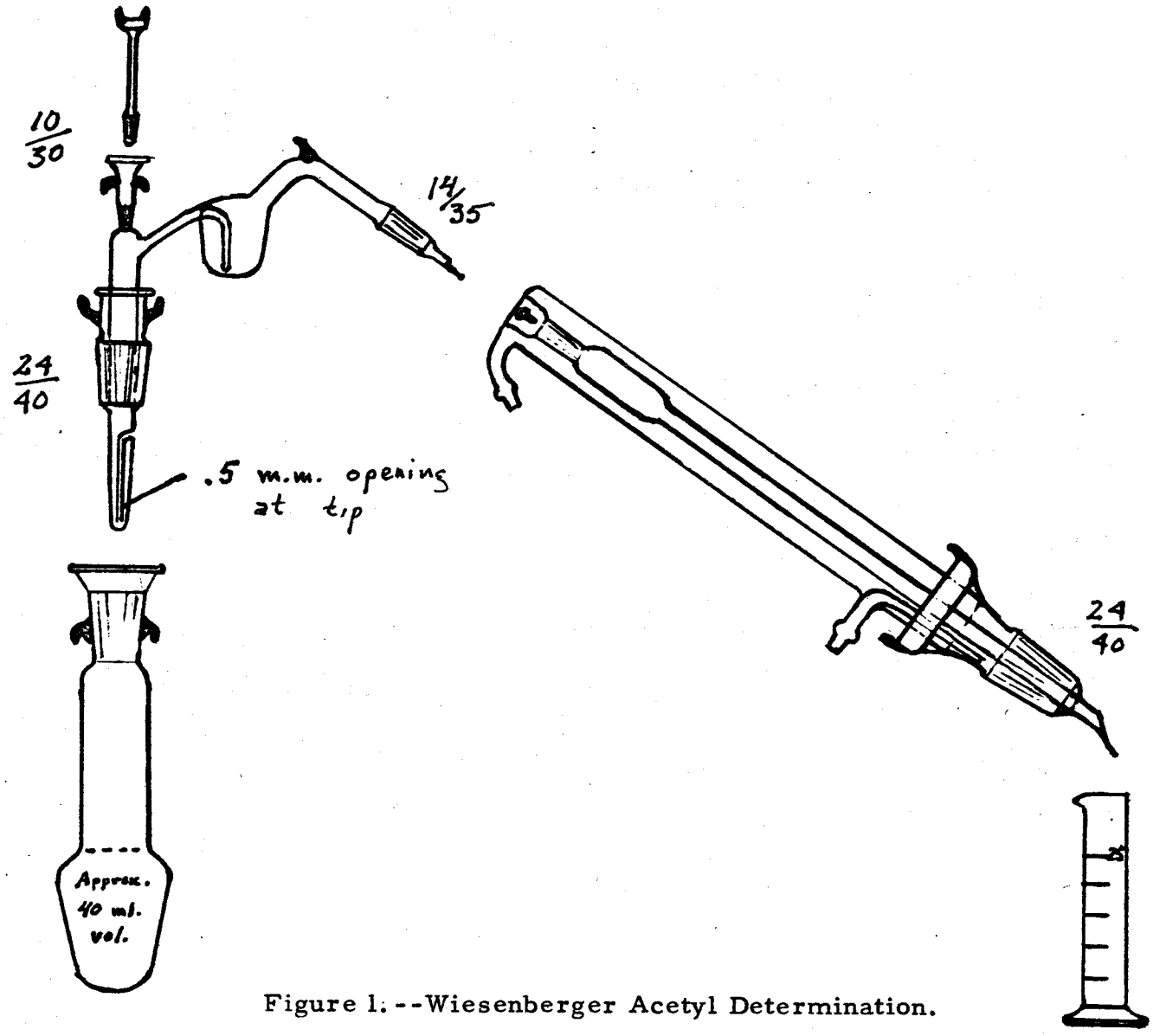


Figure 1.--Wiesenberger Acetyl Determination.

2. A ground-joint still-head, with two steam traps and removable plunger to permit addition of solutions to the reaction flask.

3. A ground-joint condenser approximately 15 in. long with a drip tip.

4. A 25-ml. graduate.

5. An Erlenmeyer flask of 125-ml. capacity.

6. Ground-joint (24:40) flasks of 500- and 750-ml. capacity, for preparation of reagents, steaming out the condenser, etc.

7. Pipets of appropriate sizes for delivery of reagents.

Reagents

Chromic Acid Method

1. 4N Chromic acid solution: 67 g. of chromic anhydride (CrO_3) is dissolved in 500 ml. of boiled distilled water. After this solution has stood for 1 day, it is filtered through a fine sintered-glass suction filter. Then it is added with cooling to 125 ml. of concentrated sulfuric acid (d. = 1.84) and is boiled an hour under reflux. The ground-joint condenser and large ground-joint flask are used for this purpose. The heated solution is allowed to cool with the condenser attached. The flask is next attached to the distillation setup and the volatile acidic constituents are distilled over. This distillation is carried out at constant volume. Before it is started, 5 ml. water is added. This quantity is distilled off; then another 5 ml. is added and distilled. This operation is repeated until 25 ml. of distillate has been collected. The total is titrated with the standard sodium hydroxide. The distillation and titration steps are repeated with consecutive 25-ml. portions of distillate until check titrations are obtained.

After the acid content of the distillate has become constant, the flask is cooled and the reagent is stored in a ground-glass stoppered bottle provided with a cap. This reagent decomposes slowly. It must be protected from dust and fumes, and should be kept in a cool, dark place.

2. Boiled distilled water. This should be freshly prepared, and should be tested for alkalinity or acidity, and be redistilled if necessary.

3. 0.01N Sodium hydroxide solution.

4. 0.01N Hydrochloric acid solution.

5. 1% Alcoholic phenolphthalein indicator.

Methyl Alcoholic 1N Sodium Hydroxide Method

1. Methyl alcoholic 1N sodium hydroxide. 40 g. sodium hydroxide is dissolved in a mixture of 500 ml. each of water and methyl alcohol. The methyl alcohol must be neutral; if necessary, it is distilled from potassium hydroxide. Store in alkali-resistant bottle for more constant blanks.

2. 1:2 Sulfuric acid.

3. Boiled distilled water (as in 2 of "Chromic Acid Method).

4. 0.01N Sodium hydroxide solution.

5. 0.01N Hydrochloric acid solution.

6. 1% Alcoholic phenolphthalein solution.

For Analysis of Substances Containing Halogen

1. Silver chromate or silver sulfate, crystals.

Procedures

Care of the Apparatus

To get accurate results, all parts of the apparatus must be thoroughly clean. All pieces are cleaned with chromic-sulfuric acid cleaning

solution and then rinsed repeatedly with tap water, special attention being paid to all ground surfaces; then they are rinsed several times with distilled water. Distillation flasks are dried in the oven; the other parts are merely allowed to drain. The distilling condenser is steamed out thoroughly at the beginning of each day's work. If at any time the inner surface of any piece becomes filmed or noticeably contaminated, it must be cleaned as described.

Chromic Acid Procedure

A sample containing 0.02 mg. or more of acetyl (usually 3- to 5-mg. sample) is weighed in a long-handled weighing tube (or a short capillary if the sample is a liquid) and deposited at the bottom of the reaction flask. (See note 1). Two or three platinum tetrahedra are added. Then 2 ml. of the 4N chromic acid reagent is added from a pipet, washing down the sides of the flask.

The flask is attached to one of the ground-joint condensers which has been clamped in a vertical position. The ground joint is sealed with a drop of distilled water, and held in place by the usual spring-and-hook device.

The flask is immersed in a vessel of 85% phosphoric acid until the two liquid levels coincide. Heating with a Bunsen burner is begun. The sample is refluxed at 165°-170° C. for 1 to 1-1/2 hr., or longer if necessary.

When the refluxing period is ended, the flask is cooled and its exterior is washed thoroughly with tap water to remove the bath liquid.

The condenser is washed down with 4 ml. distilled water. The flask is then disengaged from the condenser and the ground surfaces are washed with 2 ml. of water; then with 1 ml.

During the reflux period, the distillation condenser has been set up and connected with the still-head and one of the larger ground-joint flasks previously mentioned. This apparatus has been steamed out for about 20-30 min.

To distill the acetic acid, the larger boiling flask is replaced by the flask containing the refluxed solution. The flask is heated with a microburner, the flame being 2-3 cm. from the bottom of the flask.

The funnel at the top of the distillation head is filled with 2 ml. of distilled water. A clean 25-ml. graduate is placed under the exit end of the condenser. The flask is heated until the vapors have begun to condense in the second steam trap. Then the flame is removed and the liquid allowed to be sucked back into the flask. This operation is repeated two or three times, until the parts of the apparatus are heated.

In all, 7 ml. of water is used in washing the reflux condenser, and this amount, plus the 2 ml. of reagent originally used, adds up to a total volume of 9 ml. in the flask. Of this, 6 ml. is distilled into the graduated cylinder. Then the flame is removed, the condensate allowed to be sucked back, and the 2 ml. of water in the funnel is fed into the flask by a cautious lifting of the plunger. The funnel is then filled with another 2 ml. of water (a little liquid should always be in the funnel during distillation).

The distillation is then carried on in 2-ml. portions, until 25 ml. has been collected. This is poured into a clean 125-ml. titration flask. Then 5 ml. is distilled into the graduated cylinder and the walls are rinsed by tipping the cylinder. This 5-ml. portion is added to the titration flask. The time for a distillation is approximately 45 min.

Phenolphthalein indicator is added to the titration flask in whole drops, six drops being used; and it is advisable to use the same dropper during a series of determinations.

The acetic acid solution is now titrated with 0.01N sodium hydroxide to a distinct pink color. Then 0.4 ml. of 0.01N hydrochloric acid is run in. The solution is boiled gently for exactly 20 sec. and back-titrated immediately with the standard alkali to the first faint pink (compare with a flask of distilled water) that is permanent for 30 sec. (It is dangerous to boil too vigorously (1,2).)

A blank determination without the sample is carried out in exactly the same manner.

Methyl Alcoholic 1N Sodium Hydroxide Procedure

This procedure differs from the previous one above in the following details:

1. The sample (25-30 mg. of wood) and reagent are heated at boiling (130°-140° C.) for 30 min. Insoluble samples are reacted in a glass "bucket." (See note 1).

2. The methyl alcohol must be removed. At the beginning of the distillation (after the apparatus has been preheated), 4 ml. is distilled into a graduated cylinder kept for this purpose. The tip of the condenser is washed thoroughly after the alcohol has been distilled. (See note 1).

3. The liquid in the flask is then acidified with 1 ml. of 1:2 sulfuric acid that is added through the funnel. Then the funnel is washed with 3 ml. of water, so that the liquid volume in the flask is 9 ml. The distillation is then carried out according to the directions given under the chromic acid procedure.

Procedure for Compounds Containing Halogen

In the chromic acid procedure, 15 to 20 mg. of silver chromate or silver sulfate is placed in the reaction flask to fix the halogen as the silver halide.

In the methyl alcoholic-sodium hydroxide procedure, silver sulfate is used. The same amount as above is placed in the funnel and, after removal of the alcohol, is washed into the flask by the sulfuric acid and water.

Calculations

$$\% \ CH_3CO^- = \frac{(\text{net titration}) \times \text{norm. factor} \times 43.03 \times 100}{\text{mg. sample}}$$

Example

A 3.108-mg. sample of acetanilide (CH_3CO^- = 31.84%) gave a total titration of 2.956 ml. of 0.00968N sodium hydroxide. The blank value was 0.118 ml. of the standard alkali. Two-tenths of a milliliter of approximately 0.02 hydrochloric acid was used to acidify the sample. This volume, by titration, was equivalent to 0.455 ml. of the standard alkali.

$$\text{Then, } \% \ CH_3CO^- = \frac{[2.956 - (0.118 + 0.455] \times 0.00968 \times 43.03 \times 100}{3.108}$$

$$= \frac{2.383 \times 0.00968 \times 43.03 \times 100}{3.108} = 31.94\%$$

Note 1:

For wood or pulp samples, or for natural products and insoluble samples in general, some container that will allow liquid to drain is necessary (See "bucket" in figure 2). This bucket must be removed with the deacetylated wood particles before distillation, or the opening in the steam trap could become clogged (explosion!). Removing the wood also prevents it from being exposed to the more concentrated acid during distillation.

After the hydrolysis (see paragraphs 2, 3, and 4 under "Chromic Acid" procedure), the bucket is lifted to the top of the flask with a wire hook and secured by a wire passed through the holes. Ten ml. of boiled, cooled distilled water is used for washing the bucket and contents, small portions at a time, allowing each portion to drain back into the flask before the next is added.

The flask is then connected to the distilling head and condenser and step 2 ("Methyl Alcoholic 1 N Sodium Hydroxide" procedure) carried out. This should be done cautiously, as the alkaline extracts of some woods foam badly, and may go through both steam traps and enter the condenser, thus spoiling the analysis.

After the alcohol has been distilled off, the liquid in the flask is acidified as in step 3. However, since 10 ml. instead of 7 was used for washing, the first distillation should be 9 ml. instead of the 6 noted under the "Chromic Acid" procedure. The remainder of the distillation is carried out as described.

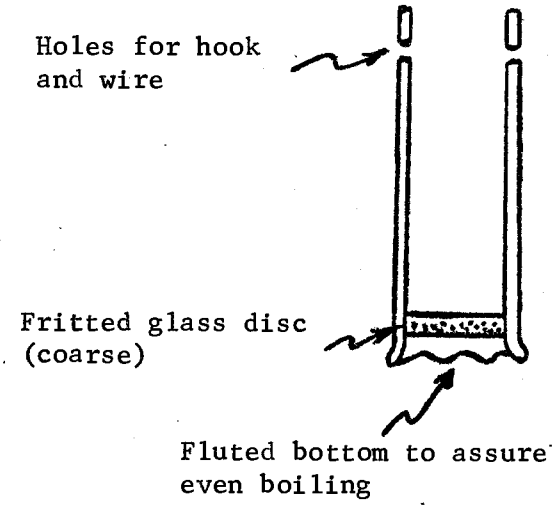


Figure 2.--Pyrex glass bucket for digesting wood samples (actual size).

Note 2: Listed below are some published values for the acetyl content of various woods.

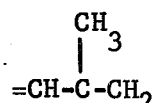
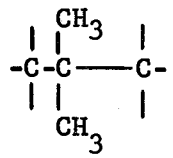
	% $CH_3\overset{O}{\overset{\|}{C}}-$	Reference
Hardwoods:		
Aspen	3.4	(3)
Balsa	4.2	(6)
Basswood	4.2	(6)
Beech	3.9	(3)
Yellow birch	3.3	(4)
White birch	3.1	(6)
Paper birch	4.4	(3)
American elm	3.9	(4)
Shellbark hickory	1.8	(6)
Red maple	3.8	(3)
Sugar maple	2.9	(4)
	3.2	(6)
Mesquite	1.5	(6)
Overcup oak	2.8	(5)
Southern red oak	3.3	(5)
Tanoak	3.8	(6)
Softwoods:		
Eastern white-cedar	1.1	(3)
Incense-cedar	.7	(6)
Western redcedar	.5	(5)
Alaska-cedar	1.1	(6)
Douglas-fir	.6	(5)
	.8	(6)
Balsam fir	1.5	(3)
Eastern hemlock	1.7	(3)
Western hemlock	1.2	(5)
Western larch	.5	(6)
Jack pine	1.2	(3)
Loblolly pine	1.1	(5)
Longleaf pine	.6	(6)
Western white pine	.7	(6)
Redwood	.8	(6)
White spruce	1.3	(3)
	1.1	(6)
Tamarack	1.5	(3)

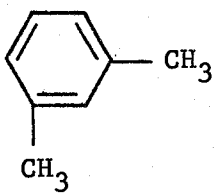
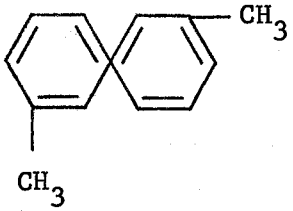
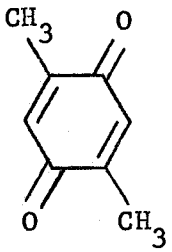
Part II--C-Methyl Groups

Principle

Substances that contain methyl groups bound to carbon often yield determinable quantities of acetic acid upon oxidative degradation by means of acid bichromate. The acetic acid thus formed is then distilled out of the reaction mixture just as in the ordinary procedure for acetyl by acid-hydrolysis, and is determined by titration.

Although it is usually easy to obtain quantitative results in acid hydrolysis of structures containing acetyl groups or in oxidation of compounds containing ethoxyl groups, acetic acid recovery figures in structures possessing methyl groups attached to carbon often fall short of theoretical values; i.e., the amount of acetic acid formed from a given carbon-methyl grouping by oxidative degradation depends upon the particular type of grouping involved and may be considerably less than expected. For instance, Kuhn and L'Orsa (7) reported the following percentages of theory that can be expected for various carbon-to-methyl groupings.

		% of the Acetyl Percentage Calculated on the Basis of Structure
CH_3-CH_2-OH	=	100
$CH_3-CO-CH_2-R$	=	85
$CH_3-CH=CH-R$	=	85
	=	90
	=	40

	Acetyl and C-Methyl % of the Acetyl Percentage Calculated on the Basis of Structure
	= 12
	= 30
	= 80

Nevertheless, the method of oxidative degradation, when used in conjunction with other analytical information, is useful in determining molecular structure (8). Partial recovery figures of an empirical nature like those listed above are usually quite reproducible under the same conditions of experiment.

Procedure

The oxidation procedure for nonvolatile substances is exactly the same as the chromic acid hydrolysis for acetyl groups. For volatile or subliming

materials, the sealed tube method of digestion may be substituted (8). Here the sample, with 2 ml. of the 4N chromic acid, is sealed in an ordinary thick-walled pyrex micro Carius tube that is heated at 120°-140° C. in a Carius furnace. (The heating time varies with the type of compound. For instance, various hydrocarbons oxidized 64 hr. at 140° C. still give low results (9).)

When the heating is finished, the tube is cooled to room temperature; then it is chilled thoroughly in an ice bath or refrigerator or before opening in the usual fashion. The contents are then rinsed into the heart-shaped distilling flask with 7 ml. of distilled water, and the subsequent distillation is carried out according to directions for the chromic acid distillation in the determination of acetyl.

Calculation

$$\% \ CH_3 \text{ to C-} = \frac{\text{(net titration)} \times \text{N.F.} \times 15.03 \times 100}{\text{mg. sample}}$$

(Same calculation as previously outlined for acetyl, but with the molecular weight of $-CH_3$ (15.03) substituted for that of CH_3-CO- (43.03).)

References

(1) Hurka, W.
1942. Iodometric determination of small quantities of acetic acid. Mikrochemie ver Mikrochim. Acta 30: 228.

(2) _____
1943. Comparative titrimetric determination of acetic acid. Mikrochemie ver Mikrochim. Acta 31: 5.

(3) Timell, T. E.
1957. Carbohydrate composition of ten North American species of wood. Tappi 40: 568.

(4) Timell, T. E.
1958. Some properties of four Canadian species of wood. Pulp Paper Mag. Canada 59(8): 139.

(5) Wise, L. E., and Ratliff, E. K.
1947. Quantitative isolation of hemicelluloses and the summative analysis of wood. Ind. & Eng. Chem., Analyt. Chem. 19: 459.

(6) U.S. Forest Products Laboratory.
1952. Tech. Note No. 235, Forest Serv., USDA.

(7) Kuhn, R., and L'Orsa, F.
1931. Analysis of organic compounds by chromic acid. Z. Angew. Chem. 44: 847.

(8) _____, and Roth, H.
1933. Microdetermination of acetyl, benzoyl, and C-methyl groups. Ber. 66B: 1274.

(9) Kirsten, W., and Stenhagen, E.
1952. The determination of C-methyl groups in aliphatic long-chain compounds. Acta. Chem. Scand. 6: 682.

ALCOHOL-BENZENE SOLUBILITY OF WOOD AND PULP[1]

The alcohol-benzene-soluble content of wood is a measure of the waxes, fats, resins, and certain ether-insoluble components, including possibly portions of some of the so-called wood gums and other water-soluble components.

Apparatus

1. Filtering crucibles, Alundum, porosity RA 98., or glass extraction thimbles with coarse, or extra coarse, porosity fritted disks.

2. Extraction apparatus, with ground glass joints. A Soxhlet apparatus is preferable, consisting of (a) Soxhlet extraction flask of 250-ml. capacity; (b) Soxhlet extraction tube, 50 mm. ID , capacity to top of siphon about 100 ml.; height of siphon tube about 55 mm. (This tube is specified because siphoning is more rapid than extractors with higher siphon tubes.) (c) condenser.

Reagents

Alcohol-benzene mixture. Mix together 1 volume of approximately 95 pct. ethyl alcohol and 2 volumes of benzene.

Test Sample

Prepare a representative sample of 40/60 mesh air-dried wood or pulp and determine its moisture content. About 4 g. is required for a determination in duplicate.

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[1] Based on: Bray, M. W., "Chemical analysis of pulps and pulpwood." Paper Trade J. 87(25): 59 (1928).

Procedure

Accurately weigh 2 ± 0.1 g. of the air-dry sample into a tared filtering crucible. Place the crucible and specimen in position in the Soxhlet apparatus and place a small cone of fine mesh wire screen in the top of the crucible to prevent any loss of the specimen. Extract with 200 ml. of alcohol-benzene mixture for 4 to 6 hr., keeping the liquid boiling briskly.

After extraction is complete, evaporate the solvent from the sample, dry in an oven for 1 hr. at 105° ± 3° C., cool in a desiccator and weigh. Continue the drying until there is no further loss in weight. Calculate the weight loss of the sample as the percentage of alcohol-benzene soluble material in the moisture-free sample.

Similar Methods

American Society for Testing and Materials	D 1107	(1956)
Technical Association of the Pulp and Paper Industry	T 6	(1959)

DETERMINATION OF ALKOXYL GROUPS[1]

Introduction

The method of determining alkoxyl groups evolved from the work of Zeisel (1) who found that the methyl group of an O-methyl compound could be measured quantitatively. The alkyl iodide, formed upon hydrolysis of the ether linkage with hydriodic acid, is distilled with carbon dioxide into a receiver containing bromine. The bromine oxidizes the iodide to iodate, which in turn is converted to iodine and titrated with thiosulfate. The accuracy of the procedure is about 0.3% (absolute basis).

Oxime ethers, alcohols, and esters, as well as ethers, react with hydriodic acid. In the procedure described here, methoxyl and ethoxyl groups would be measured quantitatively and at least some propoxyl or butoxyl groups would be measured, if present. The higher alkoxyls are done in exactly the same manner as the methoxyl but, due to the lower volatility of their iodides, temperatures and reaction or distillation times would have to be increased. There are also methods for "mixed alkoxyls" (2).

Sulfur, halogens, and nitrogen do not interfere with the results of this procedure; however, the method can be modified to measure alkyls attached to sulfur (3) or nitrogen (4).

Reactions

$$ROCH_3 + H_2O \longrightarrow ROH + CH_3OH$$

[1] Based on "Determination of Alkoxyl Groups" by Adalbert Elek in "Organic Analysis," Volume I, pages 67-126. Interscience Publishers, Inc., New York, N.Y. 1953. Additional references are listed at the end of this section.

$$CH_3OH + HI \longrightarrow CH_3I + HOH$$

$$CH_3I + Br_2 \longrightarrow CH_3Br + IBr$$

$$IBr + 3H_2O + 2Br_2 \longrightarrow HIO_3 + 5HBr$$

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$I_2 + 2S_2O_3 = \longrightarrow 2I^- + S_4O_6$$

Applications

The alkoxyl group determination is of special value in elucidating the structure and molecular weight of organic compounds. It is also used for the detection of alcohol in human organs. The quantitative determination of minute amounts of ethyl alcohol (in steam distillates of human brains) has been reported by Gettler, Niederl, and Benedetti-Pichler (5).

Reagents and Solvents

1. Preparation and Purification of Hydriodic Acid

The preparation of the important reagent hydriodic acid is described by Clark (6):

254 g. of iodine and 185 g. of water are heated to 50° C. in a 500-ml. flask, to which a ground-glass condenser is attached. Sixty-six g. of 50% sulfate-free hypophosphorous acid is added in small portions until the iodine is completely reduced. The mixture is heated for 3 hr. more while a current of carbon dioxide or nitrogen is passed through the liquid. The constant-boiling solution is distilled, discarding the first 10-15 ml. of distillate. The hydriodic acid, sp. gr. 1.69-1.70, is stored in small, dark, glass-stoppered bottles with the addition of 0.1 ml. of 50% hypophosphorous acid.

Friedrich (7) gives the following description for the preparation of hydriodic acid:

One-hundred g. of iodine and 100 ml. of water are added to a flask having a ground-in glass cooler. A suspension of 5 g. of red phosphorus in 100 ml. of water is poured into the flask in small portions and with constant stirring. Then the cooler is attached to the flask. Nitrogen gas is now bubbled through the mixture by means of an inserted glass tube which reaches the bottom of the flask. The nitrogen gas is purified through a washer containing sulfuric acid. The contents of the flask are now heated while nitrogen is bubbling through the mixture. The heating is continued until the solution becomes colorless. The heating is then discontinued and the liquid is cooled to room temperature, during which time the water is running in the cooler.

To remove the red phosphorus, the hydriodic acid is quickly filtered (to avoid prolonged contact with the air) through a clean and dry glass-sintered filter and stored in small, dark, glass-stoppered bottles sealed with melted paraffin.

The quality of the hydriodic acid (sp. gr. 1.7) is important. The general contention that the hydriodic acid should be colorless has not been substantiated by some of the investigators, for example, Elek and Steyermark (8,9).

A simple method for obtaining a good quality of hydriodic acid is described by Steyermark (9): The hydriodic acid generally purchased is unsuitable for use without purification, whether reagent grades or those designated as special for micro determination of methoxyl are used. They often contain hydrogen sulfide, phosphine, and possibly alkyl iodides formed from reaction of the acid vapors on the material used to cover the bottle stoppers. These contaminants result in large blanks.

The reagent grade hydriodic acid, sp. gr. 1.7, is refluxed in the usual all-glass apparatus with an air condenser for about 2 hr. while a stream of carbon dioxide or nitrogen is passing through it. The color of the acid is of no importance. Even the rather dark-colored acid gives no blank, according to Steyermark. When stored in brown, glass-stoppered bottles at laboratory temperature, the acid continues to give good results for a number of weeks. Blanks should be run at frequent intervals. Though it is repeatedly stated in the literature that the acid used should be colorless and fresh (6,10,11, 12,13,14,15), any dissolved iodine present in the hydriodic acid should be advantageous, since Elek (8) showed that the addition of a few crystals of iodine to the reaction mixture converts organically bound sulfur to the elementary form, thus preventing its interference.

The acid vapors should never be allowed to come in contact with organic vapors or material which would cause recontamination. When heating is stopped, the flow of gas likewise is discontinued as fuming acid is formed by passing the gas through at room temperature. A blank determination should then be carried out to test the quality of the acid. If ever a trace of silver iodide precipitate is obtained, the refluxing of the acid with carbon dioxide or nitrogen should be repeated.

If the determination is done by the volumetric method using 2 ml. of hydriodic acid, the blank should not use more than 0.3 ml. of 0.01N sodium thiosulfate, otherwise the acid must be repurified until the blank is satisfactory. The acid is stored in small, dark, glass-stoppered bottles in the usual way. Blanks should be run at frequent intervals, especially when only occasional analyses are made.

Pure, constant-boiling (b.p. 126°-127° C.) 57% hydriodic acid can also be prepared as described by Samsel and McHard (16):

Place 70 g. of C.P. red phosphorus in a 2-liter, three-necked, round-bottomed flask fitted with a reflux condenser, dropping funnel, and thermometer. The flask is half-filled with distilled water and heated on a low flame. A solution of 800 g. of iodine in 800 ml. of hydriodic acid is poured slowly through the dropping funnel into the flask and heated until the mixture boils (127° C.). The boiling is continued for 0.5 hr. After a while, when the red phosphorus has settled, the acid is decanted into a suitable distillation flask equipped with an efficient fractionation column. An all-glass apparatus equipped with ground-glass joints is used. A stream of carbon dioxide is passed through the apparatus during distillation. The inlet tube should dip beneath the surface of the liquid. The distillation is continued and the portion of the distillate having a specific gravity between 1.69 and 1.70 is used and stored in the usual way in small, dark, glass-stoppered bottles previously swept out with carbon dioxide or nitrogen.

The hydriodic acid which has been thus purified is ready for use and is kept by some in the laboratory (9) and by others (8) in a cool, dark place.

The effect of the hydriodic acid concentration on the alkoxyl values is shown in table 1. The data in table 1 show that small variations in the concentration of hydriodic acid do not affect the reproducibility of values, especially with compounds which are easily dissolved by the hydriodic acid, but give low values for some compounds (ethylcellulose) if the specific gravity of the hydriodic acid used is below 1.69 at 25° C.

Table 1.--Effect of hydriodic acid concentration on alkoxyl content (16)

Hydriodic acid		Ethyl-cellulose[1]	Methyl-cellulose[2]	p-Ethoxybenzoic acid[3]	
Specific gravity at 25° C.	Concentration, percent by weight			Found	Calculated
1.694	56.7	48.6 48.4	31.0 31.1	27.0 27.1	27.1
1.672	55.8	47.1 47.4	31.0 30.9	26.9 26.9	27.1
1.665	55.5	47.7 47.5	31.1 30.9	26.9 27.0	27.1

[1]Ethocel, lot 4521.

[2]Methocel, lot M-1097.

[3]Eastman C.P., m.p. 196°-198° C.

Using the same hydriodic acid for several determinations is not the safest procedure to follow, especially when unknown samples are analyzed or when the sample is resistant to hydrolysis, in which case a longer period of hydrolysis is necessary. When the hydriodic acid becomes permanently darkened, fresh reagent should be taken. (When sample is added to the hydriodic acid reagent, there is usually a darkening, but this will clear during distillation unless the reagent is exhausted or contaminated.)

2. Use of Cadmium Sulfate

A 5% aqueous solution of reagent quality cadmium sulfate is prepared by dissolving 5 g. of cadmium sulfate in 100 ml. of water. The solution is conveniently kept in a dropping bottle.

The cadmium sulfate solution is used in the washer (scrubber) to retain any hydrogen sulfide which the hydriodic acid contained originally, or which might be formed by the reducing action of the hydriodic acid on a sulfur-containing sample.

3. Use of Sodium Thiosulfate

The use of sodium thiosulfate in the washer serves to retain any iodine, hydriodic acid, and hydrogen sulfide which may be carried over during distillation.

A 5% solution of reagent quality sodium thiosulfate is prepared in the same manner as the 5% cadmium sulfate and is kept in a dropping bottle.

4. Combined Use of Cadmium Sulfate and Sodium Thiosulfate

These two chemicals have been used in the washer solution, in equal proportions, in a quantity sufficient to fill about half the volume of the washer. The inlet tube leading into the washer should always end much below the surface of the liquid to effect sufficient scrubbing of the gases passing through. White (17) has found that the use of sodium thiosulfate alone in the washer leads to low results, as the alkyl iodide partially dissolves and reacts with the sodium thiosulfate. Red phosphorus suspension and cadmium sulfate solution form a very satisfactory combination for the "scrubber" solution. Some workers use cadmium sulfate and sodium thiosulfate, sodium thiosulfate alone, or red phosphorus suspension alone.

5. Use and Purification of Red Phosphorus

An aqueous suspension of red phosphorus in the washer serves to retain any iodine and hydriodic acid which may be carried over with the carbon dioxide stream during distillation.

The red phosphorus is purified by being heated with strongly ammoniacal water in a porcelain dish on a boiling water bath for 1 hr. It is then filtered and washed thoroughly with water until the supernatant liquid is no longer acid to litmus. It is stored in a dropping bottle under water.

The phosphorus suspension develops acidity on standing. Therefore, before each use, the supernatant liquid should be decanted and the phosphorus suspended in fresh water.

6. Preparation of Buffered Glacial Acetic Acid Solution

Ten g. of reagent grade sodium acetate is dissolved in reagent quality glacial acetic acid in a glass-stoppered bottle. The mixture is shaken (preferably in a shaking machine) for a few minutes to complete solution of the sodium acetate. After the sodium acetate is completely dissolved, the reagent is ready for use. It can be kept at room temperature.

7. Bromine

To effect complete oxidation of the alkyl iodide which distills into the receiver, bromine is added to the sodium acetate-glacial acetic acid solution in the receiver. Bromine should always be in excess. Kinsman and Noller (18) recommend the addition of 0.1 ml. of bromine for an alkoxyl content equivalent to 20 ml. of 0.1N sodium thiosulfate. Elek (8) adds 6 to 8 drops of bromine, which has always been found sufficient. This amount of bromine corresponds

with about 0.2 ml. used by Hoffman and Wolfrom (13), who found that this amount of bromine is adequate for methoxyl or ethoxyl contents equivalent to 40 ml. of 0.1N sodium thiosulfate.

It has been experienced by Houghton and Wilson (19) that bromine vapors in the laboratory atmosphere affect the accuracy of the titration of the iodine. This can be remedied by placing a piece of cotton or glass wool moistened with a few drops of formic acid in the exit of the receiver. Hoffman and Wolfrom (13) use a modified calcium chloride tube filled with cotton which retains and reduces the amount of bromine that might escape into the atmosphere.

8. Preparation of Standard Sodium Thiosulfate

Sodium thiosulfate solution, 0.1N or 0.01N is prepared by weighing out and dissolving 24 g. of sodium thiosulfate thiosulfate for the 0.1N or 2.4 g. for the 0.01N for 1 liter of solution. The 0.02N or 0.01N solution is best prepared from the 0.1N solution which has been aged for some time.

The sodium thiosulfate solution is not too stable and changes titer quite quickly without a preservative. If, however, the solution is aged (for 1 to 2 wk.), it can be well preserved with 1% of amyl alcohol, and even better by adding to the solution 1% of chloroform; the titer then remains constant for a long time. The addition of sodium carbonate as a preservative has been recommended by Slotta and Haberland (20).

General Comments

The method and apparatus to be chosen depend largely on: The size of sample available, the type of compound (if known), the number of alkyl groups

in the molecule, whether the alkyl is linked to oxygen, nitrogen, or sulfur, and the number of carbon atoms in the alkyl group.

The distinction between macro, semimicro, and micro methods is largely based on the weight of sample used for the determination, and the size of the apparatus. With great advances and refinements in apparatus, technique, and balances, the macro methods have become obsolete and are replaced by the more elegant and reliable semimicro and micro methods.

Dissolution of the sample is the safest procedure to follow, especially when dealing with unknown compounds.

Lubrication of the ground-glass joint on the reaction flask is accomplished with a little SISCO 300 (Swedish Iron and Steel Company), molten phenol, a drop or two of hydriodic acid.

It is an essential part of a successful analysis to ensure smooth boiling of the reaction mixture. The following means have been used:

(a) Pregl (15) and Friedrich (11) used a small cup made of tinfoil, weighing 10-20 mg., to prevent bumping by the formation of tin iodide.

(b) Elek (8) used a few Alundum grains (size 14-16) to ensure smooth boiling, with satisfactory results.

(c) Furter (12) recommends platinum tetrahedra.

(d) Steyermark (9) used the platinum boat containing the weighed sample to reduce bumping; or a few platinum tetrahedra may be inserted to prevent bumping.

(e) Glass beads, small glass rods, or boiling rods are also useful.

Hoffman and Wolfrom (13) did not use boiling chips, since they "tend to increase the size and irregularity of the bubbles formed in the receiver.

Bumping was not a problem if reasonable care was exercised in turning on the stream of carbon dioxide gradually."

Peniston and Hibbert (21) add Nichrome wire about 1 cm. in length, bent into a loop, to the reaction mixture to prevent bumping.

Neuman (22) reports that highly methylated carbohydrates often give values which are 1 to 2% low when the reaction flask is heated over a free flame and the substance in the reaction flask becomes more or less "caked" (resinified). This caked portion of the substance does not react with the hydriodic acid. The variation in the degree of caking is responsible for the nonreproducibility of values obtained with the same substance. Hence, he uses an oil bath with gradual elevation of the temperature to circumvent caking. The complete solution of the compound before addition of the hydriodic acid is the safest way to prevent caking. If the compound is first completely dissolved, then "caking" does not occur whether the heating is done over a free flame, oil bath, or metal bath or electrically.

Use of the initial milliliters of hydriodic acid for several determinations is feasible when very low alkoxyl values are expected (paper and pulp materials) and just a small fraction of the hydriodic acid is used up for each determination.

The use of fresh hydriodic acid for each determination is highly recommended when the expected alkoxyl values are several percent or even up to 70%; when dealing with unknown compounds, and the compound is resistant to hydrolysis, a longer period of hydrolysis is necessary.

A blank determination is always in order with all the chemicals used in the reaction flask.

Positive blank determinations can be almost eliminated by refluxing the reagents for about 60 min.

Bailey (23) reports that in the volumetric procedure a component of the blank is due to the instability of the potassium iodide solution. He finds that "even standing for a short time the solution becomes slightly yellow due to the formation of free iodine." This can easily be eliminated by adding the required amount of solid potassium iodide to the flask in which the final titration is performed, dissolving it in a few milliliters of distilled water just before the contents of the receiver are emptied into it.

Precaution

Lower alcohols react in the same way as the ethers and esters; therefore, they must be excluded from glassware and samples. Glassware should not be rinsed in alcohol and samples must have had all residual alcohol removed (especially checking those recrystallized from alcohol).

Apparatus

The apparatus of Clark (6b) shown in fig. 1, which employs essentially the method of Vieböck and Schwappach (24), is used for the volumetric determination of alkoxyls at the Forest Products Laboratory. It is an all-glass apparatus consisting of a reaction flask A, scrubber B, and two receivers C and D. The joint on the reaction flask (the other glass joint is not sealed) is sealed with SISCO 300 stopcock grease. Carbon dioxide is used as it comes from the cylinder with no extra purification being necessary.

Procedure

Enough of a slurry of red phosphorus in 5% cadmium sulfate is added to the scrubber to cover the tip of the entrance tube. Receivers C and D are

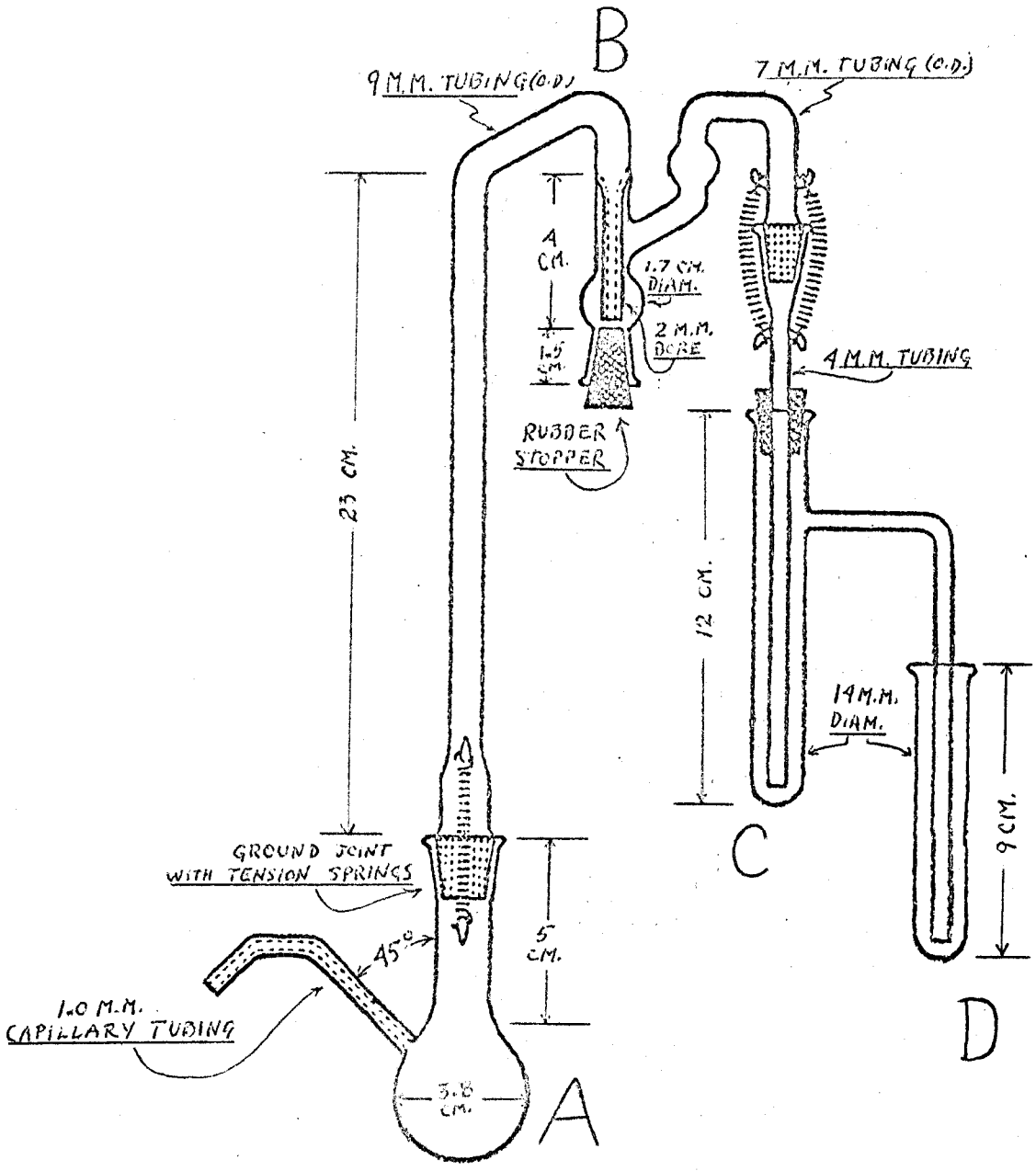

FIG. 1
Methoxyl Apparatus

M 132 205

each half-filled with a 10% solution of potassium acetate in acetic acid, and then a few drops of bromine (enough to make the receiver solution a strong red) are added to each receiver.

Into the reaction flask are placed some platinum boiling chips, about a gram of phenol, and 10 ml. of hydriodic acid. At this point, the apparatus is ready for a sample run. However, first the contents of the flask are gently boiled with a microburner flame while a slow stream of carbon dioxide passes through the sidearm of the flask. This pretreatment should be continued for about 1 hr.; its purpose is to lower the test blank value and to ensure thorough mixture of the hydriodic acid and phenol.

After the pretreatment, a blank is run according to the procedure given below for samples. A reaction time of 1 hr. is used. The amount of thiosulfate used for the titration of the blank is subtracted from the titration of the sample.

To determine the methoxyl content of an unknown sample, from 2 to 5 mg. (0.02 or more milligrams of methoxyl) of the sample are weighed in a porcelain boat and placed into the reaction flask with the phenol and hydriodic acid. The flow of carbon dioxide into the sidearm is adjusted to give a slow stream which will not allow any pulling back or vigorous splashing. The reaction flask is then heated for 1 hr. under these conditions, after which the contents of the two receivers are transferred to a 250-ml. Erlenmeyer flask into which has been placed 5 ml. of 25% aqueous sodium acetate solution. The volume is brought up to about 100 ml. with water, and then the excess bromine is destroyed by adding 6 or 7 drops of 90% formic acid. The residual bromine vapors are pulled off by suction from an aspirator. Approximately 1 g. of potassium

iodide and 1.5 ml. of 10% sulfuric acid are then added, after which the liberated iodine is titrated with 0.01N sodium thiosulfate to a starch end point. A 10-ml. microburette is used for the titration.

$$\%OCH_3 = \frac{(\text{ml. thio} - \text{blank})\ \text{N. thio} \times 0.031 \times 100}{6 \times (\text{weight of sample})}$$

Note: Listed below are some published values[2] for the methoxyl content of various woods.

	% Methoxyl
Hardwoods:	
Balsa	5.68
Basswood	6.00
Yellow birch	6.07
Shellbark hickory	5.63
Sugar maple	7.25
Mesquite	5.55
Tanoak	5.74
Softwoods:	
Incense-cedar	6.24
Alaska-cedar	5.25
Douglas-fir	4.95
Western larch	5.03
Longleaf pine	5.05
Western white pine	4.56
Redwood	5.21
White spruce	5.30

References

(1) Zeisel, S.
1885. A procedure for the quantitative determination of methoxyl. Monatsh. 6: 989.

(2) Steyermark, A.
1951. Quantitative organic microanalysis. P. 241. The Blakiston Company, New York, N.Y.

[2] U.S. Forest Products Laboratory. Technical Note No. 235. Forest Service, USDA. 1952

(3) Mitchell, J., Kolthoff, I. M., Proskauer, E. S., and others.
1961. Organic analysis. Vol. I, p. 92. Interscience Publishers, Inc., New York, N.Y.

(4) _____, Kolthoff, I. M., Proskauer, E. S., and others.
1961. Organic analysis. Vol. I, p. 81. Interscience Publishers, Inc., New York, N.Y.

(5) Edlbacher, S.
1918. On the pregl micro method for the determination of methyl groups attached to nitrogen. Z. Physiol. Chem. 101: 278.

(6a) Clark, E. P.
1938. Preparation of hydriodic acid suitable for alkoxyl and Friedrich-Kjeldahl nitrogen determinations. Indus. Engin. Chem., Analyt. Ed., 10: 677.

(6b) _____
1929. Studies on Gossypol. VI The action of boiling hydriodic acid as used in the Zeisel method upon Gossypol and some of its derivatives. A semi-micro Zeisel methoxyl method. J. Am. Chem. Soc. 51: 1479.

(6c) _____
1932. The Biebäck and Schwappach method for the determination of methoxyl and ethoxyl groups. J. Assoc. Official Agr. Chem. 15: 136; Report on microchemical methods: Alkoxyl determination. 1939. J. Assoc. Official Agr. Chem. 22: 622.

(7a) Friedrich, A.
1929. The quantitative micro determination of methoxyl and ethoxyl groups. Mikrochem. 7: 185.

(7b) _____
1929. A modified apparatus for the quantitative micro determination of alkyls attached to nitrogen. Mikrochem. 7: 195.

(7c) _____
1927. On the micro determination of methoxyl. Z. Physiol. Chem. 163: 141.

(8) Elek, A.
1939. Micro determination of alkoxyl groups. Indus. Engin. Chem., Analyt. Ed., 11: 174.

(9) Steyermark, A.
1948. Micro determination of alkoxyl groups. Analyt. Chem. 20: 368.
1951. Quantitative organic microanalysis. Pp. 203-243. The Blakiston Company, New York, N.Y.

(10) Clark, E. P.
1943. Semimicro quantitative organic analysis. P. 70. Academic Press, New York, N.Y.

(11) Friedrich, A.
1933. The practice of quantitative organic microanalysis. Pp. 133-160. Deuticke, Leipzig, and Vienna.

(12) Furter, M.
1938. The micro Zeisel method. Parts I, II, and III. Helv. Chim. Acta 21: 872, 1144, 1151.

(13) Hoffman, D. O., and Wolfrom, M. L.
1947. Determination of methoxyl and ethoxyl groups in acetals and easily volatile alcohols. Analyt. Chem. 19: 225.

(14) Niederl, J. B., and Niederl, V.
1942. Micromethods of quantitative organic analysis. 2d Ed., p. 241. Wiley, New York, N.Y.

(15) Pregl, F., and Roth, H.
1937. Quantitative organic microanalysis based on the methods of Fritz Pregl. 3d Eng. Ed., translated from 4th revised edition by E. B. Daw, p. 171. The Blakiston Company, New York, N.Y.

(16) Samsel, E. P., and McHard, J. A.
1942. Determination of alkoxyl groups in cellulose ethers. Indus. Engin. Chem., Analyt. Ed., 14: 750.

(17) White, E. P.
1944. Thiosulfate washers in alkoxy micro determinations. Indus. Engin. Chem., Analyt. Ed., 16: 207.

(18) Kinsman, S., and Noller, C. R.
1938. Determination of alkoxyl by the method of Vieböck and Schwappach. Indus. Engin. Chem., Analyt. Ed., 10: 424.

(19) Houghton, A. A., and Wilson, H. A. B.
1944. Micro determination of alkoxyl values. Analyst 69: 363.

(20) Slotta, K. H., and Haberland, G.
1932. Micro analytical determination of methoxyl and methylimide groups. Ber. 65B (1): 127.
1932. Mikrochem. 11: 157.

(21) Peniston, Q. P., and Hibbert, H.
1939. The determination of methoxyl groups in pulp and plant materials. Pap. Trade J. 109 (17) Tech. Assoc. Sect.: 46.

(22) Neumann, F.
1937. Methoxyl determinations in highly methylated hydrocarbons. Ber. 70B: 734.

(23) Bailey, A. J.
1942. An improved methoxyl apparatus. Indus. Engin. Chem., Analyt. Ed., 14: 181.

(24) Vieböck, F., and Schwappach, A.
1930. New method for the volumetric determination of the methoxyl and ethoxyl groups. Ber. 63B: 2818.

(25) American Society for Testing and Materials.
1960. ASTM D1166. Philadelphia, Pa.

DETERMINATION OF ASH IN PULP

Introduction

If the ash in pulp is considered to be the "mineral salts and inorganic foreign matter"[1] present, almost any relatively simple method for measuring the amount of ash must be only empirical. The primary reasons for this are: (1) The volatility of the inorganics, (2) the thermal instability of the inorganics, and (3) the difficulty involved in determining the original anions associated with the ash.

The composition, stability, and volatility of pulp ash have been reviewed by Bethge and Troëng.[2] In their review they state: "Analyses have shown that the most common metal cations in pulp ash are calcium, magnesium, and sodium. Potassium, which is one of the main constituents of wood ash, is usually only a minor component of pulp ash. These elements are present as oxides, carbonates, sulfates, and in some cases as chlorides. In addition to these compounds which are soluble in hydrochloric acid, there is also an acid fraction, mainly silicon dioxide. The thermostability and other properties of interest of the most important compounds have been investigated, and the results may be summarized as follows:

"(a) Calcium oxide. Calcium oxide is formed when the pulp is incinerated and is also obtained from calcium carbonate on ignition. It absorbs carbon dioxide and moisture from the air at room temperature and makes it difficult to obtain constant ash weights. Calcium oxide does not decompose at temperatures below 850° C.

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[1] Technical Association of the Pulp and Paper Industry. TAPPI Standard Method T 211. New York, N.Y.

[2] Bethge, P. O., and Troëng, T. Determination of the ash content of pulp. Svensk. Papperstid. 61: 1016. 1958.

"(b) Calcium carbonate. When calcium carbonate is ignited in a muffle furnace on platinum dishes, it loses carbon dioxide rather rapidly. Fig. 1 shows the weight loss plotted against ignition time for different furnace temperatures. At 700° the carbonate is completely converted to oxide after 6 hr.; at 800° the transformation is complete after 90 min.

"(c) Calcium sulfate. Calcium sulfate is completely stable up to at least 850° C.

"(d) Magnesium oxide. Magnesium oxide, like calcium oxide, absorbs carbon dioxide and moisture from the air at room temperature, but the weight increase is much slower than that for calcium oxide. It is stable at 850° C.

"(e) Magnesium carbonate. Magnesium carbonate is also converted to the oxide at ignition temperatures. The decomposition into magnesium oxide and carbon dioxide is more rapid than the decomposition of calcium carbonate and is completed in less than 30 min. at a furnace temperature of 600°.

"(f) Magnesium sulfate. Magnesium sulfate slowly decomposes at 850°. The loss of weight is of the order of 1.5% per hour; at 800° it is 0.05 to 0.1%.

"(g) Sodium carbonate. Sodium carbonate is also slowly decomposed at 850°. The weight loss is of the order of 1 to 2% per hr. (fig. 2). Sodium carbonate will damage platinum vessels at 850°.

"(h) Sodium chloride. Sodium chloride evaporates at 850° quite rapidly (fig. 2). At 750° its weight seems to be constant.

"(i) Sodium sulfate and silicon dioxide are stable at 850°."

Bethge and Troëng[2] then conclude that there is no ignition temperature which will give reproducible or more than relative results. Even a

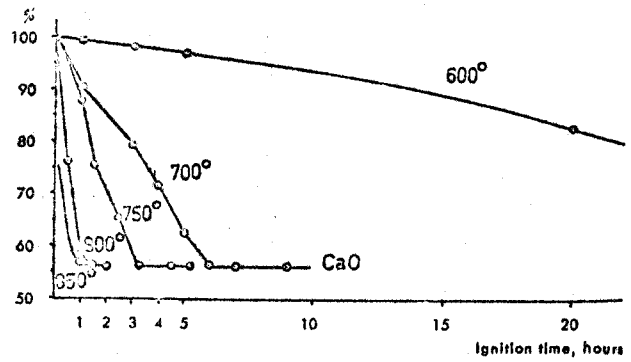

Fig. 1. The thermostability of calcium carbonate.[2]

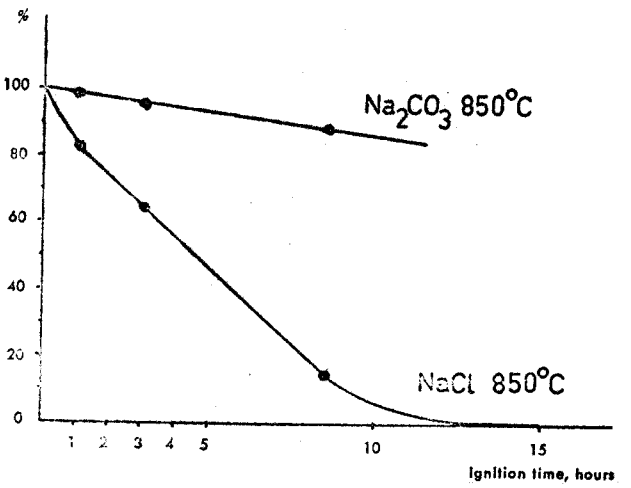

Fig. 2. The thermostability of sodium salts.[2]

temperature of 600° C., where the calcium carbonate is fairly stable and magnesium carbonate is quickly and completely converted to magnesium oxide, is not satisfactory. This is because, as is shown in fig. 3, the heat generated by the burning paper may raise local temperatures to the point where calcium carbonate decomposes rapidly and sodium salts evaporate. The volatility of sodium (and potassium) compounds has been demonstrated by Bethge and Troëng[2] and by Grove, Jones, and Mathews.[3]

To overcome the above difficulties, Bethge and Troëng[2] suggest converting the ash constituents of pulp to sulfates after incineration of the sample. The addition of sulfuric acid, removal of the excess, and igniting at 700° C. accomplish this. This procedure gives more easily reproducible (but still empirical) results.

Phifer and Maginnis[4] have made a detailed investigation of the variables in ashing methods. They found that dry ash values decreased with temperature and that this dependency varied with different pulps. During dry ashing, they measured large losses in sodium, calcium, iron, and copper, but no loss in silica. Sulfated ash determinations reflected the same strong dependency on temperature. In addition to studying the dependency of ash values on temperature and pulp, they studied the differences due to using platinum or porcelain crucibles, flame or furnace, and variations with time. They concluded that dry ashing methods should not be used as a control, except in special cases. They recommended, instead, a wet ashing followed by a flame photometric determination of sodium and calcium. They recommend that wet ashing be used as the method of sample preparation when sodium, calcium, iron, and copper analyses are to be run. Phifer's wet-ashing procedure is given in paragraph 5 of "Additional Information."

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[3] Grove, E. L., Jones, R. A., and Mathews, W. The loss of sodium and potassium during the dry ashing of animal tissue. Analyt. Biochem. 2: 221. 1961.

[4] Phifer, L. H., and Maginnis, J. B. Dry ashing of pulp and factors that influence it. Tappi 43: 38. 1960.

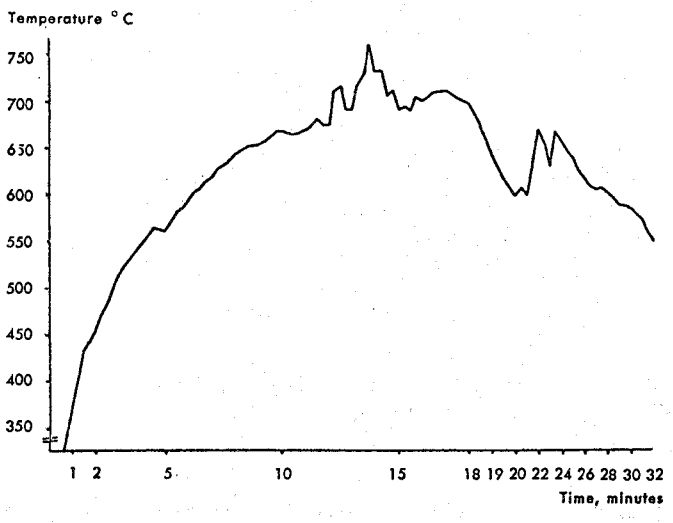

Fig. 3. Temperature changes in pulp ash determination.[2]

Despite the advantages of determining ash as sulfated ash or by wet ashing, common (nonsulfated) dry ashing procedures are still most widely used. The one given here and used at the Forest Products Laboratory is the current TAPPI Standard.[1] In this, the ash content of pulp is defined as the residue remaining after ignition at 575° ±25° C. (1067° ±45° F.) for 3 hr., or longer if necessary, to burn off all the carbon. It is a measure of mineral salts and inorganic foreign matter in the pulp, but it is not necessarily quantitatively equal to them. Pulp, like wood, is ashed at lower temperature than is paper (925° C.) to minimize the volatilization of inorganic compounds.

Apparatus

1. Crucible. A platinum crucible or dish with lid or cover is recommended. If platinum is not available, silica may be used.

2. Analytical balance having a sensitivity of 0.1 mg. with class S weights.

3. Electric muffle furnace adjusted to maintain a temperature of 575° ±25° C.

Test Specimen

Obtain a representative sample of the pulp. Weigh out a representative portion of the sample for ashing, preferably in duplicate. The size of the specimen required for each ash determination depends on its ash content and should be adjusted so that the weight of the ash will not be less than 10 mg.

and, preferably, not over 20 mg. (table 1). If the amount of moisture in the sample is not known, determine it by drying a representative portion to constant weight at 105° ±3° C.

Table 1.--Approximate size of specimen

Percentage ash	Moisture-free pulp or wood
	G.
Over 0.5	5
0.20 to 0.50	10
0.12 to 0.20	20
0.08 to 0.12	30
0.04 to 0.08	40
Less than 0.04	50

Procedure

Take a clean crucible and cover and ignite them to constant weight in a muffle furnace at 575° ±25° C. After ignition, cool slightly and place in a desiccator, preferably containing indicating-grade anhydrous alumina. When cooled to room temperature, weigh the ignited crucible on the analytical balance to the nearest 0.1 mg.

If of a suitable size, place all the specimen in the crucible and burn the pulp directly over a low flame or a bunsen burner (or preferably on the hearth of the furnace) until it is well carbonized.[5] If the crucible is too small to hold the entire specimen, gently burn the portion added and add

[5]Cover crucible during charring step if sample tends to lose ash.

more as the flame subsides. Take care not to blow portions of the ash from the crucible. Continue heating with the burner only as long as the residue burns with a flame. When the flame has died down, place the crucible in the furnace at 575° ±25° C. for a period of at least 3 hr., or longer if needed, to burn off all the carbon.

When ignition is complete, as indicated by the absence of black particles, remove the crucible from the furnace, replace the cover, and allow the crucible to cool somewhat. Then place in a desiccator and cool to room temperature. Reweigh with the ash to the nearest 0.1 mg. and calculate the percentage based on the moisture-free weight of the sample.

Report

Report the ash as a percentage of the moisture-free sample to two significant figures or to only one figure if the ash is less than 0.1%.

Precision

The results of duplicate ash determination should be suspected if they differ by more than that indicated in table 2.

Table 2.--Permissible deviations between duplicates

Weight of ash	Maximum permissible difference
Mg.	Mg.
20-50	2
5-20	1
Less than 5	.5

Additional Information

1. The ash, so obtained, usually is not fused to the platinum dish and is easily dissolved in dilute hydrochloric acid. It may be used subsequently for the determination of such constituents as iron, copper, and calcium. Such results would represent the inorganic matter in the ash and not necessarily the inorganic content of the original sample.

2. Iron chlorides, if present, may be partially volatilized under the conditions specified.

3. Since the ignition temperature affects the weight of ash, only values obtained at 575° ±25° C. should be reported as being in accordance with this method.

4. Similar method: Australian, APPITA, P 3m. Related methods: Canadian, C.P.P.A., G. 10; Swedish, C.C.A.I., ASTM D 1102.

5. As is evident from the introductory discussion, there are many ways to run an ash determination, and all are empirical in nature. The method used depends on the purpose of the test. When results are to be compared, the same method, of course, must be employed throughout. If the ash is to be analyzed for its constituent components, the only satisfactory ashing procedure is wet ashing. By this means, no inorganic cations are lost. A wet ashing procedure, as described below, is the one proposed by Phifer.[6] Birnoe and Hansen[7] have also proposed a wet ashing procedure which they report to be rapid and applicable to materials ordinarily difficult to decompose.

Accurately weigh approximately 10 g. of air-dried sample and place it into a Berzelius beaker. Add 40 ml. of 72% sulfuric acid and break up the pieces of sample with a stirring rod. Place the beaker on a hotplate and heat until

[6]Phifer, L. H. Flame photometric determination of calcium in cellulose. Analyt. Chem. 29: 1528. 1957.

[7]Birnoe, K. E., and Hansen, H. E. Rapid method for wet ashing of organic matter. Chem. Indus. 504. 1962.

the foaming ceases and the black residue has a solid granular appearance. Remove the beaker from the hotplate and add 30% hydrogen peroxide solution dropwise until the reaction slows or ceases. Return the beaker to the hotplate and heat until fumes of sulfur trioxide are given off. If any solid material remains, or if the solution is dark, remove the beaker from the hotplate and add more peroxide, following each addition with an evaporation step. It may be necessary to repeat this step several times. Do not add peroxide while the samples are on the hotplate or when sulfur trioxide fumes are evolving. The acid is then evaporated to approximately 8 ml., adding peroxide occasionally, if necessary, to remove the brown or yellow color. The sample is finally transferred quantitatively to a volumetric flask and diluted to volume. Subsequent analyses are performed on this solution.

Summary

Dry ashing of pulps give results which are empirical and very difficult to reproduce. These results vary with temperature, composition of the ash, and type of crucible.[2] Variability of ash determinations due to the volatility and thermal instability of ash components can be diminished by "sulfating" the ash. If ashing is to be followed by chemical analysis of the ash, a wet ashing procedure must be used.

DETERMINATION OF ASH IN WOOD[1]

The ash content of wood is defined as the residue remaining after ignition at 575° ±25° C. (1067° ±45° F.) for 3 hr., or longer if necessary to burn off all the carbon. It is a measure of mineral salts in the wood, but it is not necessarily quantitatively equal to them. Wood, like pulp, is ashed at a lower temperature than is paper (925° C.) to minimize the volatilization of inorganic compounds.

Apparatus

1. Crucible. A platinum crucible or dish with lid or cover is recommended. If platinum is not available, silica may be used.

2. Analytical balance having a sensitivity of 0.1 mg.

3. Electric muffle furnace adjusted to maintain a temperature of 575° ±25° C.

Test Specimen

Obtain a representative sample of the wood, preferably ground to pass a 40-mesh screen. Weigh, to 5 mg. or less, a specimen of about 5 g. of moisture-free wood for ashing, preferably in duplicate. If the moisture in the sample is not known, determine it by drying a corresponding specimen to constant weight at 105° ±3° C.

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[1]From TAPPI Standard T 15. Technical Association of the Pulp and Paper Industry, New York, N.Y.

Procedure

Carefully clean the empty crucible and cover, and ignite them to constant weight in a muffle furnace at 575° ±25° C. After ignition, cool slightly and place in a desiccator. When cooled to room temperature, weigh the crucible and cover on the analytical balance to the nearest 0.1 mg.

Place all, or as much as practicable, of the weighed specimen in the crucible. If the crucible is large enough, the entire specimen may be weighed in it. Burn the wood directly over a low flame of a bunsen burner (or preferably on the hearth of the furnace) until it is well carbonized.[2] If the crucible is too small to hold the entire specimen, gently burn the portion added and add more as the flame subsides. Take care not to blow portions of the ash from the crucible. Continue heating with the burner only as long as the residue burns with a flame. When the flame has died down, place the crucible in the furnace at 575° ±25° C. for a period of at least 3 hr., or longer if needed, to burn off all the carbon.

When ignition is complete, as indicated by the absence of black particles, remove the crucible from the furnace, replace the cover and allow the crucible to cool somewhat. Then place in a desiccator and cool to room temperature. Reweigh with the ash to the nearest 0.1 mg. and calculate the percentage based on the moisture-free weight of the wood.

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[2] If a sample tends to flare up or lose ash during charring, the crucible should be covered, or at least partially covered during this step.

Report

Report the ash as a percentage of the moisture-free wood to two significant figures, or to only one figure if the ash is less than 0.1%.

Precision

The results of duplicate determinations should be suspect if they differ by more than 0.5 mg.

Additional Information

1. Since the ignition temperature affects the weight of the ash, only values obtained at 575° ±25° C. should be reported as being in accordance with this method.

2. In this procedure, the temperature of ignition has been specified at 575° ±25° C., being the same as in TAPPI Standard T 211, "Ash in Pulp."

3. Similar Method: Australia, APPITA, P 3m. Related Methods: ASTM D 1102; Canadian, C.P.P.A., G-10.

4. Porcelain crucibles can also be used in most cases for the determination of ash.

5. Special precautions are required in the use of platinum crucibles; a list of rules to follow is given by Pierce and Haenish (1).

6. Listed below are some published values for the ash content of various woods.

	Ash (%)	Reference
Hardwoods:		
Aspen	0.2	(2)
Beech	.4	(2)
Yellow birch	.3	(3)
Paper birch	.2	(2)
American elm	.3	(3)
Red maple	.2	(2)
Sugar maple	.3	(3)
Overcup oak	.4	(4)
Southern red oak	.2	(4)
Softwoods:		
Eastern white-cedar	.2	(2)
Western redcedar	.3	(4)
Douglas-fir	.3	(4)
Balsam fir	.2	(2)
Eastern hemlock	.2	(2)
Western hemlock	.5	(4)
Jack pine	.2	(2)
Loblolly pine	.3	(4)
White spruce	.3	(2)
Tamarack	.2	(2)

7. If the wood ash is to be analyzed to determine its various constituents, wet ashing is recommended by Phifer (6).

8. Data on the volatility of some ash constituents of wood pulp are reported by Bethge and Troëng (7), Grove, Jones and Mathews (8), and Phifer and Maginnes (9). They report significant losses in sodium, calcium, iron, and copper at temperatures over 600° C.

9. A tree will contain at least traces of all of the elements found in the soil in which it is growing but the principal inorganic constituents

present are calcium, potassium, magnesium, phosphorus, and manganese compounds (10). Calcium and potassium compounds make up the bulk of the inorganics with the other constituents being present in relatively minor amounts. Some tropical woods are notably high in silica content (11).

References

(1) Pierce, W. G., and Haenish, E. L.
1948. Quantitative analysis. J. Wiley & Sons, N.Y., p. 328.

(2) Timell, T. E.
1957. Carbohydrate composition of ten North American species of wood. Tappi 40: 568.

(3) _____
1958. Some properties of four Canadian species of wood. Pulp and Paper Mag. of Canada 59(8): 139.

(4) Wise, L. E., and Ratliff, E. K.
1947. Quantitative isolation of hemicelluloses and the summative analysis of wood. Analyt. Chem. 19: 459.

(5) U.S. Forest Products Laboratory
1952. Tech. Note No. 235. Forest Service, USDA.

(6) Phifer, L. H.
1957. Flame photometric determination of calcium in cellulose. Analyt. Chem. 29: 1528.

(7) Bethge, P. O., and Troëng, T.
1958. Determination of the ash content of pulp. Svensk Paperstid. 61: 1016.

(8) Grove, E. L., Jones, R. A., and Mathews, W.
1961. The loss of sodium and potassium during the dry ashing of animal tissue. Analyt. Biochem. 2: 221.

(9) Phifer, L. H., and Maginnes, J. B.
1960. Dry ashing of pulp and factors that influence it. Tappi 43: 38.

(10) Young, H. E.
1966. Chemical elements in complete mature trees of seven species in Maine. Tappi 49: 190.

(11) Amos, G. L.
1952. Silica in timbers. Bull. 267, CSIRO, Melbourne, Australia.

CHARCOAL ANALYSIS

PART I--A METHOD OF CHARCOAL ANALYSIS[1] [2]

A practical standard method for the analysis of charcoal is desired by producers and laboratories that are concerned with the production and marketing of charcoal. The method described here employs equipment found in most laboratories, and is adapted to routine analyses of a large number of samples. It is designed to give accurate results for moisture, volatile matter, ash, and fixed carbon in briquetted wood charcoal of the type used for cooking.

Using this method, data for duplicate determinations on 100 samples were analyzed statistically. The samples of charcoal were obtained at random from various sources in different parts of the country. The results were obtained by two analysts over a period of several months. Standard deviations of duplicates were: moisture, 0.06; volatile matter, 0.25; and ash, 0.07. Moore and Beglinger also demonstrated[3] that the accuracy and reproducibility of the method are acceptable for establishing the quality of charcoal and for determining the effects of variables in production.

By this procedure, 30 samples in duplicate (60 complete determinations) can be carried out by one analyst in 2 days.

Wood charcoal of the cooking type generally contains from 2 to 4 percent moisture, 18 to 23 percent "volatiles," 1 to 4 percent ash, and 74 to 81 percent fixed carbon.

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[1] Taken from: A method of charcoal analysis, by W. E. Moore and E. Beglinger. Forest Prod. J. 11: 17. 1961.

[2] Adopted as ASTM Standard D 1762. 1964.

[3] U.S. Forest Products Laboratory. Charcoal production, marketing, and use. Forest Prod. Lab. Rep. No. 2213. 1961.

Sample Preparation

Samples are selected to be representative of the lot. Normally they will be air-dry charcoal lumps or briquets. Rain-soaked or wet samples should be spread out to air dry before they are analyzed. For determining the moisture content of the charcoal as received, the sample should be ground to pass a coarse screen such as No. 20 U.S. Standard Sieve, since heat generated by excessive grinding would cause loss of moisture. For the determination of volatile matter, a more finely ground sample is required. All of the selected sample should be ground; no part of the sample is to be rejected.

A Wiley mill, size No. 2 with a 1-mm. screen, is used for grinding. The charcoal should be ground in less than 5 min. Longer grinding times resulting from the use of a finer screen or dull knives should be avoided because of the possibility of generating heat, which in turn causes a loss of volatile material. Excessive grinding will also produce a large amount of fine particles (smaller than 100 mesh, U.S. Standard Sieve). These fine particles may be swept out of the crucible during the rapid evolution of gases in the determination of volatile matter, and thus cause errors. Because over-size particles can result in low values for volatiles, particles larger than No. 20 U.S. Standard Sieve should be avoided. The ground samples should be stored in airtight containers such as screw-top bottles. The samples should be well mixed by shaking before they are weighed. "Jack stones" can be used to facilitate mixing.

Procedure

Analyses are carried out in duplicate.

A muffle furnace that will control temperatures of 750° ±5° C. and 950° ±5° C. is required. Heat the muffle furnace to 750° C. and place previously ignited porcelain crucibles (41 mm. x 37 mm.) and lids in furnace for 10 min. Cool the crucibles in a desiccator for 1 hr. and weigh.

For the determination of moisture, add to a tared crucible an accurately weighed (to 1/10 mg.) sample of approximately 1 g. of charcoal. Place the sample in the oven at 105° C. and dry for 2 hr. Remove the dried sample from the oven, cool in desiccator for 1 hr., and weigh.[4]

For the determination of volatiles, heat the muffle furnace to 950° C. The crucible, with lid in place and containing the sample used for the moisture determination, is preheated by placing it for 2 min. on the outer ledge of the furnace (300° C., with furnace door open), and then 3 min. on the edge of the furnace (500° C.). Individual nichrome wire baskets to hold the crucibles are convenient (fig. 1).

The sample is then moved to the rear of the furnace for 6 min. with the muffle door closed. The sample is watched through a small peephole in the door of the furnace. If sparking occurs, results will be in error. If the sparking sample does not check the results of its nonsparking duplicate within ±0.5%, the analyses must be repeated.

[4]The sample shall be considered ovendry when the decrease in weight of consecutive weighings is 0.0005 g. or less. Successive drying periods shall not be less than 1 hr.

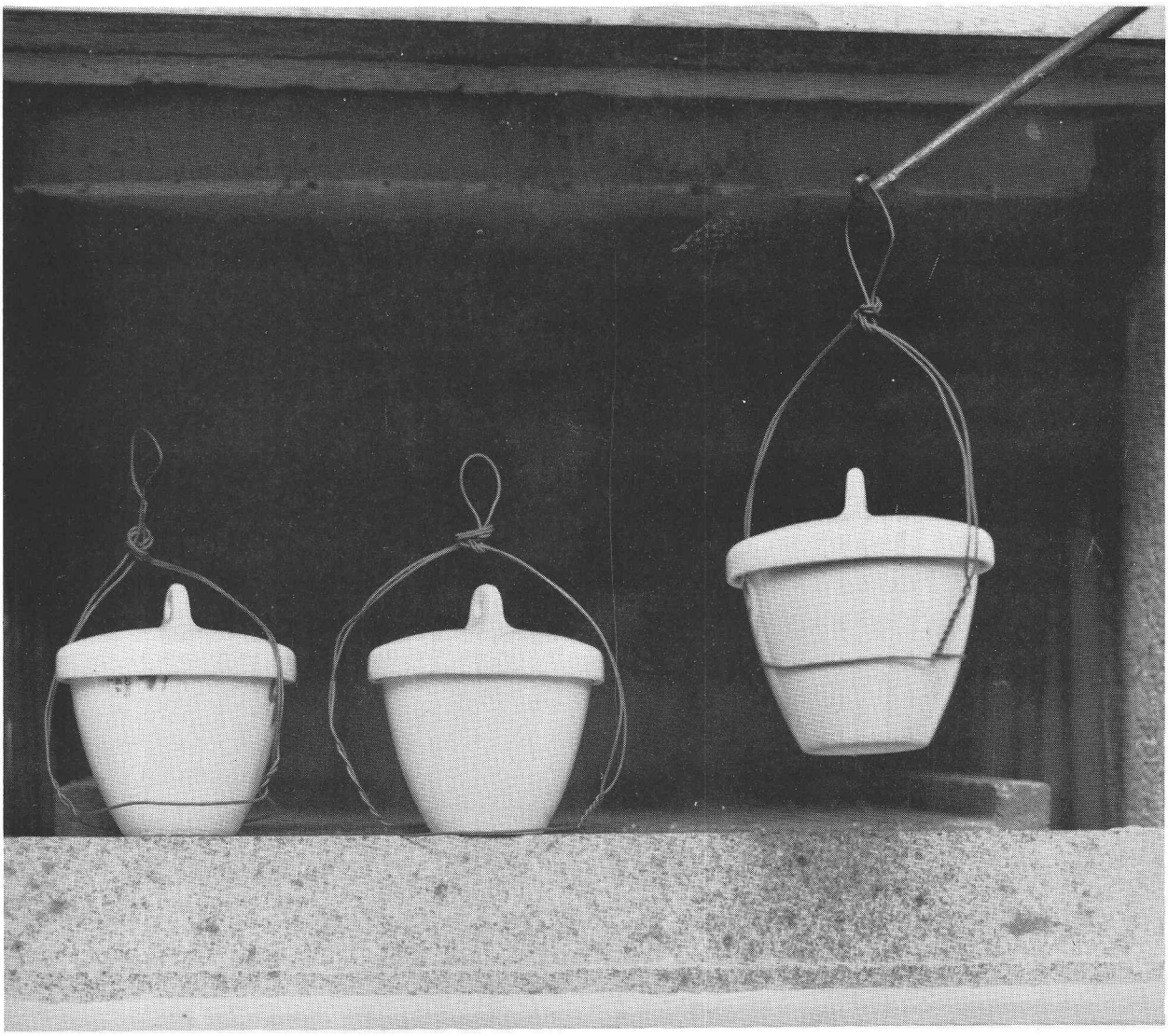

Figure 1.--Nichrome wire baskets for handling covered crucibles.

ZM 114 219

The sample is then removed and placed in a desiccator for 1 hr. and weighed.

For ash determination, place the lid and the uncovered crucible containing the sample used for the volatile determination in the muffle furnace at 750° C. for 6 hr.

The crucible with lid in place is cooled in a desiccator for 1 hr. and weighed. Burning of the sample is repeated until successive 1-hr. periods of heating result in a loss of less than 0.0005 g.

Computations

A. Moisture percent = $\frac{\text{loss in weight x 100}}{\text{weight of air-dry sample}}$

Report results to first decimal place. Moisture values for duplicates should agree within 0.1%.

B. Volatile percent = $\frac{\text{loss in weight x 100}}{\text{weight of ovendry sample}}$

Ovendry weight = weight of air-dry sample minus moisture. Report results to first decimal place.

Volatile values for duplicates should agree within 0.5%.

C. Ash percent = $\frac{\text{weight residue x 100}}{\text{weight of ovendry sample}}$

Report results to first decimal place.

Ash values for duplicates should agree within 0.1%.

D. Fixed carbon percent = 100 - (percent ash + percent volatiles)

All results except moisture are reported on an ovendry basis.

PART II--ADSORPTIVITY (ACTIVITY) OF CHARCOAL

Methylene Blue Test for Decolorization by Active Carbon[5]

Place 1 g. of the carbon (dried at 120° C.) in a 250-ml. beaker. Fill a large burette with aqueous methylene-blue solution (1 g. per liter in distilled water). Add the solution to the carbon a few milliliters at a time, stirring 1 min. after each addition. Transfer a drop of liquid from the beaker to a white spot plate after each addition and stirring. When the liquid on the spot plate shows a blue color, record the volume of the methylene-blue solution used. Calculate the grams of methylene-blue adsorbed by 1 g. of carbon.

Calculation:

Titration x g. methylene-blue per ml. = adsorption by 1 g. carbon

Benzene Saturation Value for Charcoal[6]

The charcoal is screened on 8-to 14-mesh screens and dried for 2 hr. at 150° C. before use.

a sample of 2-5 g. of the dry charcoal is weighed into a weighing bottle. It is placed, uncovered, on the plate of a desiccator which contains dry benzene, and is left to adsorb the vapor until constant weight is attained. The room temperature should be 25° C. ±2°. Before weighing, the cover is replaced and the outside of the bottle and cover wiped with a dry chamois. The weight is taken after the sample has stood 5 min. on the balance. Calculate the saturation value as milligrams per gram of sample.

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[5]Taken from test No. 139 from: Industrial carbon, by C. L. Mantell, 2d Ed. Van Nostrand, New York, N.Y. 1946.

[6]Taken from: Heat of wetting of charcoal and activity, by R. Macy. J. Phys. Chem. 35: 1398. 1931.

Calculation:

$$\frac{\text{Total weight gain in mg.}}{\text{grams charcoal}} = \text{Sat. Val.}$$

Allow about 2 days for saturation.

Iodine Adsorption Test for Active Charcoal[7]

Reagents:

1. Iodine solution, 2.7 g. per liter.

Dissolve 2.7 g. of iodine crystals and 4.1 g. of potassium iodide in 1 liter of distilled water and mix well. Store in a brown bottle in a cool, dark place and standardize at time of use.

2. Sodium thiosulfate solution, 0.01N, freshly prepared (may be diluted from stronger stock).

3. Hydrochloric acid solution, 5% by volume.

4. Starch indicator.

Check the strength of the iodine solution. To 10 ml. of the iodine solution add 1 ml. of the 5% hydrochloric acid and titrate with the 0.01N sodium thiosulfate to the usual starch end point. Record the volume of the titration (1 ml. 0.01N $Na_2S_2O_3$ = 1.27 mg. iodine).

Procedure

Weigh 0.5 g. of the carbon to be tested (dried at 120° C.) into a 250-ml. Erlenmeyer flask with a ground-glass stopper. Add 10 ml. of the 5% hydrochloric acid and stir until the carbon is well wetted. Bring it cautiously just to the

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[7] Taken from test No. 136 from: Industrial carbon, by C. L. Mantell, 2d Ed. Van Nostrand, New York, N.Y. 1946.

boiling point, then cool to room temperature. Add 100 ml. of the iodine solution, shake vigorously for 30 sec., and filter immediately through a fast filter (Whatman No. 5 or its equivalent). Allow to drain thoroughly, but do not wash. Stir the filtrate and take a suitable aliquot, depending on observed iodine color, for titration. Titrate this aliquot with the thiosulfate solution to a starch end point.

From the titration and the aliquot volume titrated, calculate the ml. of thiosulfate required to titrate the entire filtrate. Then from the standardization, calculate the thiosulfate equivalent to the 100 ml. of iodine added to the charcoal (first titration x 10).

This figure, minus the value for the entire filtrate, gives the thiosulfate equivalent to the adsorbed iodine. Then, $\frac{\text{adsorbed value x } 1.27}{\text{sample weight in grams}}$ = milligrams of iodine adsorbed per gram of charcoal.

DETERMINATION OF THE ETHER SOLUBILITY OF PULP OR WOOD[1]

Weigh 2 g. of air-dry material (pulp need not be ground, but wood should be to pass 40 mesh), the moisture content of which has been previously determined on a separate sample, into a tared alundum crucible (porosity R.A. 98) contained in a glass-stoppered weighing bottle. The crucible is removed from the bottle and placed in a Soxhlet apparatus. A small cone of fine mesh screen wire is set in the top of the crucible to prevent loss of sample by splashing. The sample is extracted for 6 to 8 hr. with approximately 200 ml. of U.S.P. ethyl ether, keeping the ether boiling briskly.

The amount of material extracted may be determined either by weighing the extracted residue after evaporation of the solvent, or by determining the loss in weight of material resulting from extraction. According to the first procedure, the ether is evaporated from the extraction flask, which is dried in an oven for 1 hr. at 105° C., cooled in a desiccator, and weighed. The drying is continued until there is no further loss in weight. The weight of the dried residue is calculated to the percentage of the ovendry material and reported as the percentage of ether-soluble matter.

According to the second procedure, the extracted material and alundum crucible are dried to constant weight in an air oven at 105° C., replaced in the glass-stoppered weighing bottle, cooled in a desiccator, and weighed.

The results of the two procedures seldom agree, and the second is the recommended method. The disagreement in the results of the two procedures is

[1]From "Methods for the Chemical Analysis of Pulps and Pulpwoods," by M. W. Bray. U.S. Forest Products Laboratory Report No. R19 (Revised 1939).

due, at least in part, to the following: (1) Some of the extracted substances volatilize during drying; (2) some of the solvent remains included within the extract and is not entirely removed during the evaporation procedure; (3) loss of sample as a result of spattering; (4) oxidation of the extracted material during drying.

Although the extracted pulp residue may be used for the determination of lignin, wood residues should first be extracted with alcohol-benzene mixture previous to the determination of lignin.

DETERMINATION OF ETHYL ALCOHOL BY MICRODIFFUSION[1]

Principle

Ethanol diffuses from the outer well of a Conway microdiffusion dish into acid dichromate solution in the center well. There it is oxidized to acetic acid by the dichromate. The unreduced dichromate remaining is measured by titration with thiosulfate, and the amount of ethanol originally present calculated from the amount of dichromate that was reduced. Acetone and 2,3-butanediol do not interfere, but volatile, oxidizable constituents, such as acetoin, diacetyl, and acetaldehyde, will intefere if present. This method is applicable to fermentation solutions.

Reactions

Oxidation of ethanol:

$$3CH_3CH_2OH + 2K_2Cr_2O_7 + 8H_2SO_4 \longrightarrow$$

$$3CH_3COOH + 2Cr_2(SO_4)_3 + 2K_2SO_4 + 11H_2O$$

Dichromate-iodine:

$$Cr_2O_7^{=} + 6I^- + 14H^+ \rightarrow 2Cr^{+++} + 3I_2 + 7H_2O$$

Iodine-thiosulfate:

$$2S_2O_3^{=} + I_2 \rightarrow S_4O_6^{=} + 2I^-$$

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[1]Based on: Winnick, T. H. Determination of ethyl alcohol by microdiffusion. Indus. Engin. Chem. Analyt. Ed. 14: 523. 1942; Conway, E. J., and Byrne, A. An absorption apparatus for the microdetermination of certain volatile substances. Part I. The microdetermination of ammonia. Biochem Jour. 27: 419. 1933: and Neish, A. C. Analytical methods for bacterial fermentations. Nat. Res. Counc. of Can., No. 2952. 1952.

Apparatus

The test requires Conway microdiffusion dishes (Conway No. 1).

Reagents

1. 10N Sulfuric acid. Cautiously add 300 ml. of concentrated sulfuric acid to 600 ml. of water, and then dilute to 1 liter.

2. Potassium dichromate. Dissolve 0.4904 g. of potassium dichromate in enough 10N sulfuric acid to give a volume of 200 ml.

3. Potassium iodide (50%). Dissolve 25 g. of potassium iodide in water and dilute to 50 ml.

4. Sodium thiosulfate (0.1N solution). Dissolve 25.0 g. of sodium thiosulfate in 1 liter of water. Let stand 3 to 4 days at room temperature, filter, add 0.5-ml. of toluene, and standardize against 0.1N iodine solution at regular intervals.

5. Iodine solution (0.1N). Weigh out 12.7 g. of resublimed iodine using a rough balance, and transfer it to a 125-ml., glass-stoppered Erlenmeyer flask which has been tared to the nearest milligram. Stopper the flask, and reweigh it to the nearest milligram to determine the weight of iodine added. Add 40 g. of potassium iodide and 25 ml. of water and stir until all iodine is in solution; then transfer quantitatively to a 1-liter volumetric flask and dilute to volume. Store in a brown, glass-stoppered bottle. Calculate the normality of the iodine from the weight added, 12.692 g. per liter, giving a 0.1N solution.

Procedure

With a pipet or accurately calibrated syringe, add exactly 1 ml. of acid dichromate solution to the center well of the Conway microdiffusion dish.

Lightly grease (with vaseline) the sealing area of the lid, so it is ready to be placed in position quickly. A sample solution (1 ml.) containing from 0.1 to 0.4 mg. of ethanol is added to the outer well and the lid is placed on immediately, being certain a good seal is obtained. Allow the sample to stand in the diffusion dish 18 hr. or more at room temperature. After diffusion of the ethanol is complete, remove the lid and add 1 ml. of water and 0.5 ml. of the 50% potassium iodide solution. The iodine released is titrated at once with 0.1N sodium thiosulfate, using a 10-ml. burette. Two drops of starch indicator are added when approaching the end point (when the brown iodine color is barely visible), and the titration continued until the blue color of the starch-iodine complex disappears. Two blanks, containing water only, in the sample section of the diffusion dish are run with each set of determinations.

Calculations

1. (Ml. thio for blank--ml. thio for sample.) Normality of thiosulfate = millequivalents of dichromate reduced.

2. $\frac{\text{Millequivalents of dichromate reduced}}{4}$ = millimoles ethanol.

3. Millequivalents of dichromate reduced x 11.5 = mg. ethanol.

4. (Combined equation.) (Ml. thio for blank--ml. thio for sample.) Normality of thio x 11.5 x dil'n factor = $\frac{\text{mg.}}{\text{ml.}}$ ethanol in sample.

DETERMINATION OF EXTRACTIVES IN WOOD[1]

Introduction

Extractives in wood consist of materials soluble in neutral solvents and not a part of the wood substance. They are largely made up of resins, tannins, waxes, gums, fats, and phenolics. In most hardwoods and softwoods, the extractives' content is from 2 to 5 percent, but in very resinous trees, or parts of trees, the level may be 25 percent or more.

The following procedure, using alcohol-benzene, alcohol, and water successively, is applicable to all North American woods. It may also be applied to bark, straw, and similar fibrous materials, and to pulps.

Apparatus

1. Soxhlet extractor, glass, of a size appropriate for the sample size. The sample is contained within the Soxhlet in a coarse porosity fritted glass or Alundum crucible. A fine wire screen cover is placed over the top of the material to prevent channeling of solvent and to prevent particles from splashing out.

2. Crucible holder and suction flask.

Reagents

1. Ethyl alcohol, 95 percent.

2. Alcohol-benzene mixture. Mix one volume of 95 percent ethyl alcohol and two volumes of benzene.

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[1]Based on TAPPI Standard Method T-12 1959.

Sample

The sample should be air dried, ground to pass a 40-mesh screen and its moisture content known.

Procedure

Accurately weigh into a tared Alundum or fritted glass crucible a suitable amount (1 to 5 g.) of wood sample. Place the crucible and sample in the Soxhlet apparatus being certain that the sample does not extend above the level of the top of the siphon tube. Extract at a rate of not less than four siphonings per hour for 4 hours with the alcohol-benzene mixture. After 4 hours, place the crucible in a crucible holder and attach to a suction flask and wash with alcohol to remove the benzene.

The alcohol wash consists of washing the sample in the crucible with alcohol (using suction) three times, then covering the sample with alcohol and letting stand for 10 minutes with the suction off. Then pull off the alcohol with suction and wash three times again. The sequence of soaking and washing is then repeated two more times.

If an alcohol extraction is necessary,[2] return the crucible to the Soxhlet and extract for 4 hours with 95 percent alcohol and follow this with a water wash. If the alcohol extraction is not necessary, the alcohol is removed directly by a water wash. The procedure for washing with water (not hot) is the same as the alcohol wash. After the water wash, the sample

[2]An alcohol extraction is necessary for woods high in tannins. Oaks, chestnut, and redwood require the alcohol Soxhlet extraction; the various species of spruce, pine, fir, hemlock, poplar, birch, beech, and maple usually do not.

and crucible are dried to constant weight at 105° C., and weighed. The loss in weight is calculated as extractives (correcting the weight of the original sample for its moisture content) and percent extractives reported on an ovendry basis.

DETERMINATION OF FORMALDEHYDE IN PAPER AND WOOD

Introduction

Formaldehyde reacts with chromotropic acid quantitatively forming a colored product, the intensity of which can be measured with a spectrophotometer. Eegriwe[1] discovered this reaction and found it to be highly selective. The following is a partial list of compounds which he found do not intefere with this reaction or its analytical applications:

Aldehydes:

- Propionaldehyde
- Butyraldehyde
- Isobutyraldehyde
- Isovaleraldehyde
- Glyceraldehyde
- Oenanthaldehyde
- Crotonaldehyde
- Chloral hydrate
- Glyoxal
- Benzaldehyde
- Oxybenzaldehyde
- Vanillin
- Salicylaldehyde
- Phthalaldehyde
- Isophthalaldehyde
- Terephthalaldehyde
- Cinnamaldehyde

Alcohols:

- Ethanol
- Glycerol

Ketones:

- Acetone

Acids:

- Levulinic
- Glycolic
- Oxalic
- Gallic
- Acetic

Sugars:

- Glucose
- Fructose
- Mannose
- Lactose
- Galactose

MacFadyen,[2] studying the same reaction, found that acetaldehyde, formic acid, and methanol were also nonreactive toward chromotropic acid.

The color-developing procedure given here is that of MacFadyen adapted to the measurement of formaldehyde in paper and wood. This procedure has

[1] Eegriwe, E. Z. Reactions and reagents for the detection of organic compounds. Analyt. Chem. 110: 22. 1927.

[2] MacFadyen, D. A., Watkins, H. D., and Anderson, P. R. Estimation of formaldehyde in biological mixtures. Jour. Biol. Chem. 158: 107. 1945.

been applied to samples which have been treated with formaldehyde and a catalyst[3] to increase their dimensional stability. The polyol oxidation procedure required in the preparation of a calibration curve is based on that of Lambert and Neish.[4]

Principle

Formaldehyde is liberated from the sample by treating with sulfuric acid. The dissolved formaldehyde is then measured by means of the color produced through its reaction with chromotropic acid. Formaldehyde concentration is computed from a standard calibration curve relating the intensity of color to formaldehyde concentration. The standard curve is made up from standard solutions of either glycerol or mannitol, which are oxidized by periodic acid to yield formaldehyde and formic acid. Both of these substances yield 2 moles of formaldehyde per mole upon oxidation.

Reactions

General reaction of polyols with periodate:

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_n \\ | \\ CH_2OH \end{array} + (n+1)\ H_5IO_6 \longrightarrow n\ HCOOH + 2HCHO + (n+1)\ HIO_3 + (2n+3)H_2O$$

Removal (reduction) of periodate, iodate, and iodine by arsenite:

$$H_5IO_6 \xrightarrow{-2H_2O} IO_4^- + H^+$$

$$IO_4^- + H_2AsO_3^- \longrightarrow H_2AsO_4^- + IO_3^-$$

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[3] Stamm, A. J. Dimensional stabilization of wood by thermal reactions and formaldehyde cross-linking. Tappi. 42: 39, 44. 1959.

[4] Lambert, M., and Neish, A. C. Rapid method for estimation of glycerol in fermentation solutions. Canad. Jour. Res., sect B., 28(3): 83 1950.

$$2IO_3^- + 5H_2AsO_3^- + 2H^+ \longrightarrow I_2 + 5H_2AsO_4^- + H_2O$$

$$I_2 + H_2AsO_3^- + H_2O \longrightarrow H_2AsO_4^- + 2I^- + 2H^+$$

Apparatus

1. Spectrophotometer. Beckman DU or equivalent equipped with 10-mm. pyrex or silica cells.

2. Hot water bath to hold test tubes during color development.

Reagents

1. 12N Sulfuric acid. Cautiously add 360 ml. of concentrated sulfuric acid to 500 ml. of distilled water and dilute to 1 liter.

2. 0.5N Sulfuric acid. Add 105 ml. of concentrated sulfuric acid to 5 liters of water and dilute up to 7.5 liters.

3. Sodium periodate (0.1M). Dissolve 46 g. of periodic acid in 1,500 ml. of water in a 2-liter volumetric flask. Neutralize with 1N sodium hydroxide until faintly acid to methyl red. Dilute to 2 liters.

4. Sodium arsenite (1M). Dissolve 90 g. of sodium hydroxide in about 1 liter of water. Weigh 200 g. of arsenious acid (AS_2O_3) into a beaker and add enough sodium hydroxide to make a paste slurry. Transfer the slurry into a 2-liter volumetric flask, completing the transfer with the rest of the sodium hydroxide. When dissolution is complete, add water to the mark, mix, and filter.

5. Chromotropic acid. Cautiously add 1,200 ml. of concentrated sulfuric acid to 600 ml. of water, and then let the solution cool. Dissolve 4 g. of reagent grade 4,5-dihydroxynaphthalene 2,7-disulfonic acid (chromotropic acid)

in 400 ml. of distilled water. If not clear, filter. Dilute to 2 liters with 2:1 sulfuric acid. Store in a brown, glass-stoppered bottle, and prepare fresh every 2 to 3 weeks.

Calibration Curve

Accurately prepare a stock solution of glycerol or mannitol and then dilute to give standard solutions which will yield formaldehyde concentrations, upon oxidation, over a range from 1 to 6 μg./ml. Table 1 shows the amounts of glycerol or mannitol that correspond to this range. Make up a standard calibration curve for formaldehyde according to the procedure that follows:

Table 1.--Amounts of mannitol and glycerol to yield proper formaldehyde concentrations

Standard solutions		Concentration of formaldehyde after oxidation
Mannitol	Glycerol	
Mg./ml.	Mg./ml.	Mg./ml.
0.79	0.40	1.0
1.58	.80	2.0
2.37	1.20	3.0
3.17	1.60	4.0
3.96	2.00	5.0
4.75	2.40	6.0

Pipet (or use a calibrated syringe) 0.5 ml. of standard solution into a 250-ml. beaker containing exactly 20 ml. of 0.5N sulfuric acid. Add 5 ml. of sodium periodate (0.1M), and after exactly 5 min., add 5 ml. of sodium arsenite (1M). Mix reactants after each addition. After adding the sodium arsenite, iodine appears in the solution and then fades.

This solution is allowed to stand for 5 to 10 min., and then 100 ml. of water is added. Mix, then pipet 1 ml. into a pyrex test tube and add exactly 10 ml. of chromotropic acid solution. Thetest tube is covered with aluminum foil, shaken, and placed in a steam bath for 30 min. The solutions are cooled by adding tapwater to the cooker. After cooling, the samples are shaken, and their absorbance measured at 570 mμ. Two blanks are run with the samples, and readings are made against a blank. A standard curve is then constructed relating absorbance at 570 mμ. to the concentration of formaldehyde in the oxidized solution (130.5 ml. total volume in a beaker of oxidized solution).

Procedure for Unknown

Divide the paper sample into small pieces (0.5 by 0.5 cm.), or grind the wood to pass a 40-mesh screen, and dry for 1 hr. at 105° C. Remove the sample from the oven and cool in a desiccator. Weigh out approximately 0.5 g. of sample into a 125-ml. Erlenmeyer flask. Add 50 ml. 12N sulfuric acid. Stopper and allow to stand 48 hr., or until the particles have disintegrated. The flask is not agitated and is kept stoppered to prevent loss of formaldehyde. Transfer (filter or decant) the solution to a 100-ml. volumetric flask and dilute to the mark with water. If necessary, dilute according to table 2. Pipet 1 ml. of the diluted solution into a test tube (approximately 19 by 150 mm.). Add 10 ml. of chromotropic acid solution, mix, and cover the test tube with aluminum foil. Heat 30 min. in a steam bath. Cool the steam bath and sample by running cold water into the cooker. Remove the sample, mix, and measure the absorbance of the solution at 570 mμ. Two blanks are run

Table 2.--Amount of dilution

Estimated formaldehyde	Dilute 1 ml. to--
%	Ml.
0.0	0
.1	0
.5	10
1.0	10
2.0	25
3.0	50
4.0	50
5.0	50
6.0	100
7.0	100

with the samples and readings are made against the blanks. The formaldehyde content of the sample is computed from the calibration curve.

DETERMINATION OF FURFURAL[1] [2]

Introduction

Dunlop and Peters[3] have reviewed the existing gravimetric and volumetric methods for the determination of furfural. The method described here is based on that of Stone and Blundell;[2] it consists of distilling the furfural from a solution of sample and quantitatively measuring the amount present spectrophotometrically. Root[1] reports a molar absorptivity for furfural of 14,850 at 276 mμ. This fairly high absorptivity, combined with the small volumes required, give this test good sensitivity as well as speed and accuracy.

Apparatus

Beckman DU spectrophotometer or its equivalent.

Procedure

A standard calibration curve is made up relating the concentration of furfural in a water solution to the absorbance of the solution at 276mμ. A solution of 6 mg. of furfural per liter has an absorbance very close to 0.9.

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[1]Root, D. F. Kinetics of the acid-catalyzed conversion of xylose to furfural. 1956. (Unpublished Ph. D. thesis on file in the Department of Chemical Engineering, University of Wisconsin.)

[2]Stone, J. E., and Blundell, M. J. A micromethod for the determination of sugars. Canad. J. Res. B 28: 676. 1950.

[3]Dunlop, A. P., and Peters, F. N. The furans. Reinhold Publishing Corp., New York. 1953.

Appropriate dilutions of a solution of this concentration will then give the other necessary points between 0 and 0.9.

Furfural is separated or isolated from interfering substances, prior to analysis, by distillation in the apparatus shown in fig. 1. Each still is made up of a 200-ml., round-bottom distillation flask; a vertical, water-cooled glass condenser; and an electric heating mantle. The distillate is collected in a volumetric flask of a size that is appropriate for the amount of furfural present. Samples containing 0.1 mg.[4] of furfural are placed in the 200-ml. distillation flasks and the volume in the flasks brought up to about 60 ml. with water. Voltage to the heating mantle is set at 70 v., and about 50 ml. of distillate is collected in the flask. The solution volume is then diluted to the mark. Then the absorbance of the test solution is measured at 276 mμ, and the furfural concentration of the test solution and sample solution is computed from the standard calibration curve.

Analytical Notes

1. Root[1] studied the effect of storage on the absorption spectrum of furfural. The results, shown in fig. 2, demonstrate the instability of such solutions. Root noted a small, but detectable, decrease in absorbance in

[4]The 0.1 mg. of furfural is required for the distillation step. For the optical measurements, 6 mg. is sufficient.

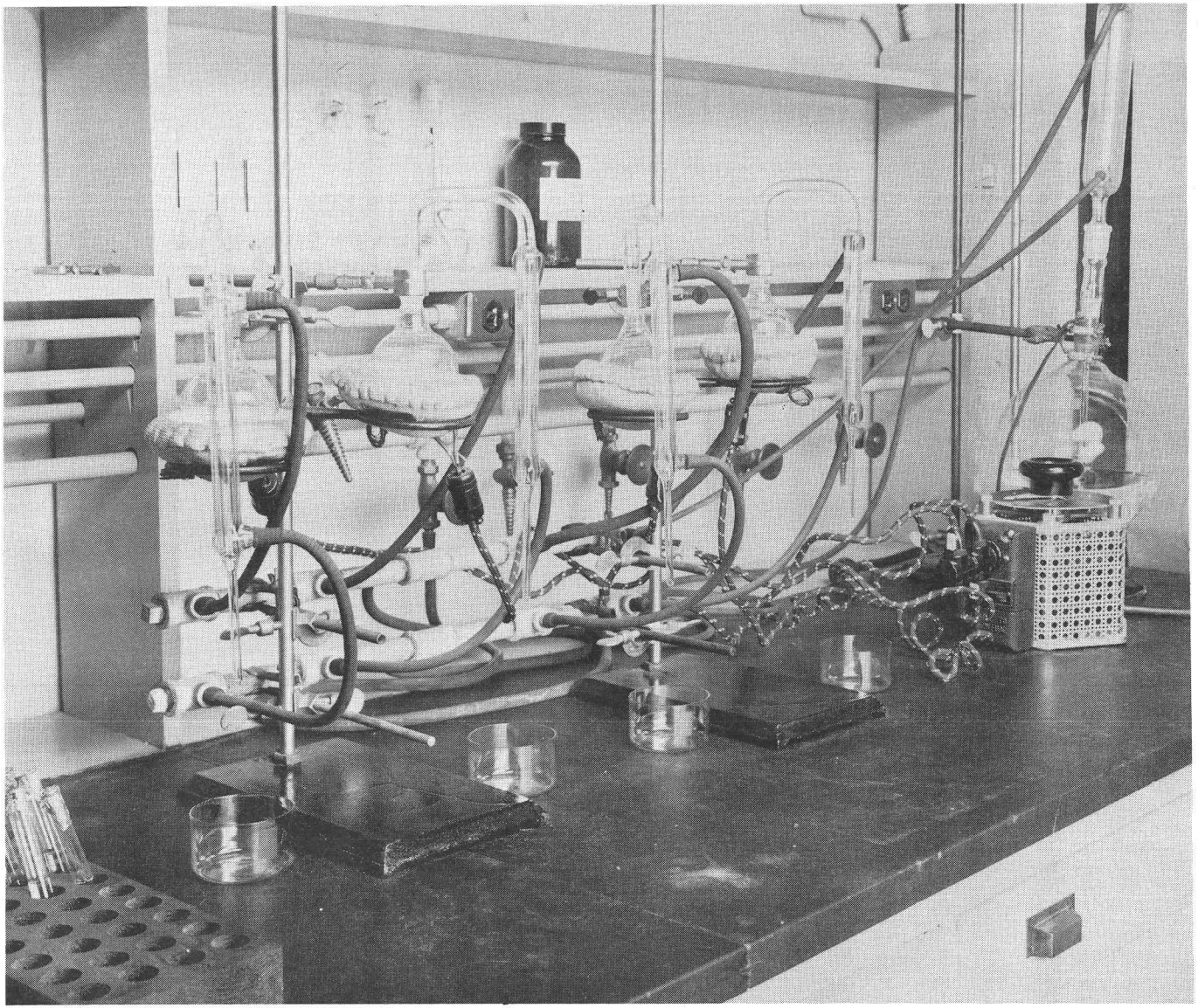

Figure 1.--Equipment for the recovery of furfural by distillation.

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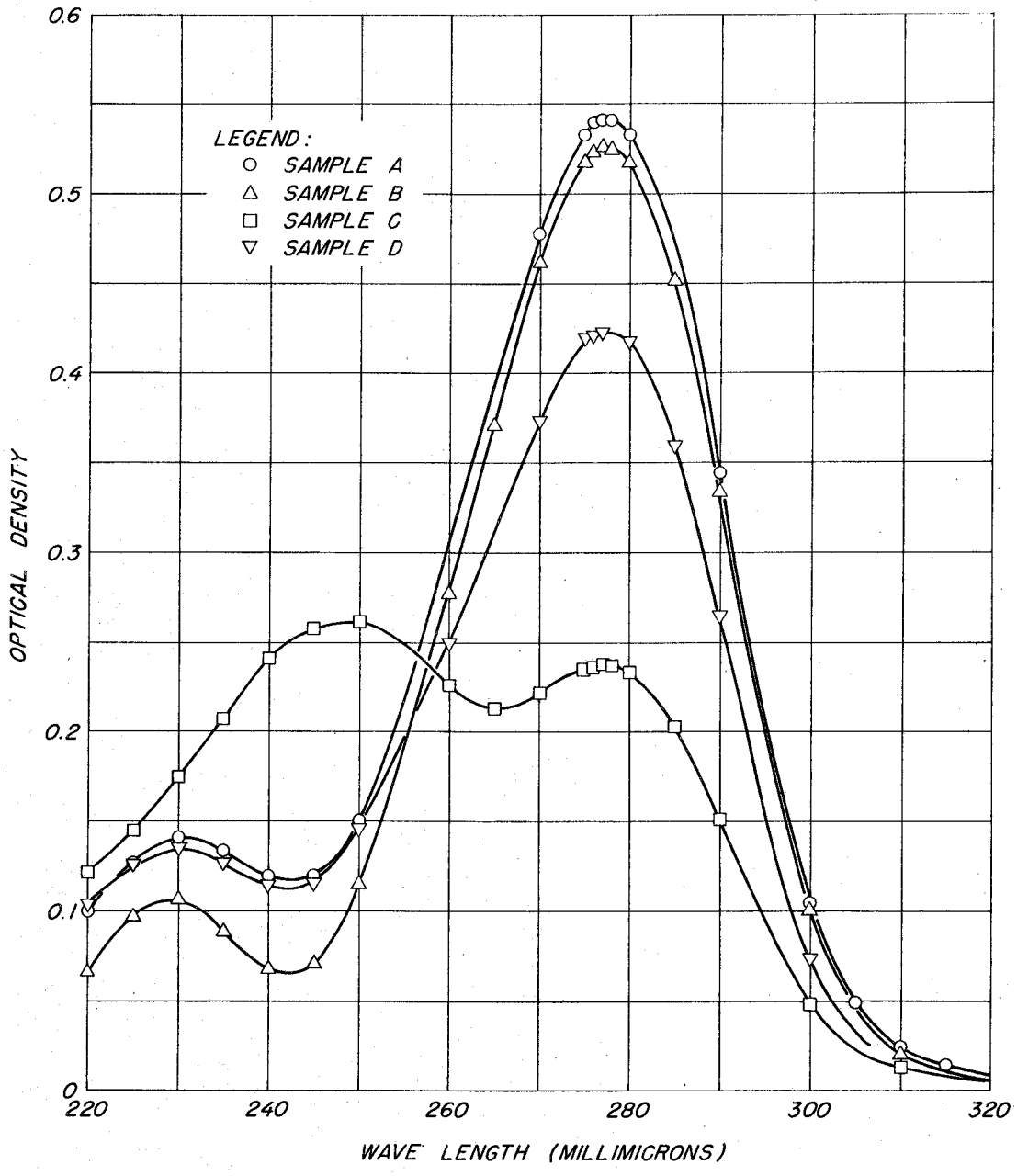

Figure 2.--The effect of storage conditions on the ultraviolet absorption spectra of dilute aqueous furfural solutions.

Curve A: Freshly prepared solution; 3.49 mg. per liter.

Curve B: Solution A stored in a refrigerator for 13 days.

Curve C: Solution A stored at room temperature away from direct sunlight for 13 days.

Curve D: Solution A stored at room temperature in absence of any light for 15 days.

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samples that stood for 24 hr. in stoppered glass bottles. Furfural studies by Dunlop[5] show that these changes are probably due to autoxidation and that they are accompanied by increasing acidity. Only a small amount of oxygen uptake results in considerable darkening, and nearly opaque furfural may still be quite pure.

2. Root[1] found that distilling 80% of the sample volume resulted in quantitative recovery of furfural with less than 1% average deviation.

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[5] Dunlop, A. P. Furfural formation and behavior. Indus. Engin. Chem. 40: 204. 1948.

DETERMINATION OF SOLUBLE HALIDES IN WOOD

Introduction

Hygroscopic chemicals are sometimes applied to green wood (especially hardwoods) in the seasoning process to prevent or reduce surface checking.[1] The most frequently used chemical agent is sodium chloride in either solid or solution form. If the salt content in the wood becomes high or is unbuffered, there is a serious corrosion problem when the wood is in contact with metal hardware and fastenings. For this reason, it is sometimes desirable to know the level of the halide (as sodium chloride) concentration in wood. Specifically, qualitative or quantitative halide tests have been used at the Forest Products Laboratory to determine the penetration of salt in the wood planking of boats, yachts, and ships that are used in sea water, and to determine the concentration of salt in wood beams and structural members of buildings for salt, fertilizer, and chemical plants and the wood floors and walls of trucks and trailers.

Qualitative Test

Reagents

1. Nitric acid, approximately 1:1.
2. Silver nitrate, 5% solution (5 g. per 100 ml. of solution).

Procedure

A small portion of wood sample (1 to 5 g. of sawdust, chips, shavings, or slices) is placed in a beaker and covered with distilled water. Soluble

[1] U.S. Forest Products Laboratory. Special methods of seasoning wood. FPL Rep. No. 1665-6. 1960.

halides are then leached out by allowing the sample to stand for 10 min., or if sample pieces are large, boiling for 5 min. Make the sample slightly acid (to litmus) with 1:1 nitric acid. Add a few drops of a 5% silver nitrate solution and observe the precipitate formed. In all wood samples, a slight turbidity will develop upon the addition of silver nitrate, but when the wood has been salt-seasoned, an easily observed, dense, white (or near white) precipitate forms.

Quantitative Test[2]

Principle

A form of the well-known Volhard titration procedure is used. The wood samples are extracted with water, diluted to suitable volume, and an aliquot is treated with excess standard silver nitrate solution. The organic matter is oxidized with potassium permanganate and nitric acid. The excess silver nitrate is titrated with standard potassium thiocyanate. Ferric ammonium sulfate is used as the indicator. Direct titration with silver nitrate is not used on extracts of wood because their color obscures the chromate end point.

Reagents

1. Approximately 0.1N silver nitrate. Reagent grade silver nitrate (16.989 g.) is dissolved in distilled water containing a few drops of nitric acid, filtered through a paper (Whatman No. 40), and made up to 1 liter with distilled water.

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[2]From Method No. 15.143. Official Methods of Analysis of the Association of Official Agricultural Chemists. 9th Ed. 1960.

2. Approximately 0.1N potassium thiocyanate. Potassium thiocyanate (9.719 g.), dried at 105° C. for 4 hr., is dissolved in distilled water and diluted to 1 liter.

3. Ferric ammonium sulfate indicator. 1N Nitric acid (100 ml.) is saturated with the salt at room temperature.

4. Nitric acid, free of nitrous acid. Equal parts of concentrated nitric acid and distilled water are mixed, boiled until colorless, cooled, and stored in a dark place.

5. Potassium permanganate (5%).

6. Nitrobenzene C.P.

Standardization

First, 15-20 ml. of silver nitrate are measured from a burette into a 250-ml. Erlenmeyer flask. Then, 5 ml. of the prepared nitric acid and 1 ml. of indicator are added. The silver nitrate is titrated with the potassium thiocyanate solution to the first brownish-red coloration which persists on shaking. The ratio $\frac{\text{ml. } AgNO_3}{\text{ml. KSCN}}$ at the equivalent point is computed.

Five to 10 ml. of a solution made from pure dry sodium chloride (0.1N, 5.845 g. per liter) is measured from a burette into a 250-ml. Erlenmeyer flask. This is acidified with 5 ml. of the nitric acid, then silver nitrate is run in from a burette until a 3-5-ml. excess is present (the silver nitrate and sodium chloride solutions are approximately equal in strength). Next, 1 ml. each of the indicator and of nitrobenzene, and the excess silver nitrate is titrated with the thiocyanate solution.

Volume thiocyanate times $\frac{\text{ml. } AgNO_3}{\text{ml. KSCN}}$ equals excess $AgNO_3$. Total volume $AgNO_3$ added to NaCl minus excess $AgNO_3$ then equals ml. $AgNO_3$ used by NaCl. Then $\frac{\text{ml. NaCl} \times 5.845}{\text{ml. } AgNO_3 \text{ used by NaCl}}$ equals mg. NaCl equivalent to 1 ml. $AgNO_3$.

Procedure

The ovendry wood sample (3-5 g., thin slices or shavings) is placed in a 500-ml. Erlenmeyer flask and 100 ml. distilled water is added. The wood is extracted at the simmering point (85° to 90° C. for 4 hr.). The sample is removed and rinsed. If the chloride concentration is known to be 1% or less, the entire extract may be titrated. If more than 1% of chloride is present, the extract is made to a convenient volume and a suitable aliquot is taken. The sample for titration is treated with 10 ml. of the nitric acid and an excess of the silver nitrate. It is heated to the boiling point, and 5% potassium permanganate is added dropwise until the brown color of the extractives fades to a clear yellow and the precipitate is well coagulated. It is then cooled to room temperature, indicator and nitrobenzene added, and the titration with thiocyanate is carried out as in the standardization.

The volume of excess silver nitrate is calculated as in the standardization, and this figure is deducted from the volume of silver nitrate added to the sample. The difference is the volume of silver nitrate required to precipitate the NaCl in the sample or the net titration.

$\frac{\text{Net titration} \times (\text{NaCl equivalence of } AgNO_3 \times 100}{\text{grams sample} \times 1000}$ equals percent soluble halide as NaCl.

Remarks

For high dilutions when the aliquot is practically water clear or for "sea water" samples, a direct titration with the silver nitrate may be used, provided that the sample is very near the neutral point (test with litmus). The method of Mohr (potassium chromate indicator) or that of Fajans (dichlorofluorescein indicator) is suitable.[3] The latter method gives the sharper end point (white to pink).

Tests with "sea water"-treated samples (slices approximately 0.075 in. thick) and matched slices ground to 40 mesh show that grinding offers no advantage over using the whole slices. In both cases, extraction of halide appears to be complete after a single extraction period; further extraction yields no detectable halogen. Thicker samples (for example, blocks) or wood of different species might require longer extraction or stepwise extraction. Likewise, the nitric acid-permanganate treatment might need to be modified or replaced by another oxidation procedure with woods which yield other types of extractives.

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[3] Pierce, W. G., and Haenish, E. L. Quantitative analysis. J. Wiley & Sons, N.Y., pp. 299-304. 1948.

HOLOCELLULOSE IN WOOD

Introduction

Holocellulose is the total carbohydrate fraction of wood.[1] It is white, cream, or straw-colored, depending upon the kind of wood it is derived from. Cross and Bevan cellulose is similar but does not contain the entire hemicellulose fraction, as some is extracted with the lignin. The chlorine-monoethanolamine method (see Part I) is most used for quantitatively measuring the amount of holocellulose in a sample. The chlorite-acetic acid method (see Part II) is usually used to prepare holocellulose because it is an easier method. However, the end point for the chlorite-acetic acid treatment is not sharply demarked; either some lignin remains in the product or some carbohydrate is lost.

I. The Chlorine-Monoethanolamine Method[2]

This method consists of treating extractive-free wood with chlorine and extracting with alcoholic monoethanolamine. The cycle is repeated until the lignin is removed (sample and extract become colorless). This method can be used for measuring the holocellulose content of a sample or for preparing holocellulose.

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[1] Ritter, G. J., and Kurth, E. F. Holocellulose, total carbohydrate fraction of extractive-free maple wood. Ind. and Eng. Chem. 25: 1250. 1933.

[2] Based on TAPPI Standard Method T9, 1954, and ASTM Standard D-1104, 1956.

Apparatus.--1. Crucibles Alundum, RA 98, or Pyrex crucibles with fritted glass bottoms of medium porosity and contained in weighing bottles. Alundum crucibles provide more filtering surface.

2. Weighing bottles of suitable size to contain the crucibles.

3. Chlorination apparatus (fig. 1).

4. A Soxhlet apparatus consisting of an extraction flask, 250-ml. capacity; a Soxhlet extractor; and a condenser.

Reagents.--1. Alcohol-benzene extraction solvent. Mix 1 volume of 95% ethyl alcohol with 2 volumes of C.P. benzene.

2. Alcohol-monoethanolamine solution. A 0.3% solution (by volume) of monoethanolamine in 95% ethyl alcohol.

3. Ethyl alcohol, 95%.

4. Chlorine gas.

5. Ethyl ether.

Test specimen.--The test specimen shall consist of at least 6 g. of air-dry extractive-free wood, ground to pass a 40-mesh sieve and be retained on a 60-mesh sieve.[3]

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[3]For extraction procedure, see: "Preparation of Extractives-Free Wood," in Appendix of this manual.

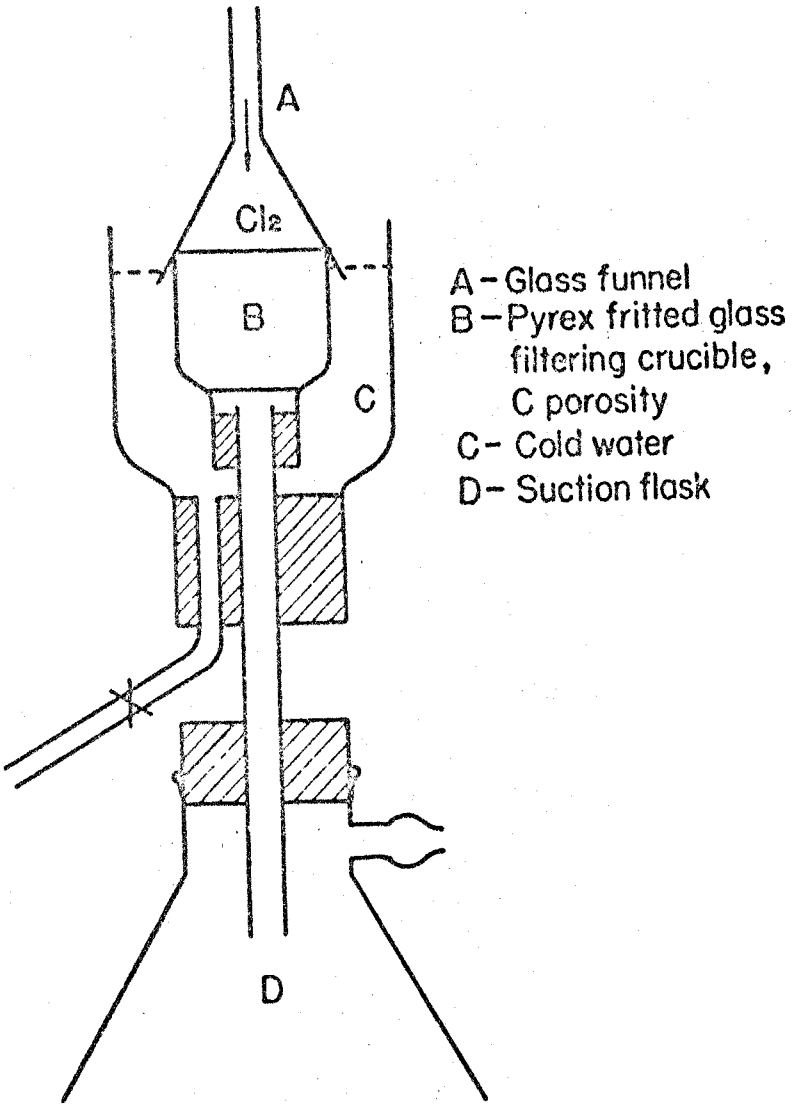

Figure 1.--Chlorinator for holocellulose.

in the Soxhlet apparatus. Transfer the wood to a Büchner funnel, remove the excess solvent with suction, and wash the thimble and wood with alcohol to remove the benzene. Return the wood to the extraction thimble and extract with 95% alcohol for 4 hr. or longer if necessary, until the alcohol siphons over colorless.

If the thimble is nearly full, place a Gooch crucible of suitable size in the rim of the thimble to keep the sample together. With each solvent, extract at a rate of not less than 4 siphonings per hour. Remove the wood from the thimble and spread it out in a thin layer until the alcohol evaporates. Transfer the material to a 1-liter Erlenmeyer or Florence flask and extract successively with three 1-liter portions of distilled water, heating the flask with each change of water for 1 hr. in the hot-water bath. Keep the water boiling before the addition of the wood and surround the flask in the bath with boiling water. After the final extraction with water is complete, filter on a Büchner funnel, wash with 500 ml. of boiling distilled water, and allow the extracted material to come to an air-dry condition.

Store the sample in an airtight container and determine the moisture content on a sample separate from the one used for holocellulose determination or isolation.

Procedure.--Weigh accurately, in duplicate, approximately 2 g. of ground, extractive-free, air-dry sample in a tared glass crucible contained in a

weighing bottle. Determine the moisture content on a separate sample taken from the remainder of the test specimen.

Using slight suction, chlorinate the sample by passing chlorine gas through a funnel inverted over the crucible which is in position on a suction flask. Keep the crucible and contents cool with ice water. After chlorinating for 3 min., remove the inverted funnel, stir the wood thoroughly, and rechlorinate for 2 min.

Add alcohol to dissolve excess chlorine and hydrochloric acid and, after 1 min., remove by suction. Release the vacuum, drain off the ice water, add sufficient hot alcohol-monoethanolamine (75° C. or higher) to cover the wood completely, and stir thoroughly. Let the solution stand 2 min.; then remove by suction. Repeat the solvent treatment. Remove any remaining solvent by washing twice with 95% alcohol; then twice with cold water. Remove the wash liquids by suction.

The above procedure is not sufficient to remove all the lignin, so repeat the treatment of the moist sample with chlorine and subsequent extraction and washing as outlined above until the color of the residue is white or fails to change with additional chlorination. The second and following treatments with chlorine (after washing the specimen in the crucible with distilled water) should not require more than 2 or 3 min. Prolonged action of chlorine gas, together with the hydrochloric acid formed in the secondary reactions, hydrolyzes the holocellulose and gives low yields.

After all the lignin has been removed, wash the fibers twice with alcohol to remove the alcohol-ethanolamine, twice with cold (10° C.) water, and again with alcohol until the residue is neutral to litmus. Finally, wash thoroughly with ether to remove all the alcohol and to facilitate drying.

Air-dry the holocellulose to remove excess ether and then dry for 2-1/2 hr. at 105°±3° C. in an oven. (If subsequent analyses or tests are to be made on the holocellulose, it should be dried at 60° C. under vacuum.) Finally place the tared crucible in the original stoppered weighing bottle, cool in a desiccator over calcium sulfate, and weigh.

Report.--The result shall be calculated and reported as percentage by weight of holocellulose in the moisture-free wood on either an unextracted or extractive-free basis.

Additional information.--1. A fraction comparable to Cross and Bevan cellulose can be obtained and measured from the holocellulose as follows:[4]

Place the crucible and contents from the holocellulose determination (dried under vacuum at 60° C.) in a 600-ml. beaker and add 200 ml. of boiling 1.3% H_2SO_4. Place the beaker and contents in a boiling water bath having a liquid level coinciding with that of the solution in the beaker, and continue hydrolysis for 2 hr. Maintain the original levels of the acid solution and the water bath by the addition of water.

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[4]Van Beckam, W. G., and Ritter, G. J., Rapid methods for the determination of holocellulose and cross and bevan cellulose in wood. Paper Trade J. 105: 18-127. 1937.

Remove the crucible from the acid and wash back into the beaker with distilled water any of the suspension remaining on its outer surface. Filter the acid mixture through the same crucible and wash the hydrolyzed holocellulose residue with hot water under suction until neutral to litmus, then with 20 ml. of alcohol, and finally with 20 ml. of ether. Air-dry the residue to remove the excess ether and then dry to constant weight in an oven at 105°±3° C. Calculate the weight as the percentage of Cross and Bevan cellulose on the basis of the moisture-free specimen.

2. The lignin content, determined by the sulfuric acid method, plus the holocellulose content of the moisture-free wood, should theoretically equal 100%. Kurth's[5] data using the chlorine-monoethanolamine method correspond to theoretical values to within less than 1.0%.

II. The Chlorite-Acetic Acid Method[6] [7] [8]

Warm acidified sodium chlorite liberates chlorine dioxide which, in turn, attacks lignin to form soluble products. Carbohydrate is attacked to a very limited degree under these conditions and can be filtered off and retained as holocellulose.

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[5] Kurth, E. F., Chemical analysis of western woods, Part I. Paper Trade J. 126(6): 56. 1948.

[6] Jayme, G., Preparation of holocellulose and cellulose with sodium chlorite. Cellulosechem., 20: 43. 1942. (Mar.-Apr.).

[7] Wise, L. E., Quantitative isolation of hemicelluloses from coniferous woods. Ind. and Eng. Chem., Anal. Ed. 17: 63. 1945.

[8] Wise, L. E., Murphy, M., and D'Addieco, A. A., Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Paper Trade J. 122(2): 35. 1946.

Test specimen.--Samples are ground and extracted in the same way as in the chlorine-monoethanolamine method described in Part I.

Apparatus.--Crucibles, Alundum RA 98, or Pyrex crucibles with fritted glass bottoms of coarse porosity.

Reagents.--1. Sodium chlorite: 1.5 g. sodium chlorite for each 160 ml. water.

2. Glacial acetic acid.

Procedure.--Five-gram samples of ground (to pass a 40-mesh and be retained on a 60-mesh screen) air-dry extractive-free wood are used. The wood is treated in a 250-ml. Erlenmeyer flask with 160 ml. of water containing 1.5 g. of sodium chlorite and 10 drops (0.5 ml.) of glacial acetic acid and heated under a hood for 1 hr. at 70° to 80° C. The flask is closed loosely by an inverted 25-ml. Erlenmeyer and rotated gently at intervals during the reaction. After 1 hr., 10 drops of acetic acid and 1.5 g. of sodium chlorite are added without cooling the mixture, and the heating continued for an additional hour.

Four such treatments are usually required for coniferous woods, whereas three treatments suffice for most hardwoods and annual plants. In testing new materials, orienting experiments may be necessary to determine the number of treatments that will be required. It is desired to reduce the lignin content of the holocellulose only to within the range of 2% to 9%. After the chloriting is completed, the residue should be white, and it should retain the original woody structure. The suspension is cooled immediately in an ice bath

and filtered through a coarse, fritted glass crucible. The residue is washed repeatedly with ice water followed by acetone, and air dried. The residue is then dried at 105° C. for 1 hr., weighed, and reported as percentage by weight of holocellulose in moisture-free wood on either an unextracted or extractive-free basis.

HYDROXYMETHYLFURFURAL ANALYSIS[1]

Introduction

5-Hydroxymethyl-2-furaldehyde is formed by the acid-catalyzed dehydration of glucose, sucrose, or fructose. The reactions involved are shown in fig. 1. In this analysis the hydroxymethylfurfural (HMF) is isolated by paper chromatography and then quantitatively measured by UV absorption spectrophotometry.

The solvent system used in the isolation of 5-hydroxymethyl-2-furaldehyde has been suggested by Buch, Montgomery, and Porter[2] and is the organic layer which results from the mixing of equal volumes of 5M aqueous formic acid and pentanol. This irrigating system results in a compact band of 5-hydroxymethyl-2-furaldehyde with an R_f value of 0.75. Levulinic acid, a byproduct of the sugar-HMF reaction, also has an R_f value of 0.75 with this solvent system but does not interfere in the analysis because its molar absorptivity is approximately 1/1000[3] that of HMF which has been determined to be 16,920[1] at 284 mμ.

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[1]Based on: McKibbins, S. W., Harris, J. F., and Saeman, J. F., A sample applicator for chromatographic paper and its use for 5-hydroxymethyl-2-furaldehyde and levulinic acid analysis. J. Chromatogr. 5: 207. 1961.

[2]Buch, M. L., Montgomery, R., and Porter, W. L., Identification of organic acids on paper chromatograms. Anal. Chem. 24: 489. 1952.

[3]McKibbins, S. W., Kinetics of the acid-catalyzed conversion of glucose to 5-hydroxymethyl-2-furaldehyde and levulinic acid. A thesis submitted in partial fulfillment of the requirements for the degree of Ph.D. (Chemical Engineering), University of Wisconsin. 1958.

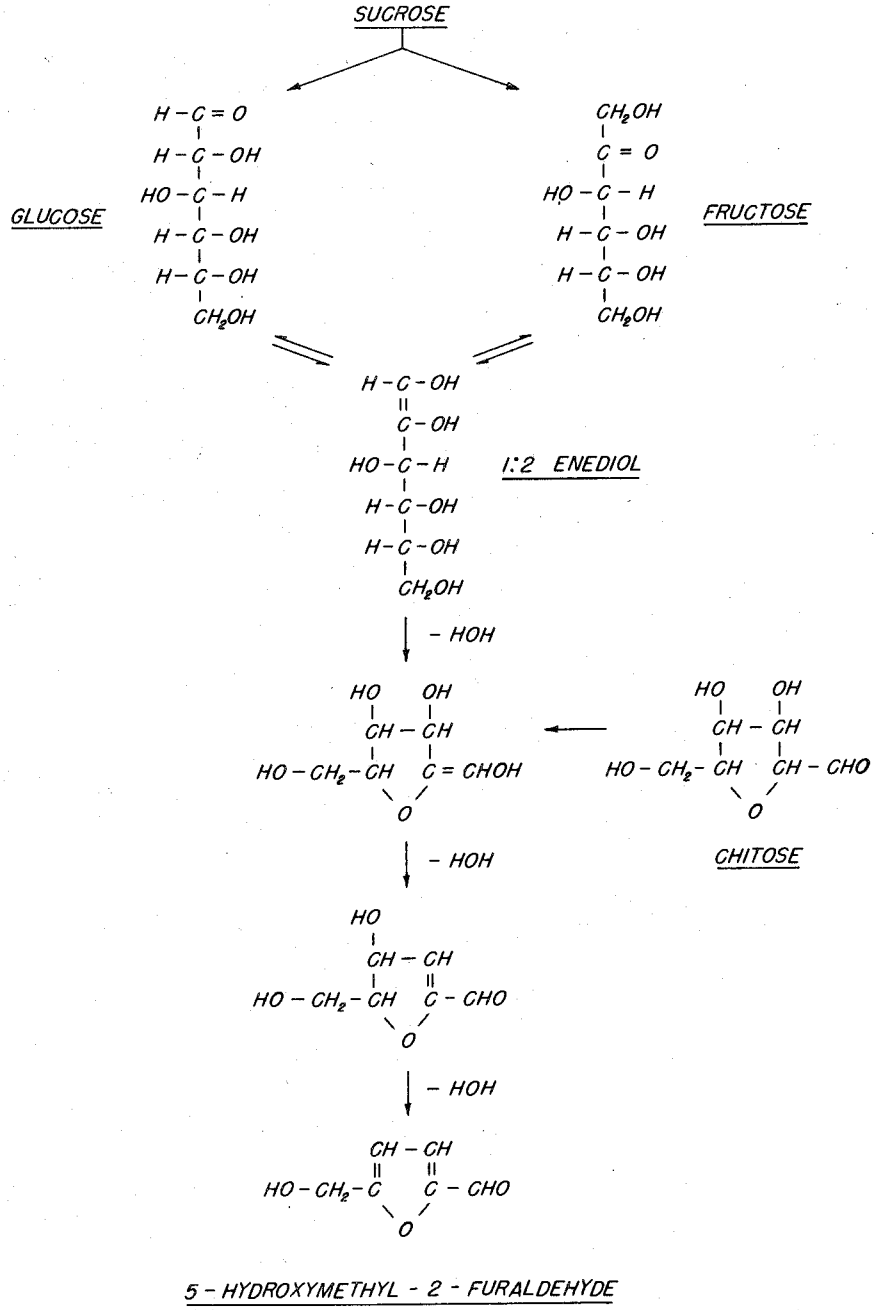

Figure 1.--Mechanism of the formation of 5-hydroxymethyl-2-furaldehyde from sucrose proposed by Wiggins (Wiggins, L., The preparation of levulinic acid from sugar, The Sugar Res. Found. Inc., England (1948)).

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Procedure

Using a pipet or the streaker (fig. 2) described by McKibbins, et al.,[1] apply solutions containing hydroxymethylfurfural to 18- by 22-in. sheets of Whatman No. 3MM chromatographic paper. Irrigate the sheets at 30° C. for 15 hr. with the formic acid-pentanol solvent. Dry the sheets and locate the band of 5-hydroxy-methyl-2-furaldehyde with the aid of a UV lamp (the hydroxymethylfurfural appears dark on the light background).

Four tabs containing samples, each 75 mm. wide, are then cut from this band and placed into individual beakers containing predetermined quantities of distilled water to elute the 5-hydroxymethyl-2-furaldehyde. (Concentrations of original samples and elution volume are adjusted to result in an absorbance reading of from 0.2 to 0.7 units.)

The hydroxymethylfurfural concentrations are calculated from the absorbance of the solutions at 284 mμ by reference to a prepared standard calibration curve made up from pure knowns. The very dilute solutions of 5-hydroxymethyl-2-furaldehyde required by the spectrophotometer show a tendency to degrade when stored in sunlight at room temperature. This degradation will not occur significantly if they are kept under refrigeration and in the dark. In practice, direct sunlight and long delays in analysis should be avoided.

Additional Information

McKibbins, et al.,[1] found that approximately 8% of the HMF applied was not recovered. A detailed investigation into the possible sources of this loss,

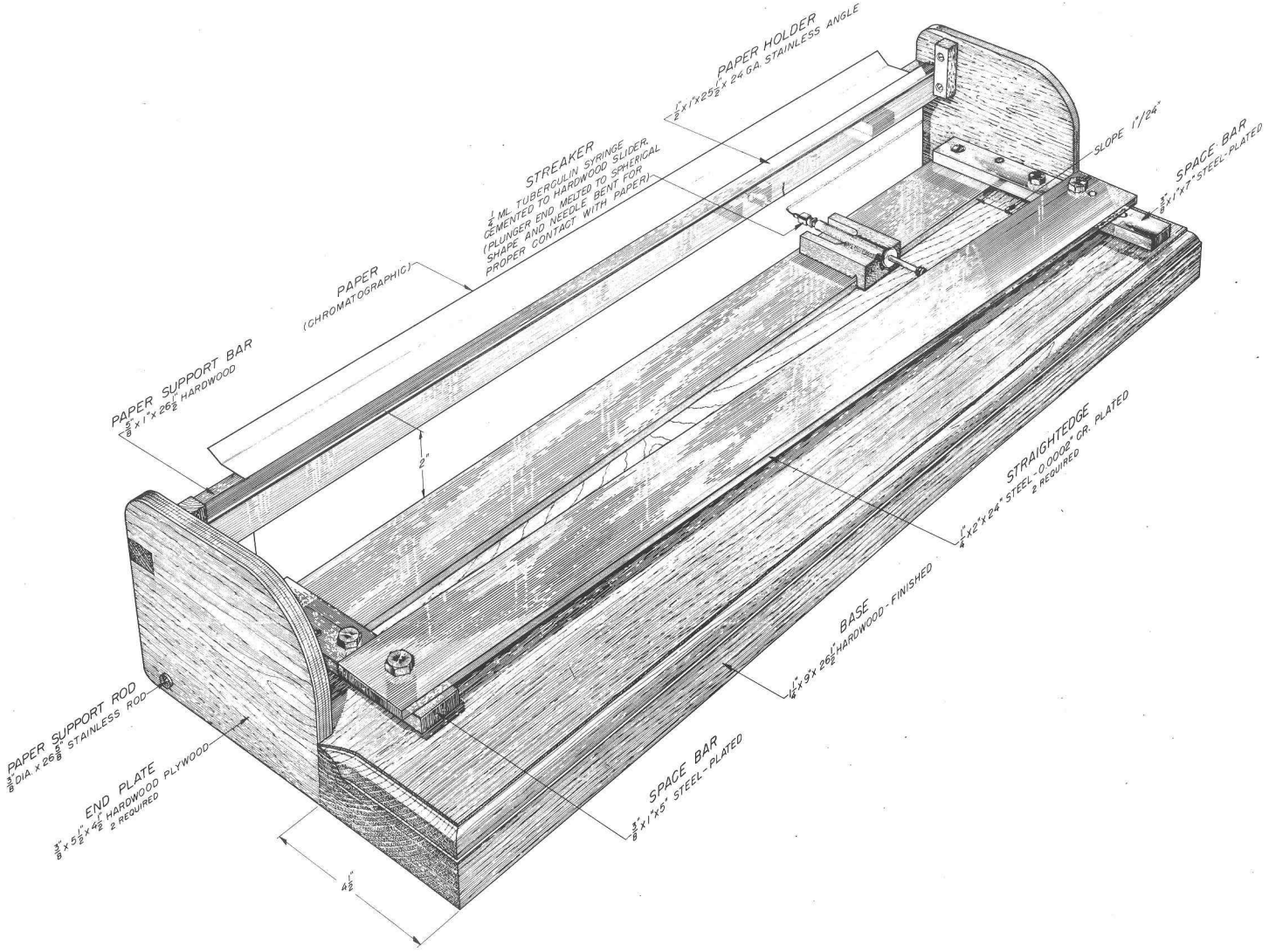

Figure 2.--Sample applicator for chromatographic paper.

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including several chromatographic separations of the same sample of 5-hydroxymethyl-2-furaldehyde, seemed to indicate that the loss is due to its volatilization during the removal of water after streak application prior to irrigation. Since the loss is constant over a several-fold change in concentration and is satisfactorily compensated for, a calibration curve may be used with confidence over the range prescribed.

McKibbins also studied the behavior of several additional similar compounds which might result from the acid-catalyzed degradation of glucose; the properties measured in this investigation are summarized in table 1 and fig. 3. It will be noted that the chromatographic behavior is quite similar for all the compounds, but that the absorption spectra differ sufficiently to at least allow differentiation.

Table 1.--Properties of 5-hydroxymethyl-2-furaldehyde and related compounds[1]

Compound	Melting point	Wavelength at major peak	Molar Absorptivity 1/mole x cm.	R_f
	°C.	Mμ		
5-Hydroxymethyl-2-furaldehyde	33.4	284	16,920	0.75
Oxy-bis-(5-methylenefurfural)	114.5	282	28,600	.84
5-Hydroxymethyl-2-furoic acid	160-161 (dec.)	251	12,420	.74
2-Hydroxyacetylfuran	82.5	276	14,090	.73
Levulinic acid	33.5	270	25.1	.75

[1]Irrigation solvent system is the organic layer from an equivolumetric mixture of 5M aqueous formic acid and pentanol.

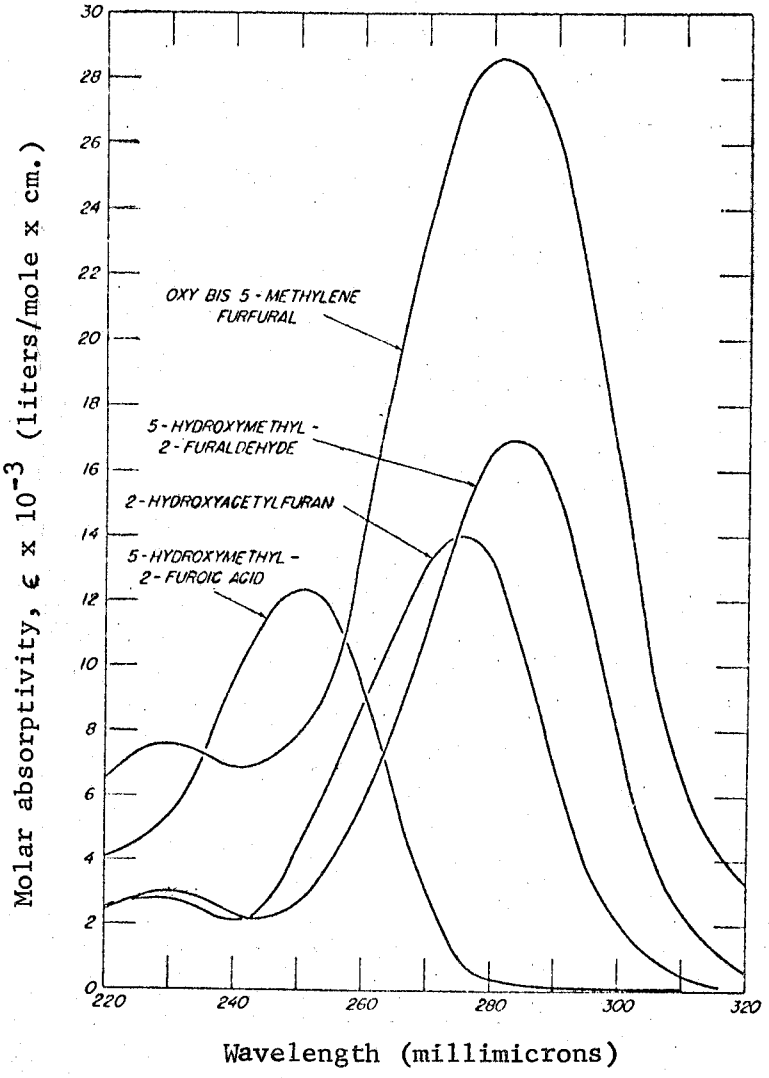

Fig. 3.--Ultraviolet absorption spectra for 5-hydroxymethyl-2-furaldehyde and related compounds.

DETERMINATION OF THE ION-EXCHANGE CAPACITY OF PULPS[1]

Introduction (2)

Both cellulose and cellulose nitrate are known to have ion-exchange capacity. In wood pulps, this exchange capacity is associated with the presence of carboxyl groups, either produced by oxidation during alkaline pulping or bleaching operations, or already present in the polyuronide portion of hemicellulose. Under certain conditions, carbonyl groups also have been shown to take part in ion-exchange. With nitropulps, the situation is more complex: carboxyl groups present in the starting pulps may persist through nitration and stabilization, additional carboxyl groups may be formed by oxidation during nitration, and acidic groups such as sulfuric acid half-esters may be produced during nitration and remain in the final nitropulp. Therefore, in the utilization of wood pulps in production of cellulose nitrates and in studying the variables of the cellulose nitrate stabilization process, it is of interest to determine the ion-exchange capacities of pulps and nitropulps.

Numerous methods have been suggested for the determination of the carboxyl groups in cellulose (3). Extensive reviews have been published by Davidson and Nevell (4) and by Jayme and Neuschaffer (5). Some of these methods have also been applied to the measurement of the ion-exchange capacity of cellulose nitrates (6,7,8).

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[1] Taken from the method of Sobue and Okubo (1) as modified by Millett, Schultz, and Saeman (2).

The more recent procedures call for a careful pretreatment of the sample with dilute mineral acid or with carbon dioxide-saturated water to remove cations already bound to the carboxyl groups. This de-ashing step is then followed by the determination of the carboxyl groups. Carboxyl content is measured in a number of ways, such as direct alkaline titration (9); addition of a salt solution followed by titration of the acid liberated by the exchange reaction (4,10,11,12,13); or measurement of the decrease in cation concentration of the added salt solution (1,14).

Chromatographic techniques are usually highly effective in establishing equilibria of the type necessary for de-ashing, washing, and saturating the exchange sites of cellulose. Chromatographic procedures also have the advantages of speed and convenience (15). The method of Millett, Schultz, and Saeman (2), which is described here, utilizes a chromatographic apparatus which is a modified version of that proposed by Sobue and Okubo (1). They evaluated the variability of this apparatus statistically. The method was applied to the study of ways in which ion-exchange capacity is influenced by pulp preparation variables. Exchange capacities of nitropulps were measured as a function of nitrogen content and stabilization level. As a contribution to the efforts of the Joint ACS-ASTM Committee on Functional Groups, exchange capacity data were also obtained on five International Committee for Cellulose Analysis (I.C.C.A.) reference pulps that were selected as "standards" for physical and chemical measurements. The exchange capacity data are presented in table 1 to give an approximate range of the carboxyl content of pulps.

Table 1.--Ion-exchange capacity data obtained by the Forest Products Laboratory on the ICCA pulps

ICCA sample No.	Description of pulp	Ion-exchange capacity (millimoles of Ca/100 gm. pulp)						
		1	2	3	4	5	6	Average
1	Acetate cotton linters	0.25	0.25	0.25	0.25	0.28	0.27	0.26
2	Acetate sulfite pulp	.89	.91	.88	.92	.94	.92	.91
3	Prehydrolysis sulfate pulp (tire cord)	.78	.77	.77	.80	.79	.77	.78
4	Rayon sulfite	1.05	1.01	1.02	1.02	1.06	1.04	1.03
5	Cellophane sulfite	1.48	1.29	1.32	1.36	1.29	1.26	1.33
6	Rayon sulfite birch	2.20						2.20
7	Paper sulfite	3.18	3.09					3.14
8	Greaseproof sulfite	3.78	3.74					3.76
9	Rayon sulfite birch	1.68	1.72	1.82				1.74

Principle

The exchange capacity (carboxyl content) is determined by passing a dilute calcium acetate solution through a previously de-ashed pulp column supported on a sintered glass filter disk, and measuring the decrease in calcium concentration by direct titration with a standard solution of ethylenediaminetetraacetic acid (EDTA). The sample size is so adjusted so that only about one-fourth of the calcium ions are bound, thus insuring complete exchange.

Procedure

Reagents

1. Deionized water.--Prepared by passing distilled water through a Barnstead Bantam demineralizer. A glass tube packed with about 2 in. of absorbent cotton is attached to the outlet side of the demineralizer. This filters out resin fines which would strongly interfere with exchange measurements.

2. EDTA solution.--Dissolve exactly 1 g. disodium ethylenediaminetetracetate dihydrate (Baker analyzed reagent) + approximately 0.025 g. $MgCl_2 \cdot 6H_2O$ in 1 liter de-ionized water.

3. Buffer solution.--Dissolve 67.5 g. NH_4Cl in 570 ml. analytical-grade NH_4OH. Make up to 1 liter with de-ionized H_2O.

4. Indicator.--Dissolve 0.5 g. Eriochrome Black T (Baker analyzed reagent) + 4.5 g. hydroxylamine hydrochloride in 100 ml. of methanol.

5. Calcium solutions.--Dissolve 0.33 g. $Ca(CH_3COO)_2 \cdot H_2O$ in 3 liters de-ionized water. Adjust pH to 6.5⟶7.0 by addition of 1 percent CH_3COOH and of supernatant liquid from a centrifuged saturated solution of $Ca(OH)_2$. This calcium acetate solution contains approximately 2500 µg. Ca^{++} per 100 ml.

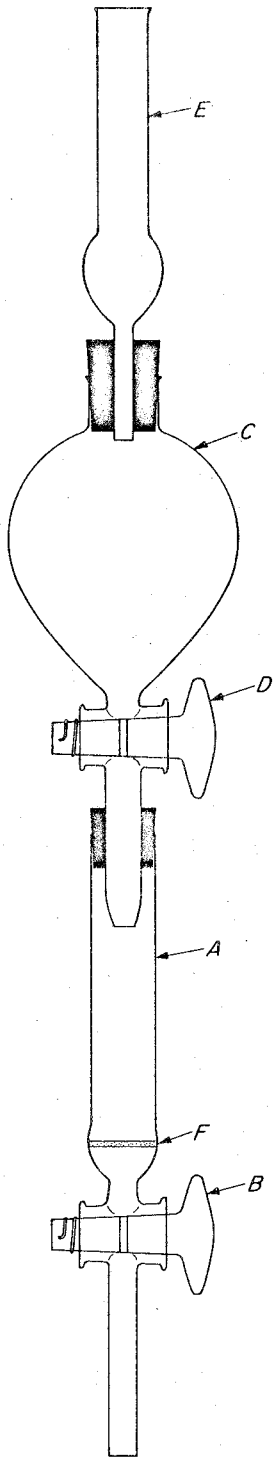
6. De-ashing solution.--Approximately 1:100 HNO_3:H_2O.

Sample Preparation

Approximately 5 g. of sheeted pulp are torn into small pieces and dispersed in 1 liter of distilled water by means of a high-speed mixer. The pulp slurry is filtered on a Büchner funnel and washed with an additional liter of distilled water. As much water as possible is removed by evacuation without squeezing. The pulp mat is then broken up and spread out on an aluminum foil sheet to dry for about 1 hr. under an electric fan, after which it is mechanically fluffed. The fluffed pulp is again spread out on an aluminum sheet and allowed to condition for 3 days in a controlled humidity room. Moisture determinations are made to ascertain the weight of air-dried sample equivalent to a desired weight of ovendry sample.

De-ashing

The empty glass exchange column (A) (fig. 1) is dried for 1 hr. at 110° C. and weighed to the nearest milligram. The column is then tightly packed with the desired dry weight of pulp. A solution reservoir (C) filled with 1:100 HNO_3:H_2O, which has previously been boiled under vacuum to remove dissolved



LEGEND:
A - GLASS EXCHANGE COLUMN (18 MM. O.D. - 4" LONG)
B - OUTLET STOPCOCK (NO. 2)
C - SOLUTION RESERVOIR (125 C.C. SEPARATORY FUNNEL)
D - INLET STOPCOCK (NO. 2)
E - ASCARITE TUBE
F - SINTERED-GLASS DISC (COARSE)

Figure 1.--Equipment for column ion-exchange capacity measurements.

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air, is inserted in the top of the column. With stopcock (D) closed, the column is evacuated for approximately 1 min. through the outlet stopcock (B). At the end of this time stopcock (B) is closed and inlet stopcock (D) is opened allowing the acid to completely fill the column. This assures complete wetting of all fibers with solution and eliminates air bubbles. The solution reservoir is removed and the pulp sample is again packed down in the column with a glass rod flattened on one end. Excess acid is allowed to flow out through (B) leaving only a head of about 0.5 cm. above the pulp column. The solution reservoir containing 125 ml. 1:100 HNO_3:H_2O is reinserted tightly into the top of the column and the inlet stopcock (D) is opened fully. The outlet stopcock (B) is opened sufficiently to allow solution to drain through the column at a rate of about 30 ml. per hour. Stopcock (B) is closed just as the last of the acid solution enters the pulp column. The solution reservoir is then removed and the pulp is washed acid-free with successive portions of de-ionized water from a wash bottle. (About 50 ml. is usually sufficient.) With stopcock (B) closed, all water is removed from its stem with a pipe cleaner. The column is then weighed to the nearest milligram to determine the weight of water retained by the pulp. A rubber stopper inserted in the open end of the column immediately after weighing prevents loss of water by evaporation.

Uptake of Calcium

Immediately after weighing, the solution reservoir containing about 125 ml. of calcium acetate solution is attached to the column. Before the reservoir is firmly seated, sufficient solution is admitted to the column to

give about a 0.5 cm. head of liquid above the pulp bed. The calcium solution is then allowed to flow through the pulp bed in the same manner as was the de-ashing solution. Exactly 100 ml. of effluent are collected in a volumetric flask.

The flask contents are thoroughly mixed, after which two 25-ml. aliquots are pipetted into 125-ml. Erlenmeyer flasks. Two aliquots of the original calcium solution are also taken. To each aliquot 1 ml. of buffer solution and 3 drops of indicator solution are added, then the samples are titrated with the EDTA solution. At the end point, the lavender color of the solution changes to turquoise. A blank of de-ionized water containing indicator and buffer serves as a color standard. The EDTA is delivered from a 5-ml. microburette fitted with a fine polyethylene tip.

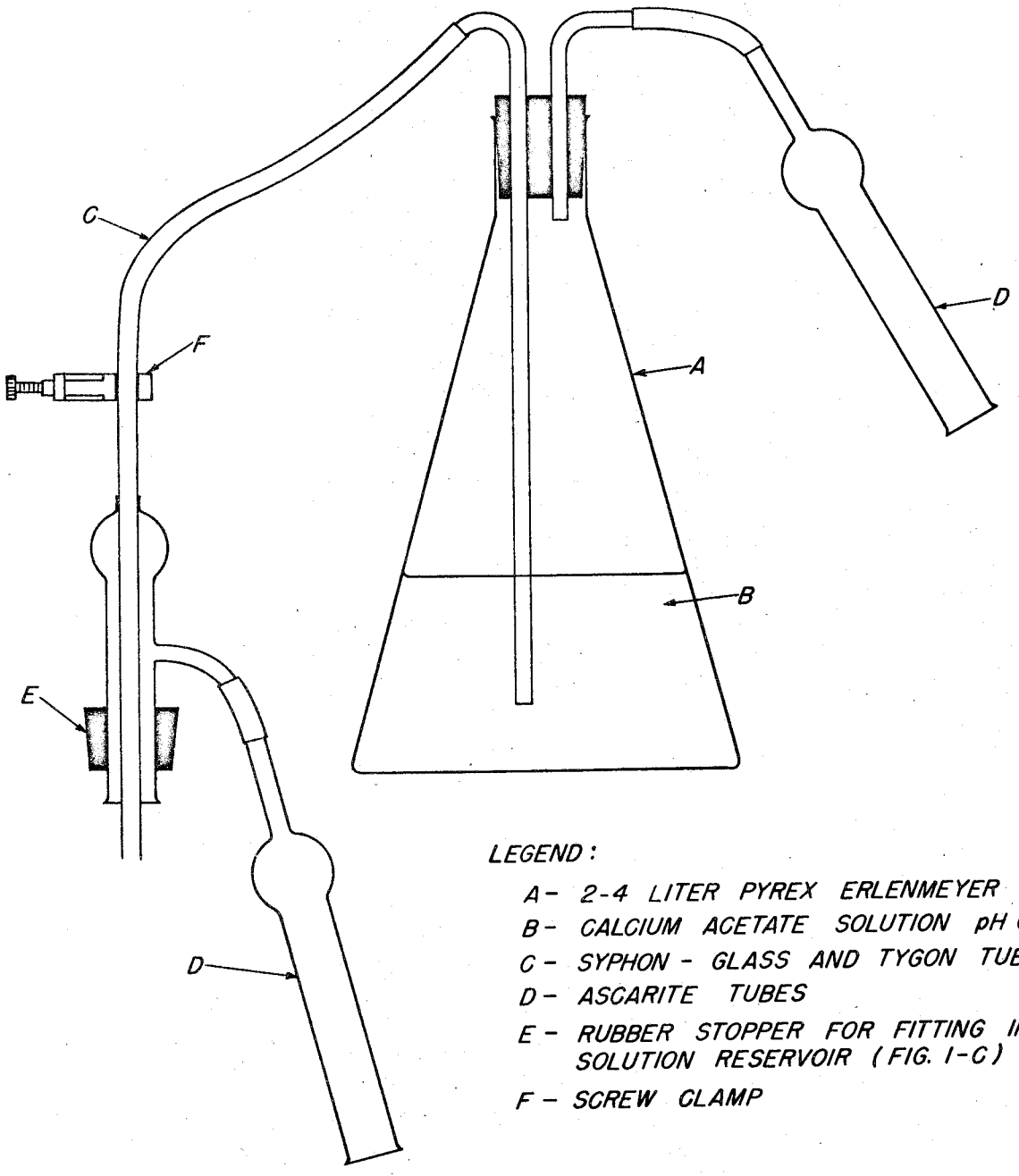
Figure 2 shows the container used for storing and dispensing the stock calcium acetate solution. In filling the column reservoirs, rubber stopper (E) is inserted in the top of the reservoir and screw clamp (F) is opened to permit the calcium solution to siphon into the reservoir. Carbon dioxide protection is afforded by ascarite tubes (D).

Calculations

The interpretation of data on the ion-exchange capacity of pulps in terms of carboxyl content, when measured by this method, is complicated by the fact that the precise exchange reaction involved is not known. Two reactions of the divalent calcium ions with carboxyl groups are possible:

1. $RCOOH + Ca(OAc)_2 \longrightarrow RCOOCaOAc + HOAc$
2. $R(COOH)_2 + Ca(OAc)_2 \longrightarrow R(COO)_2Ca + 2HOAc$

FIGURE 2 - CATION SOLUTION CONTAINER



LEGEND:

A - 2-4 LITER PYREX ERLENMEYER
B - CALCIUM ACETATE SOLUTION pH 6.5-7.0
C - SYPHON - GLASS AND TYGON TUBING
D - ASCARITE TUBES
E - RUBBER STOPPER FOR FITTING INTO SOLUTION RESERVOIR (FIG. 1-C)
F - SCREW CLAMP

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By reaction No. 1, 1 calcium ion is bound per carboxyl group. By reaction No. 2, 1/2 calcium ion is bound per carboxyl group. The relative proportion of calcium that is consumed by reaction No. 2 depends upon the number of carboxyl groups that are sufficiently close for reaction No. 2 to occur. If all carboxyl groups are isolated, only reaction No. 1 is involved.

It is evident that this difficulty is avoided in the calcium acetate method in which ion-exchange capacity is determined by titration of the liberated acetic acid; 1 acetic acid group corresponds to 1 carboxyl group regardless of which reaction is involved. The use of a monovalent salt in the cation depletion method for determining ion-exchange would also avoid this complication.

Because of the uncertainties in the exchange reactions, the ion-exchange capacities obtained by this method are expressed in terms of millimoles of calcium bound per 100 g. of material, whether pulp or nitropulp. The calculations employed are as follows:

x = milliliters EDTA solution required to titrate 25 ml. of $Ca(OAc)_2$ solution before ion exchange. 1 ml. of the EDTA solution is equivalent to 0.1076 mg. of calcium.

y = milliliters EDTA solution to titrate 25 ml. of $Ca(OAc)_2$ solution after ion exchange.

The volume y must be corrected for the water content of the sample at the time of treatment with the $Ca(OAc)_2$ solution. For 100 ml. of $Ca(OAc)_2$ solution, the correction is:

$$\frac{\text{ml. } H_2O \text{ in tube}}{4} \cdot \frac{x}{25}$$

The working equation for 100 ml. of $Ca(OAc)_2$ solution is:

$$\frac{x - y + \frac{\text{ml. } H_2O \text{ in tube} \cdot x}{100}}{\text{sample weight}} \cdot 4.100 =$$

ml. EDTA equivalent to Ca bound by 100 g. of sample

Multiplying this by $\frac{0.1076}{40}$ gives the results in terms of millimoles of calcium bound per 100 g. of sample.

Notes

1. Using this modified method, 1 operator can conveniently take care of 12 exchange columns at a time.

2. A complete or leveling-off exchange value is attained only at calcium ion exchange of less than 30 pct. of that available. To provide a margin of safety, sample size should be so adjusted that no more than one-fourth of the total available calcium ion content is consumed during ion exchange.

3. A major part of the variability of the method can be ascribed to the titration step. A suitable refinement of this step would lead to a substantial improvement in the precision of the method.

References

(1) Sobue, H., and Okubo, M.
1956. Determination of carboxyl group in cellulosic materials with the "Dynamic ion-exchange method." Tappi 39: 415.

(2) Millett, M. A., Schultz, J. S., and Saeman, J. F.
1958. Measurement of ion-exchange capacity of some wood pulps and nitro pulps. Tappi 41: 560.

(3) TAPPI-ASTM-ICCA Task Group
1962. Carboxyl content of cellulose. C.A. No. 8017. Tappi 45(10): 142A.

(4) (a) Davidson, G. F., and Nevell, T. P.
1947. Acidic properties of cotton cellulose and derived oxycelluloses. Part I. Shirley Inst. Mem. 21: 41.

(b) _____ and Nevell, T. P.
1948. Acidic properties of cotton cellulose and derived oxycelluloses. Part I. J. Text. Inst. 39: T59.

(5) Jayme, G., and Neuschaffer, K.
1955. Determination of carboxyl groups in pulps. Das Papier 9: 143.

(6) Keirstead, K., and Myers, J.
1954. A study of the sulfate ester of unstabilized cellulose acetate. Can. J. Chem. 32: 566.

(7) Gagnon, P. E., Keirstead, K. F., Walker, J., and Kavanaugh, K.
1956. Hydrolysis of cellulose acetate sulfate in acetone-water solutions. Can. J. Technol. 34: 71.

(8) _____, Keirstead, K. F., and Yamasaki, D.
1957. Determination of sulfate acid ester in unstabilized cellulose nitrate. Can. J. Technol. 34: 477.

(9) Yackel, E. C., and Kenyon, W. O.
1942. The oxidation of cellulose by nitrogen dioxide. J. Amer. Chem. Soc. 64: 121.

(10) (a) Ant-Wuorinen, O., and Visapää, A.
1956. Application of an automatic titration method in the study of the behavior of cellulose towards different salt solutions. Pap. and Timber (Finland) 38: 327.

(10) (b) Ant-Wuorinen, O., and Visapää, A.
1956. The dependence of the functional group determination results of cellulose on its state of accessibility. Pap. and Timber (Finland) 38: 479.

(c) _____ and Visapää, A.
1957. The effect of steeping cellulose in acid on the accessibility of its functional groups. Pap. and Timber (Finland) 39: 229.

(11) (a) Samuelson, O., and Wennerblom, A.
1955. Determination of carboxyl groups in cellulose. Svensk Papperstid. 58: 713.

(b) _____ and Wennerblom, A.
1957. Note on the determination of carboxyl groups in cellulose. Svensk Papperstid. 60: 706.

(12) Wilson, K.
1948. Determination of carboxyl groups in cellulose. Svensk Papperstid. 51: 45.

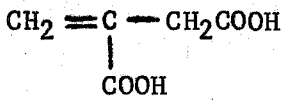
(13) Lüdtke, M.
1935. The acid content of rayons and its determination. Angew. Chem. 48: 650.

(14) Doering, H.
1956. Determination of carboxyl groups in pulps with complexon. Das Papier 10: 140.

(15) Ultee, A. J., and Hartel, J.
1955. Chromatographic determination of carboxyl groups in filter paper. Anal. Chem. 27: 557.

DETERMINATION OF ITACONIC ACID[1]

Itaconic acid is a dibasic acid with the structure:



This acid is produced by certain organisms in the fermentation of the wood sugars glucose and xylose.

Apparatus

1. A spectrophotometer equipped to measure color intensities at 385 mμ.

2. A water bath set at 32° ±0.25° C., equipped with a stirrer to dissipate the heat generated by the reaction.

3. Automatic pipets for dispensing pyridine and acetic anhydride.

Reagents

Itaconic acid (or its salt) for preparing the standard curve.

Procedure

To a test tube add 1.00 ml. of standard, water (for reagent blank), or sample (containing 1 to 100 μg. of itaconic acid. Add 1.30 ml. pyridine and swirl the tube. Add 5.70 ml. of acetic anhydride, swirl the tube once, and immediately place it in the constant temperature bath at 32° C. Color development is complete after 30 min. and remains relatively constant for

[1] Taken from Hartford, C. G. "Rapid spectrophotometric method for the determination of itaconic, citric, aconitic, and fumanic acids." Anal. Chem. 34: 426 (1962).

25 min. at 32° C. or in air at 22° to 32° C. Read the absorptivity of the solution at 385 mμ correcting for the blank and any interfering substances. Itaconic acid content of the sample is computed from the prepared calibration curve. Results are reproducible within 0.3 percent.

DETERMINATION OF LACTIC ACID[1] [2] [3]

Introduction

This method is designed for the measurement of lactic acid in wood sugar fermentation solutions that contain acetaldehyde, sodium bisulfite, and acetaldehyde-bisulfite complex, as well as lactic acid. The lactic acid present is measured by oxidizing it with sulfuric acid and copper sulfate to form acetaldehyde. The liberated acetaldehyde, when treated with p-phenyl phenol, gives a violet color. The intensity of this violet color increases linearly with the acetaldehyde concentration at low concentrations.

Propylene glycol interferes with the test, as does acetaldehyde from the acetaldehyde-bisulfite complex. Acetaldehyde from the complex is removed by breaking up the complex by adding barium hydroxide, which raises the pH and precipitates sulfite. Free acetaldehyde is then boiled off.

In fermentation solutions, clarification to remove protein is necessary prior to analysis. If barium is used to break up acetaldehyde-bisulfite complex, it will also remove protein so no further clarification is necessary.

Apparatus

Beckman DU spectrophotometer or equivalent equipped with 10-mm. silica cells.

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[1] Neish, A. C., Analytical methods for bacterial fermentations. Nat. Res. Counc. of Can., No. 2952. (2nd rev.). 1952.

[2] Barker, S. B., and Summerson, W. H., Colorimetric determination of lactic acid in biological meterial. J. Biol. Chem. 138: 535. 1941.

[3] Markus, R. L., Colorimetric determination of lactic acid in body fluids utilizing cation exchange for deproteinization. Arch. Biochem 29: 150. 1950.

Reagents

1. Copper sulfate-sulfuric acid. Dissolve 2 g. of $CuSO_4 \cdot 5H_2O$ in 10 ml. water and add to 600 ml. of concentrated sulfuric acid (or add 40 ml. of a 20% $CuSO_4$-H_2O solution to one 9-lb. bottle of concentrated sulfuric acid).

2. 1.5% solution of p-phenol in 0.05% NaOH. (This should be made up every 30-40 days.)

3. 0.1N iodine solution. Weigh out 12.7 g. of resublimed iodine, using a rough balance, and transfer it to a tared, 125-ml., glass-stoppered Erlenmeyer flask. Stopper the flask and reweigh it on the analytical balance to determine the weight of iodine accurately. Add 40 g. of KI crystals and 25 ml. water and shake until iodine is in solution. Transfer to a 1-liter volumetric flask and dilute to volume. Store in glass-stoppered brown bottles.

4. 0.5% starch solution.

5. 10% barium hydroxide solution.

Procedure

Into a 150-ml. beaker weigh sample equivalent to 0.1 to 1 mg. of acetaldehyde. Add 50 ml. of water and 2 ml. of 10% barium hydroxide solution and boil for 1/2 hr. Cool solution and add one drop of a starch solution, one-half drop of 0.1N iodine solution, and swirl. If the starch-iodine blue color that is formed persists, the removal of the acetaldehyde-bisulfite complex is complete, and the sample solution is then boiled again until the blue color fades.

If the blue color does not persist after swirling, boil solution for 10 min. more and test with iodine. Repeat this until blue color persists.

When the complex has been removed as indicated by the persistence of a blue color, bring the sample solution to a boil again and continue boiling until the blue fades. Filter the solution through Whatman No. 40 or 42 filter paper into a 100-ml. volumetric flask, wash, dilute to volume, and mix.

Into a wide-mouth vial (16 ml. ID 95 ml. long) fitted with a screw cap, pipet 1 ml. of the test solution. Add 6 ml. of the $CuSO_4$-H_2SO_4 solution and mix thoroughly. The vial is immersed in boiling water for exactly 2 min., then cooled to 15°-18° C. (below 20° C.). When the vial is cool, 0.1 ml. of p-phenyl phenol reagent is added and mixed in. The color is developed by placing the tube in a constant-temperature bath at 30° C. for 1/2 to 1 hr. It is immersed in boiling water for 90 sec. and cooled to room temperature. The violet color is read at 560 mμ on the spectrophotometer. Three blanks are run in the same way, substituting 1 ml. of distilled water for the sample. Lactic acid is calculated from a prepared calibration curve.

Precaution

This method is extremely sensitive. The temperatures are critical and every care must be exercised to avoid touching the inner surfaces of glassware or tips of pipets. All glassware is cleaned with cleaning solution, followed by trisodium phosphate. Rubber gloves should be worn during washing to avoid contamination by the hands.

DETERMINATION OF LEVULINIC ACID[1]

Introduction

This procedure is designed to be applied to solutions derived from the acid-catalyzed reaction of glucose or fructose in which levulinic acid is formed with hydroxmethylfurfural as an intermediate (figs. 1 and 2). The procedure involves isolating levulinic acid by paper chromatography as the ammonium salt and measurement of the isolated compound by the iodoform method.

Paper Chromatographic Separation Procedure

Free levulinic acid is too volatile to yield a reproducible, quantitative chromatographic separation, but the ammonium salt of levulinic acid will chromatograph into a compact, quantitative band when a mixture of ethanol (95%), ammonia (29% NH_3), and water (100:5:5 volumetric ratio) is used as an irrigating solvent. This solvent system results in the following R_f values for the major components of the glucose reaction:

Component	R_f
Glucose	0.30
Ammonium formate	.35
Ammonium levulinate	.40
5-hydroxymethyl-2-furaldehyde	.75

[1]Based on: McKibbins, S. W., Harris, J. F., and Saeman, J. F. "A sample applicator for chromatographic paper and its use for 5-hydroxymethyl-2-furaldehyde and levulinic acid analysis. J. Chromatogr. 5: 207. 1961.

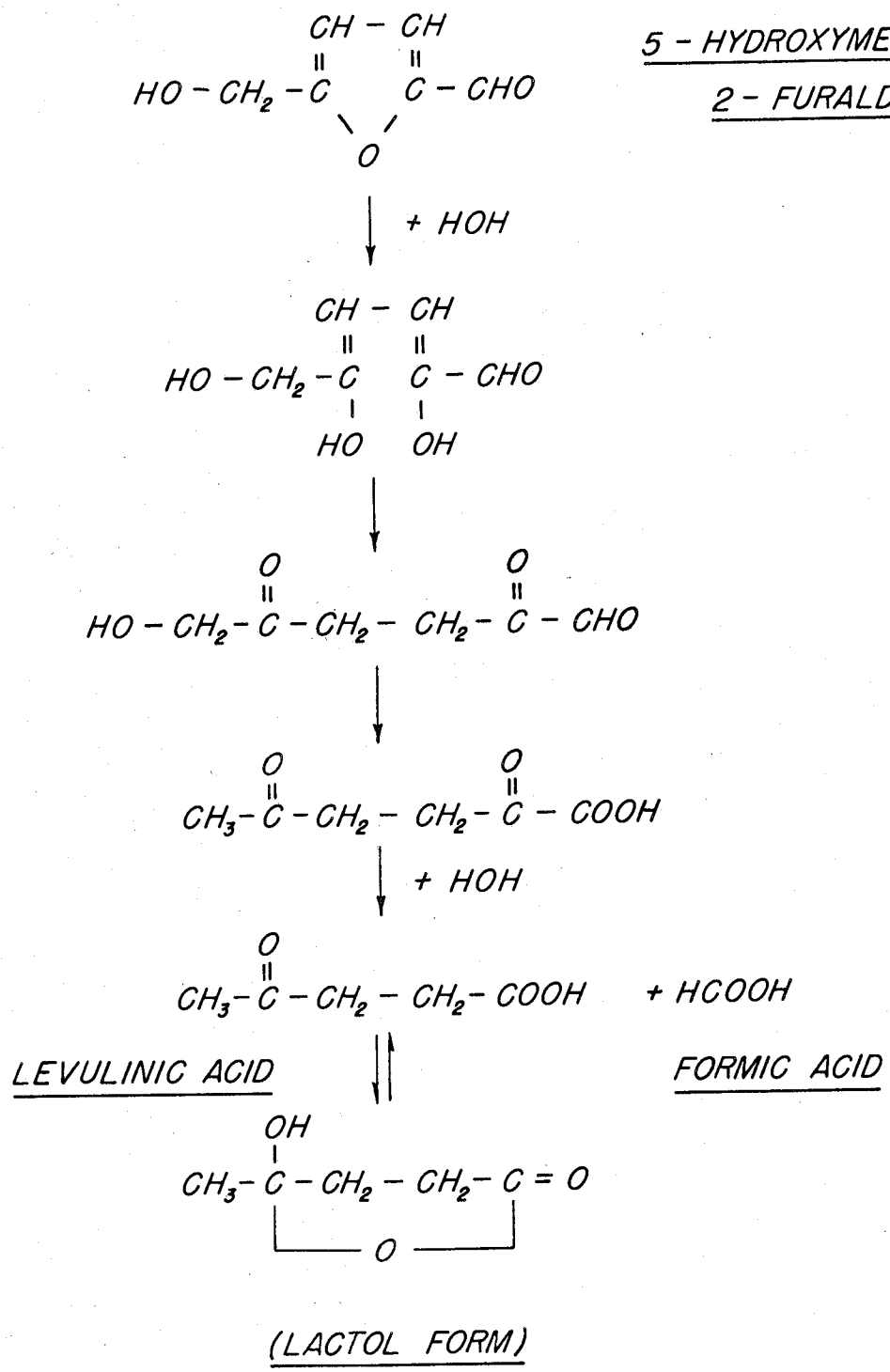

Figure 1.--Mechanism for the formation of levulinic acid from 5-hydroxymethyl-2-furaldehyde proposed by Wiggins (Wiggins, L., "The Preparation of Levulinic Acid from Sugar," The Sugar Res. Found. Inc., England (1948)).

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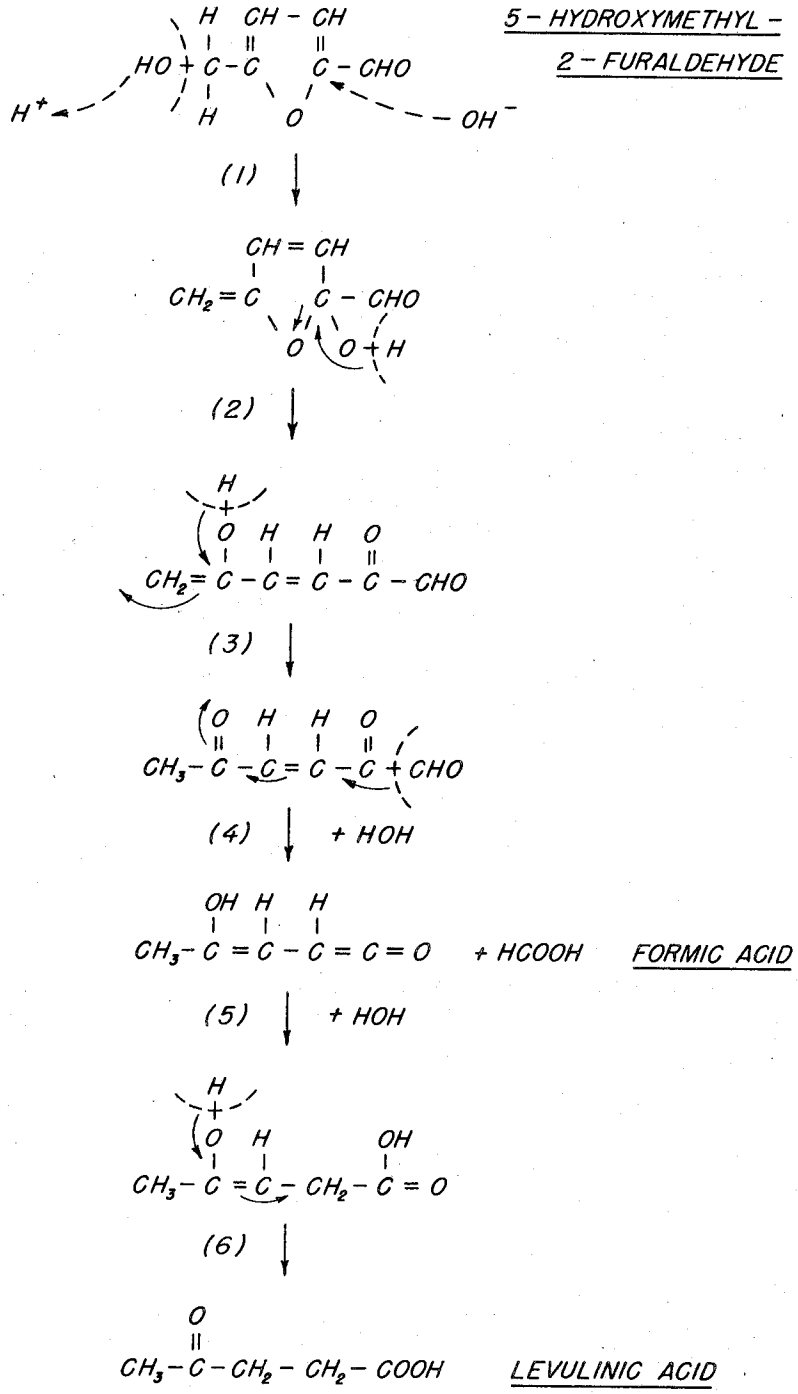

Figure 2.--Electronic interpretation of the formation of levulinic acid from 5-hydroxymethyl-2-furaldehyde proposed by Isbell in J. Res. Nat. Bur. Standards, R. P. No. 1573, 32: 45. 1944.

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Sheets 18 x 24 in. of Whatman No. 3MM chromatography paper are used. Samples are applied in spots with a pipet or preferably, streaked with the streaker-type applicator described by McKibbins et al.[1] (fig. 3). A streak of 1.0N ammonium carbonate is first applied to the paper at the starting line and then dried. Levulinic acid solution (standard or sample solution) is then applied over the first streak and thus converted to ammonium levulinate *in situ*.

The general chromatographic techniques are those described by Saeman, Moore, Mitchell, and Millett.[2] In this case, sheets are irrigated for 15 hr. with the ethanol, ammonia, and water solvent previously described. After 15 hr., the papers are dried, and four 75-mm. vertical strips removed for analysis. The remaining guide strips are sprayed with a 2, 4-dinitrophenylhydrazine indicator (2 g. dissolved in 400 ml. of 10% hydrochloric acid) to locate the position of the levulinate band. The position of the band is marked on the 75-mm. test strips and the corresponding area containing the levulinate cut out for analysis.

The ammonium levulinate is eluted from the paper tabs generally using the procedure described by Saeman et al.[2] The only exception is that, to accommodate the thick 3MM paper, the lower glass plate is replaced with a stainless steel plate with a notch (0.125 in. wide by 0.008 in. deep) milled into the front edge. By this system, then, the material is eluted from the paper tab and collected completely in a 0.75-ml. capillary pipet. The solution is then transferred to a 50-ml. Erlenmeyer flask for analysis.

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[2] Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millett, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. Tappi 37: 336. 1954.

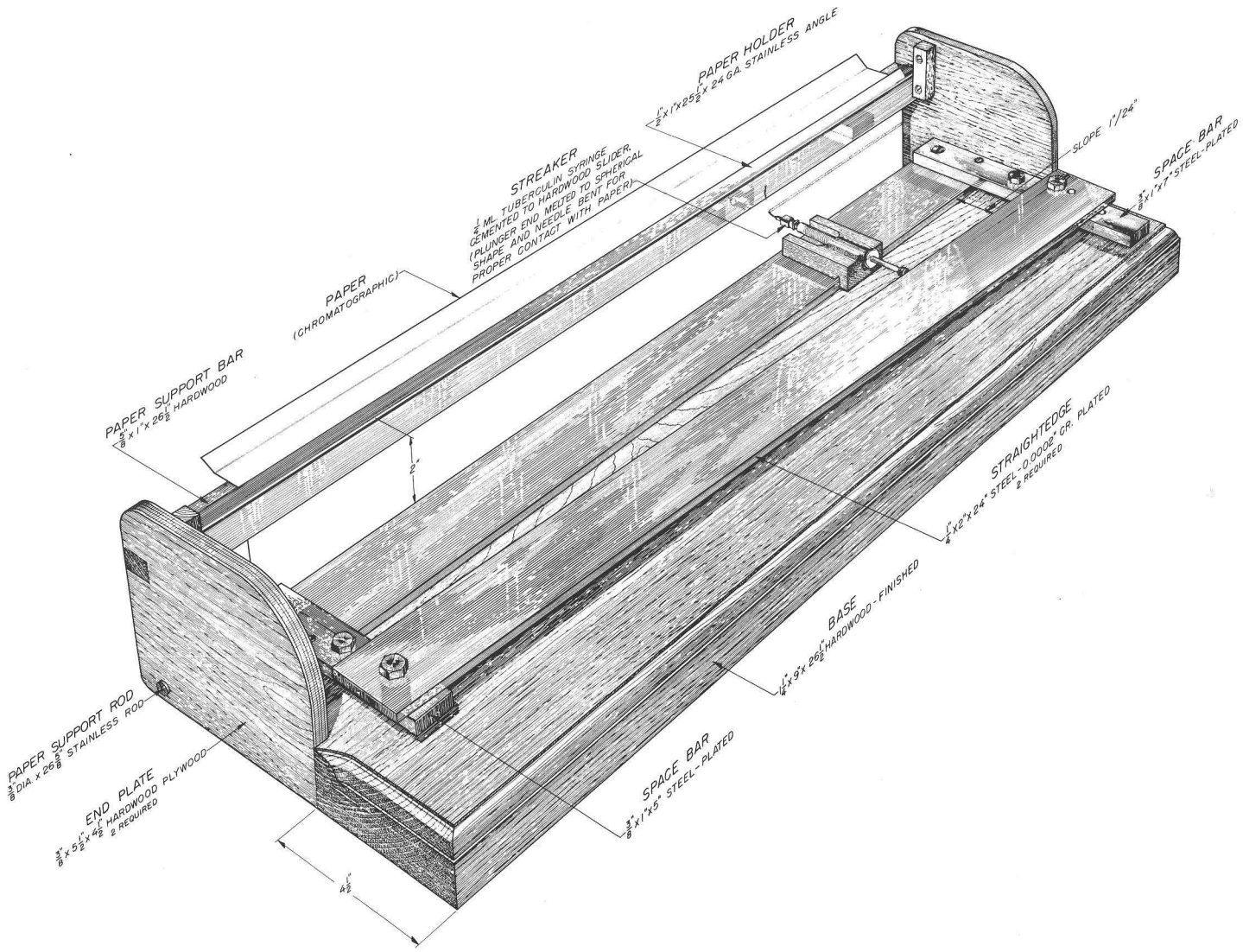

Figure 3.--Sample applicator for chromatographic paper.

M 117 216

Since ammonium ion interferes with the iodometric analysis, it is necessary to remove it by adding 0.25 ml. of 0.5N sodium carbonate to the flask and evaporating the solution to dryness. This is done at room temperature under reduced pressure on a vacuum (aspirator) manifold arrangement capable of handling several samples at one time (fig. 4). The dried sodium levulinate is then dissolved in 1 ml. of distilled water and the analysis carried out as described below.

Determination of Levulinate

The method used to measure the amount of the separated levulinic acid was suggested by Ploetz and Bartels[3] and Frost and Kurth.[4] This method depends on the presence of the acetyl which undergoes the iodoform reaction in the presence of alkaline iodine solutions. This reaction is not stoichiometric so measurements must be made from a calibration curve.

Reactions[5]

$$CH_3C(=O)CH_2CH_2O(=O)\text{-}OH + 3I_2 + 5NaOH \longrightarrow$$

$$CHI_3 + 3NaI + (CH_2COONa)_2 + 4H_2O$$

Procedure.--A calibration curve is made up from solutions of known levulinic acid concentration, and samples are run in the same manner along with two blanks.

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[3]Ploetz, T., and Bartels, H. Quantitative determination of levulinic acid. Bericht 74B: 1456. 1941.

[4]Frost, T. R., and Kurth, E. F. Levulinic acid from wood cellulose. Tappi 34: 80. 1951.

[5]Haughton, C. O. Estimation of acetone. Ind. Eng. Chem. Anal. Ed. 9: 167. 1937.

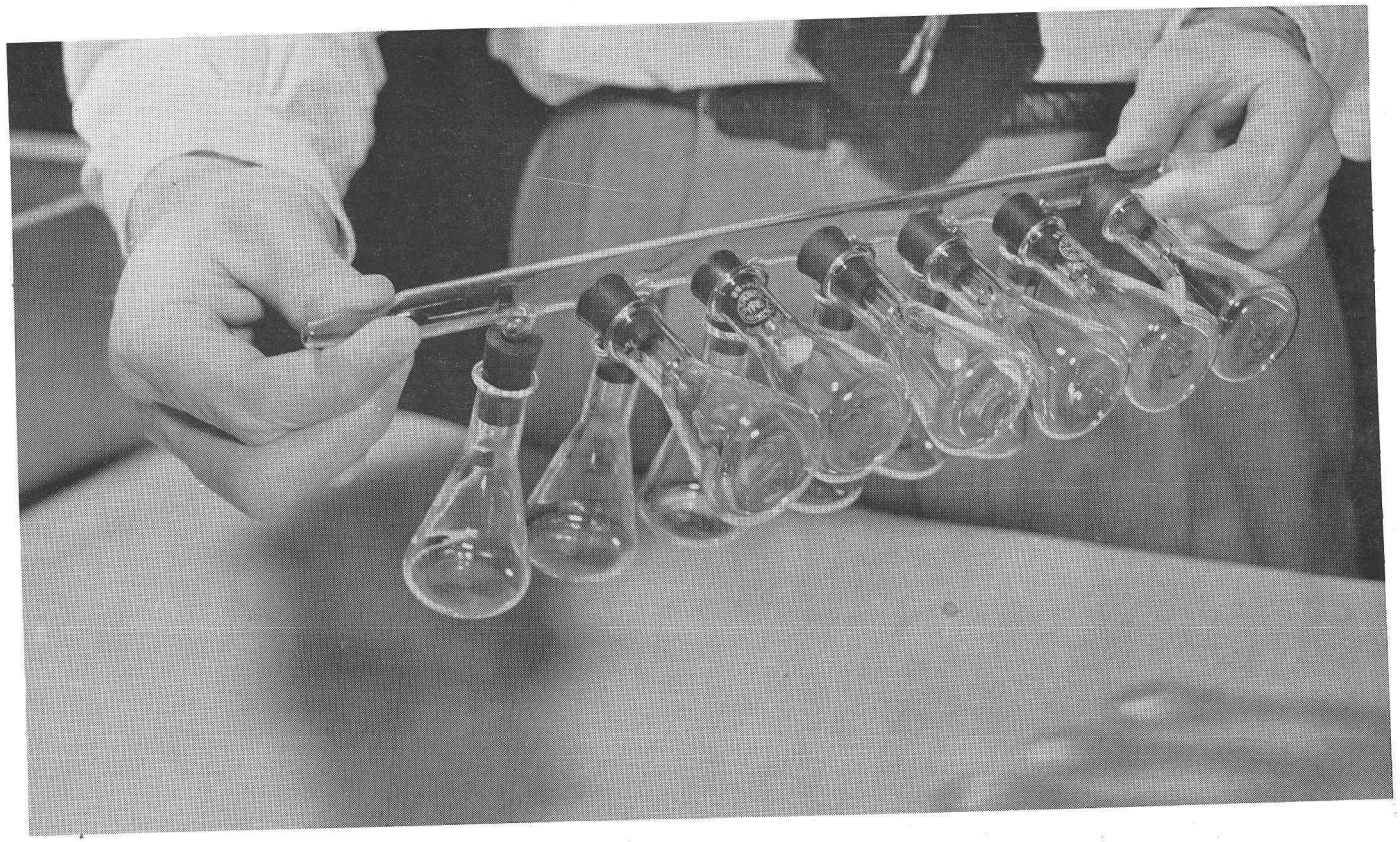

Figure 4.--Vacuum manifold and flasks used to dry solutions at room temperature.

M 95439 F

For the calibration curve, take a series of 1-ml. aliquots containing from 0 to 5 mg. of levulinic acid and transfer each to a 50-ml. Erlenmeyer flask; add 5 ml. 0.1N iodine solution followed by 5 ml. of 1.0N potassium hydroxide, stopper, swirl, and place for 10 min. in a water bath thermostated at 30° C. A yellow flocculent precipitate of iodoform is produced. After 10 min., remove the flasks, add 5 ml. of 1.1N hydrochloric acid to make the solutions acidic. The free iodine in the samples is then titrated with standard 0.1N sodium thiosulfate using starch as an indicator.

Unknowns are run in the same manner, taking the levulinic acid concentration off the calibration curve and running blanks with each set to check reagents. McKibbins[1] data on standard solutions showed a standard deviation of 1.7% in the 0-5 mg. per ml. range.

ACID-INSOLUBLE LIGNIN IN PULP--STANDARD METHOD[1,2]

Introduction

When wood pulp is treated with strong acids, the carbohydrates are hydrolyzed and solubilized. The insoluble residue remaining is measured gravimetrically as lignin.

It is recommended that the pulp sample be given a preliminary extraction with hot water to remove last traces of cooking liquor and with alcohol-benzene to remove resins; some of these resins, if allowed to remain in the pulp, would remain insoluble and be measured as lignin.

Chemical wood pulps, especially sulfite, may contain materials derived from the lignin originally present in the wood, but soluble under the treatment employed in this method. Therefore, the values obtained for acid-insoluble lignin should be regarded as minimal values for the lignin content of chemical wood pulps.

Apparatus

1. Filtering crucibles.--Alundum (RA98) or Gooch crucibles with an acid-washed asbestos mat are recommended for filtering the separated lignin.

2. Extraction apparatus.--A Soxhlet apparatus is preferable, consisting of (a) Soxhlet extraction flask, 250-ml. capacity; (b) Soxhlet extractor, ID ca. 45 to 50 mm., capacity to top of siphon approximately 100 ml.; (c) condenser; (d) extraction thimbles or crucibles of alundum or fritted

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[1] Klason, P., The lignin content of spruce wood. Cellulosechemie 4: 81. 1923.
[2] Similar method: TAPPI Standard T-222, 1954.

glass and of coarse or medium porosity. The extractors specified siphon more frequently than extractors with greater capacity.

Reagents

1. Alcohol-benzene solution.--Mix 1 volume of 95% ethyl alcohol and 2 volumes of C.P. benzene.

2. Sulfuric acid, 72%.--Carefully pour 665 ml. of concentrated H_2SO_4 into about 300 ml. of water and, after cooling, dilute to 1 liter. Standardize against standard sodium hydroxide solution, using methyl orange indicator. Adjust the acid to a strength of 72%±0.1%, by addition of water or concentrated H_2SO_4 as may be found necessary.

Note: As concentrated H_2SO_4 varies somewhat in strength, the measurement does not have to be made accurately; it may be done with a liter graduate. The proportions given are for concentrated H_2SO_4 of 96% strength and 1.84 specific gravity.

If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72% H_2SO_4 the specific gravity at 20°/4° C. is 1.6338; and at 60°/60° F. or 14.56°/15.56° C. it is 1.6389. A variation of 0.1% in the strength of acid at this concentration causes a change of 0.0012 in the specific gravity; 72% H_2SO_4 is equivalent to 24.1 N.

Test Specimen

The test specimen shall consist of a representative portion of the pulp which is extractive-free and air-dried. Any large pieces should be broken down by disintegration or torn apart by hand. Prior to hydrolysis, pulps should

be brought to an air-dry condition so that residual moisture does not significantly change the hydrolyzing acid concentration. However, pulps dried to this extent are difficult to dissolve. To overcome this difficulty, pulps are "fluffed" before attempting to hydrolyze them.

Fluffing is accomplished by either grinding or scraping the sample off a sheet with a blade or by air-drying from acetone. Obtaining a fluffy sample by scraping is perhaps satisfactory but practical only in the case of sheeted material.

Acetone displacement is the procedure generally used. Here the sample is stirred or beaten into slurry in water (a Waring Blender is not used because of too much cutting) and then filtered through a Büchner funnel (with suction) pressing the pulp finally with a rubber dam. The cake is washed with acetone on the filter and then stirred (by hand) in a beaker of acetone. The pulp is filtered out of the slurry, washed with acetone, removed from the filter, spread out, and air-dried. This air-dried sample us used for hydrolysis.

The dry weight of the sample is determined by drying a separate sample for 2 hr. at 105°±3° C. If there is not enough sample to determine the volatiles on a separate sample, the sample itself can be dried prior to hydrolysis if the pulp is extremely clean. However, if any residual acid or alkali is present, a sort of hornification occurs, and the dried sample becomes resistant to hydrolysis.

Procedure

If the sample may contain residual cooking liquors, wash it thoroughly with hot water by adding hot water, mixing, and decanting several times.

Air-dry the sample and then place a suitable quantity in an extraction thimble for the alcohol-benzene extraction. A porous crucible or fritted glass crucible may also be used, but the sample must be kept below the level of the siphon tube. Extract at a rate of not less than 4 siphonings per hour for 4 hr. with alcohol-benzene mixture in the Soxhlet apparatus. Transfer the pulp to a Büchner funnel, remove the excess solvent with suction, and wash the thimble and pulp thoroughly with alcohol to remove the benzene. The alcohol wash is followed by a thorough washing with water to remove the alcohol. The water wash consists of covering the sample on the Büchner funnel with water (not necessarily hot) and letting it stand for 10 min. with the suction off. The suction is then turned on, pulling off most of the water, and the sample is then washed three more times with the suction on. The suction is then turned off again and the same sequence is repeated two more times.

The pulp sample is then fluffed by the acetone-displacement procedure, which was described previously, and then air-dried.

Weigh accurately into weighing bottles (40 x 50 mm.) two samples of extracted, fluffed, air-dried pulp of approximately 2 g. each for hydrolysis and lignin determination. Weigh, at the same time, two air-dried samples of approximately 1 g. each into glass-stoppered weighing bottles for determination of the moisture content. Dry the moisture samples in an oven for 2 hr. at 105°±3° C., replace the stoppers, cool in a desiccator, loosen the stoppers to equalize the pressure, and weigh. Continue the drying for 1-hr. periods until the weight is constant (±0.1% change on reheating). (If preferred, the moisture samples may be dried overnight at 105°±3° C. and a constant weight assumed.) Calculate the percentage of moisture-free extracted pulp.

To the aid-dried samples for lignin determination, add slowly, with stirring, 40 ml. of cold (15° C.) 72% H_2SO_4. The sample must be well mixed and free of lumps by stirring constantly for at least 1 min. and longer if necessary. Allow to stand at a temperature of 20°±0.1° C. for 2 hr. after the pulp has completely dispersed in the acid, and stir frequently. After 2 hr. wash the material into a 2-liter Erlenmeyer flask, dilute to a 3% acid concentration with 1,500 ml. of distilled water, and boil for 4 hr. under a reflux condenser.

After 4 hr., allow the insoluble material to settle and filter[3] through a filtering crucible which has previously been dried (at 105°±3° C.) and weighed in a glass-stoppered weighing bottle. Wash the residue free of acid with 500 ml. of hot water, dry the crucible and contents in an oven for 2 hr. at 105°±3° C., and place in the weighing bottle. Cool in a desiccator, loosen the stopper of the bottle, and weigh as lignin. Repeat the drying and weighing until the weight is constant.[4]

Report

The weight of acid-insoluble lignin shall be reported as a percentage by weight of the moisture-free pulp.

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[3] If sugar analysis is required on the same sample, the hydrolysate is made up to an appropriate (0.4-1.5 mg./ml.) volume after filtering; then the liquid is filtered, and the filtrate taken for sugar analysis.

[4] Alundum crucibles are cleaned by igniting, and back-washing with hydrochloric acid. Fritted glass crucibles are cleaned by back-washing with nitric acid.

Additional Information

(1) Bray, M. W.
1928. Chemical analysis of pulps and wood. Paper Trade J. 87(25): 59 (Dec. 20).

(2) Ritter, G. J., Seborg, R. M., and Mitchell, R. L.
1932. Factors affecting quantitative determination of lignin by 72 percent sulfuric acid method. Ind. Eng. Chem. Anal. Ed. 4: 202.

ACID-INSOLUBLE LIGNIN IN PULP--MODIFIED HYDROLYSIS METHOD[1]

Introduction

When wood pulp is treated with strong acids, the carbohydrates are hydrolyzed and solubilized. The insoluble residue that remains is measured gravimetrically as lignin.

The method described here differs from our "Standard Method" in that a smaller sample size is used and the hydrolysis procedure is modified. The two methods do give equivalent results. (Tables 1 and 2 compare results obtained on wood samples by the two methods.)

It is recommended that the pulp sample be given a preliminary extraction with hot water to remove last traces of cooking liquor and with alcohol-benzene to remove resins, some of which, if allowed to remain in the pulp, would remain insoluble and be measured as lignin.

Chemical wood pulps, especially sulfite, may contain materials derived from the lignin originally present in the wood, but which are soluble under the treatment employed in this method. Therefore, the values obtained for acid-insoluble lignin should be regarded as minimal values for the lignin content of chemical wood pulps.

Apparatus

1. Extraction apparatus.--A Soxhlet apparatus is preferable, consisting of (a) Soxhlet extraction flask, 250-ml. capacity; (b) Soxhlet extractor,

[1]Based on: Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millett, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. Tappi 37: 336. 1954.

Table 1.--Lignin analysis on matched wood samples and methoxyls on lignin prepared by "Modified Hydrolysis" and "Standard" methods[1]

Sample	Lignin method			Methoxyl determinations		
	Modified hydrolysis	Standard	Difference	On modified hydrolysis "lignin"	On standard "lignin"	Difference
	%	%		%	%	
Red gum	24.99	25.02		20.37	20.50	
	25.15	24.93		20.41	20.46	
Ave.	25.07	25.00	+0.07	20.39	20.48	-0.09
Maple	23.70	23.73		20.74	20.60	
	23.71	23.70		20.80	20.71	
Ave.	23.71	23.72	-0.01	20.77	20.66	+0.11
Aspen	18.91	18.90		21.13	21.00	
	18.85	18.75		21.13	21.20	
Ave.	18.88	18.83	+0.05	21.13	21.10	+0.03
Douglas-fir	29.56	29.27		15.25	14.76	
	29.48	29.32		15.05	14.95	
Ave.	29.52	29.30	+0.22	15.15	14.86	+0.29
Sitka spruce	25.18	24.65		13.75	15.42	
	25.00	24.67		13.86	15.41	
					15.48	
					15.44	
Ave.	25.09	24.66	+0.43	13.81	15.43	-1.6
Black spruce	27.49	27.69		15.82	14.60	
	27.59	27.69		15.75	14.57	
Ave.	27.54	27.69	-0.15	15.79	14.59	+1.20
White spruce	26.46	26.75		15.40	15.93	
	26.36	26.68		15.48	15.96	
Ave.	26.41	26.72	-0.31	15.44	15.95	-0.51
Western hemlock	31.31	31.51		15.39	14.72	
	31.35	31.58		15.38	14.58	
Ave.	31.33	31.55	-0.22	15.39	14.65	-0.74

[1] U.S. Forest Products Laboratory, Wood Chemistry Division Quarterly Report No. 12, Dec. 1, 1959.

Table 2.--Repeated lignin and sugar determinations on aspen wood.[1] One-half gram samples were used and results based on extractive-free ovendry wood (by modified hydrolysis method)

Sample	Lignin		Sugar	
	Results	Average	Results	Average
	%		%	
1	17.90 17.95	17.93	74.96 75.33	75.15
2	18.09 18.09	18.09	75.03 74.89	74.96
3	17.92 17.87	17.90	75.10 75.20	75.15
4	17.87 18.06	17.97	74.75 75.17	74.96
5	18.03 18.02	18.03	75.48 74.86	75.17
6	18.18 18.02	18.10	74.84 74.91	74.88
7	17.87 18.00	17.94	74.86 74.78	74.82
8	18.22 18.27	18.25	74.99 75.60	75.30
9	18.33 18.55	18.44	74.86 74.90	74.88
10	18.62 18.74	18.68	75.81 76.04	75.93
11	18.17 18.04	18.11	75.62 75.45	75.54
12	18.82 18.94	18.88	74.85 75.08	74.97
Grand Ave.		18.19		75.14
Standard deviation		0.31726		0.32093
Standard error of mean		0.09158		0.09264

[1] U.S. Forest Products Laboratory, Wood Chemistry Division Quarterly Report No. 12, Dec. 1, 1959.

ID 45 to 50 mm., capacity to top of siphon approximately 100 ml.; (c) condenser; and (d) extraction thimbles or crucibles of alundum or fritted glass and of coarse or medium porosity. The extractors specified siphon more frequently than extractors with greater capacity.

2. Constant temperature bath set at 30°±.5° C.

3. Autoclave.

4. Filtering crucible.--Alundum (RA98) or Gooch crucibles with an acid-washed asbestos mat are recommended for filtering the separated lignin.

Reagents

1. Alcohol-benzene solution.--Mix 1 volume of 95% ethyl alcohol and 2 volumes of C.P. benzene.

2. Sulfuric acid, 72%.--Carefully pour 665 ml. of concentrated H_2SO_4 into about 300 ml. of water and after cooling dilute to 1 liter. Standardize against standard sodium hydroxide solution using methyl orange indicator. Adjust the acid to a strength of 72%±0.1% by addition of water or concentrated H_2SO_4 as may be found necessary.

Note: As concentrated H_2SO_4 varies somewhat in strength, the measurement does not have to be made accurately; it may be done with a liter graduate. The proportions given are for concentrated H_2SO_4 of 96% strength and 1.84 specific gravity.

If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72% H_2SO_4 the specific gravity at 20°/4° C. is 1.6338, and at 60°/60° F. or 15.56°/15.56° C. it is 1.6389. A variation of 0.1% in the strength of acid at this concentration causes a change of 0.0012 in the specific gravity; 72% H_2SO_4 is equivalent to 24.1 N.

Test Specimen

The test specimen shall consist of a representative portion of the pulp which is extractive-free and air-dried. Any large pieces should be broken down by disintegration or torn apart by hand. Prior to hydrolysis, pulps should be brought to an air-dry condition so that residual moisture does not significantly change the hydrolyzing acid concentration. However, pulps dried to this extent are difficult to dissolve. To overcome this difficulty, pulps are "fluffed" before attempting to hydrolyze them.

Fluffing is accomplished by either grinding or scraping the sample off a sheet with a blade or by air-drying from acetone. Obtaining a fluffy sample by scraping is perhaps satisfactory but practical only in the case of sheeted material.

Acetone displacement is the procedure generally used. Here the sample is stirred or beaten into slurry in water (a Waring Blender is not used because of too much cutting) and then filtered through a Büchner funnel (with suction), pressing the pulp finally with a rubber dam. The cake is washed with acetone on the filter and then stirred up (by hand) in a beaker of acetone. The pulp is filtered out of the slurry, washed with acetone, removed from the filter, spread out, and air-dried. This air-dried sample is used for hydrolysis.

The dry weight of the sample is determined by drying a separate sample for 2 hr. at 105°±3° C. If there is not enough sample to determinethe volatiles on a separate sample, the sample itself can be dried prior to hydrolysis if the pulp is extremely clean. However, if any residual acid or alkali is present,

a sort of hornification occurs, and the dried sample becomes very resistant to hydrolysis.

Procedure

If the sample may contain residual cooking liquors, wash it thoroughly with hot water by adding hot water, mixing, and decanting several times. Air-dry the sample and then place a suitable quantity in an extraction thimble for the alcohol-benzene extraction. A porous crucible or fritted glass crucible may also be used, but the sample must be kept below the level of the siphon tube. Extract at a rate of not less than 4 siphonings per hour for 4 hr. with alcohol-benzene mixture in the Soxhlet apparatus. Transfer the pulp to a Büchner funnel, remove the excess solvent with suction, and wash the thimble and pulp thoroughly with alcohol to remove the benzene. The alcohol wash is followed by a thorough washing with water to remove the alcohol. The water wash consists of covering the sample on the Büchner funnel with water (not necessarily hot) and letting it stand for 10 min. with the suction off. The suction is then turned on, pulling off most of the water, and the sample is then washed three more times with the suction on. The suction is then turned off again and the same sequence is repeated two more times.

The pulp sample is then fluffed by the acetone-displacement procedure, which was described previously, and air-dried. This sample is then used for the hydrolysis step.

Weigh accurately into weighing bottles two samples of extracted, air-dried pulp of a size appropriate for the lignin content of the sample and the filtering and weighing techniques used.

Weigh, at the same time, two samples of appropriate size into glass-stoppered weighing bottles for the determination of moisture content. Dry the moisture samples in an oven for 2 hr. at 105°±3° C., replace the stoppers, cool in a desiccator, loosen the stoppers to equalize the pressure, and weigh. Continue the drying for 1-hr. periods until the weight is constant (±0.1% change on reheating). (If preferred, the moisture samples may be dried overnight at 105°±3° C. and a constant weight assumed.) Calculate the percentage of moisture-free extracted pulp.

To the sample, add 1 ml. of 72% H_2SO_4 for each 0.1 g. of sample. After adding the acid, place the sample in the 30° C. bath, allow it to stand for 3 min., then stir it thoroughly. Stirring should be repeated periodically to assure complete mixing and dispersion of lumps. Allow the sample to remain in the 30° C. bath for 1 hr.

After 1 hr., remove the sample from the bath and transfer it to an Erlenmeyer flask, using 28 ml. of water for each milliliter of 72% H_2SO_4 added. This results in a 4% acid solution for the secondary hydrolysis. Cover the sample with a watch glass.

The secondary hydrolysis is accomplished in an autoclave according to the following procedure:

1. Open the valve in the distilled water line to the reboiler.

2. Preheat autoclave to 220° F. and adjust the supply steam pressure to 60 p.s.i.g. (pressure to reboiler).

3. Close supply valve and vent autoclave.

4. Place samples in autoclave and admit steam with vent open, allowing escape of air, and raising temperature to 220° F. by the end of 4 min.

5. Close bypass and allow temperature to increase to 246° F., adjusting supply steam pressure as required to maintain autoclave temperature at 248° F. This should be completed in 7 min. from the time samples are introduced. The autoclave is maintained at this temperature for 1 hr. by proper adjustment of steam pressure regulation valve, then vented through the orifice (a gate valve with a 3/32-in.-diameter hole drilled through the gate). Venting should be completed in 6 min. This results in a nearly linear temperature decrease, and the rate of pressure release is such that no sample loss occurs through excessively rapid boiling.

Remove the samples from the autoclave and filter off the lignin,[2] keeping the solution hot. Filtering is done with either a Gooch, Alundum (RA98), or fritted glass crucible with an asbestos mat, using suction. The residue is thoroughly washed with hot water then dried at 100° to 105° C. for 4 hr.

Weigh crucibles and calculate the percent lignin on a moisture-and extractive-free basis.

Alundum crucibles are cleaned by igniting and then back-washing with hydrochloric acid.

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[2] If sugars are to be measured on the same sample, the hydrolysate is made up to an appropriate (0.4-1.5 mg./ml.) volume after filtering and this filtrate taken for sugar analysis.

ACID-INSOLUBLE LIGNIN IN WOOD--STANDARD METHOD[1,2]

Introduction

When wood is treated with strong acids, the carbohydrates are hydrolyzed and solubilized. The insoluble residue is measured gravimetrically as lignin.

Some wood samples must be extracted with alcohol-benzene solvent prior to lignin analysis to remove the extractives that are acid-insoluble and would be measured as lignin. In the extraction procedure, the sample is extracted: (1) With alcohol-benzene to remove resins, fats, and waxes, (2) with alcohol to remove catechol tannins and benzene, and (3) with hot water to remove alcohol and other remaining water solubles.

The alcohol extraction is necessary in analysis of woods high in tannin--i.e., oak, chestnut, redwood, etc. It has not been shown necessary in the more common pulpwoods, such as the various species of spruce, pine, fir, hemlock, poplar, birch, beech, and maple. For these woods, the alcohol extraction may be omitted, and only an alcohol wash is necessary to remove benzene. In analysis of woods not listed, the desirability of the alcohol extraction depends upon the purpose of the analysis, and the report should state whether or not alcohol extraction was used.

Apparatus

1. Filtering crucibles.--Alundum (RA98), fritted glass crucibles (medium porosity), or Gooch crucibles with an acid-washed asbestos mat are

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[1] Klason, P. The lignin content of spruce wood. Cellulosechemie 4: 81. 1923.

[2] Similar to TAPPI Standard T 13, 1954.

recommended for filtering the separated lignin. (Glass crucibles cannot be used if the lignin is to be ashed.)

2. Extraction apparatus.--A Soxhlet apparatus is preferable, consisting of (a) Soxhlet extraction flask, 250-ml. capacity; (b) Soxhlet extractor, ID 45 to 50 mm., and capacity to top of siphon approximately 100 ml.; (c) condenser; (d) extraction crucibles of alundum or fritted glass and of medium porosity. The extractors specified siphon more frequently than extractors with greater capacity.

Reagents

1. Alcohol-benzene solution.--Mix 1 volume of 95% ethyl alcohol and 2 volumes of C.P. benzene.

2. Sulfuric acid, 72%.--Carefully pour 665 ml. of concentrated H_2SO_4 into about 300 ml. of water and, after cooling, dilute to 1 liter. Standardize against standard sodium hydroxide solution, using methyl orange indicator. Adjust the acid to a strength of 72%±0.1%, by addition of water or concentrated H_2SO_4 as may be found necessary.

Note: As concentrated H_2SO_4 varies somewhat in strength, the measurement does not have to be made accurately; it may be done with a liter graduate. The proportions given are for concentrated H_2SO_4 of 96% strength and 1.84 specific gravity.

If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72% H_2SO_4 the specific gravity of 20°/4° C. is 1.6338; and at 60°/60° F. or 15.56°/15.56° C. it is 1.6389. A variation of 0.1% in the strength of the acid at this concentration causes a change of 0.0012 in the specific gravity; 72% sulfuric acid is equivalent to 24.1 N.

Test Specimen

The test specimen shall consist of wood that has been ground to pass a 40-mesh sieve, extracted and air-dried.

Extraction Procedure

Place a suitable amount of ground sample into an extraction thimble and extract it at a rate of not less than four siphonings per hour for 4 hr. in a Soxhlet apparatus with the alcohol-benzene solvent. If an alcohol extraction is necessary (see Introduction), transfer the sample to a Büchner funnel, remove excess solvent with suction, wash with alcohol, then replace in the extractor and extract for 4 hr. with 95% alcohol. If the alcohol extraction is not necessary, a thorough washing with alcohol to remove the benzene is sufficient. In either case, follow the alcohol wash by a thorough washing with water to remove the alcohol.

The water wash consists of covering the sample on the Büchner funnel with water (not necessarily hot) and letting it stand for 10 min. with the suction off. The suction is then turned on, pulling off most of the water, and the sample is washed three more times with the suction on. The suction is then turned off again and the same sequence is repeated two more times. The sample is then air-dried.

Lignin Procedure

Accurately weigh two ground, extracted, wood samples of approximately 1 g. each into tared, glass-stoppered weighing bottles for moisture determination. Accurately weigh two wood samples of approximately 1 g. each into weighing bottles (40 x 50 mm.) for lignin determination. Dry the moisture

samples in an oven for 2 hr. at 105°±3° C.; replace the stopper; cool in a desiccator; loosen the stopper to equalize the pressure; and weigh. Continue the drying for 1-hr. periods until the weight is constant (±0.1% change on reheating for 1 hr.). Calculate the percentage of moisture-free wood.

To the samples for lignin determination, slowly add, with stirring, 15 ml. of cold (15° C.) 72% H_2SO_4. Stir constantly for 1 min., being sure to break up all lumps in the sample. Allow to stand for 2 hr. with frequent stirring at a temperature of 20°±0.1° C. Wash the material into a 1-liter Erlenmeyer flask, dilute to a 3% acid concentration with 560 ml. of distilled water, and boil for 4 hr. under a reflux condenser.

After allowing the insoluble material to settle, filter[3] into a filtering crucible which has previously been dried at 105°±3° C. and weigh in a glass-stoppered weighing bottle. Wash the residue free of acid with 500 ml. of hot water, dry the crucible and contents in an oven for 2 hr. at 105°±3° C., and place in the weighing bottle. Cool in a desiccator, loosen the stopper of the bottle, and weigh as lignin. Repeat the drying and weighing until the weight is constant (±0.1% change in reheating).

If a correction for ash is desired, transfer the lignin to a tared platinum crucible and determine the ash content in the usual manner. If the lignin cannot be quantitatively transferred, it may be ashed in the filtering

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[3] If sugar analysis is required on the sample, the hydrolysate is made up to an appropriate (0.4-1.5 mg./ml.) volume after filtering; this filtrate is then neutralized and used for the sugar analysis.

crucible, provided the latter has been ignited and heated to constant weight before filtration of the lignin residue. (Ignition cannot be performed in fritted glass crubicles.)[4]

Report

The weight of lignin shall be reported as a percentage by weight of the moisture-free extracted wood. If the wood was extracted with alcohol, or if the lignin was corrected for ash, the report shall so state.

Additional Information

(1) Bray, M. W.
1928. Chemical analysis of pulps and wood. Paper Trade J. 87(25): 29 (Dec. 20).

(2) Ritter, G. J., Seborg, R. M., and Mitchell, R. L.
1932. Factors affecting quantitative determination of lignin by 72 percent sulfuric acid method. Ind. Eng. Chem. Anal. Ed. 4: 202.

(3) _____, and Barbour, J. H.
1935. Effect of pretreatments of wood on the lignin determination. Ind. Eng. Chem. Anal. Ed. 7: 238.

[4]Alundum crucibles are cleaned by ignition and back-washing with hydrochloric acid. Fritted glass crucibles are cleaned by back-washing with nitric acid.

ACID-INSOLUBLE LIGNIN IN WOOD--MODIFIED HYDROLYSIS METHOD[1]

Introduction

When wood is treated with strong acid, the carbohydrates are hydrolyzed and solubilized. The insoluble residue is, by definition, lignin and can be measured gravimetrically.

The method described here differs from our "Standard Method" in sample size and in the hydrolysis procedure, but the two methods give equivalent results (Tables 1 and 2). Some wood samples must be extracted with solvent prior to lignin analysis to remove the extractives that are acid-insoluble and would be measured as lignin. In the extraction procedure, the sample is extracted: (1) With alcohol-benzene to remove resins, fats, and waxes; (2) with alcohol to remove catechol tannins and benzene; and (3) with hot water to remove alcohol and other remaining water-solubles.

Apparatus

1. Soxhlet extraction apparatus.
2. Shell vials, 22-mm. ID, 95-mm. length.
3. Constant temperature bath set at 30°±0.5° C.
4. Autoclave.
5. Crucibles: Alundum (RA98); Gooch with thin asbestos mat; or fritted glass with thin asbestos mat.

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[1]Based on: Techniques for the determination of pulp constituents by quantitative paper chromatography, by J. F. Saeman, W. E. Moore, R. L. Mitchell, and M. A. Millett. Tappi 37: 336. 1954.

Table 1.--Lignin analysis on matched wood samples and methoxyls on lignin prepared by "Modified Hydrolysis" and Standard methods[1]

Sample	Lignin method			Methoxyl determinations		
	Modified hydrolysis	Standard	Difference	On modified hydrolysis "lignin"	On standard "lignin"	Difference
	%	%		%	%	
Red gum	24.99	25.02		20.37	20.50	
	25.15	24.93		20.41	20.46	
Ave.	25.07	25.00	+0.07	20.39	20.48	-0.09
Maple	23.70	23.73		20.74	20.60	
	23.71	23.70		20.80	20.71	
Ave.	23.71	23.72	-0.01	20.77	20.66	+0.11
Aspen	18.91	18.90		21.13	21.00	
	18.85	18.75		21.13	21.20	
Ave.	18.88	18.83	+0.05	21.13	21.10	+0.03
Douglas-fir	29.56	29.27		15.25	14.76	
	29.48	29.32		15.05	14.95	
Ave.	29.52	29.30	+0.22	15.15	14.86	+0.29
Sitka spruce	25.18	24.65		13.75	15.42	
	25.00	24.67		13.86	15.41	
					15.48	
					15.44	
Ave.	25.09	24.66	+0.43	13.81	15.43	-1.6
Black spruce	27.49	27.69		15.82	14.60	
	27.59	27.69		15.75	14.57	
Ave.	27.54	27.69	-0.15	15.79	14.59	+1.20
White spruce	26.46	26.75		15.40	15.93	
	26.36	26.68		15.48	15.96	
Ave.	26.41	26.72	-0.31	15.44	15.95	-0.51
Western hemlock	31.31	31.51		15.39	14.72	
	31.35	31.58		15.38	14.58	
Ave.	31.33	31.55	-0.22	15.39	14.65	-0.74

[1]U.S. Forest Products Laboratory, Wood Chemistry Division Quarterly Report No. 12. Dec. 1, 1959.

Table 2.--Repeated lignin and sugar determinations on aspen wood.[1] One-half gram samples were used and results based on extractive-free ovendry wood (by modified hydrolysis method)

Sample	Lignin		Sugar	
	Results	Average	Results	Average
	%		%	
1	17.90 17.95	17.93	74.96 75.33	75.15
2	18.09 18.09	18.09	75.03 74.89	74.96
3	17.92 17.87	17.90	75.10 75.20	75.15
4	17.87 18.06	17.97	74.75 75.17	74.96
5	18.03 18.02	18.03	75.48 74.86	75.17
6	18.18 18.02	18.10	74.84 74.91	74.88
7	17.87 18.00	17.94	74.86 74.78	74.82
8	18.22 18.27	18.25	74.99 75.60	75.30
9	18.33 18.55	18.44	74.86 74.90	74.88
10	18.62 18.74	18.68	75.81 76.04	75.93
11	18.17 18.04	18.11	75.62 75.45	75.54
12	18.82 18.94	18.88	74.85 75.08	74.97
Grand Ave.		18.19		75.14
Standard deviation		0.31726		0.32093
Standard error of mean		0.09158		0.09264

[1] U.S. Forest Products Laboratory, Wood Chemistry Division Report No. 12, Dec. 1, 1959.

Reagents

1. Alcohol-benzene solvent.--One volume 95% alcohol to 2 volumes benzene.

2. Sulfuric acid, 72%.--Carefully add 665 ml. concentrated H_2SO_4 to 300 ml. water, and after cooling dilute to 1 liter. Standardize against standard sodium hydroxide solution using methyl orange indicator. Adjust the acid to a strength of 72%±0.1% by addition of water or concentrated H_2SO_4 as required; 72% H_2SO_4 is equivalent to 24.1 N.

Sample

The wood sample shall be ground to pass a 40-mesh screen and be extractive-free and air-dried.

Extraction Procedure

Place a suitable quantity of ground sample in an extraction thimble and extract it at a rate of not less than 4 siphonings per hour for 4 hr. in a Soxhlet apparatus with the alcohol-benzene solvent. Transfer the wood to a Büchner funnel, remove the excess solvent with suction, and wash the thimble and sample with alcohol to remove the benzene. If an alcohol extraction is necessary,[2] return the sample to the extractor and extract with 95% alcohol for 4 hr. or until the alcohol siphons over colorless. If the alcohol extraction is not necessary, a thorough washing with alcohol to remove the benzene is sufficient. In either case, the alcohol wash is followed by a thorough washing with water to remove the alcohol.

[2]The alcohol extraction is necessary for woods high in tannin. Oaks, chestnut, and redwood require the alcohol extraction; the various species of spruce, pine, fir, hemlock, poplar, birch, beech, and maple do not.

The water wash consists of covering the sample on the Büchner funnel with water (not necessarily hot) and letting it stand for 10 min. with the suction off. The suction is then turned on, pulling off most of the water, and the sample is then washed three more times with the suction on. The suction is then turned off again and the same sequence repeated two more times. The sample is then air-dried.

Lignin Procedure

Accurately weigh out approximately 0.3 g. of ground air-dried, extractive-free wood into a shell vial. At the same time, weigh out another sample for a moisture determination. Dry the moisture sample in a weighing bottle at 105° C. until constant weight is reached. (This is approximately 2 hr. or until the weight does not change more than 0.1% upon 1 hr. of reheating.)

To the sample in the shell vial add 1 ml. of 72% H_2SO_4 for each 0.1 g. of sample. After adding the acid, place the vial in the 30° C. bath, allow it to stand for 3 min., then stir it thoroughly. Stirring should be repeated periodically to assure complete mixing and reaction. Allow the sample to remain in the 30° C. bath for 1 hr.

After 1 hr., remove the sample from the bath and transfer it to a 125-ml. or 250-ml. Erlenmeyer flask, using 28 ml. of water for each milliliter of 72% H_2SO_4 added. This results in a 4.0% solution for the secondary hydrolysis. Cover the sample with a watch glass.

The secondary hydrolysis is accomplished in an autoclave according to the following procedure:

1. Open the valve in the distilled water line to the reboiler.

2. Preheat autoclave to 220° F. and adjust the supply steam pressure to 60 p.s.i.g. (pressure to reboiler).

3. Close supply valve and vent autoclave.

4. Place samples in autoclave and admit steam with vent open, allowing escape of air, and raising temperature to 220° F. by the end of 4 min.

5. Close bypass and allow temperature to increase to 246° F., adjusting supply steam pressure as required to maintain autoclave temperature at 248° F. This should be completed in 7 min. from the time the samples are introduced. The autoclave is maintained at this temperature for 1 hr. by proper adjustment of steam pressure regulation valve, then vented. Venting should be completed in 6 min. This results in a nearly linear temperature decrease, and the rate of pressure release is such that no sample loss occurs through excessively rapid boiling.

Remove the samples from the autoclave and filter off the lignin,[3] keeping the solution hot. Filtering is done with either a Gooch, alundum (RA98), or fritted glass crucible (with an asbestos mat) using suction. The residue is thoroughly washed with hot water, then dried at 100° to 105° C. for 4 hr.

Weigh back crucibles and calculate the percent lignin on a moisture and extractive-free basis.

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[3] If sugars are to be measured on the same sample, the hydrolysate is made up to an appropriate (0.4-1.5 mg./ml.) volume after filtering; then the liquid is filtered and the filtrate taken for sugar analysis.

Alundum crucibles are cleaned by igniting and then back-washing with hydrochloric acid.

SPECTROPHOTOMETRIC DETERMINATION OF LIGNIN IN SMALL WOOD SAMPLES[1]

Introduction

It is sometimes desirable to determine the lignin content of very small wood samples, such as might be obtained from segments of growth rings or parts of cell walls. This procedure is applicable to that type of problem. With this procedure, (1) wood can be brought into solution without apparent serious degradation, (2) precise measurements of the absorbance of the solutions can be obtained that can be related to lignin concentration, and (3) work can be done in the microsize sample range.

The method consists of treating an extractive-free sample of wood meal, ground to pass an 80-mesh screen, with a reagent of acetyl bromide in acetic acid. When dissolution is complete, excess reagent is removed by adding water, and bromine and polybromide are removed by adding hydroxylamine hydrochloride. The sample is diluted to volume with acetic acid, and the absorbance of the resulting solution is measured at 280 mμ. The lignin concentration is calculated from this absorbance and a previously established lignin absorptivity value in accordance with the Beer-Lambert relationships.

The absorptivity of a particular lignin is calculated from the absorbance of a known standard lignin solution by again applying the Beer-Lambert relationships. The known lignin solution can be made up by acetyl bromide dissolution of weighed amounts of an isolated lignin like native, enzyme, or

[1] Johnson, D. B., Moore, W. E., and Zank, L. C. The spectrophotometric determination of lignin in small wood samples. Tappi 44: 793. 1961.

Bjorkman lignin or by acetyl bromide dissolution of wood for which the lignin content is known by virtue of some standard method.

Experimental

Apparatus

1. Reactor used is a glass-stoppered test tube made by the Kimble Glass Company. It is 19 mm., outside diameter, 150 mm. long, with a capacity of 28 ml. Stoppers are notched to allow escape of expanding and evolved gases.

2. Constant temperature bath regulated at 70° ±0.1° C.

3. Spectrophotometer used is a Beckman Model DU equipped with 10-mm. silica cells or its equivalent.

Reagents

The reagents used are acetyl bromide, reagent grade, redistilled; sodium hydroxide solution, 2 molar; hydroxylamine hydrochloride solution, reagent grade, 7.5 molar; glacial acetic acid, reagent grade, redistilled; and 25% (by volume) acetyl bromide in acetic acid.

Samples

The wood sample is ground to pass an 80-mesh screen, extractive-free, and air-dried. The moisture content is determined on a separate sample, preferably, but if the amount of sample is limited, the test can be run on an oven-dry sample to eliminate the need for a moisture determination.

Procedure

To determine the absorptivity of a standard lignin, a sample having an accurately known weight between 3.0 and 6.0 mg. is placed into a reaction

tube. Then, 10 ml. of the 25% acetyl bromide reagent is added to the sample. The tube is stoppered with a notched stopper and then heated for 30 min. in a water bath maintained at 70° ±0.1° C. The test tube is gently swirled at each 10-min. interval to mix reactants and promote dissolution. After 30 min. of heating, the sample is removed from the heating bath and placed in a beaker of water at 15° C. to cool. The cooled sample is transferred to a 200-ml. volumetric flask in which has been placed 9 ml. of 2M sodium hydroxide and 50 ml. of acetic acid. A minimum amount of acetic acid is used as a washing solvent to complete the transfer. Then, 1 ml. of 7.5M hydroxylamine hydrochloride is added. The contents are mixed, cooled, and diluted to the desired volume with acetic acid. The absorbance of this solution at 280 mμ is measured, and the absorptivity of the lignin at this wavelength is calculated as follows from the amount added:

$$\text{Absorptivity (lignin)} = \frac{\text{absorbance standard - absorbance blank}}{\text{concentration lignin (grams per liter)}}$$

The blank is run along with the sample in the same manner, omitting only the sample.

To determine the lignin content of an unknown specimen, the same experimental procedure is followed, and the lignin content is computed as follows:

$$\text{Percent lignin} = \frac{\text{(absorbance of sample - absorbance of blank) liters x 100}}{\text{absorptivity of lignin standard x sample weight (grams)}}$$

Samples containing more than 6 mg. of lignin are diluted with blank solution to give a concentration that has an absorbance between 0.3 and 0.7; if the sample contains less than 3 mg. of lignin, all volumes, including that of the acetyl bromide reagent, are reduced proportionally to give a satisfactory

absorbance reading. If blanks are greater than 0.010 absorbance units, tests are repeated using freshly prepared reagents.

Wood samples ranging from 2 to 100 mg. in weight and containing from 0.6 to 21.3 mg. of lignin have been analyzed in this way at the Forest Products Laboratory. These weights, however, do not necessarily represent sample-size limits.

Results and Discussion

Wood is almost wholly soluble in a reagent of 25% acetyl bromide, but a trace of material always remained insoluble in the experiments here reported.

This insoluble residue was identified as the membranous substances described by Pew[2,3] which originate as insoluble films deposited on the inner surfaces of ray cell parenchyma. This material, according to Pew, is found only in heartwood. In spruce, it amounts to a mere trace, but it comprises as much as 1% of the wood in Douglas-fir. After wood solutions are diluted to the required concentration for making absorbance measurements, the effect of undissolved particles in negligible.

Solutions of wood that have been treated with acetyl bromide have a brown color, but the color is not intense. A wood solution diluted to a concentration suitable for analysis (approximately 20 mg. of lignin per liter) is almost colorless. The ultraviolet absorption spectrum of wood treated with acetyl bromide is almost identical to those of both treated and untreated enzyme lignin.[1] This suggests that (1) the basic lignin chromophore is relatively

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[2] Pew, J. C. Membranous substances in common heartwoods. J. Forestry 47: 196. 1949.

[3] Marton, R. Brightness of high yield pulps. Part I. Morphology and nature of coloring materials in wood. Tappi 43: 826. 1960.

unaltered by treatment with acetyl bromide, and (2) the absorption in the ultraviolet of acetyl bromide-treated wood solutions is due to lignin, with little or none contributed by the nonlignin fraction. Negative aniline acetate tests for furfural support this second conclusion. Additional evidence was obtained by determining the absorptivity of the essentially nonlignin wood substance removed from spruce wood by cellulytic enzyme action.[1] When approximately 60% of the wood substance was removed, the absorptivity of the material removed was found to be 0.29, using the equation below:

$$K = \frac{K_1 - K_2 f}{1 - f}$$

where

K = absorptivity of the material removed by enzyme action

K_1 = absorptivity of original wood

K_2 = absorptivity of wood remaining after enzyme treatment

f = fraction of wood remaining after enzyme treatment (yield)

The true absorptivity of the nonlignin fraction is probably much smaller than the 0.29 for material removed by enzyme action, because the ultraviolet absorption curves of the enzyme liquors revealed that some lignin was removed in the enzyme treatment. An estimate of the amount of lignin lost indicated that it was enough to account for all of the loss in absorptivity of the residue. The absorptivity of the nonlignin fraction in spruce is then certainly not more than 0.29 (compared to more than 20 for lignin) at 280 mμ, and it is probably so much smaller as to be insignificant.

DETERMINATION OF MOISTURE IN WOOD AND WOOD PULP

Because wood is a hygroscopic material, it can contain large and varying amounts of moisture. The moisture of North American woods may vary from 20 to 75 percent (pct. of original sample).[1] To have meaning, the results of chemical analyses of wood are reported on an ovendry basis, and it is usually for this reason that the moisture in a wood sample is measured. Moisture in wood can be measured by ovendrying or an azeotrope (toluene-water) distillation method. Moisture may also be measured by the Karl Fischer method, electrical moisture meters, or isotope dilution methods. A bibliography of methods for determining moisture in pulp and paper is given in NBS Report No. 7064.[2]

At the Forest Products Laboratory, the ovendrying method for the determination of moisture is generally used. An azeotrope method would be used if extraneous volatiles were present which would cause an error in the moisture values. The ovendrying procedure used follows that described by Bray.[3]

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[1] Rasmussen, E. F. Dry kiln operators' manual, p. 197. U.S. Dept. of Agr. Handb. No. 188. 1961.

[2] Forshee, B. W. Bibliography on the interaction of cellulose and moisture. National Bureau of Standards Rpt. No. 7064. 1961.

[3] Bray, M. W. Methods for chemical analysis of pulps and pulpwoods. U.S. Forest Prod. Lab. Rpt. No. R19 (rev.) 1939.

Procedure

First, 2-4 g. of sample (pulp or sawdust) are weighed into a tared, glass-stoppered weighing bottle. The sample (with the stopper removed) is placed in a convection oven at 105° ±3° C.[4] and dried to constant weight (±0.1% change in the amount of moisture present upon 1 hr. reheating). Usually, 3-5 hr. is required for drying sawdust. Larger samples and larger particle sizes may take longer. When samples are removed from the oven for weighing, they are placed in a desiccator, cooled, and then weighed. Moisture is reported as percent moisture in the original sample.

Broughton and Hobbs[5] made a study of the variables and precision of the ovendrying of pulp. Their precision data are in table 1.

From the data in table 1, Broughton and Hobbs concluded that duplicate results of ovendrying moisture determinations should be within 0.2%. Further data from the Technical Section of the Paper Makers Association[6] supporting this conclusion are given in tables 2 and 3. They show temperature variation to have some effect on final moisture values, but extended drying times have very little effect.

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[4] 105° ±3° C. is recommend in TAPPI Standard T 18 for the drying of pulpwood, in TAPPI Standard T 210 for the drying of pulp, and in TAPPI Standard T 412 OS-63 for the drying of paper. ASTM Tentative Standard D2016-62T, 1962, for the determination of the moisture content of wood specifies a 103° ±2° C. temperature for drying.

[5] Broughton, G., and Hobbs, A. K. Moisture determination in paper by ovendrying. Tappi 35: 217. 1952.

[6] Committee report, Sampling and testing paper for moisture content. Paper Maker and British Paper Trade Jour. 119(1): 32. 1950.

Table 1.--Precision of ovendrying method

Sample	Results	Average	Standard deviation
Wrapping paper (kraft)	8.42, 8.24, 8.52, 8.32, 8.11, 8.33, 8.24, 8.44, 8.19, 8.32	8.32	0.12
Bound paper (25% rag)	7.64, 7.63, 7.83, 7.61, 7.53	±/7.65	±/.11
Blotting paper	5.93, 6.10, 6.26, 6.13, 6.41, 6.37	6.20	±/.18

1/ Calculated from Broughton and Hobbs' data.

Table 2.--The effect of temperature in ovendrying upon moisture test results1/

Sample	98° ±2° C.	107° ±2° C.	123° ±3° C.
	%	%	%
Kraft wrapping	8.1	8.5	8.8
Newsprint	7.7	8.3	8.6
Sulfite wrapping	7.4	7.9	8.1
Esparto wrapping	7.1	7.4	7.7

1/ See footnote 5 of text.

Table 3.--The effect of prolonged drying upon moisture test results[1]

Sample	2 hr.	6 hr.	14 hr.
	%	%	%
Kraft	8.73	8.73	8.88
Newsprint	8.22	8.22	8.51
Sulfite	7.93	7.88	7.93
Esparto	7.51	7.50	7.59

[1] See footnote 6 of text.

DETERMINATION OF NICKEL IN G-16-TYPE CATALYST[1]

Introduction

G-16-type nickel catalyst is used in the hydrogenation of wood sugars in their conversion to glycols and sugar alcohols.[2] The catalyst runs from 50% to 52% nickel on a weight basis. In this method, the sample is dissolved in hydrochloric acid and the nickel is precipitated as nickel dimethyl glyoxime, which is filtered out and weighed.

Apparatus

1. Tared Gooch crucibles.
2. Whatman No. 2 filter paper.

Reagents

1. Concentrated hydrochloric acid.
2. Tartaric acid.
3. Ammonium chloride.
4. Concentrated ammonium hydroxide.
5. Dimethyl glyoxime; 1% in methanol.
6. Methyl red indicator.

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[1] Standard method SM-C-8G, Technical Service Department, The Girdler Company, Louisville, Ky.

[2] Clark, I. T. Hydrogenolysis of sorbitol. Indus. Engin. Chem. 50(8): 1125-1126.

Procedure

A 0.1-g. sample (see Analytical Note 1) is accurately weighed and transferred into a 250-ml. beaker. Add 15 to 20 ml. of concentrated hydrochloric acid and boil gently until the nickel is dissolved. Filter the solution through No. 2 Whatman filter paper into a 250-ml. volumetric flask. Dilute to the mark with water, and mix. Pipet a 50-ml. aliquot of this solution into a 400-ml. beaker and dilute with 200 ml. of distilled water. Add 3 or 4 g. each of tartaric acid and ammonium chloride, then several drops of methyl red indicator, and make basic with concentrated ammonium hydroxide. Add 15 ml. of dimethylglyoxime solution and stir. Allow the solution to stand 10 to 15 min. (see Analytical Note 2) and filter through a tared Gooch crucible.[2] Wash the precipitate thoroughly and dry for 1-1/2 hr. at 110° C. Remove the crucibles from the oven, cool in a desiccator, and weigh.

Calculation

$$\text{Percent nickel} = \frac{\text{Weight of precipitate} \times 101.60}{\text{Weight of sample}}$$

Analytical Notes

1. Because of the high nickel content and powdery nature of the G-16 catalyst, greater accuracy is obtained by weighing a large sample and taking an appropriate aliquot portion rather than attempting to weigh a small enough sample and determining the nickel directly.

2. If the solution does not separate with a clear bottom layer, further stirring and 2 to 3 ml. more dimethyl glyoxime are needed.

DETERMINATION OF NITROGEN BY THE KJELDAHL[1] METHOD

(Includes Total, Ammoniacal, and Urea Nitrogen)

Introduction

The Kjeldahl method is used to determine the nitrogen in foodstuffs, fertilizer, yeast, fermentations, etc. It is directly applicable to amines and amides but not to nitro, azo, and azoxy compounds. These latter compounds must be reduced (Zn - Hg amalgam and acid or salicylic acid, sodium thiosulfate and acid) before the Kjeldahl treatment.

The early history of this method has been presented by Vickery (1) and reviews by Friedrich (2) and Bradstreet (3) give bibliographies. Hiller, Plazin, and Van Slyke (4) have discussed the method, providing background information and references. Procedures are given here for the determination of total nitrogen, ammoniacal nitrogen, and urea nitrogen.

The Kjeldahl method consists of three steps: (1) The organic nitrogen is converted to an ammonium salt by digestion with concentrated sulfuric acid. (2) The ammonium salt is converted in basic solution to ammonia, which is distilled and recovered quantitatively in an acid solution.

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[1] Kjeldahl, J., A new method for determining nitrogen in organic materials. Z Anal. Chem. 22:366. 1883.

(3) The ammonia in the distillate is determined by titration of the excess acid with standard base.

A commonly used modification of the above-described procedure is to distill the ammonia into a boric acid solution and to titrate the ammonium borate formed (5). The advantage of this is that the only standard solution necessary is the standard acid.

The principal reactions involved in the Kjeldahl determination are given below.

Amines:

$$RCH_2NH_2 + H_2SO_4 + H_2O \longrightarrow RCH_2OH + NH_4HSO_4$$

(The alcohol is further oxidized to carbon dioxide and water.)

Amides:

$$RC(=O)NH_2 + H_2SO_4 + H_2O \longrightarrow RC(=O)OH + NH_4HSO_4$$

(The carboxylic acid is further oxidized to carbon dioxide and water.)

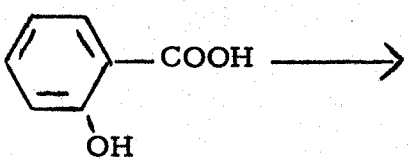
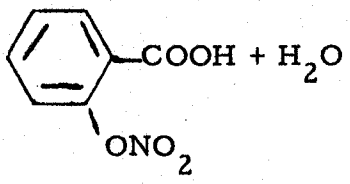
Nitro Compounds:

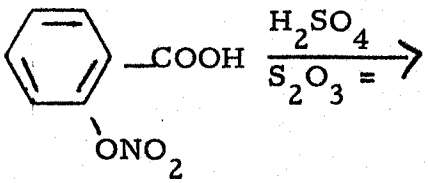
$$(1)\ RNO_2 + 6H^+ \xrightarrow{Hg\text{-}Zn} RNH_2 + 2H_2O$$

(The amine is decomposed by sulfuric acid as before.)

or:

$$(2)\ RNO_2 + H_2SO_4 \longrightarrow RHSO_3 + HNO_3$$

(3) HNO_3 +  

(4) 

(The reduced nitrated salicylic acid is decomposed by sulfuric acid and Kjeldahl procedure is continued.)

Total Nitrogen

Reagents

1. Concentrated C. P. sulfuric acid.
2. Potassium sulfate.
3. Copper sulfate.
4. N/14 Sulfuric acid.
5. N/14 Sodium hydroxide.
6. 49 pct. Sodium hydroxide (saturated).
7. Mossy zinc.

Procedure. --Do samples in duplicate.

A blank is always run along with the sample and the titration value of the sample is subtracted from that of the blank.

Place an accurately weighed or pipetted sample containing from 10 to 40 mg. of nitrogen into a 300-ml. Kjeldahl digestion flask. Add 1 ml. C. P. concentrated sulfuric acid and boil off any water.

For each 0.3 g. (approximate) of yeast or for each gram (approximate) of wood, add 10 ml. C. P. concentrated sulfuric acid to the flask. If more than 25 ml. H_2SO_4 is required, use a 500-ml. digestion flask. Then add 5 g. potassium sulfate and approximately 0.25 g. of hydrated (5 moles water) copper sulfate for each 10 ml. H_2SO_4 added. Digest mixture over a Bunsen burner, beginning with low heat. The digestion of a sample is complete when the carbon has all disappeared. This may take from 30 min. to several hours according to the sample being treated.

After the digestion is complete, cool the sample and then slowly (without swirling) add 70 ml. of 40 pct. sodium hydroxide solution for each 10 ml. H_2SO_4 originally added. Add a few pieces of mossy zinc, connect flask to condenser (the condenser tube should be below the surface of the acid), swirl gently, and apply heat immediately to prevent the acid

in the receiving flask from backing up into the condenser. The acid in the receiver will also back up into the condenser tube at the end of the digestion period if the receiver is not lowered so that the tip of the condenser is above the surface of the acid before the heating is discontinued.

Distill ammonia out of the alkaline solution into 50 ml. (this amount can be adjusted to the sample size) N/14 sulfuric acid containing a few drops of methyl red indicator.[2] This distillation should not be too rapid or (1) alkali may be carried over in spray or (2) the ammonia may not be completely absorbed in the acid. Descriptions of the distillation apparatus can be found in most analytical reference books. A spray trap is always a part of the apparatus, again to prevent sodium hydroxide from being carried over in the spray. Distill over about one-half the liquid. Titrate the excess acid in the receiver with N/14 sodium hydroxide. Compute the nitrogen content of the sample.

$$(1)\ \text{Percent nitrogen} = \frac{\text{me's } H_2SO_4 \text{ neutralized by } NH_3 \times 14 \times 100}{\text{grams sample} \times 1,000}$$

With N/14 reagents this becomes:

$$(2)\ \text{Percent nitrogen} = \frac{\text{Blank titration - Sample titration}}{\text{grams sample} \times 10}$$

---

[2] An antifoaming agent must sometimes be added if protein materials are present.

Notes on Procedure

1. After adding the alkali, all operations should be performed rapidly in order to prevent the loss of ammonia. For the same reason, solutions should be cold before mixing.

2. The mossy zinc helps to prevent bumping during distillation.

3. A piece of litmus paper can be used to be sure that the solution is alkaline before distillation.

4. During distillation, the temperature must be kept constant because, if it cools, the contents of the receiver will be sucked back.

5. High blanks may be due not only to nitrogen in reagents, but to absorption of ammonia from the air during analysis.

6. Results on duplicates should agree within 0.3 pct. of each other (absolute value), for example, 2.00 and 2.30 pct. would be acceptable.

7. Upon cooling after digestion, there is sometimes a tendency for the reaction mixture to solidify. This should be prevented, or at least the solids broken up, by shaking the flask so dissolution in alkali will be easier.

8. The ammonia can be steam distilled if desired.

Ammoniacal Nitrogen

Clarification. --If the samples contain a substantial amount of protein, they are clarified (to remove protein) to reduce foaming during distillation. The clarification procedure used is based on those of Somogyi[3] and Neish.[4] In this procedure, 100 to 200 ml. of fermentation solution is placed in a tared 250-ml. centrifuge bottle and then the bottle and sample weighed. Zinc sulfate solution (250 g. per liter) is then added in the ratio of 15 ml. for each 200 ml. of sample. The pH is adjusted to between 7.6 and 7.8, then the bottle and its contents weighed again to determine the dilution factor. The solids are then brought down by centrifuging. An aliquot of the supernate can then be taken for analysis.

In this method no acid digestion is necessary. If foaming becomes serious even after clarification, an antifoaming agent should be added.

Reagents

1. Magnesium oxide.
2. Mossy zinc.
3. 49 pct. Sodium hydroxide.
4. N/14 Sulfuric acid.

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[3] Somogyi, M. A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem. 86: 655 1930.

[4] Neish, A. C. Analytical methods for bacterial fermentations. Nat. Res. Counc. Can. No. 2952 (1952).

5. N/14 Sodium hydroxide.

6. Zinc sulfate solution (250 g. per liter).

7. Antifoaming agent (dissolve 50 g. diglycol stearate in 375 ml. benzene, 75 ml. alcohol, and 250 ml. dibutyl phthalate, with warming if necessary).

Procedure. --Determinations are done in duplicate.

A blank is run along with the sample and its acid consumption value subtracted from that consumed by the sample.

If clarification is not necessary, weigh or pipette the sample containing from 10 to 40 mg. of nitrogen into a 300-ml. Kjeldahl flask and proceed with distillation as described below. If clarification is necessary, accurately weigh a convenient amount of sample into a centrifuge bottle (weight bottle, then bottle plus sample). To the bottle then add 15 ml. zinc sulfate solution for each 200 ml. of sample. Adjust the pH level between 7.6 and 7.8 and then centrifuge. Take an aliquot of the supernate for analysis.

Place the aliquot containing from 10 to 40 milliequivalents of nitrogen in a 300-ml. Kjeldahl flask. Add 2 g. of magnesium oxide, mossy zinc, and 50 ml. of 49 pct. sodium hydroxide. Shake the mixture. Distill the ammonia present into 50 ml. of N/4 sulfuric solution which also contains

a few drops of methyl red indicator. Distill over about one-half the liquid, then determine the amount of acid neutralized by ammonia by titrating excess acid with standard sodium hydroxide.

If samples were not clarified, the computation of the nitrogen content is the same as for total nitrogen.

$$(1)\ \text{Percent nitrogen} = \frac{\text{ml. } H_2SO_4 \text{ neutralized by } NH_3 \times 100}{\text{grams sample} \times 1,000}$$

or:

$$(2)\ \text{G. nitrogen per 100-ml. sample} = \frac{\text{ml. } H_2SO_4 \text{ neutralized by } NH_3}{\text{ml. sample} \times 10}$$

If samples were clarified, the computation shown in equation (2) is used except that a dilution factor is needed to account for the dilution made during clarification. Then the result given by equation (2) must be multiplied by the weight of sample after clarification and divided by the weight of the sample before clarification. Of course, in these calculations the blank value must also be included (subtracted from acid consumed by the NH_3 from the sample).

Urea Nitrogen

To determine the nitrogen in a sample which is due to the presence of urea, or to determine urea itself, an aliquot is treated with urease

which decomposes the urea to form carbon dioxide and ammonia. An ammoniacal nitrogen is then run and the ammonia found is attributed to the nitrogen from the urea.

Reagents

1. Magnesium oxide.
2. Mossy zinc.
3. 49 pct. Sodium hydroxide.
4. N/14 Sulfuric acid.
5. N/14 Sodium hydroxide.
6. Zinc sulfate solution (250 g. per liter).
7. Urease tablets or powder.
8. 0.1 N Hydrochloric acid.
9. 0.01 Sodium hydroxide.
10. Calcium chloride solution (250 g. per liter).
11. Antifoaming solution (dissolve 50 g. diglycol stearate in 375 ml. benzene, 75 ml. alcohol, and 250 ml. dibutyl phthalate, with warming if necessary).

Procedure. --The samples are clarified to reduce foaming if substantial amounts of protein are present using the procedure described in the method for ammoniacal nitrogen.

The urease must first be standardized. The object of this is to measure the capacity of the urease and then make up a solution for use such that 10 ml. will decompose at least 0.1 g. urea. To do this, dissolve 0.5 g. urease in 50 ml. water and add enough 0.1 N hydrochloric acid to make it neutral (methyl red end point).

To each of four Kjeldahl flasks add 0.1 g. urea. Then add 5, 10, 15, and 20 ml. of the urease solution to each respective flask. Let the flasks stand 1 hr. at room temperature and then distill the ammonia formed into 50 ml. of N/14 sulfuric acid. Calculate the percent decomposition from the amount of acid used up by the ammonia (see total nitrogen analysis). Calculate the concentration of urease that will give solution such that 10 ml. will decompose 0.1 g. urea. This solution, then, is the standard urease solution.

To determine the urea in a fermentation solution, place an aliquot containing approximately 0.1 g. urea in a 500-ml. Kjeldahl flask. Bring up to about 250 ml. with water. Add 10 ml. standard urease solution, stopper tightly, and let stand at room temperature 1 hr. After 1 hr., rinse stopper and neck with a few ml. of water. Add 2 g. heavy-type magnesium oxide, 1 ml. calcium chloride solution, and 5 ml. of the anti-foam solution. Connect the flask to the condenser and distill 100 ml.

into 50 ml. of N/14 sulfuric acid. Titrate remaining acid with N/14 sodium hydroxide using methyl red as an indicator. Samples are run in duplicate and a blank is run with the samples. The percent nitrogen due to urea is calculated as it is in the total nitrogen analysis. If urea content is desired, the nitrogen value is multiplied by 2.143.

Blanks are run along with samples, but in this case the blank contains reagents and sample but no urease. As measurements of both ammoniacal and urea nitrogen are desired, the blank contains reagents with no sample and then ammoniacal nitrogen tests run on two different aliquots, one having been treated with urease and one not; the difference in nitrogen then being attributed to the urea.

References

(1) Vickery, H. B.
1946. The early years of the Kjeldahl method to determine nitrogen. Yale J. Biol. Med. 18: 473.

(2) Friedrich, A.
1933. The quantitative determination of nitrogen by the Kjeldahl method. Microchemie 13: 91.

(3) Bradstreet, R. B.
1940. A review of the Kjeldahl determination of organic nitrogen. Chem. Rev. 27: 331.

(4) Hiller, A., Plazin, J., and Van Slyke, D. D.
1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. J. Biol. Chem. 176: 1401.

(5) Meeker, E. W., and Wagner, E. C.
1933. Titration of ammonia in presence of boric acid (macro and micro Kjeldahl procedures). Indus. Eng. Chem. Anal. Ed. 5: 396.

DETERMINATION OF PENTOSANS IN PULP[1]

I. Introduction

The method described is for determining pentosans in pulp by the orcinol colorimetric method in the range from less than 1% to about 16%. An alternate method for determining the furfural by bromination is also given. The bromination method has a more limited range but does not require a spectrophotometer.

In both methods, the pulp is treated with boiling hydrochloric acid (approximately 12 % or 3:7) to hydrolyze the pentosans to pentoses, which are then converted to furfural. The furfural is then distilled, collected, and determined by either the orcinol or bromination methods. Hydroxymethyl furfural is also produced during the distillation, but does not interfere in the colorimetric determination and is corrected for in the bromination method.

II. The Orcinol Method

An aliquot of the distillate is treated with orcinol reagent and, after it stands for 2 hr., its absorbance is measured at 630 mμ. By referring to a calibration curve prepared from known furfural solutions, and using a pentosan-to-furfural conversion factor, the percentage of pentosans may be calculated.

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[1]This method is an adaptation of the method designated CCA 24:56 by the Analysis Committee of the Cellulose Industry of the Swedish Association of Pulp and Paper Engineers and is also ASTM Standard Method D 1787, 1962, for pentosans and TAPPI Tentative Standard T 223, 1963.

Apparatus

1. Flask, two-neck round-bottom, 500-ml. standard taper 24/40.

2. Dropping funnel, 50-ml., with standard taper 24/40 neck, with the lower section graduated (with glass-marking pencil or ink) into six 50-ml. intervals.

3. Distillation connecting tube, with standard-taper 24/40 inner joints to fit the round-bottom flask and Graham condenser, with the proper angles to allow for the vertical position of the condenser. If a thermometer is to be used, the adapter should have a 10/30 standard-taper thermometer well.

4. Condenser, Graham, water-cooled, with standard-taper 24/40 connections having an effective length of at least 50 cm.

5. Adapter, with standard-taper 24/40 joints with gas inlet.

6. Receiving flask, 500-ml., with 24/40 standard-taper joint, to collect the distillate.

7. Ice bath, for receiving flask to help prevent loss of furfural.

8. Heating mantle, for reaction flask, controlled by a variable transformer. A gas burner may be substituted for the heating mantle.

9. Constant-temperature bath, capable of maintaining a temperature within ±0.5° C. of the temperature selected for development of the color with orcinol, which should be between 15° and 30° C.

10. Volumetric flasks, 50-, 100-, and 500-ml. capacity.

11. Pipets, transfer, 5-, 10-, 15-, 20-, 25-, and 50-ml. capacity.

12. Photometer, filter, or spectrophotometer, suitable for measuring absorbance at 630 mμ.

Reagents

1. Hydrochloric acid (3:7). Mix 3 volumes of concentrated hydrochloric acid (sp. gr. 1.18 to 1.19) with 7 volumes of water. The concentration will be approximately 12%.

2. Furfural. Distill furfural immediately before use. Use only freshly distilled pale yellow material with a boiling point of 162° C. It is preferable to distill in vacuo.

3. Orcinol solution. Dissolve 0.40 g. of orcinol and 0.5 g. of $FeCl_3 \cdot 5H_2O$ in 1 liter of 11.0N HCl. Discard solutions more than 1 wk. old.

4. Ethanol. 95% aldehyde-free.

Preparation of Calibration Curve

Dissolve about 0.5 g. furfural, weighed to an accuracy of ±0.2 mg., in HCl (3:7) and dilute to 500 ml. with HCl (3:7) in a volumetric flask. After mixing thoroughly, pipet 5, 10, 15, 20, 25, 30, and 50 ml. into 100-ml. volumetric flasks and dilute to the mark with HCl (3:7). This gives solutions with 50, 100, 150, 200, 250, 300, and 500 mg. of furfural per liter. (Higher or lower ranges of concentrations can be prepared to fit specific conditions.)

Pipet 10 ml. of each solution into a 50-ml. volumetric flask and add 25 ml. of orcinol solution. Place the flasks in a constant temperature bath.[2]

Prepare a blank containing all the reagents, so that it is complete except for the furfural solution.

[2]The color intensity is dependent on the temperature during the reaction of furfural with orcinol. Any temperature between 15° and 30 ° C. may be used, provided the temperature is the same during the pentosans determination as in the preparation of the calibration curve.

After 60±5 min., almost fill the volumetric flasks with ethanol, mix, and bring to the temperature of the bath; then fill to the mark with ethanol, and again mix thoroughly. Place in the bath for a second 60±5-min. period. Measure the absorbance at 630 mμ. and correct for the reading on the reagent blank.

Plot absorbance versus milligrams of furfural to give a calibration curve.

Procedure

The size of sample to be taken depends on its pentosan content. Select the proper size of sample and suitable aliquot for the subsequent colorimetric measurement from the following tabulation:

Pentosans Content (%)	Sample Size (g.)	Size of Aliquot (ml.)
1	4	10
1-3	3	10
3-5	2	10
5-10	1	10
10	1	5 (plus 5 ml. HCl (3:7))

Allow the sample to come to moisture equilibrium with the atmosphere of the balance. Weigh to the nearest milligram and transfer it to the 500-ml. round-bottom flask. At the same time weigh a sample for moisture determination and make the determination according to standard procedure. Add 100 ml. of hydrochloric acid (3:7) to the sample flask, washing down any fibers adhering

to the sides of the flask. Assemble the distillation apparatus and mark the flask to indicate the liquid level. If the room air temperature is above 20° C., cool the receiver with an ice bath.

Add 300 ml. of HCl (3:7) to the dropping funnel.

Start timing the distillation when the distillate first starts to appear and distill for 90 min., collecting in that time 300 ml. of distillate in the receiver. Allow the acid to drop into the distillation flask from the dropping funnel at such a rate that the original 100-ml. level is maintained.[3] It is important that the proper rate of distillation be maintained and that 300 ml. of distillate be collected. The receiver should be marked at the 300-ml. level.

Transfer the contents of the receiver to a 500-ml. volumetric flask. Rinse the receiver[4] with HCl (3:7) and add the rinsings to the volumetric flask. Dilute to the mark with HCl (3:7) and mix well.

Pipet 10 ml. of the diluted distillate into a 50-ml. volumetric flask, add 25 ml. of orcinol solution, and place the flask in the constant temperature bath. After 60±5 min. almost fill the flask with ethanol, mix, and bring to the temperature of the constant temperature bath; finish filling to the mark with ethanol and mix thoroughly. Place in the bath for a second 60±5-min. period.

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[3] It is important to avoid overheating of the flask above the liquid level.

[4] If a 500-ml. volumetric flask, marked at the 300-ml. level, is used as the receiver, transfer of the distillate and rinsing of the receiver are avoided. Proper choice of sample size and aliquot will allow the use of a 300-ml. volumetric flask as the receiver. The distillation can be stopped just short of the mark to allow precise adjustment of the volume.

Measure the absorbancy of the blue color at 630 mμ., using a blank containing 10 ml. (or whatever size aliquot is used) of HCl (3:7), instead of the distillate, as a reference solution. Read the furfural concentration of the distillate from the calibration curve.

Calculations

The theoretical factor for converting pentosans to furfural is 0.727, which is the quotient of the molecular weight of furfural, 96, divided by the formula weight of anhydroxylose, 132. Assuming that the pentosans content of wood consists principally of xylan that is converted to furfural with a yield of 88 pct. the empirical factor for converting furfural values to pentosans is $\frac{1}{0.727 \times 0.88} = 1.563$

Calculate the pentosans content of the sample by the formula:

$$\text{percent pentosans} = \frac{\text{grams furfural in distillate} \times 1.563 \times 100}{\text{wt. of sample in grams, corrected for moisture}}$$

Report

Report the pentosans content of the cellulose as a percentage of the moisture-free sample. Make all determinations at least in duplicate, and report the average value.

Precision

In general, the range of duplicates should not exceed about 10 pct. of the amount of pentosans present.

III. Bromination Method (Alternate Method)[5]

This procedure is the same as the orcinol procedure up through the distillation step except that the receiver used is a 1000-ml., glass-stoppered flask instead of a 300-ml. volumetric flask. The furfural is determined by reaction with bromine, the excess bromine being measured by titration with thiosulfate.

Reagents

1. Ten pct. potassium iodide solution.

2. Potassium bromate-bromide solution, 0.2 N. Dissolve 5.57 g. of $KBrO_3$ and 50 g. of KBr in water, add 1 g. of Na_2CO_3 and dilute to 1 liter. This solution is standardized indirectly by means of a blank determination against standardized thiosulfate.

3. Sodium thiosulfate solution, 0.1 N. Dissolve 25 g. $Na_2S_2O_3.5H_2O$ in 1 liter and standardize with potassium dichromate.

4. Starch indicator solution.

Procedure

After 300-ml. of distillate is collected in the 1-liter flask, add 50 ml. of water and 250 g. of crushed ice. When the temperature of the mixture has fallen to 0° C. or lower, gently add 20 ml. of 0.2N bromide from a pipet with a minimum of agitation. Close the bottle promptly with its ground-glass stopper, shake well, and let stand for exactly 5 min. with occasional shaking. The temperature should remain at 0° C. or lower. Remove the stopper, add 10 ml. of 10% KI solution from a small graduate, and replace the stopper as quickly as possible. Shake the mixture thoroughly to allow absorption of the bromine

[5] Taken from TAPPI Standard T 223 (Tentative 1963).

vapor; then titrate with standardized 0.1N $Na_2S_2O_3$ until almost colorless using starch indicator toward the end of the titration.

Perform a blank titration in exactly the same manner, using all reagents, including the ice, except start with 270 ml. of 12% HCl and dilute to 350 ml., instead of using 30 ml. of distillate plus 50 ml. of water.

Calculation:

$$\text{Pentosans, \%} = \frac{7.50\ (V_2 - V_1)N}{W} \text{ -1.0}$$

where:

N = normality of the thiosulfate solution

V_1= volume of thiosulfate used in the test

V_2= volume of thiosulfate solution used in the blank

W = weight of the moisture-free specimen in grams.

Precision

A general guide for the agreement between duplicate tests is as follows:

Pentosans	Suggested agreement
%	%
2	0.1
2 - 10	.4
10	1.0

(see table page 7)

IV. Additional Information

1. The theoretical factor for converting furfural to pentosans is 1.375, the molecular weight of the xylan unit 132, divided by the molecular

weight of furfural, 96. Assuming that the pentosan content of wood consists principally of xylan that is converted to furfural with a yield of 88%, the empirical factor for converting furfural values to pentosans is:

$$\frac{1.375}{0.88} = 1.563$$

2. In the bromination method, the milliequivalent weight of furfural is 0.048. The formula for calculating the pentosans as a percentage is:

$$\frac{1.563 \times 0.048 \times 100 \times \underline{N} \times (V_2 - V_1)}{W} - 1.0 = \frac{7.50 \times \underline{N} \times (V_2 - V_1)}{W} - 1.0$$

The correction 1.0 is subtracted to compensate for the hydroxymethylfurfural produced during the distillation. This correction has been discussed in detail for several pulps by Bethge;[6] its value may be more accurately determined for specific pulps.

3. An interlaboratory testing program[7] has been conducted comparing the orcinol, bromination, and aniline acetate methods for measuring furfural. Standard deviation values for a group of 10 pulps analyzed for pentosans by the orcinol method are as follows:

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[6] Bethge, P. O., Svensk Paperstid. 59: 372. 1956.

[7] Wilson, W. K., and Mandel, J., ACS-ASTM-TAPPI-ICCA Pentosans Task Group. Tappi 43: 998. 1960.

Pentosans	Standard deviation	
	Within laboratory	Between laboratories
%		
0.41	0.069	0.10
.88	.080	.10
1.13	.086	.11
1.27	.097	.12
1.98	.11	.12
4.18	.16	.19
4.69	.17	.21
5.18	.18	.23
10.40	.30	.57
16.36	.45	1.06

Standard deviation values for a group of four pulps analyzed for pentosans by the bromination method on an interlaboratory basis are as follows:

Pentosans	Standard deviation	
	Within laboratory	Between laboratories
%		
4.49	0.22	0.35
5.02	.23	.36
10.01	.27	.55
15.94	.32	.82

The figures in these two tabulations were derived from the analyses of three combined distillates and therefore do not include the variations among individual distillates.

DETERMINATION OF THE pH OF WOOD

Introduction

Wood is generally somewhat acidic. The pH, or degree of acidity, is of interest because acids promote the corrosion of metals that come in contact with the wood. Metal fastenings and metal items crated in wood are important examples of this. If wood is kept dry, very little corrosion of metals will take place; but at high moisture levels, corrosion may be rapid.

The predominant acid found in wood is acetic, but small amounts of other substances, such as formic acid, thujic acid, tropolones, and tannins, may also contribute to acidity. As most of the acetic acid present is the result of the hydrolysis of acetyl groups that are attached to the wood substance, moist conditions, especially at elevated temperatures, will increase the acetic acid content (1). Such conditions might be encountered during kiln drying, steam bending, or storage of wood. Acetic acid liberated during the steam bending of woods with high acetyl content may be sufficient to cause considerable wood degradation and result in a significant loss in strength.

The pH of woods varies widely even within species. The oaks, Douglas-fir, cedars, and pines tend to be relatively acidic, with maple, ash, elm, and aspen less acidic. Metals will be attacked more rapidly by more acidic

woods. Various kinds of metals are attacked differently, depending on the type of acid present, but only extremely small amounts of acid are necessary to promote corrosion of metals (2).

There is no widely accepted method for the determination of the pH of wood. Gray (3) measured the pH of sawdust in a minimum amount of water and used this as an indication of the acidity of the wood. This is not the pH of the wood at its moisture content, but the values obtained are a useful index. He lists pH values for a large number of woods. His data again show considerable variation (up to 3 pH units) within a species, but some uniformity within families. The pH of most specimens falls within a range of 3.5 and 5.5.

In Gray's data, the difference between sapwood and heartwood did not show a consistent trend. In oak, however, the sapwood is consistently less acid than the heartwood. Gray pointed out that his samples were of unknown age and that aging could have a pronounced influence on pH. He made his pH reading when a steady value was reached (usually about 20 min.).

Campbell (4) determined the pH of wood by suspending sawdust in solutions of various pH. The pH of the wood was determined to be the pH of the water solution that did not change upon the addition of the sawdust.

Sandermann and Rothkamm (5) reviewed the value of pH measurements on wood and the different methods used. They preferred the method of Campbell and Bryant (4) and reported the pH of 80 different woods by this method. They also measured buffering capacities and compared heartwood and sapwood pH.

Hudson and Milner (6) and Stamm (7) measured the pH of paper by moistening it and using a flat-headed glass electrode. They found this system to be rapid and easy, and to give close agreement with extraction procedures. This method requires rigid standardization to obtain reproducible results, and time must be allowed for the acids to diffuse out of the wood to establish an equilibrium condition. During the equilibration period, the distilled water may absorb carbon dioxide, lowering the pH of the water.

Packman (1) studied the acids evolved upon storage of wood and found that acidity increased with storage time and temperature. The predominant acid was acetic, and a corresponding loss in acetyl in the wood was noted.

Clarke and Longhurst (2) exposed various metals to the vapors evolved from wood and measured the effect quantitatively. They found that very small amounts (0.5 p.p.m.) of acetic acid could promote corrosion at high moisture levels.

All of the methods previously mentioned give results that are reasonably comparable. The measurement of pH in minimum distilled water is used at the Forest Products Laboratory because it is simpler. The ratio of wood to water is 1:3 to 1:5. However, this does not give the pH of the wood at a lower moisture content. This can be shown by pH measurements of a sawdust suspension in a minimum amount of water and then in stepwise dilutions of this suspension. A curve can be constructed relating pH to dilution. Extrapolation will indicate a slightly lower pH of the wood at its moisture content.

The difficulty lies in how to extrapolate correctly at the lower moisture levels.

Procedure Used at the Forest Products Laboratory

One part by weight of freshly ground (to pass 40-mesh screen) sawdust is placed in three parts of freshly boiled and cooled distilled water. The sample and water are mixed until the sawdust is wet, and the pH is then measured with a glass electrode pH meter after 5 minutes.

References

1. Packman, D. F.
1960. The acidity of wood. Holzforschung 14: 178.

2. Clarke, S. G., and Longhurst, E. E.
1961. The corrosion of metals by acid vapors from wood. Jour. Appl. Chem. 11: 435.

3. Gray, V. R.
1958. Jour. Inst. Wood Sci. 1: 58.

4. Campbell, W. G., and Bryant, S. A.
1941. Determination of pH in wood. Nature 147: 357.

5. Sandermann, W., and Rothkamm, M.
1959. On the determination of pH values in timbers and their practical importance. Holz als Roh und Werkstoff 17: 433.

6. Hudson, F. L., and Milner, W. D.
1959. The use of flat headed glass electrodes for measuring the pH of paper. Svensk Papperstidn. 62: 83.

7. Stamm, A. J.
1961. A comparison of three methods for determining the pH of wood and paper. Forest Prod. Jour. 11: 310-312.

DETERMINATION OF POLYETHYLENE GLYCOL IN WOOD

Wood is sometimes impregnated with polyethylene glycol to increase its dimensional stability. In research on this technique, it becomes necessary to know how much polyethylene glycol is in the wood. In the analysis, polyethylene glycol is taken as the hot-water-soluble extract of the wood sample minus the hot water-soluble extract of a control sample.

Apparatus

Weighing bottle: I D, 50 mm.; height, 60 mm.; volume, approximately 85 ml.

Sample

Thin (1 mm. or less) cross sections of wood.

Procedure

A control containing no polyethylene glycol is run along with the sample, following the same procedure as is used for the sample.

Tare a weighing bottle by drying it for 3 hr. at 67° C. under vacuum, cooling 30 min. in a desiccator, and weighing.

Take approximately 3 g. of sliced sample and break it up with a paper cutter and pliers. Dry the sample for 5 hr. at 67° C., under vacuum, cool, and accurately weigh approximately 3 g. into a 250-ml. beaker.

Add 75 ml. of water to the beaker, cover with a watch glass, and allow the sample to simmer on a hotplate at about 95° C. (adding water if necessary to maintain the water level) for 5 hr. Near the end of the extraction, allow

the volume to decrease to 40-50 ml. so that it can be conveniently contained in the weighing bottle. Filter the hot contents of the beaker through fluted filter paper, washing the residue with 10 ml. of hot water. Collect the filtrate in the tared 85-ml. weighing bottle.

Evaporate the extract to dryness on a steam chest. Complete the drying by placing the weighing bottle in the oven at 67° C. under vacuum for 3 hr. After 3 hr., cool the sample and weigh.

The control run on untreated wood is handled the same as the sample.

The percent of polyethylene glycol is calculated as percent of ovendry weight corrected for the extract obtained on the untreated sample.

Appendix

1. Moisture-free polyethylene glycol-treated wood loses weight significantly upon continued heating at 100° C. at atmospheric pressure due to chemical decomposition (not simply volatilization). Loss of polyethylene glycol does not occur under vacuum at 67° C.; therefore drying polyethylene glycol-treated wood and polyethylene glycol extracts under vacuum at 67° C. is recommended.

2. Polyethylene glycols can also be measured by the method of Shaffer and Critchfield[1]. This method is based on the reaction of polyglycols with the heteropoly acids, silicotungstic and phosphomolybdic. After reaction, the measurement of the polyglycol can be made by either gravimetric or colorimetric means.

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[1]Shaffer, B. C., and Critchfield, F. H. Solid polyethylene glycols (carbowax compounds), quantitative determination in biological materials. Analyt. Chem. 19: 32. 1947.

DETERMINATION OF PROPYLENE GLYCOL

Propylene glycol is one of the products formed upon hydrogenation of wood sugars[1]. This method for the determination of propylene glycol is based on that of Barker and Summerson[2] and of Markus[3]. It depends upon the conversion of propylene glycol to acetaldehyde by heating with concentrated sulfuric acid and copper sulfate. The liberated acetaldehyde, when treated with p-phenylphenol, gives a violet color, which follows Beer's Law over a narrow range at extremely low concentrations. Lactic acid, acetaldehyde, and sodium bisulfite-acetaldehyde complex interfere, since they undergo the same reaction. Ethylene glycol and glycerol do not interfere.

Reagents

1. Copper sulfate-sulfuric acid. Dissolve 2 g. of $CuSO_4 \cdot 5H_2O$ in 10 ml. of water and add to 600 ml. of concentrated sulfuric acid (or add 40 ml. of a 20% $CuSO_4 \cdot 5H_2O$ solution to one 9-lb. bottle of concentrated sulfuric acid).

2. p-Phenylphenol, a solution of 1.5% in 0.05% NaOH. Mix 3 g. of p-phenylphenol, 1.5 g. NaOH pellets, and a little water, then heat and stir until they dissolve. Make this up to 200 ml. with distilled water. New reagent should be made up every 30-40 days.

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[1] Clark, I. T. Hydrogenolysis of sorbitol. Indus. and Eng. Chem. 50: 1125. 1958.

[2] Barker, S. B., and Summerson, W. H. Colorimetric determination of lactic acid in biological material. Jour. Biol. Chem. 138: 535. 1941.

[3] Markus, R. L. Colorimetric determination of lactic acid in body fluids utilizing cation exchange for deproteinization. Arch. Biochem. 29: 159. 1950.

Apparatus

Beckman DU spectrophotometer or equivalent.

Procedure

Into a wide-mouth vial (16 ml. ID; 95 ml. length) fitted with a screw cap, pipet 1 ml. of a solution which will yield 1-10 μg. of acetaldehyde (corresponding to 1.7-17.3 μg. propylene glycol). Add 6 ml. of the $CuSO_4$-H_2SO_4 solution and mix thoroughly. The vial is immersed in boiling water for exactly 2 min., then cooled to 15°-18° C. When the vial is cool, 0.1 ml. of p-phenylphenol reagent is added and mixed in. The color is developed by placing the tube in a constant temperature bath at 30° C. for 1/2 to 1 hr. It is then immersed in boiling water for 90 sec. and cooled to room temperature by immersing in cold water. The violet color is read at 560 mμ on the spectrophotometer. Three blanks are run in the same way, substituting 1 ml. of distilled water for the sample. Propylene glycol is calculated from a prepared calibration curve.

Precaution

This method is extremely sensitive. Temperatures are critical; and every care must be exercised to avoid touching or otherwise contaminating the inner surfaces of glassware or tips of pipets. Glassware should be cleaned with cleaning solution, trisodium phosphate, and hot water. Rubber gloves and tongs should be used to avoid contamination from the hands.

PYROLIGNEOUS ACID ANALYSIS[1]

Introduction

Pyroligneous acid is a product of the destructive distillation of wood. It consists of the material making up the aqueous fraction of the wood distillate. It contains solvents such as methanol and acetic acid and other volatile constituents that are soluble in water containing these solvents. Since the pyroligneous acid layer is in contact with the tar layer, there is an equilibrium condition: The tar fraction will contain small amounts of methanol, acetic acid, and water, and the pyroligneous acid fraction will contain some phenol ethers.[2]

Determining Total Volatile Acid

Place five or six glass beads or porcelain chips in a 200- or 300-cc. Jena or Pyrex distilling flask fitted with a two-hole rubber stopper. Weigh flask and beads without stopper. In one hole of the rubber stopper put a 100-cc. dropping funnel and in the other hole a 250° C. thermometer adjusted so that it touches the bottom of the flask. Pipet 100 cc. of the crude pyroligneous acid into the dropping funnel and place flask in a sand bath. The temperature of the sand bath should not exceed 225° C.

The crude acid is distilled by dropping it into the flask at a rate just enough faster than the rate of distillation to prevent the temperature (as shown by the thermometer) in the flask from rising above 140° C. When all of

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[1] Based on Klar, M., "The technology of wood distillation" pp. 388-424, D. Van Nostrand Company. 1925.

[2] Wise, L. E. Wood Chemistry, p. 684, Reinhold Publishing Corp., New York, N.Y. 1944.

the crude acid has been added, place 50 cc. of distilled water in the dropping funnel and, as soon as the thermometer in the flask reads 140° C., begin adding the water at the same rate as used for the acid. The distillation is complete when all the water has been added and the thermometer reads 150° C.

Remove the stopper at once and wipe the bulb of the thermometer on the inside of the neck of the flask to remove adhering tar. Remove flask from sand bath, wipe clean, let cool, and weigh. The gain in weight of the flask is the grams of dissolved tar per 100 cc. of crude acid.

Make up distillate to 250 cc., mix thoroughly, and titrate a 50-cc. portion with normal sodium hydroxide, using phenolphthalein as indicator. The titration should be made cold with six or seven drops of indicator. The endpoint should be carried to the first permanent red; however, the endpoint is not very sharp and usually goes through a reddish-orange stage to permanent red. Record as total acid.

The titration should be made as soon as possible after distillation and must be made the same day as the distillation. If not, there is usually considerable oxidation and darkening of the tar-free distillate, even on standing overnight in a well-stoppered flask, which makes the determination of the endpoint difficult.

The total acid titration value is used later to compute the concentrations of acetic and formic acids.

Determining Acetic Acid

Add an excess (25g.) of finely powdered mercuric oxide to 50 cc. of the distillate obtained in total acid determination. The mixture is made up to about 100 cc., well stirred, and placed on a steam bath. At the end of

2 hrs. enough additional mercuric oxide is added so that the mercuric acid precipitated in the bottom gives a layer of about 15 g. in weight. Allow to remain on the steam bath 1 hr. longer. The total is then transferred to a 500-cc. distilling flask and an excess (40 cc.) of sirupy 85% phosphoric acid is added (to decompose any mercuric acetate formed); a few drops of paraffin oil are also added to minimize frothing. The mixture is distilled until a slight frothing is noticed, and the residue is washed by distillation with 100 cc. of water dropped in from a dropping funnel just fast enough to prevent frothing. The whole distillate is then titrated with normal sodium hydroxide, using phenolphthalein as indicator.

Compute the percentages of formic and acetic acids present as follows:

cc. NaOH for total acid − cc. NaOH for acetic acid = cc. NaOH for formic acid

$$\frac{5 \times \text{cc. alkali} \times 0.06005}{\text{Sp. gr. pyroligneous acid}} = \% \text{ acetic acid}$$

$$\frac{5 \times \text{cc. alkali} \times 0.04602}{\text{Sp. gr. pyroligneous acid}} = \% \text{ formic acid}$$

Wood Alcohol

Measure 500 cc. of crude pyroligneous acid into a 1-liter distilling flask placed on an asbestos gauze. Distill over 300 cc.; make the distillate strongly alkaline with strong sodium hydroxide[3] (35% to 40% solution of commercial sodium hydroxide is satisfactory and 75 cc. is ample), and distill

[3] The dark color produced on adding the alkali prevents the use of litmus paper, and phenolphthalein cannot be used on account of the alcoholic solution.

the neutralized distillate to a volume of 225 cc. The same flask used in the first distillation may be used for the second after simply emptying out the contents. A sand bath should be used for this distillation to permit complete hydrolysis of methyl acetate. Add to the second distillate an excess of the strong alkali used for the first distillate (10 cc. is sufficient) and distill over 65%, corresponding to a volume of approximately 155 cc. A fourth distillation of 65% should be made, adding about 2 cc. of concentrated sulfuric acid before making the distillation. The last distillation should be made with a well-cooled condenser and 90 ml. collected in a 100-ml. volumetric flask. For the third and fourth distillations, use 500-cc. distilling flasks placed on asbestos gauze. Glass beads or porcelain chips should be used in all distilling flasks to prevent bumping and frothing. Adjust the temperature of the distillate to 20° C., dilute to 100 ml., and mix. Measure the specific gravity of the distillate with a pycnometer. The alcohol content of the distillate is derived from concentration density correlation tables and used (expressed as a decimal) in the calculation of alcohol in the original pyroligneous acid sample.

Compute the percent alcohol in the pyroligneous acid as follows:

$$\frac{\text{Volume of distillate (final) x specific gravity of distillate}}{\text{Volume of original pyroligneous acid}} \times \frac{\text{alcohol content of distillate x 100}}{\text{specific gravity of original pyroligneous acid}} = \text{\% alcohol in original pyroligneous acid}$$

Acetone

In the presence of alkali, iodine reacts with acetone to form iodoform.

$$2NaOH + I_2 \longrightarrow NaI + NaOI + H_2O$$

$$CH_3COCH_3 + 3NaOI \longrightarrow CH_3COCI_3 + 3NaOH$$

$$CH_3COI_3 + NaOH \longrightarrow CHI_3 + CH_3COONa$$

In this method, acetone is measured by adding excess iodine and titrating the excess with thiosulfate.

$$I_2 + 2S_2O_3^{=} \longrightarrow 2I^- + S_4O_6^{=}$$

To a 25-ml. portion of the final alcohol distillate, add 10 ml. 2N sodium carbonate, then add 50 ml. 0.1N iodine. Count 3 min., beginning when the pipet is half empty. At the end of 3 min., add 10 ml. 3N H_2SO_4. Titrate the whole with 0.1N thiosulfate using starch as an indicator. The difference between 50 and the number of milliliters of thiosulfate used equals the number of milliliters of iodine solution reacting with acetone.

The general formula for the computation of acetone content is given below.

$$\frac{(50 \times \underline{N}\ (\text{iodine}) - \text{ml. thio.} \times \underline{N}_{(\text{thio.})}) \times \frac{\text{Mol. wt. acetone}}{6000} \times \text{aliquot factor} \times 100}{\text{vol. orig. sample (500 ml.)} \times \text{sp. gr. orig. sample}}$$

= % acetone

In this specific application, the general formula becomes:

$$\frac{(50\text{-ml. thio}) \times 0.000774}{\text{sp. gr. sample}} = \%\ \text{acetone by weight}$$

SIEVE ANALYSIS

This is a general procedure applicable to any granular sample, including sawdust and charcoal. A frequent use for this type of analysis is to evaluate grinding procedures by giving the amounts of the various particle sizes present after grinding.

Apparatus

1. A set of U.S. Standard Sieves or equivalent, having the appropriate range of mesh openings (table 1).

2. A shaker (Ro-Tap) for the sieves.

Sample

Dry wood builds up considerable static charge; therefore, air-dry samples or samples conditioned in 80% RH should be used for sieve analysis.

Procedure

Stack the required number of U.S. Standard Sieves covering the required range of mesh openings and attach a receiving pan to the bottom. Sieve openings should be progressively smaller from top to bottom. Place 100 g. of sample on the top sieve, cover, and shake (mechanically on a Ro-Tap or by hand) for 5 min. or until separation is complete.[1] With the aid of a fine brush, transfer the sample retained on each sieve to a tared beaker or aluminum container and weigh.

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[1] One criterion for sufficient shaking is given in TAPPI Standard T16, where shaking is continued until a 20% increase in shaking time does not increase the amount passed through the finest screen more than 1.5%.

Table 1.--U.S. Standard Sieve information

Sieve No.	Sieve opening	
	In.	Mm.
2.5	0.315	8.00
3.0	.265	6.73
3.5	.223	5.66
4	.187	4.76
5	.157	4.00
6	.132	3.36
7	.111	2.83
8	.0937	2.38
10	.0787	2.00
12	.0661	1.68
14	.0555	1.41
16	.0469	1.19
18	.0394	1.00
20	.0331	.84
25	.0280	.71
30	.0232	.59
35	.0197	.50
40	.0165	.42
45	.0138	.35
50	.0117	.297
60	.0098	.250
70	.0083	.210
80	.0070	.177
100	.0059	.149
120	.0049	.125
140	.0041	.105
170	.0035	.088
200	.0029	.074
230	.0024	.062
270	.0021	.053
325	.0017	.044

Report

Report the particle size distribution as is illustrated in the following example. Approximate particle sizes in each cut can also be included in the report for completeness, convenience, and clarity.

Passed by--	Retained by--	% of sample
	Sieve A	10
Sieve A	Sieve B	30
Sieve B	Sieve C	40
Sieve C	Sieve D	10
Sieve D	Sieve pan	10
	Total	100

DETERMINATION OF SILICA IN ORGANICS

Silica is a common constituent of wood ash. Most woods have only traces (0.01% of the wood), but many tropical woods may have silica contents of 1.0 or more (see note).[1] Silica is determined by ashing the sample, treating the ash with hydrofluoric acid, and measuring the loss of silicon as the volatile silicon tetrafluoride. Using this procedure, samples containing 1 mg. or more of silica can be analyzed with an accuracy of ±1%.

Reagents and Apparatus

1. Platinum crucible of a 10- to 15-ml. capacity with cover.
2. Forceps with platinum tips.
3. Sulfuric acid 1:1, no residue.
4. Hydrofluoric acid, approximately 50%, no residue.

Procedure[2]

Clean and ignite a platinum crucible and cover. Cool to room temperature in a desiccator and weigh the crucible (not the cover). Insert an amount of sample estimated to contain 0.1 mg. or more of silica and weigh the crucible and sample. Place the cover on the crucible and ignite the crucible and contents in a muffle furnace at 600°-700° C. Cool and weigh; then repeat the heating-cooling-weighing cycle until a constant weight is reached.

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[1] Amos, G. L., and Dadswell, H. E. Siliceous inclusions in wood in relation to marine borer resistance. J. Austral. Council Sci. Indus. Res. 21: 190. 1948.

[2] Based on: Benedetti-Pichler, A. A. Introduction to the microtechnique of inorganic analysis. John Wiley and Sons, New York. 1942.

Wet the remaining ash with a drop of 1:1 sulfuric acid (this does not tend to spatter as much as concentrated sulfuric acid does). The sulfuric acid is then slowly fumed off by heating under a hood on a hotplate set at medium or low heat.

The crucible is then ignited in a muffle furnace at 850° C. for 10 min. Remove the crucible using platinum-tipped tongs, place it on a porcelain triangle, and allow it to cool to about 100° C.; then bring it to room temperature in a desiccator and weigh. Ignition, cooling, and weighing are repeated until constant weight is attained.

Add 0.5 ml. of 1:1 sulfuric acid and 0.5 ml. hydrofluoric acid (48%). Again slowly evaporate off the acids under the hood at low heat on a hotplate. Ignite the residue in the muffle furnace at 850° C. for 5 min., then cool and weigh as before. The difference in weight corresponds to the weight of silica in the sample.

A blank can be run to determine the effect of solid residue from the acid reagents and of volatilization of platinum. The latter is not likely to be significant, however, since only losses that occur during the last ignition would affect the results.

$$\% \text{ silica } (SiO_2) = \frac{\text{loss in weight after HF addition}}{\text{sample weight}} \times 100$$

Note

Amos[3] has compiled silica analysis results on more than 400 siliceous timbers. His compilation indicates that most species contain from traces to 1.6% silica, but values of 2 and 3% are not uncommon.

He reports that a specimen of Leukosyke capittelata Wedd. from New Guinea was found to contain between 9 and 10% silica.

[3] Amos, G. L. Silica in timbers. Bulletin 267, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia. 1952.

DETERMINATION OF SODIUM IN FERMENTATION SOLUTIONS

Sodium is usually present in fermentation solutions, either from the nutrients added, from chemicals added for pH control, or some similar purpose. In some fermentation samples, the sodium concentration will be high enough and interfering substances low enough so that the only isolation step required is to dilute and to filter. In any individual problem, however, possible interferences must be checked. In this procedure, samples are made up to contain approximately 50 p.p.m. of sodium. The emission energy of the solutions is measured at the sodium line at 590 mμ using an oxygen-acetylene flame photometer. The concentration of sodium is found by comparing with standard solutions run along with the sample.

Apparatus

1. Beckman DK-2 recording spectrophotometer with a flame attachment or its equivalent.

Reagents

1. Standard sodium solutions made up (using the predominant sodium form present in the sample) to contain 5, 10, 30, 50, 70, 90, and 100 p.p.m. of sodium.

Procedure

Accurately weigh a sample containing approximately 5 mg. of sodium into a 100-ml.-volume flask. Dilute the sample to 100 ml. with distilled water, mix thoroughly, and filter through filter paper.

With the flame photometer, measure the emission energy (repeat scan five or six times and take an average) of the sample and the energy of standard solutions of immediately higher and lower sodium concentration. By plotting the emission energy (in any units) of the standards versus their concentration (p.p.m.), the concentration of the sample can be read from the graph by interpolation:

$$\% \text{ sodium} = \frac{\text{graph reading (p.p.m.)}}{\text{sample weight (g.)} \times 100}$$

DETERMINATION OF SOLIDS IN WATER

This test is used as a rough check on the hardness qualities of distilled and untreated water. In checking still operation, samples are usually taken from (1) untreated water, (2) water from the still, and (3) water from the distilled water tap.

Principle

A portion of water is taken and evaporated to dryness. The residue is weighed and reported as p.p.m. of solids. Distilled water usually contains 5 or less p.p.m. of solids.

Procedure

Do the analysis in duplicate.

Tare a 250-ml. beaker by heating for 5 min. on a hotplate at medium heat, drying for 1 hr. in an oven at 105° C., cooling 1 hr. in a desiccator, and weighing.

Measure (graduated cylinder) 450 ml. of sample water into a 1-liter Erlenmeyer flask and set aside an additional 50 ml. of sample for assisting in making a subsequent transfer. Place the 1-liter Erlenmeyer on a hotplate at medium heat and boil the sample down to approximately 100 ml. Transfer the remaining 100 ml. to the tared, 250-ml. beaker, completing the transfer with the 50 ml. of sample which was set aside for this purpose. Boil the contents of the beaker down to about 10 ml. on the hotplate at medium heat, then turn the heat to low and carefully heat to dryness. Dry the beaker further in an oven at 105° C. for 1 hr., cool 1 hr. in a desiccator, and weigh. Weigh

beakers in the same order as they were when taring. Calculate p.p.m. of solids in the original 500 ml. from the gain in weight of the beakers.

$$\text{p.p.m.} = \frac{\text{wt. of solids (g.)} \times 10^6}{\text{g. or ml. of sample}}$$

The preceding procedure gives only approximate results (perhaps within 5 p.p.m.). If greater precision is desired, the following additional precautions or refinements can be applied:

1. Use larger water samples to give greater solids residue.
2. Ground beakers before weighing to remove static electrical charges.
3. Use smaller containers for collecting and weighing the residues.
4. When taring beakers, start with a quantity of water equal to that present when doing samples, and carry this through the same procedure as the sample using the final weight as the tare weight of the beaker. Then use the same beaker for the sample.
5. Correct for changes in room conditions, such as temperature and humidity by running two tared beakers (empty) along with the beakers that contain the samples. Any large changes in these control beakers would suggest either rejecting the analyses or correcting for the change.

DETERMINATION OF SUGAR ALCOHOLS, GLYCEROL, AND GLYCOLS

I. Introduction

The principal products formed upon hydrogenation and in some fermentations of glucose are sorbitol, xylitol, erythritol, glycerol, and propylene and ethylene glycol (1). The analysis of mixtures of these compounds is, therefore, of interest in wood conversion studies involving both hydrogenation and fermentation of wood sugars.

In the analysis of mixtures of these compounds, the chromatographic procedures of Moore, et al. (2) are used to isolate the various components prior to determining them individually. Paper sheet chromatography is used for isolating sorbitol, xylitol, erythritol, and glycerol, but propylene and ethylene glycol are too volatile and are partially lost from paper so are isolated by cellulose column chromatography. Column chromatography, however, is not readily adaptable for handling large numbers of samples; so a faster, alternate procedure is included for determining glycols by colorimetric methods when a great number of analyses are required.

The method by which the isolated polyols are determined is that described by Jackson (3) and Neish (4). This method is based upon cleavage of the polyol by periodate; the excess periodate being determined by adding excess sodium arsenite and titrating the excess arsenite with iodine. The thiosulfate method described by Siggia (5) can also be used, but the arsenite method gives a better end point and can be used over a greater range.

The chromatographic separation techniques are included in this procedure so that mixtures can be analyzed; however, if the sample to be analyzed contains only one polyol or if only some sort of "total polyol" is sought, then the separation step can be omitted. Total polyol or individual polyol can then be determined directly by periodate oxidation, followed by either the arsenite method or the appropriate colorimetric method (described in the alternate procedure).

II. Chromatographic Method Procedure

This determination is usually based on reference to standard calibration curves. In preparing calibration curves, the purity of the standard reagents must be determined. Moore et al. (2) evaluated their reagents using six different methods. They found that their commercial reagent-grade sorbitol, xylitol, erythritol, and propylene and ethylene glycol were close enough to 100% pure to assume that figure, and their glycerol was found to be 95.6% pure. Karl Fisher moisture determinations on the glycerol indicated a moisture content of 5.06%. This agrees with the value for glycerol obtained by the several methods of analysis used.

A. Analysis for Sugar Alcohols and Glycerol

Reagents:

1. Ammoniacal silver nitrate. Add two drops of a 10% sodium hydroxide solution to 15 ml. of a 2-1/2% silver nitrate solution and then enough ammonia to redissolve the precipitate.

2. Periodic acid (0.1M). Dissolve 4.6 g. of periodic acid in 200 ml. of water. Store in a brown glass-stoppered bottle.

3. Potassium iodide (50%). Dissolve 25 g. of potassium iodide crystals in water and adjust the volume to 50 ml.

4. Sodium arsenite (0.11N). Dissolve 2.5 g. sodium hydroxide, 5.5 g. arsenic trioxide, and 20 g. sodium bicarbonate in about 800 ml. of water. Adjust the volume to 1 liter after the bicarbonate has dissolved.

5. Sodium bicarbonate (6.8%). Dissolve 34 g. of sodium bicarbonate in water and adjust the volume to 500 ml.

6. Standard iodine solution (0.1). Weigh out 12.7 g. of resublimed iodine, using a rough balance and transfer it to a 125-ml. glass-stoppered Erlenmeyer flask, previously tared to the nearest milligram. Stopper the flask and reweigh it on the analytical balance to determine the weight of iodine accurately. Add 40 g. of potassium iodide crystals and 25 ml. of water; shake until all the iodine is in solution. Transfer quantitatively to a 1-liter volumetric flask and make up to volume. Store in a brown glass-stoppered bottle. 12.692 g. of iodine per liter gives a 0.1000N solution.

7. Starch solution. Mix 2 g. of soluble starch with sufficient water to make a paste, dilute to 200 ml., and heat to the boiling point. Cool, add 0.2-0.4 ml. toluene as a preservative. Store in a glass-stoppered bottle.

8. Sulfuric acid (1N). Pour 30 ml. of concentrated sulfuric acid into 800 ml. of water and adjust the volume to 1 liter when cool. If desired, this may be standardized against standard sodium hydroxide by titration to the methyl red end point.

Reactions.--General reaction of polyols with periodate.

$$\begin{matrix}CH_2OH\\|\\(CHOH)_n\\|\\CH_2OH\end{matrix} + (n+1)\ IO_4^- \longrightarrow n\ HCOOH + 2HCHO + (n+1)IO_3^- + H_2O$$

Upon the addition of potassium iodide, the excess periodate reacts to form iodine.

$$IO_4^- + 2I^- + 2H^+ \longrightarrow IO_3^- + H_2O + I_2$$

It is important in the above reaction that the pH be above 5 or else iodate will also oxidize iodide. For this reason the solution is buffered by adding excess sodium bicarbonate. The iodine released must then be titrated by arsenite since the pH is too high for the use of thiosulfate. If the arsenite is added before the iodide, there is never any appreciable amount of free iodine released into the medium, and the iodide then serves to catalyze the reduction of the periodate by arsenite.

Arsenious acid in alkaline solution gives:

$$As_2O_3 + H_2O + 2OH^- \longrightarrow 2H_2AsO_3^-$$

which reacts with the liberated iodine as follows:

$$I_2 + H_2AsO_3^- + H_2O \longrightarrow 2I^- + H_2AsO_4^- + 2H^+$$

The arsenite is added in excess and the excess measured by titration with standard iodine.

Chromatographic separation procedure.--Sample solutions are made up to contain from 0.5% to 10.0% of the product. The various polyols present are isolated by paper chromatography. Sheets of Whatman No. 1 chromatography paper, 18 x 24 in., are used with the long dimension vertical, so the developing solvent flows in the machine direction of the paper.

The sample may be applied to the paper in a spot or a streak. At the Forest Products Laboratory, the samples are streaked, using the apparatus and technique described by McKibbins, Harris, and Saeman (6) (fig. 1). The volume applied is 0.03 ml. per 75 mm. of paper.

Irrigation and elution techniques that follow are similar to those suggested by Saeman, Moore, Mitchell, and Millett (7). After the sample has dried, the sheet is hung in a chromatographic cabinet and brought to equilibrium with the vapors from the irrigating solvent in the bottom of the cabinet. The sheet is then irrigated with the butanol, pyridine, water (10:3:3) solvent. Development, separating sorbitol, xylitol, erythritol, and glycerol, is complete after 19 or 20 hr. of irrigation at 30° C. After development is complete, the sheets are removed from the cabinet and air dried.

Four evenly spaced, 75-mm. sample strips are then cut lengthwise down the sheet, starting from the line where the sample was applied. Spray the remaining "indicator strips" with ammoniacal silver nitrate and heat at 110° C. for 10 min. Upon heating, the alcohols appear as dark bands on the indicator strips; by replacing the sample strips into the cut out areas of the original sheet, the bands of sample material are located. The identity of the polyol in a particular band is established by comparing its R_f value with knowns.

On paper sheets and columns, the polyols come off in the following order: Propylene glycol moves fastest, followed by ethylene glycol, glycerol, erythritol, xylitol, and sorbitol. After the normal 20-hr. development on a sheet to separate the sugar alcohols and glycerol, the ethylene and propylene glycol are still on the sheet; but some losses have occurred through

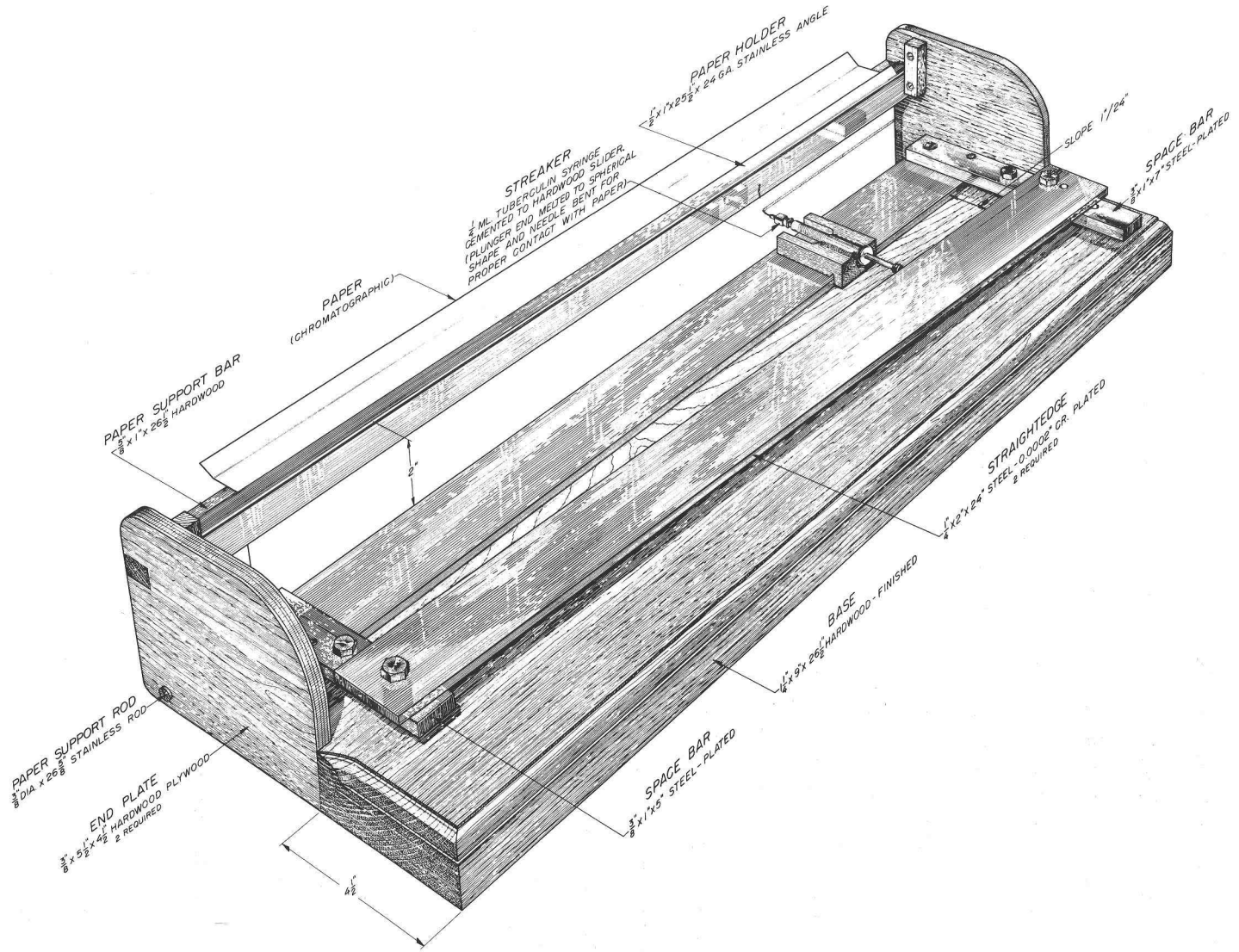

Figure 1.--Apparatus for applying samples to paper.

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evaporation, so this system is not used for the quantitative measurement of these glycols. Instead, the glycols are separated on a column where the propylene and ethylene glycol are taken off. Other constituents are left on to be washed off later with water. If sugars are present, they appear above the polyols on the paper, with glucose coinciding with sorbitol. It is presumed that glucose would interfere with the determination of sorbitol by periodate.

When the bands of the sample have been located, the 75-mm. tabs containing the various isolated polyols are cut out and the sample materials eluted with water. Elution is done by the method described by Saeman et al. (7), collecting the eluate in 3/8-ml. micropipets.

Polyol determination.--Two milliliters of 1N sulfuric acid is pipeted into a 50-ml. Erlenmeyer flask and exactly 1 ml. of 0.1M periodic acid added. The chromatograph eluate, containing up to 3.5 mg. of polyol, is then added to the Erlenmeyer and enough water to make a volume of approximately 10 ml. The contents of the flask are then mixed and allowed to stand 1 hr. at room temperature. Add 5 ml. bicarbonate solution and, after thorough mixing, add exactly 2 ml. of the 0.11N sodium arsenite solution. Two drops of 50% potassium iodide are mixed in and the flask allowed to stand 10 min. or more at room temperature. Four drops of starch indicator are added and the solution titrated with 0.1N iodine solution to the blue starch-iodine end point.

Calculations.--The amount of periodate used by the sample can be calculated from the difference in the amount of iodine consumed in titrating the blank and

the sample. One ml. of 0.1N iodine is equivalent to 0.0095965 g. periodic acid or 0.1 mg. of periodic acid.

One mg. of periodic acid is reduced by:

18.217 mg. sorbitol

19.016 mg. xylitol

20.353 mg. erythritol

23.023 mg. glycerol

38.045 mg. propylene glycol

31.035 mg. ethylene glycol

The alternative to this calculation is to work from a standard calibration curve. This latter procedure is preferred if suitable standard compounds are available.

B. Analysis for Glycols

Propylene ethylene glycol are isolated by column chromatography rather than paper chromatography to minimize losses due to evaporation. The column used is glass, 7 mm. ID, 32 in. long, and packed with powdered cellulose. To speed up flow rates, a packing material coarser than the commercial material is used. It is prepared by grinding high alpha, bleached sulfate Douglas-fir pulp in a Wiley mill to pass a 1-mm. screen. Columns are packed to a length of 30 in. to give a flow rate of 5 mm. per hr. when an air pressure of 10 (p.s.i.) is applied. Prior to use, packed columns are prewashed with water for 14 to 18 hr. and then with solvent (84% butanol, 16% water) for several days. Prewashing is repeated until good separations can be obtained on the column.

To determine elution volumes, apply from 0.4 to 2.0 mg. of glycol mixtures (in solution) to a small filter-paper disk resting on the top of the cellulose. Irrigate the column then with 100 ml. of irrigating solvent (84% butanol, 16% water). Collect 5-ml. fractions; analyze them by the periodate method used for sugar alcohols, and construct a graph of the type illustrated by fig. 2, from which elution volumes can be determined. Samples of unknowns are run by collecting the appropriate fractions and analyzing. Analysis of the eluates is accomplished by the periodate-arsenite method in the same manner as the sugar alcohols.

A column can be reused by washing with 15 ml. of water, followed by 15 ml. of solvent. The column is kept wet when not in use.

III. Chromatographic-Colorimetric Combination Method (Alternate Procedure)

This alternative to the preceding procedure has been described by Clark(8). In this procedure sorbitol, xylitol, erythritol, and glycerol are isolated and measured in the same way as previously described, but the glycols are done colorimetrically. First, total formaldehyde formed by periodate oxidation is measured by the chromotropic acid method and propylene glycol is measured by the p-phenylphenol method. Ethylene glycol concentration can then be calculated by subtracting the formaldehyde formed by constituents other than ethylene glycol from the total formaldehyde value and converting to ethylene glycol content. The chromotropic acid method described for determining total

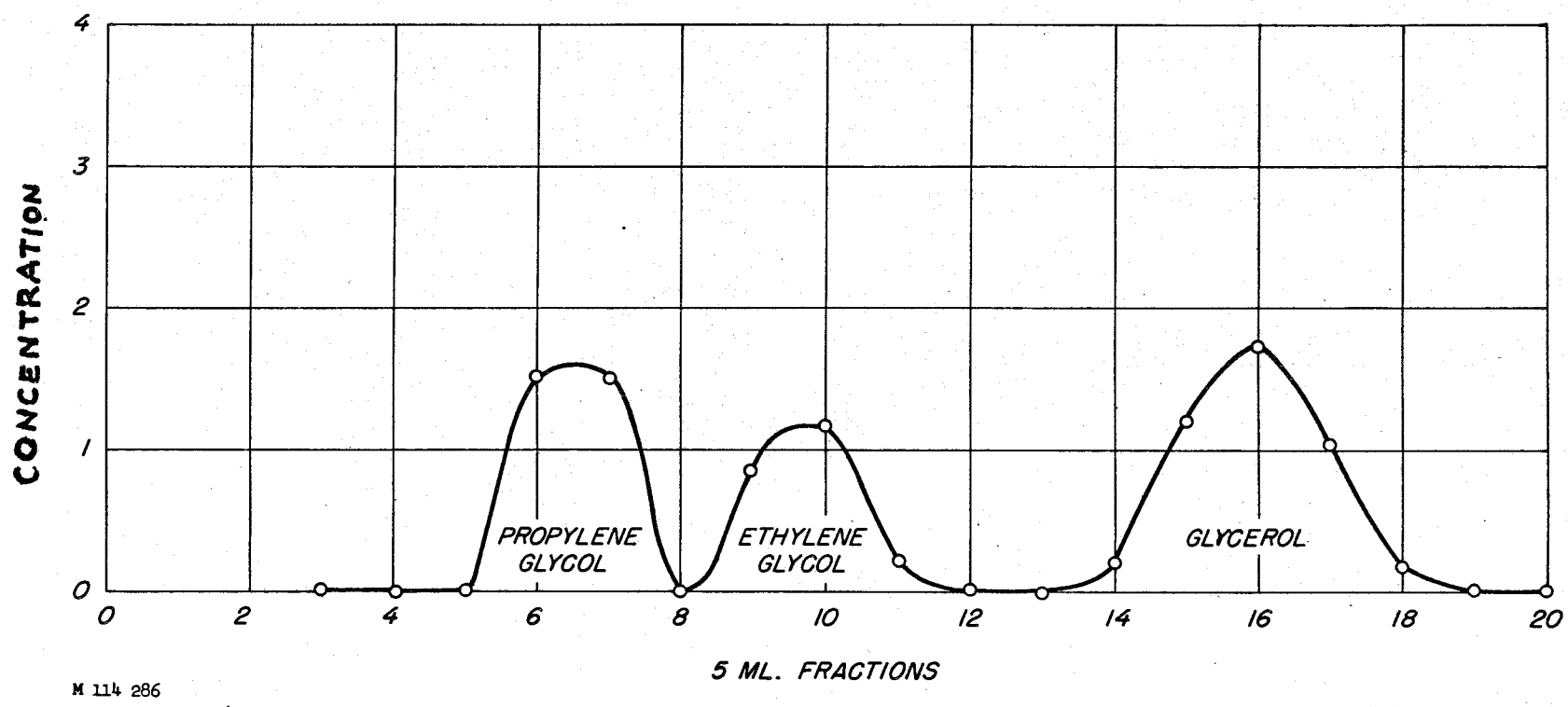

Figure 2.--Separation of glycols in butanol and water solvent system on cellulose column.

formaldehyde can also be used directly on a sample containing any individual polyol by the usual colorimetric procedure of working from a calibration curve made up from knowns.

Determination of total formaldehyde.--In this procedure, polyols are oxidized, by periodic acid, to formic acid and formaldehyde. The formaldehyde formed is measured colorimetrically by means of the colored compound it forms with chromotropic acid (9). A partial review of the literature concerning periodic acid oxidations has been reported by Bradford, Pohle, Gunther, and Mehlenbacher (10).

The reactions describing the periodate oxidation have already been given in A.

The reaction of formaldehyde with chromotropic acid to form a colored compound was discovered by Eegriwe(11). He found it to be highly selective. The following is a partial list of compounds which he found do not interfere with this reaction or its analytical applications.

Aldehydes	Acids
Propionaldehyde	Levulinic
Butyraldehyde	Glycolic
Isobutyraldehyde	Oxalic
Isovaleraldehyde	Gallic
Glyceraldehyde	Acetic
Oenanthaldehyde	
Crotonaldehyde	Sugars
Chloral hydrate	
Glyoxal	Glucose
Benzaldehyde	Fructose
Oxybenzaldehyde	Mannose
Vanillin	Lactose
Salicylaldehyde	Galactose
Phthalaldehyde	
Isophthalaldehyde	

Aldehydes cont.

Terephthalaldehyde
Cinnamaldehyde

Ketones

Acetone

Alcohols

Ethanol
Glycerol

MacFadyen(12), studying the same reaction, found that acetaldehyde, formic acid, and methanol were also nonreactive toward chromotropic acid.

The color-producing procedure given here is that of MacFadyen (12).

Apparatus.--Spectrophotometer. Beckman DU spectrophotometer or equivalent equipped with 10-mm. pyrex or silica cells.

Reagents.--1. Sulfuric acid (0.5N). Add 105 ml. of concentrated sulfuric acid to 5 liters of water and then dilute up to 7.5 liters.

2. Sodium periodate (0.1M). Dissolve 46 g. of periodic acid in 1,500 ml. of water in a 2-liter volumetric flask. Neutralize with 1N of sodium hydroxide until faintly acid to methyl red. Dilute to 2 liters.

3. Sodium arsenite (1M). Dissolve 90 g. of sodium hydroxide in about 1 liter of water. Weigh 200 g. of arsenious acid (As_2O_3), and add enough sodium hydroxide to make a paste slurry. Transfer the slurry into a 2-liter volumetric flask, completing the transfer with the rest of the sodium hydroxide. When dissolution is complete, add water to mark, mix, and filter.

4. Chromotropic acid. Cautiously add 1,200 ml. of concentrated sulfuric acid to 600 ml. of water and let cool. Dissolve 4 g. of reagent-grade 4,5-dihydroxy-2,7-naphthalenedisulfonic acid (chromotropic acid) in 400 ml.

of distilled water. If not clear, filter. Dilute to 2 liters with the 2:1 sulfuric acid. Store in brown, glass-stoppered bottle, and prepare fresh every 2 to 3 wk.

Procedure.--The procedure followed is based on that of Lambert and Neish (9) for the determination of glycerol with the exception of using a 30-min. oxidation time instead of the 5-min. oxidation time required for glycerol.

Prepare a calibration curve relating absorbance at 570 mμ. to formaldehyde concentration as follows.

Accurately prepare a stock solution of glycerol or (preferably) mannitol, and then dilute to give standard solutions which will yield formaldehyde concentrations, upon oxidation, over a range from 1 to 6 mg. per ml. The following tabulation shows the amounts of glycerol or mannitol that correspond to this range.

Mannitol	Glycerol	Equivalent concentration of formaldehyde
μg./ml.	μg./ml.	μg./ml.
3.04	1.54	1.0
6.07	3.08	2.0
9.11	4.61	3.0
12.15	6.16	4.0
15.18	7.69	5.0
18.22	9.21	6.0

Pipet (or use a calibrated syringe) 0.5 ml. of standard solution into a 250-ml. beaker containing exactly 20 ml. of 0.5N sulfuric acid. Add 5 ml. of sodium periodate (0.1M), and after exactly 30 min. add 5 ml. of sodium arsenite (1M). Mix reactants after each addition. After adding the sodium arsenite, iodine appears in the solution and then fades. This solution is

allowed to stand for 5 to 10 min., and then 100 ml. of water are added. Mix, and then pipet 1 ml. into a Pyrex test tube (approximately 19 x 150 ml.) and add exactly 10 ml. of chromotropic acid solution. The test tube is covered with aluminum foil, shaken, and placed in a steam bath for 30 min. The solutions are cooled by adding tap water to the cooker. After cooling, the samples are shaken and their absorbance measured at 570 mμ.

Two blanks are run with the samples, and readings are made against a blank. A standard curve is then constructed relating absorbance at 570 mμ. to the concentration of formaldehyde in oxidized solution (130.5 ml., total volume, in beaker of oxidized solution).

Estimated formaldehyde (%)	Dilute 1 ml. to (ml.)
0.0	0
.1	0
.5	10
1.0	10
2.0	25
3.0	50
4.0	50
5.0	50
6.0	100
7.0	100

Unknowns are run in the same manner as the standards, and the concentrations of formaldehyde formed is computed from the standard curves.

Determination of propylene glycol.--The method (13,14) is based upon the conversion of propylene glycol to acetaldehyde by heating with concentrated sulfuric acid and copper sulfate. The liberated acetaldehyde, when treated with p-phenylphenol, gives a violet color which follows Beer's Law over a

narrow range at extremely low concentrations. Lactic acid and sodium bisulfite-acetaldehyde complex interfere since they undergo the same reaction.

Reagents.--1. Copper sulfate-sulfuric acid. Dissolve 2 g. of $CuSO_4 \cdot 5H_2O$ in 10 ml. of water and add to 600 ml. of concentrated sulfuric acid (or add 40 ml. of 20% $CuSO_4 \cdot 5H_2O$ solution to one 9-lb. bottle of concentrated sulfuric acid).

2. 1.5% solution of p-phenylphenol in 1.5% NaOH (3 g. of p-phenylphenol, 1.5 g. NaOH pellets, and a little water are heated and stirred until they dissolve. This is then made up to 200 ml. with distilled water. New reagent should be made up every 30-40 days).

Apparatus.--Beckman DU Spectrophotometer or its equivalent.

Procedure.--Into a wide-mouth vial (16-ml. ID, 95-ml. length) fitted with a screw cap, pipet 1 ml. of a solution which will yield 1 to 10 gammas of acetaldehyde (corresponding to 1.7 to 17.3 gammas of propylene glycol). Add 6 ml. of the $CuSO_4 \cdot H_2SO_4$ solution and mix thoroughly. The vial is immersed in boiling water for exactly 90 sec., then cooled to 15° to 18° C. (below 20° C.) by immersing in cold water. When the vial is cool, 0.1 ml. of p-phenylphenol reagent is added and mixed in. The color is developed by placing the tube in a constant temperature bath at 30° C. for 1/2 to 1 hr. It is then immersed in boiling water for 90 sec. and cooled to room temperature. The violet color is read at 560 mμ on the spectrophotometer. Three blanks are run in the same way, substituting 1 ml. of distilled water for the sample. Propylene glycol is calculated from a prepared calibration curve.

Precaution.--This method is extremely sensitive. Temperatures are critical and every care must be exercised to avoid touching the inner surfaces of glassware or tips of pipets. Glassware should be cleaned with cleaning solution, trisodium phosphate, and hot water. Rubber gloves and tongs should be used to avoid contamination from the hands.

Determination of Ethylene Glycol

Procedure.--The ethylene glycol content of a sample is computed from the amount of formaldehyde it forms upon periodate oxidation. The formaldehyde attributed to the oxidation of ethylene glycol is obtained by taking the "total formaldehyde" minus that contributed by the oxidation of sorbitol, xylitol, erythritol, glycerol, and propylene glycol. Factors for converting polyol weight to formaldehyde yield are given in the following tabulation.

Sorbitol	0.3297
Xylitol	.3943
Erythritol	.4918
Glycerol	.6522
Propylene glycol	.3947
Ethylene glycol	.9676

References

(1) Clark, I. T.
1958. Hydrogenolysis of sorbitol. Ind. Eng. Chem. 50: 1125.

(2) Moore, W. E., Effland, M. E., Johnson, D. B., Daugherty, M. A., and Schwerdtfeger, E. J.
1960. Chromatographic analyses of sugar alcohols and glycols. Appl. Microbiol. 8: 170.

(3) Jackson, E. L.
1944. Periodic acid oxidation. In Org. Reactions, Vol. II (R. Adams, editor-in-chief). John Wiley and Sons, Inc., New York, N.Y.

(4) Neish, A. C.
1952. Analytical methods for bacterial fermentations. Nat. Res. Coun. Can. No. 2952.

(5) Siggia, S.
1959. Quantitative organic analysis via functional groups. John Wiley and Sons, Inc., New York, N.Y.

(6) McKibbins, S. W., Harris, J. F., and Saeman, J. F.
1961. An applicator for chromatographic paper and its use for 5-hydroxymethyl-2-furaldehyde and levulinic acid analysis. J. Chromatogr. 5: 207.

(7) Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millett, M. A.
1954. Techniques for the determination of pulp constituents by quantitative paper chromatography. Tappi 37: 336.

(8) Clark, I. T.
1958. Separation of glycerol for a polyhydric alcohol mixture by nonionic exclusion. Anal. Chem. 30: 1676.

(9) Lambert, M., and Neish, A. C.
1950. Rapid method for estimation of glycerol in fermentation solutions. Can. J. Res. 28B: 83 (Mar.).

(10) Bradford, P., Pohle, W. D., Gunther, J. K., and Mehlenbacher, V. C.
1942. Determination of glycerol by oxidation with periodic acid. Oil and Soap 19: 189 (Nov.).

(11) Eegriwe, E.
1937. Reactions and reagents for the detection of organic compounds. Zh. Anal. Chem. 110: 22.

(12) MacFadyen, D. A.
1945. Estimation of formaldehyde in biological mixtures. J. Biol. Chem. 158: 107.

(13) Barker, S. B., and Summerson, W. H.
1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138: 535.

(14) Markus, R. L.
1950. Colorimetric determination of lactic acid in body fluids utilizing cation exchange for deproteinization. Arch. Biochem. 29: 159.

DETERMINATION OF SULFATE IN CELLULOSE NITRATE[1]

Introduction

When cellulose is nitrated using a sulfuric acid catalyst, some sulfate is usually present in the end product. This method is for the determination of small amounts of sulfate in cellulose nitrate, present either as residual acid or as the cellulose ester. The cellulose nitrate is digested with a mixture of nitric and perchloric acids. Barium chromate is added to the resulting solution, precipitating sulfate as barium sulfate. The original sulfate concentration is calculated from the amount of chromic acid liberated, which is measured iodometrically.

Reactions

$$2SO_4^{=} + 2BaCrO_4^{=} \longrightarrow 2BaSO_4 + 2CrO_4^{=}$$

According to Sherrill,[2] the following equilibria control the composition of chromate-dichromate solutions:

$$2CrO_4^{=} + 2H^{+} \rightleftharpoons 2HCrO_4^{-}$$

$$2HCrO_4^{-} \rightleftharpoons Cr_2O_7^{=} + H_2O$$

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[1]Based on: Hoffpauir, C. L., and Guthrie, J. D. Determination of small amounts of sulfate in cellulose nitrate and other cellulose esters. Indus. Engin. Analyt. Ed., 16: 391-392. 1944.

[2]Sherrill, M. S. The equilibrium relations of chromates in solution. J. Amer. Chem. Soc. 29: 1641-1675. 1907.

Lower pH conditions favor the existence of the dichromate ion. The stoichiometry of this analysis is not affected by the equilibrium conditions, however, and the reduction of each form by iodide ion is illustrated below:

$$Cr_2O_7^{=} + 14H^{+} + 6I^{-} \longrightarrow 3I_2 + 2Cr^{+++} + 7H_2O$$

$$2CrO_4^{=} + 16H^{+} + 6I^{-} \longrightarrow 3I_2 + 2Cr^{+++} + 8H_2O$$

The iodine is measured by titration with thiosulfate.

$$3I_2 + 6S_2O_3^{=} \longrightarrow 6I^{-} + 3S_4O_6^{=}$$

Reagents

1. Barium chromate. Dissolve 2.53 g. of reagent-grade barium chromate in 100 ml. of 2N hydrochloric acid and dilute to 1 liter.

2. Potassium iodate, 0.01N. Dissolve 0.3567 g. of reagent-grade potassium iodate in water and dilute to 1 liter. This is for standardizing the thiosulfate.

3. Sodium thiosulfate solution, 0.002N. Dissolve 1.4910 g. reagent-grade sodium thiosulfate pentahydrate in 3 liters of water. Standardize against standard potassium iodate.

4. Perchloric acid, 60%.

5. Nitric acid. Concentrated, reagent grade.

6. Starch indicator. Dissolve 1 g. of soluble starch in 100 ml. of boiling water.

7. Ammonium hydroxide. Concentrated, reagent grade.

8. Sulfuric acid. Make up a 10% solution by mixing 6 ml. of concentrated acid with 96 ml. of water.

9. Potassium iodide. Reagent grade (no free iodine).

Apparatus

1. A sandbath of sufficient size to accommodate several sample flasks at one time.

2. A glass manifold system that directs a gentle stream of air into each flask during reaction and also draws off the reaction gases by suction.

Procedure

Before weighing, the cellulose nitrate sample should be dried according to the following procedure: Allow the sample to air-dry in an aluminum foil tray for 16 hr. After 16 hr., place the cellulose nitrate in a forced-draft oven for 1 hr. at 105° C. The samples are then cooled in a desiccator.

After drying, weigh rapidly and accurately into a 50-ml. Erlenmeyer flask a sample containing between 0.4 and 1.2 mg. of sulfate. The sample should not exceed 2 g. Add 20 ml. of concentrated nitric acid to the sample flask and place the flask on the heated sandbath. Position the manifold and turn on air and vacuum systems in the manifold to draw off the fumes generated by the decomposition of cellulose nitrate.

After the cellulose nitrate is dissolved, add 3 ml. of 60% perchloric acid and continue the heating so that the solution boils gently. Digest the sample until copious white fumes are evolved. If the solution is colored, add about 10 ml. of water and again digest until white fumes appear. Repeat the digestion step until the solution becomes colorless and then concentrate the solution in the flask to a volume of 1 ml. or less. Do not allow the solution to go to dryness.

Transfer the solution to a centrifuge tube that has been marked and calibrated to contain a volume of 15 ml. Complete the quantitative transfer, using not more than 5 ml. of water for washing. Add 5 ml. of barium chromate reagent and allow the precipitate to form for at least 4 hr. or, preferably, overnight. After the standing period, make the solution alkaline with concentrated ammonium hydroxide (about 3 ml.). Add water to make the total volume exactly 15 ml.; then mix and let stand for 1 hr. Centrifuge for 10 min.

Pipet 5 ml. of the supernatant liquid into a 50-ml. Erlenmeyer flask. Add approximately 50 mg. of potassium iodide and eight drops of starch indicator. Acidify with 10% sulfuric acid; then add an excess of 2 ml. of sulfuric acid. Let the solution stand in the dark for 5 min.; then titrate with sodium thiosulfate solution to the disappearance of the starch-iodine color.

If the amount of sulfate in the sample is less than 0.4 mg. or more than 1.2 mg., the determination should be repeated, because such results fall outside the range where the stoichiometric factors can be applied. Within the range of 0.4 to 1.2 mg., 1 ml. of 0.002N sodium thiosulfate is equivalent to 0.064 mg. of sulfate in the 5-ml. aliquot. The thiosulfate should be standardized against standard iodate at the time of the titration.

Analytical Notes

1. When there is less than 0.4 mg. of sulfate in the sample, titration values are not reproducible and are usually considerably above theoretical values. In such cases, the titration value is affected by the amount of

perchloric acid remaining after digestion. If more than 1.0 ml. of perchloric acid remains after digestion, titration values tend to be high. If more than 1.2 mg. of sulfate is present, the titration values tend to be low.

2. When new reagents are made up, a blank should be run to be sure that blank values are not significant.

Safety Precautions

When drying cellulose nitrate, a warning sign should be placed on the oven, and the oven door should be left slightly ajar.

Considerable care should be exercised in handling perchloric acid. If it is both hot and concentrated and comes in contact with organic material, a definite explosion hazard exists. By initially degrading the cellulose nitrate with concentrated nitric acid and then adding a small amount of perchloric acid, this danger is diminished.

Brabson, Stein, and Jacob[3] recommend that persons using perchloric acid be thoroughly familiar with its hazards and that safety practices include the following precautions:

1. Remove spilled perchloric acid by immediate and thorough washing with large quantities of water.

2. Provide hoods and ducts for perchloric acid vapor made of chemically inert materials and so designed that they can be thoroughly washed with water. Arrange for the exhaust system to discharge in a safe location, and see that the fan is accessible for cleaning.

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[3]Brabson, J. A., Stein, C., and Jacob, K. D. Use of perchloric acid in analysis. J. Assoc. Off. Agr. Chem. 41: 76-77. 1958.

3. Avoid the use of organic chemicals in hoods employed for perchloric acid digestions.

4. In wet combustions with perchloric acid, treat the sample first with nitric acid to destroy easily oxidizable organic matter.

5. Darkening of the vessel's contents during a digestion with perchloric acid indicates the imminence of an explosion and dictates immediate withdrawal of persons from the area.

6. Contact of perchloric acid solution with strong dehydrating agents such as phosphorus pentoxide or concentrated sulfuric acid may result in formation of explosive anhydrous perchloric acid. Some analytical methods require the use of perchloric acid in the presence of strong dehydrating agents. Although no hazard has been experienced with these methods when the details are carefully followed, the use of protective shields, goggles, and similar safety devices is recommended.

7. Observe also the precautions outlined in (a) "Perchloric Acid Solution," Chemical Safety Data Sheet SD-11, Manufacturing Chemists Association of the United States, 1625 I Street, NW., Washington, D.C. (1947) and (b) "Applied Inorganic Analysis," W. F. Hillebrand, G. E. F. Lundell, H. A. Bright, and J. I. Hoffman, Second Edition, John Wiley and Sons, New York, N.Y., pp. 39-40 (1953).

DETERMINATION OF TOTAL ORGANIC MATTER[1,2]

Introduction

An oxidizing reagent made up of sodium dichromate and strong sulfuric acid will oxidize most organic compounds to carbon dioxide and water. (Acetic acid is a notable exception and is affected very little by the reagent.) This reaction can be made the basis of a quantitative measure of the organic material present in a sample by colorimetrically determining the reduced trivalent chromium ion formed. If only one organic compound is present in a sample and its identity is known, the reduced chromium is a good measure of its concentration; however, the organics present in a sample may be a mixture with some components being unknowns. In such cases, the total organic material is usually reported as the major constituent.

Principles

Two general procedures can be followed in the measurement of total organic material by wet oxidation. One is to calculate the milliequivalents of organic from the milliequivalents of dichromate reduced, and the other is to work from a calibration curve. With the calibration curve procedure, the normality and volume of dichromate do not have to be known, but a new curve must be made up for each batch of reagent.

The system of calculating organics from dichromate reduction is the one generally used at the Forest Products Laboratory. In this procedure, the

[1] Based on: Neish, A. C. Analytical methods for bacterial fermentations. National Research Council of Canada Report No. 46-8-3 (2d Rev.), p. 43. Saskatoon, November 25, 1952.

[2] Johnson, M. Rapid micromethod for estimation of nonvolatile organic matter. Biol. Chem. 181: 707. 1949.

normality of the dichromate must be known and either the oxidation of the organic must be quantitative and complete or its reducing power must be established by experiment. Table 1 lists the theoretical equivalent weights of some compounds (in the reduction of dichromate).

In the procedure that requires the milliequivalents of dichromate reduced to be known, a blank containing a known amount of standard dichromate is completely reduced by bisulfite (see "Reactions") and the absorbance of the resulting solution measured at 650 mμ. A factor relating concentration of trivalent chromium ion to absorbance is then derived from this measurement; subsequently, this factor is used to compute the reduced chromium content of sample solutions.

Reactions (Dichromate Oxidations)

1. Bisulfite.

$$6HSO_3^- + 2Cr_2O_7^= + 5H_2SO_4 \longrightarrow 5SO_4^= + 2Cr_2(SO_4)_3 + 8H_2O$$

2. Ethyl alcohol.

$$3CH_3CH_2OH + 2Cr_2O_7^= + 8H_2SO_4 \longrightarrow 2Cr_2(SO_4)_3 + 3CH_3COOH + 2SO_4^= + 11H_2O$$

3. Ethylene glycol.

$$3C_2H_6O_2 + 5Cr_2O_7^= + 20H_2SO_4 \longrightarrow 6CO_2 + 5Cr_2(SO_4)_3 + 5SO_4^= + 29H_2O$$

4. Propylene glycol.

$$3C_3H_8O_2 + 2Cr_2O_7^= + 8H_2SO_4 \longrightarrow 9CH_3COOH + 2Cr_2(SO_4)_3 + 2SO_4^= + 2H_2O$$

5. Glycerol.

$$3C_3H_8O_3 + 7Cr_2O_7^= + 28H_2SO_4 \longrightarrow 9CO_2 + 7Cr_2(SO_4)_3 + 7SO_4^= + 40\ H_2O$$

6. Erythritol.

$$C_4H_{10}O_4 + 3Cr_2O_7^{=} + 12H_2SO_4 \longrightarrow 4CO_2 + 3Cr_2(SO_4)_3 + 3SO_4^{=} + 17H_2O$$

7. Xylitol.

$$3C_5H_{12}O_5 + 11Cr_2O_7^{=} + 44H_2SO_4 \longrightarrow 15CO_2 + 11Cr_2(SO_4)_3 + 11SO_4^{=} + 62H_2O$$

8. Sorbitol.

$$3C_6H_{14}O_6 + 13Cr_2O_7^{=} + 52H_2SO_4 \longrightarrow 18CO_2 + 13Cr_2(SO_4)_3 + 13SO_4^{=} + 73H_2O$$

9. Glucose.

$$C_6H_{12}O_6 + 4Cr_2O_7^{=} + 16H_2SO_4 \longrightarrow 6CO_2 + 4Cr_2(SO_4)_3 + 4SO_4^{=} + 22H_2O$$

Apparatus

A spectrophotometer capable of measuring absorbances in the visible region of the spectrum.

Reagents

1. Sodium bisulfite. Dissolve 5.2 g. of sodium bisulfite (or 4.0 g. of sodium metabisulfite) in 100 ml. of water. Prepare fresh each week.

2. Concentrated sulfuric acid.

3. Sodium dichromate oxidizing mixture (approximately 0.1N). Dissolve 5 g. of sodium dichromate in 20 ml. of water and dilute to 1 liter with concentrated sulfuric acid. The normality of the dichromate is determined by diluting exactly 5 ml. with 100 ml. of water to lower the acid concentration, adding about 0.5 to 1.0 g. of potassium iodide crystals and titrating the iodine formed with standard thiosulfate solution to the starch-iodine end point.

$$K_2Cr_2O_7 + 6KI + 7H_2SO_4 \longrightarrow 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3I_2$$

$$I_2 + 2S_2O_3^{=} \longrightarrow 2I^{-} + S_4O_6^{=}$$

Procedure

Pipet 1 ml. of a solution containing from 0.5 to 2.5 mg. of organic material into a 25- by 200-ml. test tube; add 5 ml. of the sodium dichromate oxidizing mixture, using a calibrated syringe. One control is run using distilled water in place of the sample and another control is run using 1 ml. of 0.5M sodium bisulfite solution instead of the sample. The contents of the tubes are mixed, then the tubes covered with aluminum foil and heated 20 min. in a boiling water bath. The samples are cooled to room temperature and their absorbance measured at 650mμ. The water control is used for a reference and the bisulfite control gives the absorbance of a solution with all of the dichromate reduced. It serves as a standard, giving the percent transmittance for a known concentration of trivalent chromium.

Concentrations can be calculated either from stoichiometric relationships (table 1) or from standard calibration curves made up from knowns.

Calculations

Taking a 1-ml. sample, the milligrams of substance sought per milliliter of sample is equal to

$$\frac{\text{Mg.}}{\text{Ml.}} = \frac{N \times V}{A_1} \times F \times A_2$$

where

N = normality of the dichromate.

V = volume of dichromate (ml.).

F = milliequivalent weight of substance sought.

A_1 = absorbance of the blank completely reduced by bisulfite.

A_2 = absorbance of the sample after oxidation.

Theoretical equivalent weights for several compounds are given in table 1. The equivalent weights of many other organic compounds are listed by Neish.[1]

Table 1.--Equivalent weight of various compounds in their oxidation by dichromate

Polyol	Molecular weight	Valence change	Theoretical equivalent weight
Erythritol	122.12	+18	6.78
Ethyl alcohol	46.07	+4	11.51
Ethylene glycol	62.07	+10	6.21
Glucose	180.16	+24	7.50
Glycerol	92.09	+14	6.58
Propylene glycol	76.09	+16	9.51
Sorbitol	182.17	+26	7.01
Xylitol	152.15	+22	6.92

Notes

1. Hexavalent chromium does not have a significant absorptivity at 650 mμ, so does not interfere with the measurement of the absorbance due to trivalent chromium.

2. The determination of cellulose by the acid dichromate is outlined and discussed by Segal.[3] Low results are caused by the formation of carbon monoxide and steps are given to overcome this.

3. Application of dichromate oxidation to the determination of several acids, aldehydes, and polyols is discussed by Cardone and Compton.[4] Measurements were made at the dichromate band at 349 mμ.

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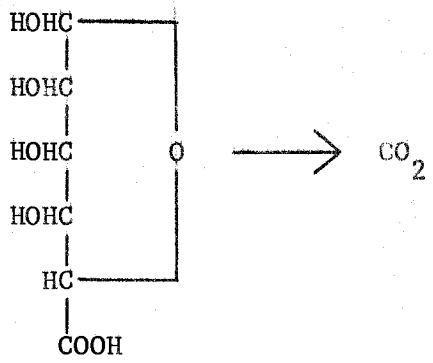
[3] Segal, L., Tripp, R. C., Tripp, V. W., and Conrad, C. M. Determination of cellulose. Anal. Chem. 21: 712. 1949.

[4] Cardone, M. J., and Compton, J. Spectrophotometric method for following dichromate reactions. Anal. Chem. 24: 1903. 1952.

DETERMINATION OF URONIC ACIDS (POLYURONIDE CARBOXYL)

Uronic acids are sugar derivatives in which the terminal (high-number end) group is a carboxyl. Upon refluxing uronic acids or polyuronides with boiling 12% hydrochloric acid, the carboxyl group decomposes to yield carbon dioxide quantitatively.[1]



This reaction is the basis for several methods for the determination of polyuronide carboxyl (1,2,3,4). The apparatus and procedure sections given here are those of Browning (5) and are modifications of an earlier micro system (6).

Apparatus (From Browning's (5) Description)

The apparatus is shown in detail in fig. 1. The guard tubes at either end of the train contain Ascarite, backed on either side with Anhydrone. Glass wool separates the materials and serves as plugs for the ends. The long drying tube immediately following the trap is filled with Anhydrone and sealed onto the delivery tube from the trap with Kroenig's cement. The following outside dimensions in millimeters are suitable for the glass tubing used for

[1]The decomposition of hexuronic acids in the presence of hydrochloric acid yields carbon dioxide, furfural, water, and other products. The furfural yield is less than theoretical (7), but in working with pure uronic acids, the yield of CO_2 is quantitative.

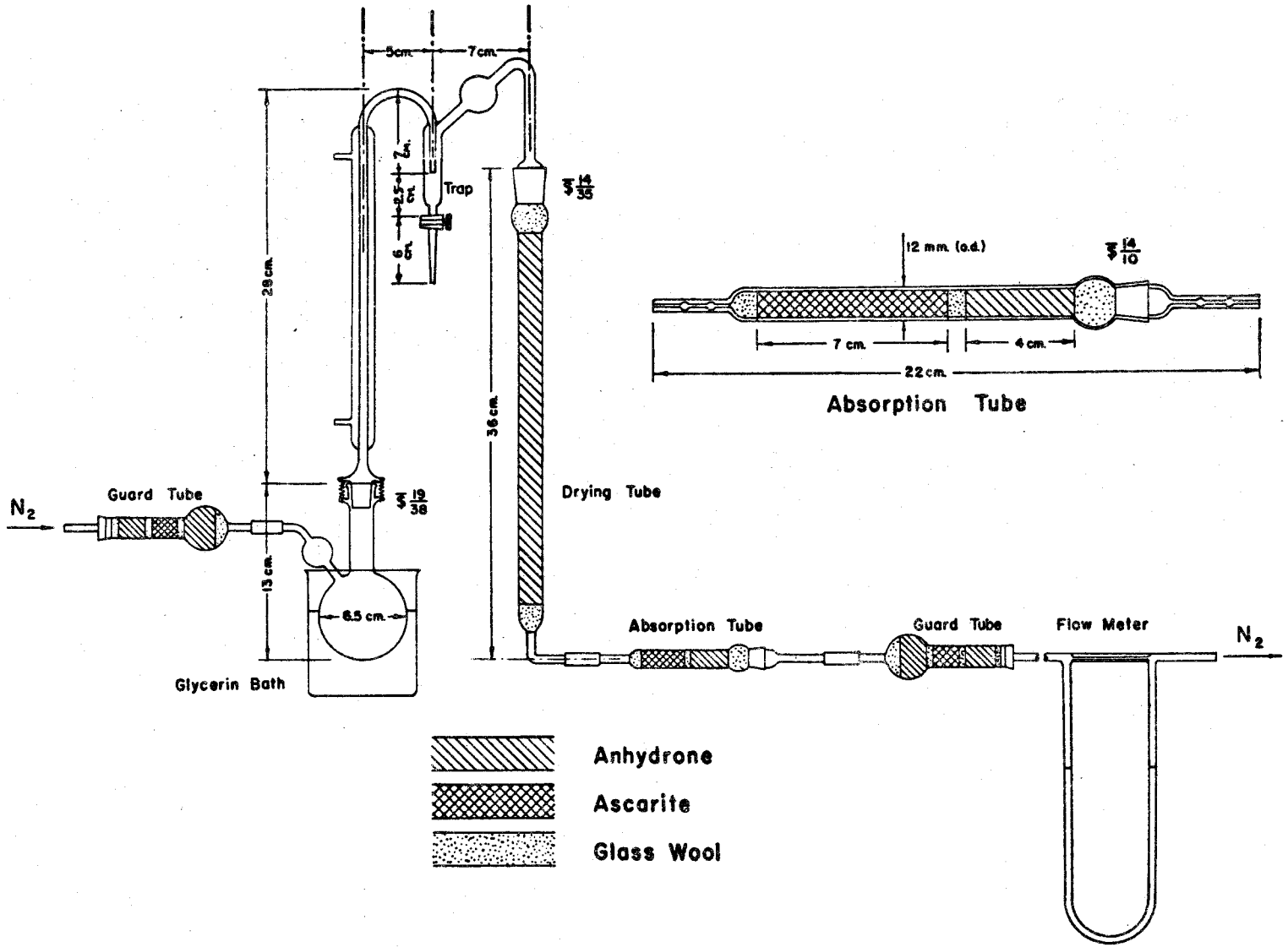


Figure 1.--Apparatus for uronic acids determination.

67-044

constructing the apparatus: Drying tube, 13 to 14; trap, 17 to 18; tubing connecting trap with condenser and drying tube, 9 to 10.

The Ascarite in the absorption tube acts as an absorbent for carbon dioxide, while the layer of Anhydrone absorbs any moisture released by the Ascarite. The capillary tubes on the ends of the absorption tube are similar to those used on tubes for microcombustions. The absorption tubes are handled only by the capillary ends, and the latter are wiped successively with a moist cloth and a dry chamois each time after removal from the apparatus. When not in use, the absorption tubes are stored in test tubes, closed, with rubber stoppers.

The trap is filled to a depth 5 mm. above the inlet tube with 85% phosphoric acid containing silver phosphate. The solution is prepared by dissolving about 10 g. of silver carbonate in 300 ml. of 85% phosphoric acid, after which it should be heated on a steam bath for 1 hr. with a stream of carbon dioxide-free nitrogen passing through the solution to remove carbon dioxide. The solution in the trap is renewed after each determination. The trap can be readily drained and rinsed with water; then refilled by the judicious application of suction at the reaction flask connection. It is necessary to thoroughly wash and clean the trap only after a number of determinations. Phosphoric acid has been found much preferable to sulfuric acid as a trap liquid because the furfural, evolved during the determination, chars in strong sulfuric acid with the formation of some carbon dioxide.

The apparatus, as described, may be applied to any of the usual modifications of the uronic acid method.

Prepurified dry tank nitrogen is used as a carrier gas. A safety bottle is provided to insure a constant pressure of nitrogen. Glycerol is used in the heating bath. The 12% hydrochloric acid used for the decarboxylation reaction is saturated with sodium chloride as recommended by Burkhart, Baur, and Link (6).

Procedure (5)

An accurately weighed sample (2 g. of wood meal or pulp, or from 25 to 100 mg. of contained uronic anhydride in substances richer in polyuronides) is placed in the reaction flask and 60 ml. of the 12% hydrochloric acid mixture added. The flask is connected to the condenser and the entire apparatus connected as shown in the diagram. Heat is applied to the glycerol bath at such a rate that the temperature is raised to 70° C. in 20 min. During this time, nitrogen is passed through the train at the rate of 1.7 liters/hr. This preliminary treatment serves to flush carbon dioxide from the apparatus and to decompose carbonates in the analysis of plant products, as recommended by Phillips, Goss, and Browne (3). The absorption tube is then removed, conditioned in a stoppered test tube near the balance for 5 min., then weighed. During this time, nitrogen is allowed to flow through the apparatus while the bath is held at 70° C.

The absorption tube is replaced and the bath temperature slowly raised to 137° to 140° C. over a period of 30 to 45 min. It is held at this temperature for 3 hr. (or longer, if necessary) after which, without stopping the flow of nitrogen, the absorption tube is removed. (The connection furthest removed from the flask is disconnected first.) The absorption tube is placed in a

stoppered test tube for 5 min., then removed, and weighed at once. The gain in weight represents the weight of carbon dioxide evolved which is multiplied by 4 to obtain the equivalent weight of uronic anhydride (Appendix I).

The operation of the apparatus has been checked by weighing 100-mg. samples of pure sodium carbonate in gelatin capsules and placing them in the reaction flask after the preliminary flushing at 70° C. The bath was then heated to 137° to 140° C. The results in table 1 show an adequate rate of removal and recovery of carbon dioxide.

Table 1.--Recovery of carbon dioxide from sodium carbonate (5)

Time at 137°-140° C.	Na_2CO_3 added (as CO_2)	CO_2 recovered
Min.	Mg.	Mg.
15	41.5	40.2
30	41.5	41.0
30	41.5	41.5

The apparatus and procedure have been checked repeatedly with specimens of pure uronic acids and their salts. The results in table 2 may be considered typical and were obtained with a sample of pure sodium galacturonate. Some representative values for uronic acids in wood and pulps are given in Appendix II.

Table 2.--Carbon dioxide from sodium galacturonate (5)

Time at 137°-140° C.	CO_2 calculated	CO_2 found	Recovery
Hr.	Mg.	Mg.	%
3	20.4	20.1	98.5
3	20.4	20.1	98.5
4	20.4	20.3	99.5
4	20.4	20.3	99.5

Although a reaction time of 3 hr. at the maximum temperature of 137° to 140° C. has been found adequate for most wood and pulp samples, the necessary time for complete evolution of carbon dioxide on a material of unknown type can be determined only by trial. The apparatus described is suitable for rate studies because the absorption tubes can be changed quickly and two tubes alternately used for absorption and weighing.

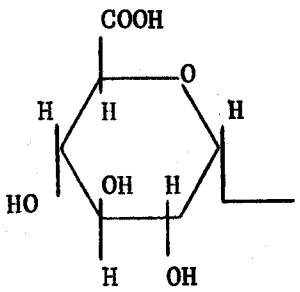
The use of 12% hydrochloric acid with sodium chloride accelerates the evolution of carbon dioxide from polyuronides, presumably because of the elevation in boiling point. At the same time, the values for carbon dioxide evolution from pure sugars which have been found are somewhat above those reported in the literature. Therefore, in some cases, the use of 12% hydrochloric acid without sodium chloride may be found preferable.[2]

[2] At the Forest Products Laboratory, Bureau of Standards dextrose has yielded approximately 0.25% carbon dioxide under the conditions of the test.

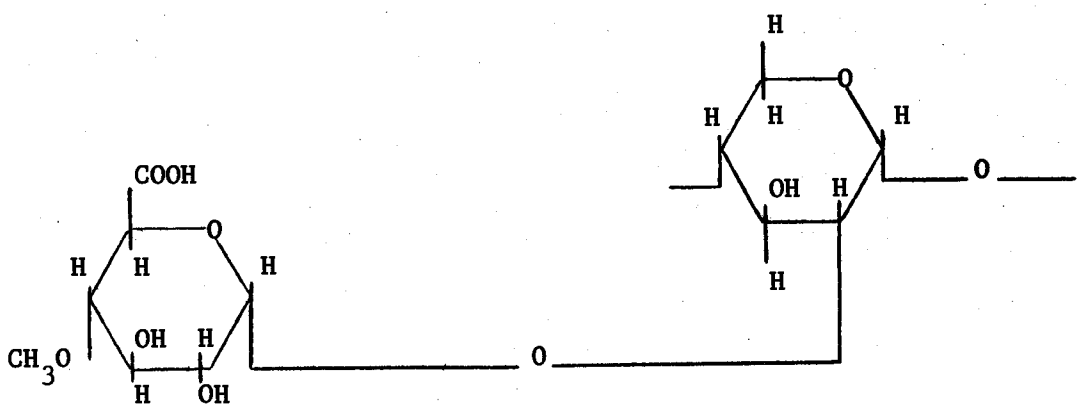
The precision of the method has been calculated from a total of 86 recent analyses in duplicate. The specimens tested covered a wide range, including woods and other fibrous materials, purified fibers, and isolated hemicelluloses and pectic preparations. The carbon dioxide values ranged from 0.3 to 16.6%. The experimental error is indicated by the standard deviation of 0.099% for each single analysis. The standard error of the average for an analysis in duplicate is $0.099\%/\sqrt{2} = 0.070\%$. For uronic anhydride, because of the unfavorable multiplying factor of 4, the standard deviation is 0.40% and the standard error is 0.28%.

Appendix I

The factor of 4.00 used in the calculations converts the carbon dioxide measured in the analysis to the uronic anhydride (glucuronic acid minus one water). Where the uronic acid group is an end group, a factor of 4.02 should be used to convert to the glucuronosyl group.



In wood, the uronic acids are predominantly in the hemicellulose fraction (8), and in deciduous trees predominantly as the 4-O-methyl glucuronic acid glycosidically linked to xylan (9,10).



To convert the carbon dioxide values obtained in the method to the 4-O-methylglucuronosyl group, a factor of 4.34 is applied. In coniferous trees, the uronic acids are present largely as the 4-O-methylglucurono-arabo-xylan (10). Small amounts of galacturonic acids are also present in wood (11,12).

The uronic acids group are always end groups and are positioned as branched units on a xylan (13) or arabinogalactan (14) chain. The average number of xylan residues per glucuronic acid unit is about 10 for hardwoods (15,16) and 5 for softwoods (17,18).

The reducing powers of uronic acids are considerably less than those of the corresponding sugars. A comparison is listed in table 3.

Table 3.--Reducing power of some uronic acids and the corresponding sugars relative to glucose as measured by the Somogyi sugar reagent[1]

Sample	Relative reducing power
Glucose	1.000
Glucuronic acid	.767
Galactose	.786
Galacturonic acid	.660

[1]Unpubl. data, U.S. Forest Products Laboratory.

Appendix II

Tabulated below are some published values for uronic anhydrides in wood and pulps.

Species	Uronic anhydrides %	Reference
Hardwoods:		
Aspen	3.3	(16)
Beech	4.8	(16)
Paper birch	4.6	(16)
Yellow birch	4.2	(19)
American elm	3.6	(20)
Red maple	3.5	(16)
Sugar maple	4.4	(20)
Overcup oak	4.5	(19)
Southern red oak	5.8	(19)
Softwoods:		
Balsam fir	3.4	(16)
Black spruce	4.1	(19)
Douglas-fir	2.8	(19)
Eastern hemlock	3.3	(16)
Eastern white cedar	4.2	(16)

Species	Uronic anhydrides %	Reference
Jack pine	3.9	(16)
Loblolly pine	3.8	(19)
Red cedar	4.2	(19)
Tamarack	2.9	(16)
Western hemlock	5.0	(19)
White spruce	3.6	(16)

Wood pulps:

Type of pulp	Uronic acid or 4-O-methylglucuronic acid %
Unbleached sulfate from pine and spruce	0
Unbleached sulfate from birch and aspen	0.69 - 2.55
Unbleached sulfite from spruce, pine, birch, and aspen	.38 - 1.30
Bleached sulfate from spruce and aspen	.37 - .96

References

(1) Lefevre, K. U., and Tollens, B.
1907. Investigation of glucoronic acid, its quantitative determination and its color reactions. Ber. 40: 4513.

(2) Dickson, A. D., Otterson, H., and Link, K. P.
1930. Method for the determination of uronic acids. Jour. Amer. Chem. oc. 52: 775.

(3) Phillips, M., Goss, M. J., and Browne, C. A.
1933. Determination of uronic acids and methoxyl in certain plants and plant materials. Jour. Assn. Official Agr. Chem. 16: 289.

(4) Whistler, R. L., Martin, A. R., and Harris, M.
1940. Determinations of uronic acids in cellulosic materials. Jour. Res. Nat. Bur. Standards 24: 13.

(5) Browning, B. L.
1949. Apparatus for determination of polyuronide carboxyl. Tappi 32: 119.

(6) Burkhart, B., Baur, L., and Link, K.
1934. A micro method for the determination of uronic acids. Jour. Biol. Chem. 104: 171.

(7) Bowman, J. R., and McKinnis, R. B.
1930. A study of the pentose and uronic acid content of orange albedo and an arabino-galacturonic acid derived from orange pectin. Jour. Amer. Chem. Soc. 52: 1209.

(8) Jayme, G., and Hahn, G.
1960. Polyuronide content of some German woods and of the holocelluloses and hemicelluloses isolated from them. Holzforschung 14(2): 52.

(9) Saarnio, J., Wathen, K., and Gustafsson, C.
1954. Uronic acids in wood pulp. Paperi ja Puu 36: 209.

(10) Hamilton, J. K., and Thompson, N. S.
1958. A comparison of the carbohydrates of hardwoods and softwoods. Pulp and Paper Mag. of Can. 59(10): 233.

(11) Scott, R.
Unpublished work, U.S. Forest Prod. Lab., Madison, Wis.

(12) Jones, J. K. N., Purves, C. B., and Timell, T. E.
1961. Constitution of a 4-O-methylglucuronoxylan from the wood of trembling aspen. Can. Jour. of Chem. 39: 1059.

(13) _____, and Wise, L. E.
1952. The hemicelluloses present in aspen wood. Part II. Jour. Chem. Soc. 3389.

(14) Urbas, B., Bishop, C. T., and Adams, G. A.
1963. Occurrence of d-glucuronic acid in arabinogalacton. Can. Jour. Chem. 41: 1522.

(15) Aspinall, G. O, Hirst, E. L., and Mohamed, R. S.
1954. Hemicellulose A of beechwood. Jour. Chem. Soc. 1734.

(16) Timell, T. E.
1957. Carbohydrate composition of ten North American species of wood. Tappi 40: 568.

(17) Aspinall, G. O., and Carter, M. E.
1956. The constitution of a xylan from Norway spruce. Jour. Chem. Soc. 3744.

(18) Dutton, G. G. S., and Smith, F.
1956. The constitution of the hemicellulose of western hemlock. Parts I and II. Jour. Amer. Chem. Soc. 78: 2505, 3744.

(19) Wise, L. E., and Ratliff, E. K.
1947. Quantitative isolation of hemicelluloses and the summative analysis of wood. Anal. Chem. 19: 459.

(20) Timell, T. E.
1958. Some properties of four Canadian species of wood. Pulp and Paper Mag. of Can. 59(8): 139.

DETERMINATION OF WOOD SUGARS

Contents

I. Introduction

Woods are approximately 75% carbohydrate, and wood pulps may be almost pure carbohydrate. These carbohydrates are polysaccharides that are made up of glucose, mannose, xylose, galactose, and arabinose monomers. Some representative analyses of wood carbohydrates are given in table 1. Since these wood sugars

Table 1.--Carbohydrate composition of some American woods (in percent of extractive-free ovendry wood)[1]

Species	Galactan[2]	Glucan	Mannan	Araban	Xylan
Aspen	0.8	57.3	2.3	0.4	16.0
Beech	1.2	47.5	2.1	.5	17.5
Paper birch	.6	44.7	1.5	.5	24.6
Red maple	.6	46.6	3.5	.5	17.3
Balsam fir	1.0	46.8	12.4	.5	4.8
Eastern white cedar	1.5	45.2	8.3	1.3	7.5
Eastern hemlock	1.2	45.3	11.2	.6	4.0
Jack pine	1.4	45.6	10.6	1.4	7.1
White spruce	1.2	46.5	11.6	1.6	6.8
Tamarack	2.3	46.1	13.1	1.0	4.3

[1]Timell, T. E. "Carbohydrate composition of ten North American species of wood." Tappi 40: 568. 1957.

[2]The "an" suffix denotes the anhydro sugar.

account for such a large fraction of the wood substance, methods for measuring their concentrations are of considerable interest to the carbohydrate and wood chemist.

Procedures used for wood sugar analyses vary with the type of sample and with the analyses required but, since many of the operations are common to more than one situation, "Determination of Wood Sugars" is divided into a "Techniques" part and a "Procedures" part.

Under "Techniques," general procedures are given, such as the hydrolysis procedure which is necessary when analyzing polysaccharides for their wood sugar makeup. In "Procedures," information is given on the particular sequence followed for obtaining particular information about particular samples.

II. Techniques

Characterizing the carbohydrate material in wood or pulp in terms of its wood sugar content requires several steps: (1) total hydrolysis to constituent sugars, (2) neutralization, (3) concentration or evaporation, (4) chromatographic separation, (5) elution, and (6) determination of the separated sugars.

In a total sugar analysis (which is reported as "glucose") the chromatographic separation is, of course, not necessary, but when information about the amounts of the various sugars is required, the separation must be made.

A. Calibrations, Calculations, and Correction Factors

1. Sugar Determination Correction Factors

(a) Relative reducing power factors.--Each wood sugar exhibits its own characteristic reducing power toward the Somogyi copper reagent. Assigning glucose a value of 1.00, the values for the other wood sugars are given in table 2. An "as glucose" value for a nonglucose sugar must be divided by the relative reducing power of the nonglucose sugar to get its correct concentration.

(b) Hydrolysis losses.--All wood sugars undergo a measurable amount of destruction during the hydrolysis treatment normally given pulps or wood. If a sugar sample has been hydrolyzed, corrections must be applied to arrive at

the original sugar concentration. Values for losses occurring during hydrolysis for the various wood sugars are relatively constant and are given in table 3.

(c) Sugar polymerization factor.--To convert a value obtained for wood sugar to the anhydro polysaccharide, the factors listed in table 4 are applied.

(d) Paper blank.--Since chromatographic paper always contains traces of soluble reducing substances which would introduce error into the sugar analysis, paper blanks must be measured and corrections made when samples have been chromatographed.

Table 2.--Relative reducing power of wood sugars (Nelson method)[1]

Sugar	Relative reducing power (weight basis)
Glucose	1.000
Mannose	.870
Galactose	.786
Arabinose	.869
Xylose	.979

[1] Nelson, N. "A photometric adaptation of the Somogyi method for the determination of glucose." J. Biol. Chem. 153: 375. 1944.

Table 3.--Percent sugar surviving hydrolysis

Sugar	Surviving hydrolysis
	%
Glucose	95.0
Mannose	91.6
Galactose	95.7
Arabinose	92.4
Xylose	86.6

Table 4.--Factors for converting sugar values to the corresponding anhydro polymer

Sugar	Anhydro-sugar conversion factor
Glucose	0.90
Mannose	.90
Galactose	.90
Arabinose	.88
Xylose	.88

For a given lot of paper, the correction for apparent reducing sugar is obtained by irrigating several randomly selected sheets in the usual way with spots of mannose and xylose applied to the starting line in the guide-strip regions. Two- by three-inch tabs of paper are then cut from the sugar-free regions adjacent to the mannose and xylose areas and eluted. The eluates are analyzed for their apparent reducing sugar content. The mean values so obtained (in micrograms) provide the desired corrections for paper blank. The differences in the blank between successive tabs from a given lot of paper are about 1 μg.

2. Pulp Analysis Calibration Curve

In the analysis of high-purity pulps where glucan, mannan, and xylan are the only significant carbohydrates present, it is convenient to account for all correction and conversion factors in one operation using empirical calibration curves. This involves taking mixtures of the three sugars in known concentrations and running them through all of the operations of a pulp analysis. The apparent percent of glucose, mannose, and xylose is calculated (as glucose) and a curve prepared relating the recovered sugar as glucose to the concentration of sugar as xylan, mannan, and glucan in the starting material.

(a) Procedure.--Prepare 0.300-g. quantities of mixtures of glucose, mannose, and xylose containing 1.00, 2.00, 3.00, 4.00, and 5.00% of both mannose and xylose as indicated in table 5. These samples will have respective mannan equivalents of 1.00, 2.00, 3.00, 4.00, and 5.00% and respective xylan equivalents of 0.00, 0.98, 1.96, 2.84, 3.92, and 4.90%. To avoid weighing

Table 5.--The composition of a set of standard sugar solutions

Sugar	Standard solutions (%)				
	1	2	3	4	5
Glucose	98	96	94	92	90
Mannose	1	2	3	4	5
Xylose	1	2	3	4	5

such small quantities of mannose and xylose, solutions of the desired mixtures may be prepared, and suitable aliquots (containing 0.300 g. of total sugar) pipeted into the vials used for primary hydrolysis and dried in a vacuum desiccator.

These sugar mixtures in duplicate are hydrolyzed, neutralized, evaporated, chromatographed, and analyzed exactly in accordance with the procedures described for pulp analysis. This yields the glucose, mannose, and xylose content of each mixture in terms of micrograms of glucose equivalent. From these values is subtracted the amount--in micrograms--of apparent reducing sugar due to the paper blank (see "Sugar Determination Correction Factors, Paper Blank"). The corrected quantities--in micrograms--of glucose, mannose, and xylose are then totaled. By dividing the corrected quantities of xylose (as glucose) by this total (all sugars as glucose), one obtains the apparent percentage of xylose in each mixture. The apparent percentage of mannose is calculated similarly.

Two curves are then constructed: one in which the apparent percentage of xylose is plotted against the calculated xylan content of the original mixtures as listed above, and a second in which the apparent percentage of mannose is plotted against the calculated mannan content of the original mixtures. These are the master calibration curves and should serve as long as the defined technique is followed.

In practice, the determination of the carbohydrate composition of a pulp becomes relatively simple. A linear streak of the neutralized and concentrated hydrolysate is placed on the paper and chromatographed. Reducing substances (as glucose) are determined in the areas occupied by glucose, mannose, and xylose, and apparent percentages of mannose and xylose are calculated. Reference to the master calibration curves then gives the composition of the pulp.

(b) Accuracy.--The described calibration procedure was designed to accommodate pulps having a mannan and xylan range between 0 and 5%. By strict adherence to the prescribed techniques and with adequate replication, errors can be held to a level of about 0.1 to 0.2% for a minor constituent, based on the total carbohydrate.[1]

For highly purified pulps with mannan and xylan contents in the 1 to 1.5% range, the master calibration curves can be made more sensitive by selecting a series of five or more calibration points over the range of 0 to 1.5% mannan and xylan.

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[1] U.S. Forest Prod. Lab., Wood Chem. Div., Analytical Sect., Quart. Rep. No. 11. 1959.

B. Hydrolysis of Polysaccharides

The total hydrolysis of a cellulose or wood requires a primary hydrolysis with strong mineral acid, followed by a secondary hydrolysis in dilute acid. The primary hydrolysis results in the formation of a mixture of oligosaccharides. It is the function of the secondary hydrolysis to complete the conversion to monomeric sugars.[2] If the lignin content of the samples is to be measured on the sample, a quantitative filtration is made after the secondary hydrolysis, as described in the procedure for the determination of lignin.

Two hydrolysis procedures are described here. The "Modified" procedure is most frequently used at the Forest Products Laboratory because it requires less time but the two are interchangeable and results are equivalent (tables 6 and 7).

1. Sample Preparation

Prior to hydrolysis, wood samples are ground to pass a 40-mesh screen, extracted, and air dried.[3]

Pulps are brought to an air-dry condition; however, pulps dried to this extent are difficult to hydrolyze. To overcome this difficulty, pulps are "fluffed" before attempting to hydrolyze them.

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[2] Some reversion products are formed during the primary hydrolysis and these are also converted to monomeric sugars during the secondary hydrolysis.

[3] If lignin or quantitative sugar values are to be determined, the moisture must be determined to get an accurate sample weight; if only the ratio of sugars is required, the samples need only be air dry so that residual moisture does not significantly change the hydrolyzing acid concentration. Moisture is determined, when necessary, by drying a separate sample at 105° ± 3° C. to constant weight.

Table 6.--Lignin analysis on matched wood samples and methoxyls on lignin prepared by modified hydrolysis and standard methods[1]

Sample	Lignin method			Methoxyl determinations		
	Modified hydrolysis	Standard	Difference	On "modified hydrolysis lignin"	"Standard lignin"	Difference
	%	%		%	%	
Red gum	24.99	25.02		20.37	20.50	
	25.15	24.93		20.41	20.46	
Av.	25.07	25.00	+0.07	20.39	20.48	-0.09
Maple	23.70	23.73		20.74	20.60	
	23.71	23.70		20.80	20.71	
Av.	23.71	23.72	-0.01	20.77	20.66	+0.11
Aspen	18.91	18.90		21.13	21.00	
	18.85	18.75		21.13	21.20	
Av.	18.88	18.83	+0.05	21.13	21.10	+0.03
Douglas-fir	29.56	29.27		15.25	14.76	
	29.48	29.32		15.05	14.95	
Av.	29.52	29.30	+0.22	15.15	14.86	+0.29
Sitka spruce	25.18	24.65		13.75	15.42	
	25.00	24.67		13.86	15.41	
					15.48	
					15.44	
Av.	25.09	24.66	+0.43	13.81	15.43	- .6
Black spruce	27.49	27.69		15.82	14.60	
	27.59	27.69		15.75	14.57	
Av.	27.54	27.69	-0.15	15.79	14.59	+1.20
White spruce	26.46	26.75		15.40	15.93	
	26.36	26.68		15.48	15.96	
Av.	26.41	26.72	-0.31	15.44	15.95	-0.51
Western hemlock	31.31	31.51		15.39	14.72	
	31.35	31.58		15.38	14.58	
Av.	31.33	31.55	-0.22	15.39	14.65	-0.74

[1]U.S. Forest Prod. Lab., Wood Chem. Div. Quart. Rep. No. 12, Dec. 1, 1959.

Table 7.--Repeated lignin and sugar determinations on aspen wood[1] by modified hydrolysis method. One-half gram samples were used and results based on extractive-free ovendry wood

Sample No.	Lignin		Sugar	
	Results	Average	Results	Average
	%		%	
1	17.90 17.95	17.93	74.96 75.33	75.15
2	18.09 18.09	18.09	75.03 74.89	74.96
3	17.92 17.87	17.90	75.10 75.20	75.15
4	17.87 18.06	17.97	74.75 75.17	74.96
5	18.03 18.02	18.03	75.48 74.86	75.17
6	18.18 18.02	18.10	74.84 74.91	74.88
7	17.87 18.00	17.94	74.86 74.78	74.82
8	18.22 18.27	18.25	74.99 75.60	75.30
9	18.33 18.55	18.44	74.86 74.90	74.88
10	18.62 18.74	18.68	75.81 76.04	75.93
11	18.17 18.04	18.11	75.62 75.45	75.54
12	18.82 18.94	18.88	74.85 75.08	74.97

Table 7.--Repeated lignin and sugar determinations on aspen wood[1] by modified hydrolysis method. One-half gram samples were used and results based on extractive-free ovendry wood--continued

Sample No.	Lignin		Sugar	
	Results	Average	Results	Average
	%		%	
Grand av.		18.19		75.14
Standard deviation		0.31726		0.32093
Standard error of mean		0.09158		0.09264

[1]U.S. Forest Prod. Lab., Quart. Rep. No. 12, Dec. 1, 1959.

Fluffing is accomplished by grinding or scraping the sample off a sheet with a blade, or by air drying from acetone.

Obtaining a fluffy sample by scraping is perhaps satisfactory but practical only in the case of sheeted material.

Acetone displacement is the procedure generally used. Here the sample is stirred into a slurry in water and then filtered through a Büchner funnel (with suction), pressing the pulp finally with a rubber dam. The cake is washed with acetone on the filter and then stirred (by hand) in a beaker of acetone. The pulp is filtered out of the slurry, washed with acetone, removed from the filter, spread out, and air dried. This air-dried sample is used for hydrolysis. The dry weight of the sample is determined by drying a separate sample overnight at 105° C. When there is not enough sample to determine the volatiles on a separate sample, the sample itself can be dried prior to hydrolysis if the pulp is extremely clean. However, if any residual acid or alkali are present, a sort of hornification occurs, and the dried sample becomes very resistant to hydrolysis.

2. Standard Hydrolysis Method

(a) Reagents.--Sulfuric acid, 72%. Carefully pour 665 ml. of concentrated sulfuric acid into about 300 ml. of water and, after cooling, dilute to 1 liter. Standardize against standard sodium hydroxide solution, using methyl-orange indicator. Adjust the acid to a strength of 72 $\pm$ 0.1%, by addition of water or concentrated H_2SO_4 as may be found necessary.

(As concentrated H_2SO_4 varies somewhat in strength, the measurement does not have to be made accurately; it may be done with a liter graduate. The proportions given are for concentrated H_2SO_4 of 96% strength and 1.84 specific gravity.)

If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72% H_2SO_4 the specific gravity at 20°/4° C. is 1.6338; and at 60°/60° F. or 15.56°/15.56° C. it is 1.6389. A variation of 0.1% in the strength of the acid at this concentration causes a change of 0.0012 in the specific gravity; 72% sulfuric acid is equivalent to 24.1N.

(b) Procedure.--Place a sample (approximately 1 g. pulp or 2 g. wood) in a weighing bottle. To the sample slowly add, with stirring, 15 ml. of cold (15° C.) 72% sulfuric acid. Stir constantly for 1 min., being sure to break up all lumps in the sample. Allow to stand for 2 hr. with frequent stirring at a temperature of 20° ± 0.1° C. Wash the material into a 1-liter Erlenmeyer flask, dilute to 3% acid concentration with 560 ml. of distilled water, and boil for 4 hr. under a reflux condenser. Remove the sample from the condenser and cool.

When many samples are being handled, primary hydrolysis of successive samples should begin at 2-min. intervals.

3. Modified Hydrolysis Method

(a) Reagents.--Sulfuric acid, 72%. Carefully pour 665 ml. of concentrated sulfuric acid into about 300 ml. of water and, after cooling, dilute to 1 liter. Standardize against standard sodium hydroxide solution, using methyl-orange

indicator. Adjust the acid to a strength of 72 ± 0.1% by addition of water or concentrated H_2SO_4 as may be found necessary.

(As concentrated H_2SO_4 varies somewhat in strength, the measurement does not have to be made accurately; it may be done with a liter graduate. The proportions given are for concentrated H_2SO_4 of 96% strength and 1.84 specific gravity.)

If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72% H_2SO_4 the specific gravity at 20°/4° C. is 1.6338; and at 60°/60° F. or 15.56°/15.56° C., it is 1.6389. A variation of 0.1% in the strength of acid at this concentration causes a change of 0.0012 in the specific gravity; 72% H_2SO_4 is equivalent to 24.1N.

(b) Apparatus.--Shell vials, 22 mm. ID; 95 mm. length. Constant temperature bath set at 30° ± 0.1° C. Autoclave.

(c) Procedure.--Weigh out approximately 0.3 g. of ground, air-dried wood or pulp sample into a shell vial. At the same time, if necessary, weigh out another sample for a moisture determination. Dry the moisture sample in a weighing bottle at 105° C. until constant weight is reached. (This is approximately 2 hr. or until the weight does not change more than 0.1% upon 1 hr. of reheating.)

To the sample in the shell vial add 1 ml. of 72% H_2SO_4 for each 0.1 g. of sample. After adding the acid, place the vial in the 30° C. bath, allow it to stand for 3 min., then stir it thoroughly. Stirring should be repeated periodically to assure complete mixing and reaction. Allow the sample to remain in the 30° C. bath for 1 hr.

After 1 hr., remove the sample from the bath and transfer it to a 125 ml. or 250 ml. Erlenmeyer flask, using 28 ml. of water for each milliliter of 72% H_2SO_4 added. This results in a 4% acid solution for the secondary hydrolysis. Cover the sample with a watch glass.

The secondary hydrolysis is accomplished in an autoclave according to the following procedure:

1. Open the valve in the distilled waterline to the reboiler.
2. Preheat autoclave to 220° F. and adjust the supply steam pressure to 60 p.s.i.g. (pressure to reboiler).
3. Close supply valve and vent autoclave.
4. Place samples in autoclave and admit steam with vent open, allowing escape of air, and raising temperature to 220° F. by the end of 4 min.
5. Close bypass and allow temperature to increase to 246° F., adjusting supply steam pressure to 30-32 lb. as required to maintain autoclave temperature at 248° F. This should be completed in 7 min. from the time samples are introduced. The autoclave is maintained at this temperature for 1 hr. by proper adjustment of steam pressure regulation valve, then vented through the orifice (a gate valve with a 3/32-in.-diameter hole drilled through the gate). Venting should be completed in 6 min. This results in a nearly linear temperature decrease, and the rate of pressure release is such that no sample loss occurs through excessively rapid boiling.
6. Remove the sample from the autoclave.

4. Neutralization

Neutralization of hydrolysates is accomplished by the addition of calcium carbonate (reprecipitated chalk), barium hydroxide, or ion-exchange resin.

Calcium carbonate is used for neutralizing hydrolysates on which only total wood sugars is to be determined (no prior chromatographic separation of the sugars). Barium hydroxide could be used on hydrolyzates of purified pulps but will give a slightly lower percent recovery of sugar.

Barium hydroxide is used for neutralizing hydrolysates which are to be chromatographed and sugars determined on a quantitative (not ratios) basis. Resin is not used when the sugars have to be recovered quantitatively because it is difficult to wash the sugars out of the resin. Barium is not used on hydrolysates of wood or impure pulps because it causes a severe foaming problem.

Resin is used to neutralize the hydrolysates of wood and impure pulps and for hydrolysates that are to be chromatographed and the ratio of the sugars present determined. Neutralization with resin is accomplished faster and more easily than by lime or barium.

(a) With calcium carbonate.--In this procedure, lime (solid) is added until a methyl-orange end point is reached.

(b) With barium hydroxide.--In this procedure, hydrolysates are titrated directly with a saturated solution of barium hydroxide (approximately 70 g. $Ba(OH)_2 \cdot 8H_2O$ per liter). The original sample, after primary hydrolysis, is washed into a tared 250-ml. wide-mouth bottle with 84 ml. of distilled water

measured in a 100-ml. graduate. The bottles should be either centrifuge bottles or of such size as to fit the cups of a laboratory centrifuge. Secondary hydrolysis is accomplished in the tared bottle.

After hydrolysis, the sample, in its tared bottle, is affixed to the titration assembly shown in fig. 1. The barium hydroxide solution is admitted directly from the stock carboy, while the bottle contents are under vigorous agitation, to a pH of approximately 5.5. The bottle is then put aside until all other samples in the series are neutralized. The barium sulfate formed in these relatively concentrated solutions occludes sulfuric acid, which is slowly released, resulting in a decreasing pH. The problem of drift is eliminated by allowing the solution to stand for about 15 min. and then readjusting the pH to the desired 5.4 with additional but more dilute barium hydroxide solution (approximately a 10:1 dilution of the original 7% solution).

The bottles containing the neutralized hydrolysates are then adjusted to a net weight of 258.4 g. by the addition of water. Each bottle now contains 250 ml. of solution and 8.4 g. of barium sulfate. The bottles are then centrifuged to settle the barium sulfate and a 200-ml. aliquot of the clear supernatant liquid is taken from each for evaporation.

(c) With ion-exchange resin.--The neutralization of sugar hydrolysates with ion-exchange resins is desirable because it is faster and easier, glassware is easier to clean, and smaller volumes are required. Contamination from prewashed resin is not a problem. When handling large numbers of samples, however, columns are inconvenient and simple batch neutralization with anion

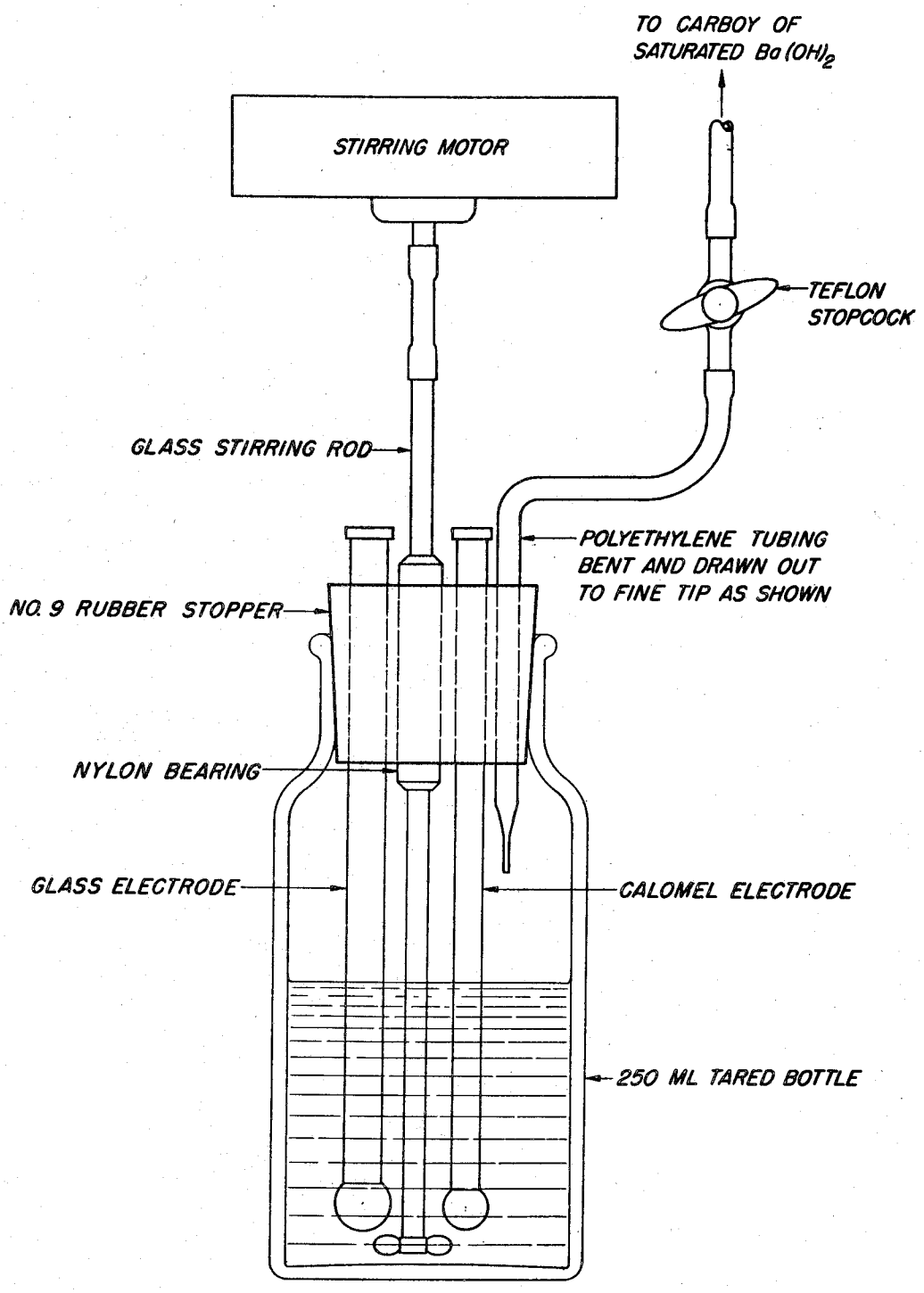

Figure 1.--Barium hydroxide titration assembly.

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resin has been found adequate (1). Neutralization is accomplished by mixing the cooled hydrolysates with approximately 25 g. of freshly washed Amberlite IR 4B ion-exchange resin (hydroxide form). This quantity of resin will raise the pH to 3.8 to 4.0 in the convenient time of 25 to 30 min., after which the solution is recovered by filtration and washing. The need for prewashing the resin arises from the fact that water-soluble materials are released by the resin during storage. If quantitative recovery is desired, it is preferable to neutralize with barium because of the difficulty in washing sugars out of the resin.

To avoid sugar losses by yeast or microbial action, store all neutralized hydrolysates under refrigeration. Adding a few drops of toluene increases protection.

C. Isolation of Wood Sugars

Isolation of individual wood sugars present in a sample in mixture is done by paper chromatography. The procedures and apparatus used are based on those described by Saeman et al. (1). An evaporation step may be required because the hydrolysate or sample may contain sugars in too low a concentration for chromatographing and measuring. After chromatographing, the sugars are located and eluted and then are ready for the determination of the individual sugars.

1. Evaporation

Sugar solutions for chromatographing should contain about 10% sugar. This requirement may necessitate concentration by evaporation. Evaporation can be

accomplished with the evaporator described by Saeman et al. (1) and illustrated in figure 2.

It is a simple, inexpensive, low-temperature evaporator that is rapid in operation (10 ml. per min.), permits high recovery of product, and causes a minimum of sugar decomposition. With a bank of three such units, one operator can conveniently evaporate 50 samples a day from 200 ml. to 3 ml.

With the aspirator turned on and the condenser in operation, the entire 200 ml. of hydrolysate is drawn into the evaporator through the capillary stopcock. Care must be taken to admit the last portion slowly and to close the cock just as air enters the bore. The solution should be cooled to a temperature below the boiling point at pressures used for evaporation. The stopcock is then adjusted to provide a very slow stream of air bubbles into the evaporator. This promotes smooth ebullition. Steam is then turned into the boiler jacket and the solution started swirling.

The last stages of evaporation should be carried out cautiously and can be accomplished with the small amount of warm condensate left in the heater jacket as the steam is turned off. The concentration required at this point depends on the composition of the sample, but a final volume of 2 to 4 ml. is common. This final volume can be read with sufficient accuracy by using calibration marks on the evaporator. The concentrate is recovered in high yield by draining it from the stopcock into a small plastic-capped vial. The solutions are now ready for chromatography and should be kept under refrigeration until so used.

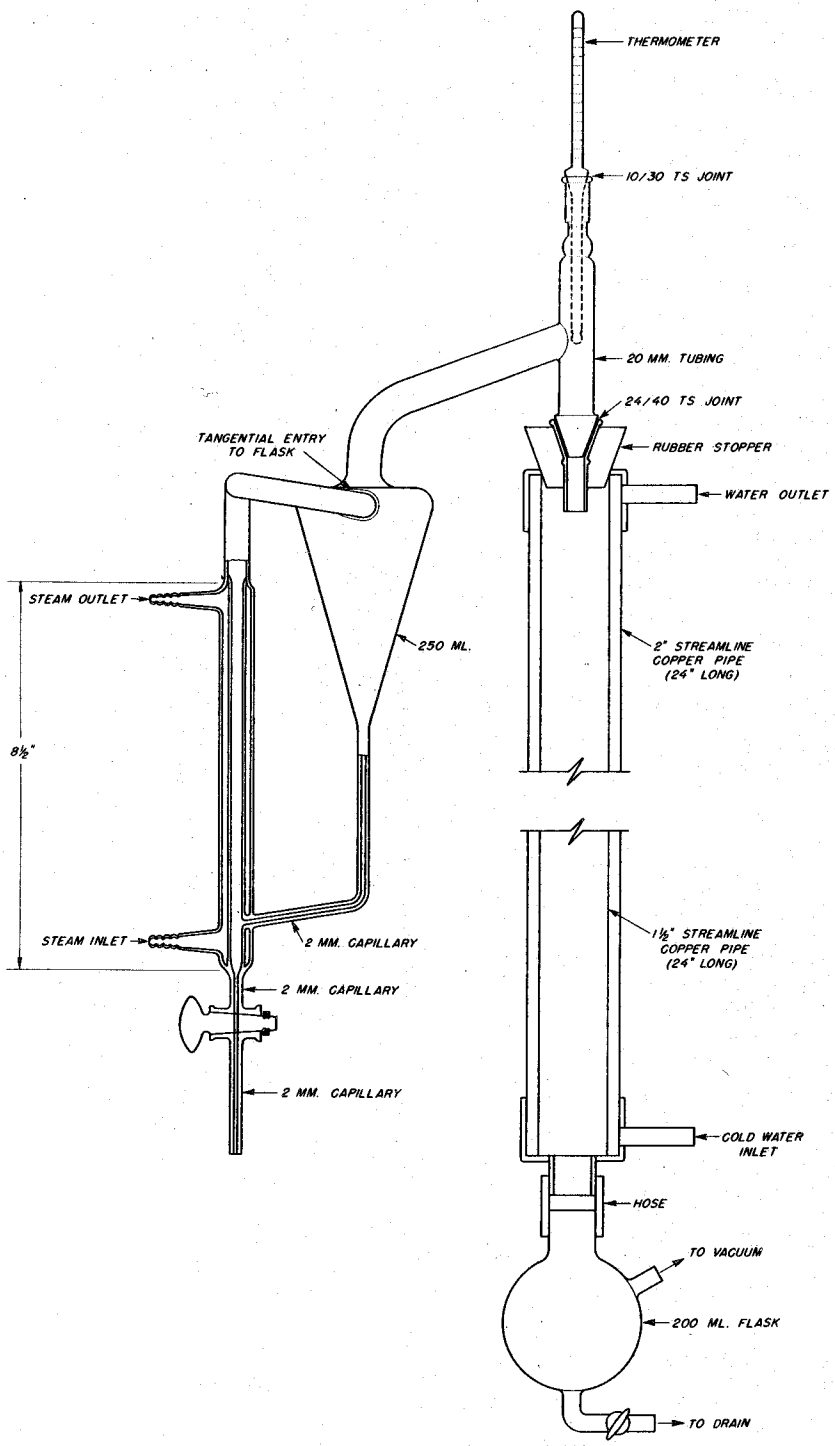

Figure 2.--Evaporator unit for concentrating sugar solutions.

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If quantitative values for the sugar content of a sample are required, quantitative removal of sugar concentrate from the evaporator is necessary. This may be effected by removing the evaporator from the condenser and fitting it to a flask containing rapidly boiling water or 95% ethanol. The vapors condensing on the cold inside surfaces wash out the sugars with a minimum of dilution. Final volume is generally 5 ml.

In unbuffered systems, a 100-fold evaporation of solutions containing a nonvolatile strong acid results in a drop of 2 units in pH. On evaporation to dryness, much greater pH changes result. Because of the buffering action of materials dissolved from the resin, resin-neutralized solutions show much less pH change on evaporation than do those neutralized with barium hydroxide. Regardless of the neutralization or evaporation techniques employed, pH changes can lead to serious error in the sugar content of hydrolysates. Too low a pH causes reversion, while too high a pH results in epimerization. Adjustments in technique should be made as necessary to insure that the pH of the concentrate be maintained in the 3.5 to 5.0 region.

2. Chromatographic Separation

In the separation procedure described below, the previously prepared sugar concentrates are applied to filter paper by streaking, the paper is irrigated by descending chromatography, and the sugar-containing areas (tabs) are cut out for chemical analysis. In the description, some working knowledge of the process is presumed. The procedures and apparatus used are generally those described by Saeman et al. (1).

(a) Sample application.--Chromatographic separations are carried out on 18-1/4- by 22-1/2-in. sheets of chromatographic paper. These sheets have folds made on a jig in positions to suit the requirements of the paper holders in the chromatographic cabinet. The long dimension of the paper is the machine direction. The folded paper is hung over the supporting bar of the streaking device of McKibbins et al. (2) illustrated in fig. 3. The wooden holder, bearing the syringe loaded with sugar concentrate (approximately 10% sugar), is then moved down the steel bar to apply a uniform streak of sample to the starting line. With Whatman No. 1 paper, the maximum loading is limited to about 400 μg. of total sugar per cm. of streak. Loading on heavier papers can be increased in proportion to paper weight. The streaked papers are then air dried. Whatman 3MM paper can be loaded to about 1,000 μg. total sugar per cm. (note C-1).

The application of sample to the paper as a continuous streak rather than as spots has the advantage, in direct photometry, of providing a uniform band of sample; when chemical methods are used, the paper is loaded to the limit of its capacity, thereby providing the most favorable ratio of applied sugar to paper blanks. The streaking device shown in fig. 3 is simple to construct, and its precision and utility have been proven in extensive use. The only critical details of construction are the two straight edges whose angular separation governs the loading of sugar concentrate on the paper. The indicated slope and syringe size are suitable for Whatman No. 1 paper. A more complete description of this device is given by McKibbins et al. (2).

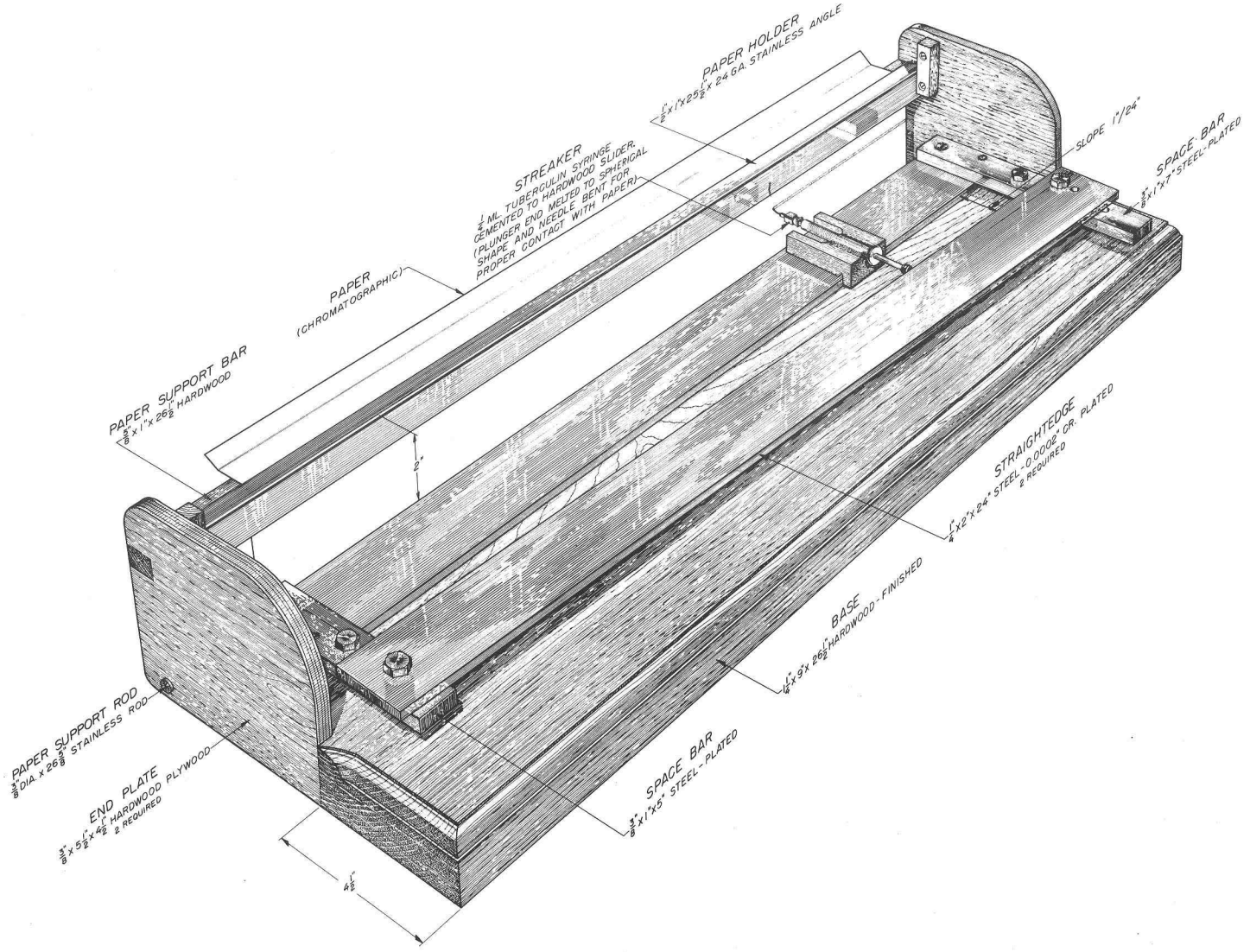

Figure 3.--Device for the precise linear application of pulp hydrolysates to chromatographic paper.

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(b) Solvent irrigation and isolation of sugars.--For irrigation, the streaked papers are hung from the supporting rods in the chromatographic cabinet. Then approximately 500 ml. of solvent is added directly to the bottom of the cabinet or to a shallow tray resting on the bottom, and the cabinet is closed for about an hour to permit vapor equilibration. Solvent is then added to the troughs (see note C-10) into which the starting edges of the papers project. Irrigation is continued for a period of time sufficient to move xylose to the bottom of the sheets. With butanol-pyridine-water in the volume ratio of 10:3:3, this time is in the neighborhood of 48 hr. for Whatman No. 1 paper at 30° C. but may vary considerably.

After irrigation, the papers are dried at room temperature in a suitably ventilated space. Four strips spaced about equally apart are then cut vertically from each chromatogram, using a steel template (75 ± 1 mm. wide by 60 cm. long) and a paper knife. With a little experience, this cutting can be done accurately to a fraction of a millimeter.

The guide strips remaining are next dipped into a fresh (prepared just prior to dipping) solution made by mixing equal volumes of ethyl ether and indicating solution (800 ml. butanol, 40 ml. water, and 20 g. aniline-acid phthalate crystals prepared as described in note C-12). The strips are allowed to drip dry, then heated in a circulating oven at 105° C. for 5 min. to color the sugar-containing areas. The previously cut-out vertical strips are then matched with the guide strips and the areas containing the glucose, mannose, and xylose are removed (with the butanol-pyridine-water system, the xylose has

the highest RF value, followed by mannose, then glucose). At this point, individual sugar components of the initial pulp hydrolysate are present on four replicate tabs of paper, each 75 mm. wide by approximately 2 in. long in the initial length direction of the sheet. (If all five wood sugars are to be resolved, follow the procedure outline in note C-2.)

(c) Notes.--(Note C-1)--Uniformity of performance and of paper blank are of critical importance in the selection of a chromatographic paper for quantitative work. Whatman No. 1 has been widely used for this purpose and can be purchased in a grade especially selected for chromatographic work. For high-purity pulps and for cotton, where minor constituents are present in very small quantities, the use of Whatman No. 3MM paper may be advantageous, since it can be loaded with three times as much solution.

(Note C-2)--Purified wood pulps generally contain only glucan, mannan, and xylan as their carbohydrate components. Irrigation with the butanol-pyridine-water system for approximately 48 hr. is adequate for such separations. This system, however, fails to resolve mannose and arabinose, and in the presence of large amounts of glucose, galactose cannot be cleanly separated from glucose. The arabinose appears below the mannose (but unresolved) and can be recognized by the characteristic pinkish color produced by pentoses when treated with aniline phthalate in contrast with the brown of the hexoses. The galactose appears above the glucose (closer to the starting point) but again is usually not resolved after only 48 hr. of irrigation. Hexoses and pentoses that have been treated with aniline phthalate reagent can also be distinguished

from each other by means of UV light. Under UV radiation, the edges of the hexose-aniline-phthalate bands fluoresce a pale yellow but pentose bands do not.

To separate all five sugars present in a hydrolysate, one large sheet (18 in.) is streaked on which galactose and glucose are separated. Four small sheets, each 6 in. wide, are streaked on which mannose, arabinose, and xylose are separated. The full-sized sheet is irrigated in a cabinet with the butanol-pyridine-water mixture for approximately 72 hr. or until everything goes off the sheet except glucose and galactose. These two sugars are separated by then and can be eluted and measured. The small 6-in. sheets are irrigated in chromatographic jars with an ethyl acetate-acetic acid-water (9:2:2) system for 22 hr., then dried and irrigated with the butanol-pyridine-water system for another 26 hr. This double irrigation separates mannose, arabinose, and xylose into well-defined bands which can then be eluted and measured. The ethyl acetate-acetic acid-water system does not perform satisfactorily in cabinets, and it is for this reason that the sheets are smaller and jars are used. Tabs are cut from these smaller sheets in the same manner as with the larger sheets. (Note C-3)--Adequate ventilation facilities should be provided to remove solvent vapors when papers are being removed from the cabinets and air dried (1). (Note C-4)--Each 75-mm. tab should contain from a minimum of 10 μg. to a maximum of 3,000 μg. of sugar. Original streaking solutions are adjusted to a concentration of approximately 10%, which in pulp analysis gives about 3,000 μg. of glucose per tab. The glucose tabs are cut up into 25 ml. of water and 0.75 ml. of this solution analyzed quantitatively for reducing sugars. Mannose,

xylose, etc., can usually be transferred from the elution pipet directly to the test tube.

(Note C-5)--Very pure pulps contain only glucose, mannose, and xylose. Most pulps contain galactose, glucose, mannose, arabinose, and xylose. Hardwoods generally contain more xylose and less mannose than softwoods, and it has been found that Douglas-fir compression wood contained approximately three times as much galactose as normal wood.[4] Some sample carbohydrate analyses are tabulated in tables 1 and 8.

(Note C-6)--Uronic acids do not move during the sugar separation and so do not interfere with the sugar analysis.

(Note C-7)--A solution of silver nitrate can also be used (sprayed on) to locate sugars (and sugar alcohols) on paper chromatograms. The solution is made up by adding 2 drops of a 10% sodium-hydroxide solution to 15 ml. of 2-1/2% silver-nitrate solution and then enough ammonia to redissolve the precipitate. A silver-nitrate solution will show the presence of sugars (brown color) without heating but sugar alcohols have to be heated before they show. Some disadvantages of silver nitrate as a sugar-locating spray are: (a) it does not distinguish between hexoses and pentoses as aniline phthalate does (pentose-aniline phthalate is pinkish) and (b) after several days, the background areas that have been sprayed with silver nitrate begin to darken.

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[4] U.S. Forest Prod. Lab., Wood Chem. Div., Analytical Sect. Quart. Rep. No. 7 1958.

Table 8.--Some examples of carbohydrate analysis of wood[1]

Wood sample	Galactose	Glucose	Mannose	Arabinose	Xylose
	%	%	%	%	%
Aspen	0.93	70.00	2.85	1.58	24.63
Douglas-fir	3.76	67.48	21.33	.95	6.48
Douglas-fir heartwood (springwood)	3.96	69.51	17.14	1.41	7.98
Douglas-fir heartwood (summerwood)	3.26	70.09	21.02	.92	4.72
Douglas-fir compression wood (springwood)	7.73	69.98	13.37	1.54	7.38
Douglas-fir compression wood (summerwood)	11.40	65.84	13.69	1.27	7.80

[1]U.S. Forest Prod. Lab., Wood Chem. Div. Analytical Sect., Quart. Rep. 7. 1958.

(Note C-8)--In practice, the irrigating solvent that is left in the trough after a separation has been carried out is poured into the bottom of the cabinet or jar to serve as equilibrating solvent for the next run. This procedure is satisfactory only to a certain point and then the reused equilibrating solvent must be replaced by fresh solvent. If this is not done, irrigation times begin to increase and times required for good separation of sugars may be 25 to 50% longer.

(Note C-9)--Temperature markedly affects the rate at which irrigants and solutes move on paper. Temperatures should be kept uniform from day to day to keep development times uniform (separations take longer at lower temperatures). For this reason, it is desirable to locate cabinets and jars in a small thermostatted room. By means of a mercury regulator, operating one or more circulating fan heaters, room temperature can be controlled to approximately 0.1° C. Temperature also must be kept uniform across a sheet. If there is a temperature gradient across a sheet, the constituents on the paper will travel at different rates, resulting in an uneven or slanted front and sugar zone. An example of this is frequently found when a chromatographic jar is placed near a cool wall. The edge of the paper nearest the wall is usually a little cooler, resulting in a slower movement of constituents down the paper in that region and nonparallel sugar zone. This problem can be eliminated (in addition to thermostatting the room) by keeping the jars and cabinets off the floor and away from walls and doors, and by maintaining active air circulation around the jars and cabinets with strong fans. Fans are generally not used or necessary

inside the jars and cabinets if there is good circulation outside the jars and cabinets.

(Note C-10)--When adding solvent to the troughs in the jars and cabinets, precautions should be taken to admit only a minimum amount of air, so as not to upset the vapor equilibrium inside the jar. In some covers for jars or cabinets, there may be small holes through which a funnel stem can be placed and the irrigating solvent poured into the trough with little air being admitted. If no such hole is provided, the covers should be opened as little as possible, a funnel stem inserted, and the irrigating solvent added to the trough, again with as little air exchange as possible.

(Note C-11)--The spotting techniques used for locating sugar areas are sensitive enough to detect from 8-10 μg. per tab width (75 mm.).

(Note C-12)--Aniline-acid phthalate crystals are prepared by dissolving, with gentle warming, 136 g. phthalic acid in 1,000 ml., 95% ethyl alcohol. Add 80 g. aniline with stirring. Chill the solution for several hours and filter by suction. Repeat the recrystallization from ethanol until the final filtrate is colorless. Wash with ether and dry 2 hr. at room temperature in vacuum. Yield is 50-60%.

3. Elution

The determination of the small quantities of sugar that have been isolated on paper requires that the individual sugars be eluted from their respective 2- by 3-in. tabs of paper and put in solution. This can be done quantatively

with little solvent and little manipulation by a combination of the techniques of Hawthorne (3) and Dimler (4).

The tabs of paper containing mannose and xylose are just caught between the edges of a pair of 2- by 2-in. glass plates and hung in an elution rack, as depicted in fig. 4, with the 3-in. dimension vertical. Elution pipets (note 3-1) of 3/4-ml. capacity are so positioned as to touch near the bottom edge of the tabs. To prevent excessive evaporation of water, the entire assembly is contained in a glass-covered humidified box.

Elution is started by filling the trough with distilled water, which rises by capillarity between the plates and then flows down the paper and into the pipets. When a pipet is filled, capillary forces prevent flow through the pipet, and surplus water flows past the tip. The volume provided by a 3/4-ml. pipet is more than adequate to remove all of the sugar from the tab, so the last water to enter is sugar free. Quantitative transfer of sugar to a test tube for analysis is achieved by draining the pipet through the end not in contact with the paper (blowing through the end that was in contact with paper).

Each paper tab containing glucose is soaked in 25 ml. of distilled water (with periodic swirling) for about 15 min. The resulting solution is filtered through glass wool to remove fibers and then 0.75-ml. aliquots are taken for analysis.

Notes.--(Note 3-1) The elution pipets are made from 3-mm. (ID) Pyrex tubing and calibrated to deliver 0.75 $\pm$ 0.005 ml. of water. This volume is suitable for use with either Whatman No. 1 paper or the heavier Whatman 3MM.

The ends of the pipet are drawn down to an inside diameter of approximately 0.020 in., one end being bent up at about a 60° angle as illustrated in fig. 4. The tips are ground flat and then given a slight bevel to reduce chipping. Capillary forces are now such that eluate is readily drawn into the straight end of the pipet but does not flow out of the bent end.

(Note 3-2)--With thicker papers, the 2- by 2-in. glass plates may become separated to such an extent that water will not rise between them. A stainless steel adapter plate, 2 in. wide and as long as the elution rack, can be made to solve this problem. The thickness of one edge of this plate is reduced by milling a cut 1/8 in. wide by 0.008 in. deep the full length of the plate. The paper tabs can now be caught in the space between this milled depression and a covering 2- by 2-in. glass slide. Elution is carried out in the usual way.

(Note 3-3)--Humidification of the elution cabinet is easily accomplished by lining the walls with strips of wet blotting paper.

(Note 3-4)--After every use, the pipets are washed by drawing through them, in succession, dilute sodium hydroxide, acid dichromate, and distilled water. They are dried by leaving them for 1/2 hr. in a bottle evacuated with an aspirator. The 2- by 2-in. slide cover glasses are cleaned in dichromate and kept wet until use.

D. Determination of Monomeric Wood Sugars

Methods used for determining "total sugars" measure simple or "monomeric sugars" in solution calculated as glucose and are based on the reducing power

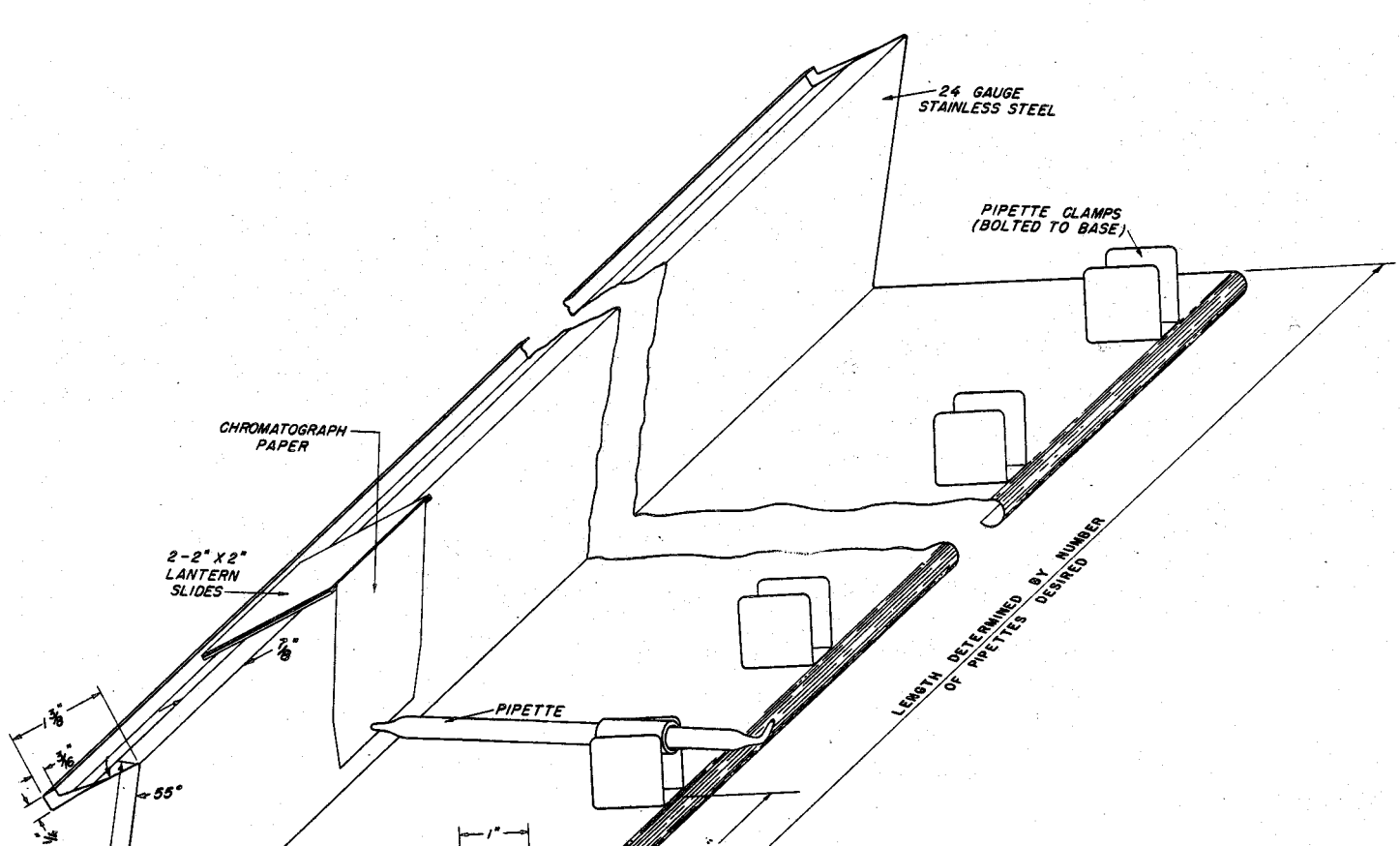

Figure 4.--Elution rack and calibrated pipet for recovering sugars from chromatogram.

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of the sugars as measured by the Shaffer-Somogyi volumetric procedure (5) or by Nelson's colorimetric modification of Somogyi's method (6).

The volumetric and colorimetric methods for the determination of sugars are interchangeable in application except where sample size or interference considerations require the use of the colorimetric method. A comparison of the sensitivity and selectivity of the two methods is given in table 9.

These methods are directly applicable to solutions of simple sugars such as polysaccharide hydrolysates, fermentation solutions, and sugar reaction mixtures.

1. Volumetric Method (5)

(a) Principle.--Sugars reduce cupric ion to cuprous ion in an amount proportional (linearly) to the amount of sugar present. Cuprous ion is then measured iodometrically (iodine and thiosulfate). The sugar concentration is derived from a prepared standard calibration curve and reported as "glucose."

(b) Reactions.--(1) Reducing sugar + Cu^{++} (tartrate) $\xrightarrow{\text{alkali}}$ Cu^{+} (ppt'd as oxide) + oxidized sugars.

Add I^- (Excess KI), IO_3^-, and H^+ (H_2SO_4).

(2) $IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$.

(3) $I_2 + I^- \longrightarrow I_3^-$.

(4) $2Cu^+$ (solubilized by acid) + $I_3^- \longrightarrow 2\ Cu^{++} + 3I^-$.

(5) I_3^- + starch $\longrightarrow$ starch $-I_2$ (blue) + I^-.

(6) Starch - I_2 (blue) + $2S_2O_3^{=} \longrightarrow S_4O_6^{=} + 2I^-$ + starch (colorless).

Table 9.--Comparison of sugar methods

Method	Minimum sample required	Interferences
Colorimetric	8 μg. per 0.75 ml.	Turbidity, color, emulsions
Volumetric	400 μg. in 1-5 ml.	Sulfite, organic acids, butanol, uronic acids

(c) Reagents.--(1) Copper reagent. This has the following composition (5):

	g. per liter
$CuSO_4 \cdot 5H_2O$ (pulverized)	7.50
$KNa(C_4H_6O_6) \cdot 4H_2O$ (pulverized Rochelle salt)	25.00
Na_2CO_3	25.00
$NaHCO_3$	20.00
KI	5.00
KIO_3	.535

The constituents are dissolved (in the order given) in about 800 ml. of water. The KIO_3 is dried for 1-1/2 hr. at 110° C., accurately weighed, dissolved separately, and quantitatively washed into the main solution. The solution is then diluted to 1 liter and thoroughly mixed.

The copper (cupric) tartrate tends to precipitate when Rochelle salt is added. This precipitate dissolves when Na_2CO_3 and $NaHCO_3$ are added.

(2) 25 $\pm$ 1% H_2SO_4. Either dilute 875 ml. concentrated C.P. sulfuric acid to 5 liters or mix 1,579 g. of C.P. sulfuric acid with 4,421 g. of distilled water.

(3) 0.50N sodium thiosulfate. This stock solution contains 125 g. $Na_2S_2O_3 \cdot 5H_2O$ per liter. It is made alkaline to phenolphthalein by titrating a 25-ml.

aliquot to a slight pink color with 0.1N NaOH and then adding the corresponding amount of alkali to the rest of the thiosulfate solution. A few drops of toluene may be added as a preservative against microbial action.

Since this solution is also decomposed by the joint action of oxygen, light, and carbon dioxide, it is stored in the refrigerator. It may be standardized against a 0.1N $K_2Cr_2O_7$ solution (4.903 g. per liter) as follows: 25 ml. of 0.1N dichromate are pipeted into a 250-ml. Erlenmeyer flask containing 25 ml., 1M H_2SO_4 and about 3 g. KI. After standing about 30 sec., the solution is titrated with the thiosulfate to be standardized. Starch indicator is added just before the end point is reached. The color change is from blue to light green.

(4) 0.01N sodium thiosulfate. This solution is made by dilution of the stock solution. It should always be fresh and used within 3 or 4 hr. after dilution. Dilution is made with CO_2-free distilled water.

(5) Starch indicator. The starch used is a soluble U.S.P. grade starch. A suspension of 10 g. of starch in 50 ml. of water is poured slowly, with stirring, into 950 ml. of boiling water. The boiling is continued for 2 or 3 min. (with stirring) after the starch has been added. A few drops of toluene are added (after cooling) as a preservative.

(d) Procedure.--This procedure is based on a calibration curve. The standard calibration curve is made up from a series of standard glucose solutions that are prepared by the procedure described in note f-1.

The aliquots (note f-1) are added by pipet or syringe to 25- by 200-mm. test tubes, and then enough water is added in to bring the total volume to

approximately 5 ml. Then 5.00 ml. of copper reagent is added, the reagents mixed, loosely stoppered with grooved corks, and then immersed in a boiling water bath for 30 min. The heat supply to the bath should be such that boiling begins again 1 or 2 min. after the tubes are immersed in it. A metal rack with a handle is convenient for holding the tubes. The water level of the bath should be above the level of the liquid in the tubes. After the tubes have been in boiling water for 30 min., they are removed and immersed in cold running water--a sink with a 3-in. overflow pipe serves for this purpose. The samples (standards) are thus cooled to room temperature in about 1-1/2 min. using water at 12° to 15° C.

One ml. of 25% H_2SO_4 is then added to each test tube and the contents shaken thoroughly several times so that the copper precipitate completely dissolves. More H_2SO_4 seems to result in higher values. Tubes must be shaken thoroughly to assure complete dissolution. Immediately after acidifying and mixing, the liberated iodine is titrated with freshly prepared 0.01N sodium-thiosulfate solution using starch indicator. It should be pointed out that all the copper must be in solution, and then the titration should be performed quickly to avoid loss of I_2 by evaporation. Blank tubes should require about 7.5 ml. of 0.01N thiosulfate as shown in table 10; this table lists approximate titration values for various amounts of glucose in the sample.

The difference between the blank titration value and the titration value for tubes containing sugar represents the iodine utilized in reoxidation of the reduced copper. A curve is constructed in which the titration difference

Table 10.--Approximate titration values for various amounts of glucose

Glucose in sample tube	Approximate titration
Mg.	Ml.
Blank	7.5
0.5	5.5
1.0	3.3
1.5	1.0

is plotted against weight of glucose. This curve is valid for only glucose and not other sugars. A new curve should be made up for each new batch of copper reagent or thiosulfate. The plot is a straight line down to about 0.4 mg. of glucose, below which the relationships cease to be linear.

In the determination of glucose in unknown samples, the same procedure is followed as in standardization. An aliquot of the solution to be analyzed is pipeted into a volumetric flask, diluted to mark, and neutralized (note f-6) if necessary (using methyl orange as an indicator). The dilution should be such that the glucose concentration in the aliquot to be analyzed (1 to 5 ml.) contains from 0.4 to 1.4 mg. of sugar. One to 5 ml. (depending on its concentration) of sugar solution are taken for analysis. It must be remembered that these dilutions should be made just before use. The glucose concentration in the unknown solution is obtained from the calibration curve. Duplicate determination should agree within 0.02 ml. or less, and the test should be repeated if the total titration volume is less than 1 ml.

(e) Procedure for determination of sugar in molasses (sucroses).--The determination of sucrose requires an additional step in that prior to analysis the sucrose must be inverted. To invert the sucrose, take 20 g. of molasses and dilute to 100 ml. with water. Pipet 10 ml. of the diluted solution into a 50-ml. Erlenmeyer flask and add 10 ml. 1.0N hydrochloric acid. Cover the flask with aluminum foil, heat in boiling water for 10 min., cool, then transfer to a 1-liter volumetric flask and dilute to mark. Use 1 ml. for analysis. The sugar content of molasses is about 50%. A calibration curve is made from pure sucrose.

(f) Notes.--(Note f-1) A sample set of the calibration solutions used at the Forest Products Laboratory is given below; they are usually made up to volume with water (note f-2) and no preservative added. Three standard solutions are made and aliquots of various sizes taken from each to give a sufficient number of points on the curve.

Standard solution	Aliquot	Mg. glucose
(A) 0.300 g. per liter.....	0.5 ml.	0.150
	1	.300
	2	.600
	3	.900
	4	1.200
	5	1.500
(B) 0.2750 g. per liter.....	0.5	.1375
	1	.275
	2	.550
	3	.825
	4	1.100
	5	1.375
(C) 0.2250 g. per liter.....	0.5	.1125
	1	.225
	2	.450
	3	.675
	4	.900
	5	1.125

Two blanks with no sugar solution added are run along with each set of samples.

(Note f-2)--If a standard glucose solution is to be used immediately, no acid need be added, and the solutions can be made up directly to the desired volume with water.

(Note f-3)--In the alkaline solution, the cuprous ion formed by the sugar reaction precipitates as an oxide. This precipitate redissolves upon acidification. If the original sugar concentration is too high (thiosulfate titration of 0.5 ml. or less), a white precipitate of cuprous iodide (insoluble in acid) forms after acidification. Apparently at low iodine concentrations, precipitation as the iodide is preferred over oxidation to cupric ion. If this precipitate can be seen and will not redissolve with shaking, the test is discarded and a new aliquot (containing less sugar) is analyzed. Tubes in which cuprous iodide has formed are cleaned with detergent and a brush to be sure all of the precipitate has been removed. If necessary, ammonia can be used to dissolve the remaining precipitate.

(Note f-4)--This method is empirical; each batch of reagent must be standardized against a pure sugar solution. Volumes must be standardized and the standards strictly adhered to.

(Note f-5)--Tartrate stabilizes cupric ion and prevents its reduction by other means than by the sugar. Iodide ion discourages self-reduction of cupric ion but also solubilizes cuprous oxide, thereby encouraging its reoxidation by oxygen. Sodium sulfate is sometimes added to the copper reagent to reduce the amount of dissolved oxygen in the sample solution, thereby preventing the reoxidation of cuprous ion by oxygen (7). The presence of fine suspended particles may encourage or catalyze reoxidation of cuprous copper to cupric copper.

(Note f-6)--Neutralization of sugar solutions for analysis with Schaffer-Somogyi sugar reagent. The sugar reagent will tolerate as many milligrams of

acid as there are milligrams of sugar present in the solution to be analyzed. Since we try to adjust the concentration of sugar in solution to 1 mg. per milliliter before analysis, the sulfuric acid concentration is adjusted to no more than 1 mg. per milliliter also.

Neutralization is accomplished by adding precipitated chalk ($CaCO_3$) after the sample has been diluted to the proper concentration for analysis (0.4 to 1.5 mg. in 1- to 5-ml. aliquot). Methyl-orange paper is used as an indicator. To make this, saturate a filter paper with a 1% solution of methyl orange in alcohol and allow it to dry. Twenty-five to 50 ml. of the acid sugar solution is neutralized by adding solid $CaCO_3$ slowly until a red color is no longer produced when the prepared indicator paper is wetted with a small drop of the solution on the end of a stirring rod.

After neutralization, allow the precipitate in the neutralized solution to settle. Then filter it through filter paper. Pour it through a second time if necessary to obtain a clear filtrate.

(Note f-7)--Hypodermic-syringe pipets greatly facilitate the precise pipeting of replicate portions required in this analysis. A description of these devices is shown in fig. 5.

2. Colorimetric Method (5)

The colorimetric method for sugars has certain advantages over the volumetric method in that it is more sensitive, is less subject to interferences (table 9), and can tolerate up to 50 mg. sulfuric acid per 100 ml. of sample (table 11), compared to about 1 mg. per milliliter for the volumetric

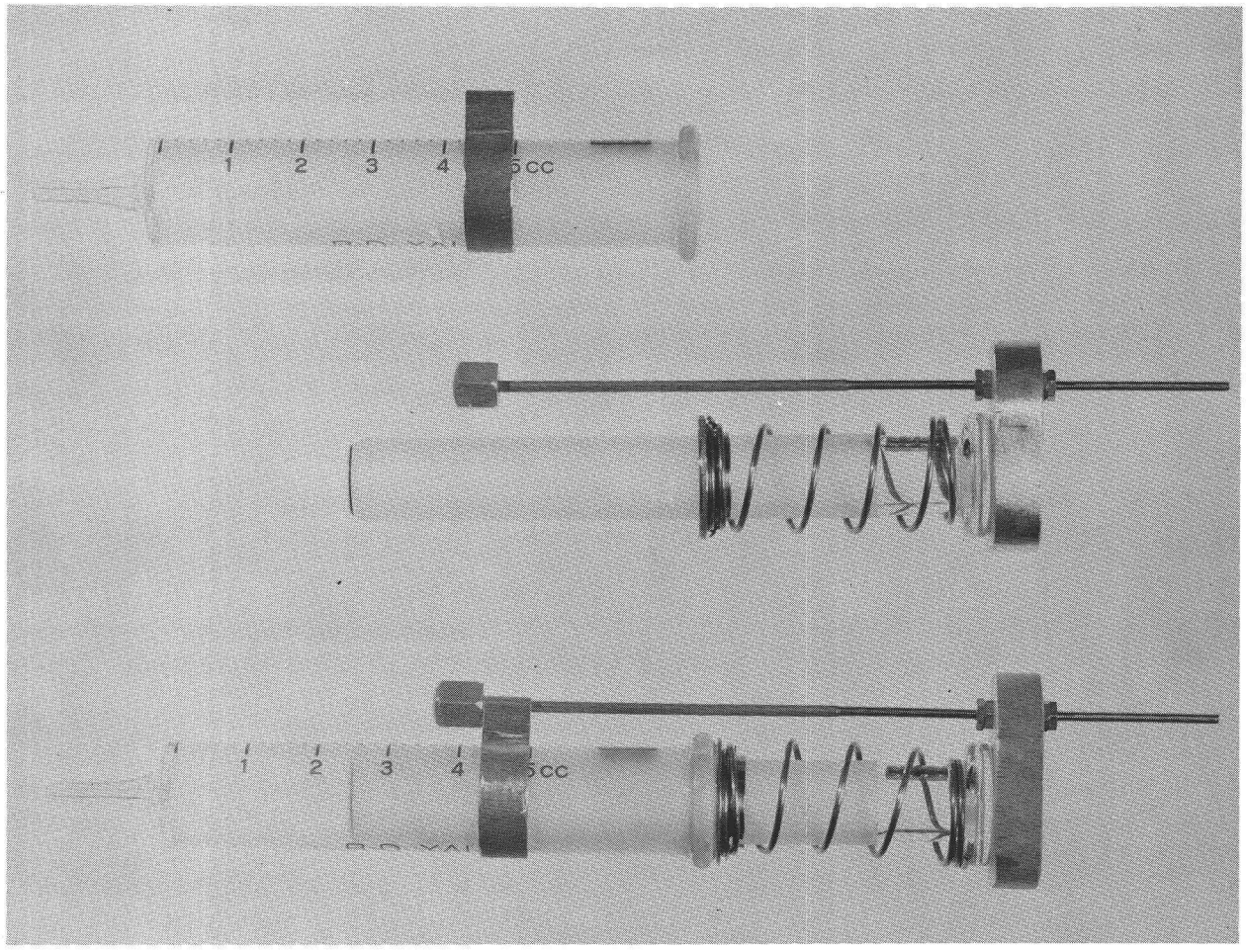

Figure 5.--Hypodermic-syringe pipet equipped with stops; pipet replicate portions of sulfuric acid with an accuracy of ± 0.0001 ml. An aluminum button on the end of the plunger and a brass band around the barrel are bonded to the glass with epoxy resin. The projection on the brass band is slotted to serve as a guide as well as a stop for the volume adjusting rod.

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Table 11.--Effect of H_2SO_4 on colorimetric glucose analyses[1],[2]

Mg. H_2SO_4 in 100 ml. solution	Absorbance reading at 520 millimicrons[3]					Average difference due to effect of acid
	Run 1	Run 2	Run 3	Run 4	Run 5	
0	0.501	0.489	0.500	0.510	0.524	
.5	.499					-0.002
1	.500					-.001
2	.501					0
5	.502	.492				+.002
10		.493				+.004
20		.490				+.001
50		.497	.502	.515	.524	+.004
100		.504	.503	.519	.528	+.008
200		.508	.508	.521	.535	+.012
300			.513	.525	.544	+.016
400			.521	.533	.547	+.022
500			.522	.532	.557	+.026
750			.520	.533	.544	+.021
1,000			.503	.520	.532	+.007

[1]Laboratory Notebook No. 533, U.S. Forest Prod. Lab., Madison, Wis.

[2]Approximately 6.5 mg. sugar per 100 ml. of solution.

[3]Each run at a different time and each result is the average of 3 samples.

method. Based on the amount of sugar in the sample, the colorimetric method will tolerate six times as much sulfuric acid as the volumetric method. For these reasons, the colorimetric method is used in the quantitative measurement of individual sugars after isolation and identification by chromatography. Although as little as 8 μg. of sugar per 0.75 ml. is sufficient, 40 to 80 μg. is more nearly ideal.

This method for wood sugars is based on a calibration curve made up of knowns. Each time new reagents are made up, a new calibration curve is required. Rather than make a calibration curve for each sugar, a new curve is made only for glucose; all sugars are determined in terms of glucose and the nonglucose sugar corrected by applying the proper factor to take into account the differences in reducing power. The reducing powers of the sugars are relatively constant; once they have been measured, they need rechecking only occasionally or if test conditions are changed.

(a) Principle.--Sugars reduce cupric ion to cuprous ion in an amount proportional (linearly) to the sugar present. The reduced copper is measured colorimetrically.

(b) Reactions.--(1) Reducing sugar + Cu^{++} (tartrate) $\xrightarrow{\text{alkali}}$ Cu^{+} (prepared as oxide) + oxidized sugars. Cuprous ion is dissolved in H_2SO_4 solution.

(2) Cu^{+} + arsenomolybdate $\longrightarrow$ blue color with an absorption maximum at about 660 mμ, but which is read at 520 mμ.

(c) Reagents.--(1) Somogyi sugar reagent (7). Dissolve 24 g. of anhydrous sodium bicarbonate and 12 g. of Rochelle salt in about 250 ml. of boiled

cooled, distilled water. To this solution add 4 g. of hydrated copper sulfate dissolved in 40 ml. of boiled, distilled water. After mixing, 16 g. of sodium bicarbonate are added and, when dissolved, the solution is poured into a 1,000-ml. graduated cylinder. Next, 180 g. of anhydrous sodium sulfate are dissolved in about 500 ml. of hot water, and the solution is boiled to expel air. After cooling, this solution is also added to the cylinder and diluted to mark.

It is preferable to prepare a 10-liter stock of the Somogyi reagent several weeks in advance of its use. This allows impurities and a slight amount of cuprous oxide to settle.

(2) Arsenomolybdate reagent (5). Dissolve 25 g. of ammonium molybdate in 450 ml. of distilled water, adding 21 ml. of concentrated sulfuric acid, mixing, and then adding 3 g. of monobasic sodium ortho-arsenate ($Na_2HAsO_4 \cdot 7H_2O$) dissolved in 25 ml. of water. This solution is placed in an incubator at 37° C. for 24 to 48 hr. before use and stored in brown bottles. For routine use, this solution should also be made up in large quantities.

(d) Apparatus.--(1) Beckman DU spectrophotometer or its equivalent.

(2) Combination steamer and test tube rack (fig. 6).

(3) Calibrated syringe pipet.

(4) 6-in. test tubes.

(e) Calibration curve.--The glucose calibration curve is made by running the sugar determination on a series of standard sugar solutions containing from 0 to 210 μg. of sugar per 3/4 ml. of solution. Glucose concentration is

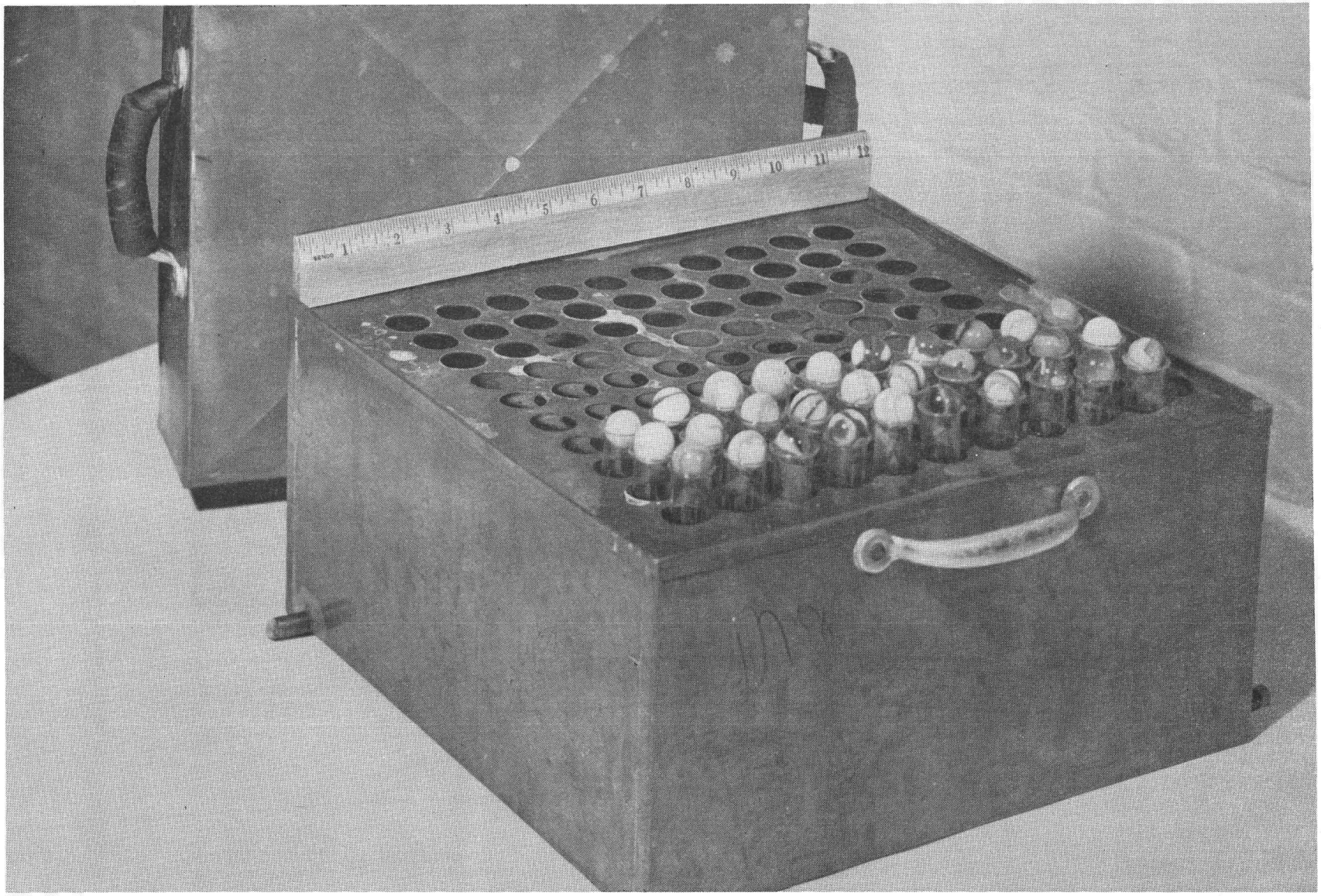

Figure 6.--Combination steam bath test tube rack for sugar analysis.

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then plotted versus absorbance at 520 mμ. The standard solutions are made up by dissolving 0.2800 g. of C.P. glucose that has been dried for 2 hr. at 50° C. in a vacuum oven in 1 liter of distilled water and accurately diluting to give the desired number of calibration points.

(f) Procedure.--Neutralize the sample, if necessary, so that it contains less than 50 mg. sulfuric acid per 100 ml. (neutralize with calcium carbonate to a methyl-orange end point). Place 3/4 ml. of sample solution containing from 20 to 160 μg. of sugar into 6-in. test tubes. Add 1.00 ml. of the Somogyi reagent and cover each tube with a marble. Heat the samples by direct steam for 20 min. in the combination steam bath and test tube rack illustrated in fig. 6. After heating, connect the steam bath to a water line through which cold water is running to provide rapid cooling. Add 1.00 ml. of the arsenomolybdate reagent to each test tube and shake to expel carbon dioxide. Add 2.25 ml. of distilled water to each tube and thoroughly mix. Two blanks are run along with the samples substituting water for the sample.

The absorbance of each sample is measured at 520 mμ, using 1.0-cm. Pyrex or silica cells against a blank for the reference. If the absorbance is greater than 1.0, 5 ml. of water are added to both the sample and blank. The amount of sugar present is computed from the measured absorbance and a previously prepared calibration curve. They are computed "as glucose" from the calibration curve corrections applied to nonglucose to compensate for differences in relative reducing power. Relative reducing power factors are given in "Calibrations, Calculations, and Correction Factors."

As a control to monitor the analysis, three samples of a glucose standard (called a "working standard") are run along with each set of samples. The glucose standard is made up by dissolving 5.000 g. ovendry glucose in 100 ml. water. One-ml. aliquots of this solution are pipeted into small polyethylene-stoppered glass vials and these standard samples are stored in a freezer. Working standard solutions are made up from these standards as they are needed. The working standard is made up by transferring the contents of a vial quantitatively to a 500-ml. volumetric flask and diluting to 500 ml. with water. This solution, when kept in the refrigerator, may be used for up to 1 month. Three 3/4-ml. aliquots of the working standard are run with each set of samples analyzed. Each aliquot contains 75 μg. of glucose.

(g) Analytical notes.--(Note h-1) The use of individually calibrated (adjustable) syringe pipets greatly facilitates the multiple pipetings encountered in the above-described sugar analysis procedure (fig. 5).

(Note h-2)--Test solutions for the colorimetric analysis should be neutral or slightly acid.

(Note h-3)--Copper and arsenomolybdate are present in quantities sufficient to oxidize over 200 μg. of sugar, but sugars present should be kept down to considerably less than that.

(Note h-4) Uronic acids interfere with this method but they can be removed chromatographically. Their relative reducing powers are much less than those of the corresponding sugars. For example, the reducing power of glucose is 1.000; glucuronic acid, 0.767; galactose, 0.786; and galacturonic acid, 0.660.[5]

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[5]Unpublished data, U.S. Forest Prod. Lab.

III. Procedures

Because of the great variety of samples that might be received for wood-sugar analysis, and because of the variety of tests run that include wood-sugar analysis, the frequently applied operations have been described in the "Techniques" part of this section; the actual analytical procedures are given here with references to those frequently used techniques rather than repeating a procedure (such as the colorimetric determination of wood sugars) each time it is applied.

The volumetric and colorimetric methods for the determination of sugars are interchangeable in application except where sample size or interference considerations require the use of the colorimetric method. A comparison of the selectivity and sensitivity of the two methods is given in table 10.

A. Determination of Total Wood Sugars

The "total sugars" determination is a measure of the simple sugars or monomeric sugars present in solution, calculated as glucose, and is based on the reducing power measured by the Schaffer-Somogyi titration procedure (5) or by Nelson's colorimetric modification of Somogyi's method (6).

The total sugars analysis is applied to samples such as nutrient solutions for micro-organisms, fermentation solutions, sugar-reaction mixtures, and pulp and wood hydrolysates. The terminology "determination of total sugars" also is applied directly to the wood or pulp but in this case, of course, the carbohydrate must be hydrolyzed prior to sugar analysis.

The simple sugars found in pulp and wood hydrolysates are galactose, glucose, mannose, arabinose, and xylose. Since each of these sugars has a different reducing power (table 3), results obtained on a mixture calculated as glucose will be subject to slight error. In most wood or pulp hydrolysates, glucose is the most abundant sugar, with smaller amounts of the other sugars. For this reason, "total sugars" as glucose is a useful index of the carbohydrate content, and errors in most cases are minor (estimated 1 to 5%).

1. Total Wood Sugars Present as Simple Sugars

The term "wood sugars" is synonymous with simple sugars or monomeric sugars. This test is a measure of the simple sugars present in a sample. The determination of "potential sugars," which are sugars present as polymers, requires a hydrolysis, or saccharification step, and then a determination of the simple sugars.

(a) Procedure.--The sample (dry weight known) containing the wood sugars is dissolved in water (or a known volume is taken and sugars reported on a milligram per milliliter basis). The concentration is adjusted to give 1 mg. of sugar per 5 ml. (or less) of solution. It is then neutralized with calcium carbonate (see "Techniques--Hydrolysis of polysaccharides--Neutralization") and filtered through coarse, fluted filter paper. A 1- to 5-ml. aliquot is then taken and the sugar determined by the volumetric method (see "Techniques--Determination of monomeric wood sugars--Volumetric method").

(b) Note.--If sucrose is present in the sample, the sucrose is inverted before neutralization. In the case of sugar cane molasses, which is about 50% sucrose, take 20 g. of molasses and dilute to 100 ml. with water. Pipet 10 ml. of the diluted solution into a 50-ml. Erlenmeyer flask and add 10 ml. of 1.0N hydrochloric acid. Cover the flask with aluminum foil, heat in a boiling water bath for 10 min., cool, then transfer to a 1-liter volumetric flask and dilute to mark. Use 1 ml. for analysis. A calibration curve is made from pure sucrose.

2. Total Wood Sugars in Pulp and Wood (Potential Sugars)

The determination of the wood sugar constituents of pulp or wood is sometimes referred to as the measurement of "potential sugars." Since the sugars are present as the anhydro polymers, the sample must be hydrolyzed to the simple sugars prior to analysis.

(a) Procedure.--The sample is ground, extracted, fluffed (pulps only), air dried, and then 0.3 g. wood or 0.1 g. pulp is weighed out. The dry weight is determined as described in "Techniques--Hydrolysis of Polysaccharides--Sample preparation." The sample is then hydrolyzed by the "modified hydrolysis" method (see "Techniques--Hydrolysis of polysaccharides--Modified hydrolysis method") and the concentration adjusted to give 1 mg. of sugar per 5 ml. (or less) of solution. It is then neutralized with calcium carbonate (see "Techniques--Hydrolysis of polysaccharides--Neutralization") and filtered through coarse, fluted filter paper. A 1- to 5-ml. aliquot is then taken and the sugar determined by the volumetric method (see "Techniques--Determination of monomeric wood sugars--Volumetric method").

(b) Note.--If the sample is to be analyzed for lignin content as well as wood sugar content, the hydrolysis and filtration procedure described in "The Determination of Lignin" is followed. The procedure (after hydrolysis and before neutralization) is to quantitatively filter off the residue (lignin) using a tared Gooch, Alundum (RA98), or medium porosity fritted glass crucible with an asbestos mat. The lignin is dried and weighed. The filtrate is transferred to a volumetric flask and diluted to mark to give a solution containing approximately 1 mg. per ml. About 50 ml. of this solution are then taken, neutralized with calcium carbonate, filtered through fluted filter paper as usual, and total sugars measured by the volumetric method. If lignin is not to be measured, it is simply removed in the filtration step following neutralization.

B. Determination of Specific Wood Sugars

The carbohydrate in pulp and wood is sometimes characterized by determining the kinds and amounts of individual sugars that make it up. This is in contrast to the "total sugar" procedure where the sugars are lumped together and measured and reported as glucose. The determination of individual sugars requires a chromatographic step whereby the individual sugars are isolated and identified prior to measurement.

The composition of a carbohydrate material may be expressed either on the basis of the total initial sample or on the basis of the carbohydrate portion of the sample. The former establishes composition on an absolute basis. It requires precise information concerning all components present in the starting material, or lacking this, it requires quantitative handling of the carbohydrate portion throughout the entire sequence of chromatographic operations.

With purified pulps, where the carbohydrate fraction comprises all but a minor part of a given sample, it is convenient to express composition on the basis of the ratio of the carbohydrate constituents; that is, the quantity of each isolated sugar is expressed as a percentage of the combined sugar content of the sample. Quantitative handling is thus not required until application of the hydrolysate to the paper. Proper correction for conversion of polysaccharide to monosaccharide and for decomposition are, of course, required in any method of analysis. These may be conveniently accounted for by calibration (for calibration procedures, see "Techniques--Calibrations, calculations, and correction factors").

Usually pulp and wood hydrolysates are analyzed for only the three predominant wood sugars--glucose, mannose, and xylose--but occasionally the determination of galactose and arabinose is also desired. The determination of all five sugars in a hydrolysate requires an extra chromatographic separation. If only three sugars are separated in a wood or pulp hydrolysate, the galactose appears with the glucose and the arabinose with the mannose.

1. On the Basis of Total Sugars
(Ratio Method)

(a) Pulp and wood samples.--(1) General. This procedure involves hydrolyzing the sample, isolating the simple sugars by chromatography, and measuring the isolated sugars by the colorimetric method.

This method is used on purified pulps where ratio values are approximately the same as absolute values and on wood when only information about the relative amounts of sugar is needed.

When determining sugar ratios, it is not necessary to know the sample weight accurately, and transfers do not have to be quantitative until the sample is applied to the paper; then all operations must be quantitative.

(2) Procedure. The sample is ground (if wood) or extracted and fluffed (if pulp), air dried, and approximately 0.3 g. wood or pulp weighed out. The dry weight is determined as described in "Techniques--Hydrolysis of polysaccharides--Sample preparation" (if lignin is not to be measured, the weight need not be known accurately). The sample is then hydrolyzed by the "modified hydrolysis" method ("Techniques--Hydrolysis of polysaccharides--Modified hydrolysis method"). From this point on, the procedure follows that given below for hydrolysates, fermentation solutions, sugar-reactions mixtures, or any sample containing simple sugars.

If lignin is to be measured on the sample, a quantitative filtration is made at this point as described in the lignin procedures and the filtrate is retained for sugar analysis. If the lignin is not to be measured, it is simply removed in the filtration step following neutralization.

(b) Simple sugar samples.--(1) General. This procedure applies to samples of pulp and wood hydrolysates, fermentation solutions, sugar-reaction mixtures, or any sample containing simple wood sugars. The sugars are neutralized, concentrated, separated, and then measured.

(2) Procedure. The sugar-containing solution (dry samples are dissolved in sufficient water to give a sugar concentration of approximately 10%) is neutralized with ion-exchange resin as described in "Techniques--Hydrolysis of polysaccharides--Neutralization." The neutralized solution is filtered through filter paper and either diluted or concentrated to give a solution containing approximately 10% sugar.

Hydrolysates are necessarily quite dilute so concentration by evaporation is usually necessary. Evaporation is accomplished using the procedure and apparatus described in "Techniques--Isolation of wood sugars--Evaporation." The sample solution is then chromatographed, the sugars separated, located, eluted; and then measured by the colorimetric method. The procedures to be followed are outlined in "Techniques--Isolation of wood sugars--Chromatographic separation" and "Elution" and "Techniques--Determination of monomeric sugars--Colorimetric method."

Corrections are then applied to account for differences in reducing power (as described in "Techniques--Calibrations, calculations, and correction factors").

2. On the Basis of Total Sample (Absolute Method)

(a) Pulp and wood samples.--(1) General. This procedure involves hydrolyzing the sample, isolating the simple sugars by chromatography, and measuring the isolated sugars by the colorimetric method. The original dry sample weight must be accurately known and all operations (transfers, neutralization volumes) must be quantitative.

This method is used when a value for total carbohydrate is desired. The individual sugars are measured and, in the case of pulp or wood, the values obtained, converted to the anhydro sugar (polymer) basis.

If lignin is to be measured also, a quantitative filtration is made after hydrolysis (see procedure for lignin) and the filtrate then made up to an appropriate volume with washing and water. If lignin is not to be measured on the sample, it is simply removed in the filtration step following neutralization.

(2) Procedure. The sample is ground (if wood) or extracted and fluffed (if pulp), and air dried as described in "Techniques--Hydrolysis of polysaccharides--Sample preparation."

A sample of approximately 0.3 g. wood or pulp is accurately weighed out (weight calculated on a dry weight basis) and the primary hydrolysis performed by the modified hydrolysis method ("Techniques--Hydrolysis of polysaccharides--Modified hydrolysis method"). After primary hydrolysis, transfer the sample into a tared, 250-ml., wide-mouth centrifuge bottle, using 84 ml. distilled water to complete the transfer. Secondary hydrolysis is then accomplished in the tared bottle as described in "Techniques--Hydrolysis of polysaccharides--Modified method." From this point on the procedure follows that given below for the determination of simple sugars in hydrolysates, fermentation solutions, sugar-reaction mixtures, etc.

(b) Simple sugar samples.--(1) General. This procedure applies to pulp and wood hydrolysates, fermentation solutions, sugar-reaction mixtures, or any sample containing simple wood sugars. The sample is neutralized, concentrated, the sugars separated chromatographically, and then measured colorimetrically. (2) Procedure. The sample is neutralized with barium hydroxide and centrifuged ("Techniques--Hydrolysis of polysaccharides--Neutralization").

Concentrate the sugar solution to about 10%, recovering the concentrate quantitatively ("Techniques--Isolation of wood sugars--Evaporation").

Adjust the concentrated sugar solution to an accurately known volume (containing approximately 5 to 10% sugar) and carry out the chromatographic separation and location of sugars as described in "Techniques--Isolation of wood sugars--Chromatographic separation."

After chromatographing the sugars and locating them, they are eluted from the paper ("Techniques--Isolation of wood sugars--Elution") and analyzed colorimetrically ("Techniques--Determination of monomeric wood sugars--Colorimetric method").

Each individual wood sugar is then measured colorimetrically ("Techniques--The determination of monomeric wood sugars--The colorimetric method") and calculations, corrections, and reports made as described in "Techniques--Calibrations, calculations, and correction factors."

(c) Note.--Absolute sugar values can also be obtained by determining total sugars and their ratios and computing the absolute sugar contents of the sample, but agreement is not as good as when it is done by quantitative chromatography. In the first method, total sugars are done by hydrolysis of an aliquot of sample, neutralization with lime, filtration, and sugar analysis by titration.

C. Miscellaneous Wood Sugar Analyses

1. Determination of Total Carbohydrate in Pulps

The preferred method for determining total carbohydrate in pulps is the quantitative paper chromatographic method. This method is described in detail under "Procedures--Determination of specific wood sugars--On the basis of total sample." This involves measuring each type of sugar quantitatively, correcting for losses incurred during testing, and converting values obtained to the anhydro-sugar (polymeric) basis.

An alternate way to get total carbohydrate is to do a total sugars ("Procedures--Determination of total wood sugars--Sugars in pulp and wood--Potential sugars") and report the total carbohydrate as the anhydro glucose polymer (cellulose). In purified pulps, results are quite accurate.

A third way to determine total carbohydrate on a purified pulp is to determine total sugars ("Procedures--Determination of total wood sugars--Total wood sugars in pulp and wood... or potential sugars") and the ratio of glucose, mannose, and xylose ("Procedures--Determination of specific wood sugars--On the basis of total sugars...or ratio basis") and compute the total carbohydrate from these values. This method has the advantage over the simple "total wood sugar" determination in that there is an allowance for taking into consideration the difference in reducing power and molecular weight of the minor constituents.

2. Determination of Total Carbohydrate in Wood

On wood samples, the preferred way to obtain a value for total carbohydrate present is to determine the extractives and lignin content and consider the remainder to be the total carbohydrate. Results obtained by this procedure agree with results obtained by quantitative chromatography to within 2% (out of approximately 70%).

The results from the determination of holocellulose can also be considered total carbohydrate but this is not recommended.

3. Determination of Fermentable Sugars in Wood Hydrolysates (8)

Wood hydrolysates contain various hexoses, pentoses, sugar decomposition products, furfural, wood extractives, and lignin. Their value for alcohol production depends, however, on the fermentable sugar content and, therefore, it is essential that fermentable sugars be differentiated from unfermentable sugars and other reducing material. This may be done by the fermentation of a sample of the sugar followed by an alcohol determination, or by a

determination of the sugar consumed in the process. These methods, however, are time consuming, especially when applied to a large number of samples. For this reason, yeast sorption, which is faster and gives equivalent results, is used in this procedure.

A high concentration of yeast will quantitatively remove sugar from dilute solutions at a rapid rate. In the examination of complex mixtures, such as blood or urine, fermentable sugars are estimated by determining the loss in reducing power of the sample after treatment with a high concentration of yeast (9). Menzinsky (10) describes the use of this rapid yeast sorption method for the evaluation of the sugars contained in sulfite waste liquors. Hägglund (11) has found sulfite liquor from pine to contain 17.0% pentoses, 42.7% mannose, 4.2% galactose, 3.2% galacturonic acid, 4.0% fructose, and 28.9% glucose. This distribution varies between species. Because galactose is not readily fermented unless the yeast has been previously acclimatized to its utilization, Menzinsky recommends that yeast for sulfite-liquor analysis be acclimatized to galactose, or that the yeast be taken from a vat in which the fermentation of sulfite liquor is taking place. In the case of wood hydrolysates, however, the proportion of galactose present is much smaller than in sulfite liquor, so in the procedure described here, an unacclimatized yeast is used.

Procedure.--The sample to be analyzed for fermentable sugar is diluted to approximately 1.0 mg. of reducing sugar per milliliter. Sulfuric acid is added to the diluted solution from a microburette in the proportions of approximately 0.1 ml. per 100 ml. Twenty ml. of this solution are then put into a 30-ml. vial, and a sufficient quantity of precipitated chalk is added to leave a small amount of undissolved excess. Approximately 1 g. of

compressed baker's yeast (Saccharomyces cerevisiae) is then added, and the vials are stoppered and shaken for 1 hr. at 30° C. At the end of this time the vials are centrifuged, and a sample of the supernatant liquor is pipeted off and analyzed for sugar by the Shaffer and Somogyi method as described in "Techniques--Determination of monomeric wood sugars--Volumetric method." If unwashed yeast is used, the value is corrected by subtracting the apparent sugar found in a blank determination which includes all constituents except the sugar sample. The difference in the sugar content of the solution before and after treatment with yeast corresponds to the fermentable sugar present.

4. Identification of Wood Sugars
By Paper Chromatography

(a) Principle.--The components of a solution of unidentified sugars are separated by paper chromatographic techniques and identified by comparing their positions on the paper (R_f values) with those of known sugars which are run simultaneously.

This test is applicable to the common wood sugars.

(b) Apparatus.--(1) Spotter. The spotter (sample applicator) used is a 2 µl. pipet.

(2) Chromatographic apparatus. The apparatus used is the same as that described in "Techniques--Isolation of wood sugars--Chromatographic separation."

(c) Reagents.--(1) Standard sugar solutions. Standard 1% (1 g. dissolved in 100 ml. of water) sugar solutions of whatever sugars are suspected of being present in the unknown are used. A preservative (benzoic acid) is added to deter microbial attack on the sugars.

(2) Chromatographic reagents. Reagents for the chromatographic separation are those described in "Techniques--Isolation of wood sugars--Chromatographic separation."

(d) Procedure.--Aliquots of standard sugar solutions and sample solutions are applied to the paper by means of the 2-µl. pipet. The spotting technique is to fill the pipet with test solution, and apply it near the top (1 in. from fold) of the chromatographic paper. This procedure is repeated, drying the spot on the paper each time with a hair dryer before applying the next aliquot until at least 10 µg. of sugar have been applied on the spot. The usual procedure is to apply several spots, each containing a different amount; this not only gives at least one spot which should fall within a good range for the separation and detection of each sugar, but also makes it easier to estimate the quantities of sugars present in the unknown by comparing the spots after separation with the standard knowns.

After the standards and unknowns are spotted on the chromatographic paper, the papers are irrigated and the sugars separated and located by the techniques described in the procedure for the quantitative analysis of pulp constituents. The sugars present are then identified by comparing their position on the paper with the position of the standards. Also of qualitative use are the facts that hexose-aniline phthalate spots fluoresce under UV light and that pentoses form a pinkish color with aniline phthalate. A rough quantitative estimate is also usually made from the intensity of the color of the spots.

References

(1) Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millett, M. A.
1954. Techniques for the determination of pulp constituents by quantitative paper chromatography. Tappi 37: 336.

(2) McKibbins, S. W., Harris, J. F., and Saeman, J. F.
1961. A sample applicator for chromatographic paper and its use for 5-hydroxymethyl-2-furaldehyde and levulinic acid analysis. J. Chromatogr. 5: 207.

(3) Hawthorne, J. R.
1947. Microestimation of sugars separated on the filter paper chromatogram. Nature 160: 714.

(4) Dimler, R. J., Schaefer, W. C., Wise, C. S., and Rist, C. E.
1952. Quantitative paper chromatography of d-glucose and its oligosaccharides. Anal. Chem. 24: 1411.

(5) Schaffer, P. A., and Somogyi, M. J.
1933. Copper-iodometric reagents for sugar determination. J. Biol. Chem. 100: 695.

(6) Nelson, N.
1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375.

(7) Somogyi, M. J.
1952. Notes on sugar determination. J. Biol. Chem. 195: 19.

(8) Saeman, J. F., Harris, E. E., and Kline, A. A.
1944. Analysis of wood sugars. Forest Prod. Lab. Rep. R1459.

(9) Somogyi, M. J.
1927. Reducing nonsugars and true sugar in human blood. J. Biol. Chem. 75: 33.

(10) Menzinsky, G.
1942. Use of micro-organisms in sugar analyses, I. A rapid microbiological method for determining the percentage of sugar in sulfite waste liquor which is available for alcoholic fermentation. Svensk. Papperstidn. 45: 421.

(11) Hägglund, E., Klingstedt, F., Rosenquist, T., and Urban, H.
1928. Carbohydrate constituents of the easily hydrolyzable hemicellulose of pine. Zool. Phys. Chem. 177: 248.

APPENDIX TO ANALYTICAL PROCEDURES

DEFINITIONS OF TERMS RELATING TO CELLULOSE AND CELLULOSE DERIVATIVES[1]

These definitions are divided into three classes of terminology as follows:

(A) Cellulosic Materials and Constituents,

(B) Chemical Modifications and Derivatives of Cellulose, and

(C) Properties of Cellulose and Associated Concepts that are applicable to both (A) and (B).

(A). CELLULOSIC MATERIALS AND CONSTITUENTS

Acetylation Pulps.--Pulps used in the manufacture of cellulose acetate or other esters and subject to various specifications by the manufacturers, including those of alpha-cellulose content and viscosity.

Alpha-Cellulose.--That portion of industrial cellulosic pulps that remains insoluble after treatment with aqueous sodium hydroxide at 20 C. The term has meaning only if there is a precise statement of the initial sodium hydroxide concentration used (usually about 18 per cent), the subsequent dilution concentrations, and other conditions employed in its isolation or determination.

Alpha Pulps.--A term used in a rather indefinite way for all those bleached wood pulps which have an alpha-cellulose content above 88 per cent. (Where possible, it is desirable to use a more specific term.)

Araban.--A pentosan yielding essentially only arabinose on hydrolysis.

Arabinogalactan.--A polysaccharide consisting of arabinose and galactose units, like the water-soluble polysaccharide of larch.

Arabinose.--A pentose which occurs as one of the sugar units in some hemicelluloses.

Arabinomethylglucuronoxylan.--A hemicellulose containing arabinose, 4-O-methylglucuronic acid and xylose groups in its structure.

Beta-Cellulose.--That portion of the pulp which dissolves in the alkaline solution under the conditions imposed in the alpha-cellulose test, but which is reprecipitated on neutralization of the alkaline solution. All conditions must be exactly specified. See Alpha-Cellulose

Carbohydrates Not Cellulose.--The non-cellulosic carbohydrates of a cellulosic material.

Cellophane Pulp.--A pulp used in the manufacture of cellophane and subject to various specifications by the manufacturers including those of alpha-cellulose content and viscosity.

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[1]Taken from ASTM Standard D 1695, 1960 revision.

Cellulose.--1. The main solid constituent of woody plants; it occurs widely elsewhere in the vegetable kingdom, and to a small extent in the animal kingdom.
2. Chemically, cellulose is β-1-4 glucan of high degree of polymerization. It is desirable to apply "cellulose" to this material only and to designate the predominantly cellulosic residue obtained by subjecting woody tissues to various pulping processes as "cellulosic residues", "cellulosic pulps", or the like.

Cellulose I.--The crystalline modification of cellulose that normally occurs in nature.

Cellulose II.--The crystalline modification of cellulose that is found in mercerized cellulose, in regenerated cellulose, and in cellulose produced by the hydrolysis of various cellulose derivatives.

Cellulose III.--A crystalline modification of cellulose produced by treatment, under certain conditions, with ammonia or sometimes by amines. The method of removing the reagent determines the modification produced.

Cellulose IV.--A crystalline modification of cellulose produced by heat treatment of Cellulose II.

Cellulose X.--A crystalline modification of cellulose produced by treatment of cellulose with strong hydrochloric acid or phosphoric acid.

Cellulose, Purified Cotton.--See Cotton Cellulose, Purified.

Cellulose, Purified Wood.--See Wood Cellulose, Purified.

Chemical Cellulose.--A chemically purified cellulosic material that is intended for chemical treatment to produce derivatives.

Chemical Cotton.--Chemical cellulose prepared from cotton; generally, but not necessarily, cotton linters.

Chemical Pulps (trade usage).--A term used in the paper industry for pulps produced by chemical processes, as contrasted to those produced by mechanical processes. See Chemical Wood Pulp.

Chemical Wood Pulp (trade usage).--A term used in the paper industry for pulps obtained by digestion of wood with solutions of various chemicals.

Note.--This term, which refers to pulp produced and purified by chemical processes, should not be confused with chemical cellulose which refers to pulp that is to be used in chemical processes.

Cotton Cellulose, Purified.--Chemical cellulose from cotton fiber or linters. See Chemical Cotton.

Cotton Linters.--See Linters.

Dissolving Pulps.--See Pulps, Dissolving.

Extractives.--Compounds occurring in plant materials but not forming part of the structural elements, which are removed with neutral solvents such as ether, alcohol, and water.

Galactan.--A polysaccharide composed essentially of galactose units. See Arabinogalactan.

Galactoglucomannan.--One of the hemicelluloses of softwoods, containing three types of sugar units, galactose, glucose and mannose.

Galactomannan.--A polysaccharide containing galactose and mannose units. Galactomannans usually have a long chain of mannose units with galactose side chains and are found in seed gums (guar, locust bean).

Gamma-Cellulose.--That portion of a cellulosic material which remains dissolved even after neutralization of tha alkaline solution from the alpha-cellulose determination. See Alpha-Cellulose.

Glucan.--A macromolecular substance which can be hydrolyzed to give almost exclusively glucose.

Glucomannan.--A hemicellulose consisting essentially of glucose and mannose.

Glucuronoxylan.--A common designation for the xylose-containing hardwood hemicelluloses. See Methylglucuronoxylan.

Hemicellulose.--Any of a number of cell-wall polysaccharides that are removable by extraction with aqueous alkali and that may be hydrolyzed by boiling with dilute acids to give constituent monosaccharide units; any of the non-cellulosic cell-wall polysaccharides.

Hexosan.--Frequently used in contradistinction to pentosan, for a polysaccharide consisting mainly of hexose units.

Holocellulose.--The total polysaccharide fraction of extractive-free wood. The method of isolation or of determination should always be given.

Kraft Pulp.--Pulp cooked by the alkaline process in which the make-up chemical is essentially sodium sulfate; sulfate pulp.

Lignin.--That part of plant material which is not saccharified by the action of 72 per cent sulfuric acid or 42 per cent hydrochloric acid, after the resins, waxes, and tannins have been removed.

Linters.--The short fibrous material adhering to cotton seed after the ginning operation. After removal from the seed it is used to a limited extent as a fibrous raw material for special papers. The principal use, however, is for chemical cellulose, that is, as the raw material for the manufacture of cellulose derivatives.

Mannan.--Strictly, a polysaccharide composed entirely of mannose units, but used conventionally to distinguish the hexosan wood hemicelluloses from the pentosans (xylan). See Galactoglucomannan and Glucomannan.

Mannogalactan.--See Galactomannan.

Methylglucuronoxylan.--The main hemicellulose of hardwood pulps; a polysaccharide containing xylose and 4-O-methylglucuronic acid groups. In the wood it is partially acetylated.

Nitrating Pulps.--Pulps used for the manufacture of cellulose nitrate and subject to various specifications by the manufacturers, including those of alpha-cellulose content and viscosity.

Oligosaccharides.--Polymeric carbohydrates containing relatively few (compared to the polysaccharides) sugar units connected by glycosidal linkages. Two to nine units has been suggested as a suitable range. For longer chains the polymers cannot be readily separated into individual molecular species.

Pectic Substances.--Complex polysaccharides containing a large proportion of galacturonic acid units.

Pectins.--Colorless, amorphous, water-soluble polysaccharides occurring in plant tissues which yield pectic acid and methanol on hydrolysis.

Pentosans.--One of the groups of amorphous carbohydrates included under the general term "Hemicellulose". Pentosans yield principally pentoses on acid hydrolysis. The principal pentosan in wood is xylan.

Polyuronides.--Polysaccharides containing uronic acid groups.

Pulps, Dissolving.--Chemical cellulose from wood pulp.

Pulps for Chemical Conversion.--Chemical cellulose from wood pulp.

Pulps for Manufacture of Cellulose Derivatives.--Chemical cellulose from wood pulp.

Purified Cotton Cellulose.--See Cotton Cellulose, Purified.

Purified Wood Cellulose.--See Wood Cellulose, Purified.

Rayon Pulps.--Pulps used in the manufacture of rayon, and subject to various specifications by the manufacturers, including those of alpha-cellulose content and viscosity.

Sulfate Pulp.--See Kraft Pulp.

Sulfite Pulp.--Wood pulp produced by cooking with a sulfite liquor made by dissolving sulfur dioxide in an aqueous base.

Wood Cellulose, Purified.--Chemical cellulose from wood.

Xylan.--A pentosan giving almost exclusively xylose on hydrolysis.

(B). CHEMICAL MODIFICATIONS AND DERIVATIVES OF CELLULOSE

Carboxymethylcellulose (CMC).--The common name for a cellulose ether of glycolic acid. It is usually marketed as a water-soluble sodium salt, more properly called sodium carboxymethylcellulose. In the early literature, it is sometimes called cellulose glycolate or cellulose glycolic acid.

Cellulose Acetate.--In the broad sense, any of several esters of cellulose and acetic acid. See Celludose Triacetate.

Cellulose Acetate Butyrate.--A mixed ester of cellulose containing both acetate and butyrate groups.

Cellulose Acetate Phthalate.--A mixed ester of cellulose containing both acetate and phthalate groups.

Cellulose Acetate Propionate.--A mixed ester of cellulose containing both acetate and propionate groups.

Cellulose Derivative.--A substance derived from cellulose by substitution of one or more of the hydroxyl groups with some other radical. Most derivatives are ethers or esters.

Cellulose Esters.--Derivatives of cellulose in which one or more of the hydroxyl hydrogens have been replaced by acyl groups.

Cellulose Ethers.--Derivatives of cellulose in which one or more of the hydroxyl hydrogens have been replaced by alkyl groups.

Cellulose Lacquer.--A liquid coating composition containing as the basic film-forming ingredients cellulose esters or ethers and plasticizers with or without resins.

Cellulose Mixed Ester.--A cellulose ester containing more than one type of acyl group.

Cellulose Nitrate.--Any of various nitrate esters of cellulose.

Note.--Cellulose nitrate is often and erroneously called "Nitrocellulose" because it was formerly considered a nitro-compound.

Cellulose Plastics.--Plastics based on cellulose compounds, such as esters (cellulose acetate) and ethers (ethylcellulose).

Cellulose Propionate.--Any ester of cellulose with propionic acid.

Cellulose Sodium Glycolate.--See Carboxymethyl Cellulose.

Cellulose Triacetate.--That form of cellulose acetate, in which the degree of substitution approaches 3 sufficiently that the product is not soluble in acetone.

Cellulose Xanthates.--The salts of cellulose xanthic acid. Commonly, cellulose xanthate refers to sodium cellulose xanthate, the essential constituent of the viscose solution, from which viscose rayon is spun.

Dope.--A composition, usually a cellulose lacquer, for application on textiles and leathers. Also a very viscous crude reaction product, as acetylation dope.

Ethylcellulose.--Any of several ethyl ethers of cellulose. The one most generally used in industry has sufficient substitution to be soluble in organic solvents.

Hydrocelluloses.--Water-insoluble products of the hydrolysis of cellulose with acids. They are molecularly heterogeneous in the sense that they are composed of molecules varying in degree of polymerization. The average degree of polymerization (DP) and the DP distribution depend on the nature of the acid

treatment and of the original cellulose. The term may also be applied to any insoluble polysaccharide so formed and separated as a more or less homogeneous fraction from the mixture of products, but the singular form "hydrocellulose" should not be used without an article, to avoid the implication of a molecularly homogeneous species.

(2-Hydroxyethyl) cellulose.--Any of several cellulose ethers in which some of the hydroxyl groups have been substituted with hydroxyethyl groups. Hydroxyethyl cellulose, except at very low degrees of substitution, is water-soluble.

(2-Hydroxyethyl) ethylcellulose.--A mixed ether of cellulose containing both hydroxyethyl and ethyl groups.

(2-Hydroxyethyl) methylcellulose.--A mixed ether of cellulose containing both hydroxyethyl and methyl groups.

(2-Hydroxypropyl) methylcellulose.--A mixed ether of cellulose containing both hydroxypropyl and methyl groups.

Ionic Cellulose Ethers.--Those water-soluble cellulose ethers which contain ionizable groups, in more than trace amounts. Sodium carboxymethyl cellulose is an example.

Lacquer.--See Cellulose Lacquer.

Methylcellulose.--Any of several methyl ethers of cellulose. Commercially, the water-soluble ether (degree of substitution approximately 1.5 to 2.0).

Methylethylcellulose.--A cellulose ether containing both methyl and ethyl groups.

Microcrystalline Cellulose.--A commercial name for cellulose which has been hydrolysed to the limiting DP and which consists essentially of microcrystals.

Nitrocellulose (trade usage).--See Cellulose Nitrate.

Nonionic Cellulose Ethers.--That class of cellulose ethers which does not contain any ionizable groups.

Oxycelluloses.--Water-insoluble substances formed by the action of oxidizing agents on cellulose. The chemical nature of oxycelluloses varies with the oxidant used, and the type is indicated by attaching the name of the oxidant adjectivally to "oxycelluloses" as in "hypochlorite oxycelluloses". Any such mixture is "an oxycellulose" and the word should not be used in the singular without either the definite or indefinite article. In many respects, the phrases "oxidized cellulose" or "partially oxidized cellulose" are preferable.

Propylene Glycol Ether of Methylcellulose.--Same as (2-hydroxypropyl) methylcellulose.

Regenerated Cellulose.--Cellulose regenerated from a solution of cellulose or from a cellulose derivative.

Sodium Carboxymethylcellulose.--See Carboxymethylcellulose.

Viscose.--A solution of sodium cellulose xanthate prepared by dissolving the reaction product formed by the interaction of carbon disulfide and alkali cellulose in an aqueous solution of sodium hydroxide. Viscose is used mainly in the manufacture of rayon or cellophane, where it is extruded through fine openings of the proper shape into a coagulating bath.

(C). PROPERTIES OF CELLULOSE AND ASSOCIATED CONCEPTS

Accessibility.--The fraction of total cellulose present which is accessible to certain reagents under certain specified conditions. The conditions of determination should always be indicated.

Acetylation.--Substitution of an acetyl radical for an active hydrogen. Specifically, formation of cellulose acetate from cellulose.

Acetyl Groups.--The characteristic groups of acetic acid; CH_3CO-.

Acyl Groups.--Radicals derived from carboxylic acids by removal of the hydroxyl group.

Acid Groups.--Functional groups having the properties of acids. In cellulose and its derivatives, these are usually carboxyl groups.

Aging.--In general, the change of properties with the passage of time. Specifically, the changes occurring in shredded alkali cellulose when allowed to stand exposed to air.

Air-dry.(trade usage).--A term applied to paper or pulp. Paper is air dry when its moisture content is in equilibrium with the atmospheric conditions to which it is exposed. According to trade custom, pulps are generally understood to be air dry when they contain 10 per cent of moisture on the oven-dry weight of the pulp.

Alcohol-Benzene Solubility.--Solubility of a cellulosic pulp in a mixture of ethanol and benzene. The term is without precise meaning unless complete specification of an analytical procedure is attached explicitly or implicitly.

Aldehyde Groups.--Carbonyl groups to which a hydrogen atom is attached; the first oxidation stage of an alcohol; -CHO.

Alkali Resistance.--For a cellulosic pulp, the fraction insoluble in alkali, usually sodium hydroxide, of a fixed concentration under specified conditions. The term is without precise meaning unless complete specification of an analytical procedure is attached explicitly or implicitly. See Alkali Solubility.

Alkali Solubility.--For a cellulosic pulp, the fraction soluble in alkali of a fixed concentration under specified conditions. This term is without precise meaning unless complete specification of an analytical procedure is attached explicitly or implicitly. Some related terms imply at least a partial specification, for example, "Ten per cent Potassium Hydroxide Solubility" or "Nondilution Alkali Solubility".

Alkali Staining.--Discoloration caused by the presence of an alkali.

Alkyl Groups.--Monovalent aliphatic radicals derived from aliphatic hydrocarbons by removal of a hydrogen.

Amorphous Regions.--Those regions within a cellulosic material which, on the basis of x-ray diffraction or other suitable technique, do not show any evidence of crystalline structure. The technique should be specified.

Anhydroglucose Units.--The repeating unit of many polysaccharides, including cellulose; since the glucose molecules have combined with elimination of water, the unit is called "anhydroglucose" rather than "glucose".

Ash.--The inorganic residue obtained by igniting a specimen of pulp, paper, or other cellulosic material in such a way that all combustible and volatile compounds are removed. Conditions of ashing should be specified.

Bleachability.--The capacity of a pulp to bleach to a given whiteness. This is approximately and indirectly related to lignin content.

Bone-dry.--See Oven-dry.

Brightness.--As commonly used in the paper industry, the reflectivity of a sheet of pulp or paper for blue light measured under standardized conditions on a particular instrument designed and calibrated specifically for the purpose.

Carbonyl Group.--The bivalent radical, -CO-, especially as it occurs in aldehydes or ketones.

Carboxyl Group.--The radical -COOH characteristic of most organic acids.

CED (Cupriethylenediamine) Viscosity.--See Viscosity, Cupriethylenediamine.

Chain Length Distribution.--In a linear polymer like cellulose, the frequency distribution of molecular size, usually expressed in units of degree of polymerization.

Chlorine Number.--The number of grams of chlorine gas or of bleaching powder (expressed as its equivalent in chlorine) that is consumed by 100 grams of ovendry pulp in a definite time under certain specified conditions. The chlorine number is an indication of the bleach requirement of the pulp and an indirect estimate of the lignin content.

Color Reversion.--A process common to almost all bleached cellulose pulps in which the color darkens to a greater or less extent on standing.

Commercial Moisture Regain.--See Moisture Regain, Commercial.

Copper Number.--The number of grams of copper in the cuprous oxide reduced from a cupric compound by 100 grams of pulp or paper (after deduction of moisture, ash, and sizing materials) treated under specified conditions with an excess of cupric solution. The copper number is an indication of the relative number of reducing groups in the pulp or paper.

Cross Linking.--The reaction of a difunctional molecule with each of two molecules of a polymer. This change of the polymer from linearity produces profound changes in the physical properties.

Crystalline Regions.--See Crystallinity.

Crystallinity.--A regular arrangement of the atoms of a solid in space. In most polymers, including cellulose, this state is usually imperfectly achieved. The crystalline regions (ordered regions) are submicroscopic volumes in which there is more or less regularity of arrangement of the component molecules. In these regions there is sufficient geometric order to enable definite x-ray diffraction patterns to be obtained. See Degree of Crystallinity; Degree of Order.

Crystallinity Index.--A number used to represent the state of crystallinity of cellulose as a whole. Unfortunately, it has been differently defined by different investigators and should not be used unless it is clearly stated which crystallinity index is meant. See Crystallinity.

Crystallite.--A term referring to a single crystalline region. See Crystallinity.

Cuam Viscosity.--See Viscosity, Cuprammonium.

Cuene Viscosity.--See Viscosity, Cupriethylenediamine.

Cuprammonium Process.--A process for making rayon by dissolving cellulose in an ammoniacal copper solution and spinning the resulting solution into an acid bath, thereby regenerating the cellulose as fibers.

Cuprammonium Viscosity.--See Viscosity, Cuprammonium.

Cupriethylenediamine Viscosity (Cuene or CED).--See Viscosity, Cupriethylenediamine.

Degradation.--In general chemical use, the conversion of a complex compound to a simpler. Specifically for cellulose, the breakdown of the polymer chain, usually by hydrolysis or oxidation. Degradation is usually applied to changes in chemical structure. See Deterioration.

Degree of Crystallinity.--The fraction by weight of a cellulose sample occurring in crystalline regions. The method of determining crystallinity must be stated. See Crystallinity.

Degree of Lateral Order.--A term representing the relative degree of molecular alignment. As for degree of crystallinity, quantitative values must be defined in terms of the experimental measurements. See Crystallinity.

Degree of Polymerization (DP).--In general, the average number of base units, or of monomeric units per molecule in linear polymers. Specifically, the average number of anhydroglucose units (or derivative units) per molecule of cellulose (or cellulose derivative). The type of average obtained depends upon the method used for the determination. Hence, the method must always be specified.

Degree of Substitution (DS).--In a cellulose derivative, the average number of hydroxyl groups substituted per anhydroglucose unit. DS varies from zero to about 3.

Density.--The mass per unit volume at a specified temperature.

Density, Apparent.--The mass per unit volume of a sheet of pulp or paper. It is commonly calculated by dividing the basis weight by the caliper, although it must be recognized that the numerical value thus obtained is dependent upon the definition of the ream.

Density, Bulk.--The mass of a cellulosic material which will fill a unit volume of a container under specified conditions.

Deterioration.--A permanent impairment of the physical properties. See Degradation.

Dry Weight.--This is usually applied to the weight of the oven-dry material, but it is ambiguous unless the method of drying is specified.

End Groups.--The functional groups at either end of the cellulose molecule. It is frequently used for the terminal glucose units which contain these groups. It is usually clear from the context which is meant and the differentiation is seldom important. One end group is reducing (probably a hemiacetal) and one nonreducing (a free hydroxyl in the 4-position).

Equilibrium Moisture Content.--The percentage weight of moisture in a material when it has attained equilibrium with water vapor at a specified pressure or partial pressure. It is determined by successive weighings of the sample, either on the adsorption or desorption curve, and plotting moisture content against the logarithm of time. It should be designated as adsorption equilibrium or desorption equilibrium.

Exchange Capacity.--See Ion Exchange Capacity.

Fiber.--The unit cell of vegetable growth, which is many times longer than its diameter and which consists largely of cellulose. It is the basic unit of pulps and papers.

Fiber Length.--When applied to a pulp, this applies to the mean fiber length and both the experimental details and the calculations must be specified.

Fiber Length Distribution.--The frequency distribution of the individual fiber lengths in a material about the mean fiber length.

Fiber Saturation Point.--The moisture content in the absorption of water by cellulose when the cell walls are saturated, but virtually no free water is present in the grosser capillary structure. The concept is based on the hypothesis that a discontinuity occurs in the sorption curve at this point. Some careful studies of the region have failed to show a discontinuity, but it is still possible to define "fiber saturation point" as an extrapolation of the adsorption curve. In any case, it should only be used with reference to the method of determination.

Fiber Structure.--The morphological structure of a fiber at the visual or microscopic level. See Fine Structure.

Fibril.--A fiberlike bundle of molecules, oriented in one direction either with the fiber axis or spirally around it. It makes up the fibrous structural unit of the single fiber. The cellulose fibrils are crystalline as shown by x-ray diffraction.

Film.--Sheeting, including that composed of cellulose or its derivatives, having nominal thickness not greater than 10 mils. See Films.

Films.--Transparent sheeting. If this is used as a generic term, regardless of sheet thickness, the plural shoudl be used and the singular restricted to a specified size. See Film.

Fine Structure.--A generic term denoting the submicroscopic structure which depends on molecular arrangement.

Fluidity.--The reciprocal of viscosity.

Gamma Number.--Degree of substitution (DS) x 100. Of widespread use in the cellulose xanthate field.

Gloss.--The geometrically selective reflectance of a surface responsible for its shiny or lustrous appearance. Surface reflectance is commonly at a maximum in or near the geometric directions in which a mirror would reflect light.

Hardness.--A term referring to the degree of cooking of a pulp. A hard pulp is one in which the residual lignin content is relatively high.

Haze.--The turbidity produced by the material in some cellulose derivatives which is not completely soluble in the medium. This turbidity may refer to solutions or to the solid plastic.

Hemiacetal Groups.--Functional groups derived from carbonyl groups by addition of one molecule of an alcohol, of the general structure. $-C\begin{matrix}OH\\OR\end{matrix}$

Hornification.--The behavior of certain forms of cellulose and related materials on drying, which results in a lowered reactivity. This is apparently caused by the formation of new hydrogen bonds with a corresponding decrease in accessibility.

Hydrodynamic Specific Surface.--The specific surface of a fibrous material as measured by the filtration resistance of a compacted pad formed from a fiber suspension under specified conditions.

Hydrophilic.--Having an affinity for water.

Hydrophobic.--Having an antagonism to water.

Hydroxyl Groups.--The monovalent group -OH, characteristic of hydroxides and alcohols.

Inherent Viscosity.--See Viscosity, Inherent.

Intrinsic Viscosity.--See Viscosity, Intrinsic.

Ion Exchange.--A reversible process by which ions are interchanged between a solid and a liquid with no substantial structural changes in the solid.

Ion Exchange Capacity.--The weight of ions that can be exchanged by a unit volume of ion-exchange materials.

Ion Exchange Materials.--Insoluble solid materials with the ability to exchange reversibly certain ions, in the structure or attached to the surface as functional groups, with ions in a surrounding medium.

Iron-Sodium Tartrate (EWNN or FeTNa).--A complex tartaric acid salt of sodium and ferric iron. Solutions in aqueous sodium hydroxide will dissolve cellulose. Both the German abbreviation (EWNN) and the English (FeTNa) are to be found in English articles. The details of preparation of the solution should always be given.

Iron-Sodium-Tartrate Viscosity.--The viscosity of a solution or dispersion of cellulose or pulp under standardized condition when dissolved in the iron-sodium-tartrate solvent. See Iron-Sodium Tartrate.

Kappa Number.--The number of milliliters of 0.1N potassium permanganate solution consumed by 1 g. of oven-dry pulp under specified conditions. It is an indication of the hardness or bleachability of a pulp. An indirect estimate of lignin content.

Lateral Order.--The degree of regularity of arrangement of atoms and atomic groups in the direction normal to the molecular chain axes in linear polymers. Quantitative evaluation is impossible without further specification and without description of the particular experimental technique.

Leveling-Off Degree of Polymerization (LODP).--The nearly constant degree of polymerization of cellulose reached after very prolonged mild hydrolysis or short drastic hydrolysis.

Limiting Viscosity Number.--See Viscosity, Intrinsic.

Luster.--In the pulp and paper industry, synonymous with "gloss" or "sheen". See Gloss.

Mercerization.--The process of subjecting a vegetable fiber to the action of a fairly concentrated aqueous solution of a strong base so as to produce great swelling with resultant changes in fine structure, dimensions, morphology, and mechanical properties.

Mesomorphous Cellulose.--Those portions of cellulose in which the segments of the individual molecules have some regularity of arrangement, but not enough to permit strict lattice order to give a distinct x-ray diffraction pattern.

Methoxyl Group.--The monovalent group $-OCH_3$, characteristic of methyl alcohol and its esters or ethers.

Moisture Content.--The moisture present in a cellulosic material, as determined by

prescribed methods, conventionally expressed as a percentage of the total weight of the "wet" material.

Moisture Equilibrium.--The condition reached by a sample when it no longer takes up moisture from, or gives up moisture to the surrounding air.

Moisture Equilibrium for Preconditioning.--The moisture equilibrium condition reached by a sample after free exposure to air controlled at the standard condition for preconditioning.

Moisture Equilibrium for Testing.--The equilibrium moisture condition reached by a sample after free exposure to moving air controlled at standard conditions.

Moisture Regain.--The moisture present in a cellulosic material, as determined by prescribed methods, expressed as a percentage of the oven-dry weight.

Moisture Regain, Commercial.--An arbitrary figure formally adopted as the regain to be used in calculating the commercial or legal weight of shipments or deliveries of any specific material.

Moisture Regain, Standard.--The moisture regain of sample brought from a lower moisture regain into equilibrium with the standard atmosphere.

Moles of Substituent Combined (M.S.).--In a cellulose derivative, the average number of substituent molecules per glucose unit. For most derivatives, M.S. = D.S., but for a few, such as hydroxyethylcellulose, the substituted group may also be reactive and M.S. may be greater than D.S.

Nonreducing End Groups.--Those terminal glucose units of cellulose or its derivatives which contain a free hydroxyl in the 4-position and do not reduce Fehling's solution or similar reagents.

Oleoresins..--Nonaqueous secretions of resin acids dissolved in terpenic hydrocarbons that are produced or exuded from the intercellular resin ducts of living trees, especially the conifers and accumulated, together with oxidation products, in the wood of weathered limbs or stumps.

Order Distribution.--See Lateral Order.

Orientation.--The angle made by the crystallites of the cellulose with the fiber axis. This is approximately the same angle as that made by the molecules or the fibrils.

Oven-dry.--The state of a cellulosic material which has been dried to constant weight at a temperature of 100 to 105 C.

Per Cent Hydrolysis-Resistant Cellulose.--The residue after treatment of cellulose with acid under specified conditions, expressed as a percentage of the original.

Permanganate Number.--The number of milliliters of tenth-normal potassium permanganate which is consumed by one gram of oven-dry pulp under certain specified and carefully controlled conditions. It is an indication of the hardness or bleachability of a pulp. An indirect estimate of lignin content.

Plastic, adj.--1. Capable of being deformed continuously and permanently in any direction without rupture, under a stress exceeding the yield value.
2. Made of, consisting of or pertaining to plastics. See Plastic, n.

Plastic, n.-- A material containing as an essential ingredient an organic substance of large molecular weight, which is solid in its finished state and, at some stage in its manufacture or in its processing into finished articles, can be shaped by flow.

Porosity.--The existence in a material of connected air voids. It is frequently expressed as the ratio of void volume to total volume.

Reactivity.--The ability to react. For proper use of the term, the reaction in question and the conditions should be stated and the parameter used in measuring reactivity indicated, such as rate, uniformity or the like.

Reduced Viscosity.--See Viscosity, Reduced.

Reducing End Groups.--Those terminal glucose units in cellulose or its derivatives in which the 1-position is not substituted or involved in a glycosidic linkage. These end groups will reduce Fehling's solution or similar reagents.

Regain.--See Moisture Regain.

Relative Viscosity.--See Viscosity, Relative.

Reversion.--In general, the tendency of pulp or paper properties to return to those of the material at some other stage. It is frequently specifically applied to the loss of brightness with time, after bleaching has produced a high brightness.

Ripening.--In the manufacture of viscose rayon, that stage where the cellulose xanthate solution is stored several days under controlled conditions to reach a state from which coagulation is easier.

Sliver.--A continuous strand of loosely assembled fibers that is approximately uniform in cross-sectional area and without twist.

Specific Gravity.--The ratio of the weight of a specimen to the weight of an equal volume of water, both at the same specified temperature. Generally, density is more useful. See Density.

Specific Surface.--The surface per unit weight (or less frequently per unit volume) of a moisture-free sample. The specific external surface is used as a measure of the degree of fineness of fibrillation.

Specific Viscosity.--See Viscosity, Specific.

Standard Moisture Regain.--See Moisture Regain, Standard.

Surface Area.--The total area of the surface. As usually used for reactive pulps, this applies not to external surface, but to the internal surface as well, the total surface available to gases or penetrating liquids. It is useful to express this as specific surface, area per gram.

Swelling.--Increase in volume and dimensions caused by penetration of a liquid.

Total Internal Surface.--The surface of a cellulosic material available for absorption of a given substance. A useful substance for this purpose is water; the value obtained with water is close to the potential maximum surface which can be developed without disruption of the crystalline structure.

Unit Cell.--A parallelopiped element of crystal structure, containing a certain number of atoms, the repetition of which through space will build up the complete crystal.

Uronic Acids.--Oxidation products of the sugars in which a primary alcohol group has been oxidized to a carboxyl without changing the reducing functional group.

Viscosity.--In general, the resistance of a fluid to flow or motion within itself. As applied to cellulose or its derivatives the viscosity of a solution of the substance.

Viscosity, Cuprammonium.--The viscosity of a solution or dispersion of cellulose or pulp in cuprammonium hydroxide under standardized conditions.

Viscosity, Cupriethylenediamine.--The viscosity of a solution or dispersion of cellulose or pulp under standard conditions when dissolved in a solution of copper hydroxide in ethylenediamine.

Viscosity in Absolute Units (CGS Poises).--The force in dynes required to move, at a velocity of 1 cm. per sec., one surface having an area of 1 sq. cm. past another parallel like surface 1 cm. away, overcoming the resistance to shear of the material filling the space between.

Viscosity Index.--The ratio of the viscosity of a highly concentrated solution to that of a dilute solution. It is a measure of solvent power and, in derivatives, of uniformity of substitution.

Viscosity, Inherent.--The quotient of the natural logarithm of relative viscosity by the concentration that is, $\ln \eta r/c$. The concentration should be specified.

Viscosity, Intrinsic.--The limiting value of reduced viscosity, $\eta sp/c$, as c (concentration) approaches zero. The IUPAC Committee of Nomenclature has recommended the expression "Limiting viscosity number" for this.

Viscosity Number.--See Viscosity, Reduced.

Viscosity Ratio.--See Viscosity, Relative.

Viscosity, Reduced.--The specific viscosity divided by the concentration. The IUPAC Committee on Nomenclature has recommended the expression "Viscosity number" for this.

Viscosity Relative.--The ratio of the viscosity of a solution to that of the pure solvent. The IUPAC Committee on Nomenclature has recommended the expression "Viscosity ratio" for this.

Viscosity, Specific.--The difference between the viscosity of a solution and that of a solvent, divided by the latter.

Viscosity, Xanthate.--For a cellulosic pulp, the viscosity of the viscose that will be obtained as a result of a specified series of processes.

Viscous Flow.--Flow, usually at low velocity, in which fluid elements flow in a straight line parallel to the direction of flow of the liquid in the absence of turbulence.

Water Vapor Permeability.--The rate of water vapor transmission per unit of thickness and per unit of vapor pressure differential. Test conditions must be specified.

Whiteness.--The degree of approach of the color of a substance to that of the ideal white, commonly represented by a standardized preparation of magnesium oxide.

Xanthate Viscosity.--See Viscosity, Xanthate.

DEFINITIONS OF TERMS RELATING TO PRECISION AND ACCURACY[1]

The following definitions are applicable when one operator provides a series of test results and no prior information on the method is used. They are endorsed by Analytical Chemistry and suggested when results reported are suitable for statistical treatment (5 or more determinations).

Series. A number of test results which possess common properties that identify them uniquely.

Mean. The sum of a series of test results divided by the number in the series. Arithmetic mean is understood.

Precision Data. Measurements which relate to the variation among the test results themselves--i.e., the scatter or dispersion of a series of test results, without assumption of any prior information. The following measures apply:

Variance. The sum of squares of deviations of the test results from the mean of the series after divisions by one less than the total number of test results.

Standard Deviation. The square root of the variance.

Relative Standard Deviation. The standard deviation of a series of test results as a percentage of the mean of this series. This term is preferred over "coefficient of variation."

Range. The difference in magnitude between the highest test result and the lowest test result in a series.

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[1]Taken from "Guide for Measures of Precision and Accuracy," Anal. Chem. 38: 2010. 1966.

Accuracy Data. Measurements which relate to the difference between the average test results and the true result when the latter is known or assumed. The following measures apply:

Mean Error. The average difference with regard to sign of the test results from the true results. Also equal to the difference between the average of a series of test results and the true result.

Relative Error. The mean error of a series of test results as a percentage of the true result.

PREPARATION OF EXTRACTIVE-FREE WOOD[1]

Introduction

Extractives in wood consist of material soluble in neutral solvents and not a part of the wood substance. They are largely made up of resins, tannins, waxes, gums, fats, and phenolics. In most hardwoods and softwoods the extractives' content is from 2 to 5 percent, but in very resinous trees or parts of trees, the level may be 25 percent or more.

The following procedure, using alcohol-benzene, alcohol, and water successively, is applicable to all North American woods. It may also be applied to bark, straw, pulps, and similar fibrous materials.

Apparatus

1. Soxhlet extractor, glass, of a size appropriate for the sample size. The sample is contained within the Soxhlet in a cellulose extraction thimble, a cotton bag, or the discharge tube of the Soxhlet can be covered with a screen or wad of cotton and the entire body of the extractor filled with the wood sample. A wire screen disk placed over the top of the sample will prevent channeling by the dripping condensate.

2. Büchner funnel and suction flask.

Reagents

1. Ethyl alcohol, 95 percent.

2. Alcohol-benzene mixture. Mix one volume of 95 percent ethyl alcohol and two volumes of benzene.

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[1]Based on TAPPI Standard Method T-12 1959.

Sample

The sample should be ground to pass a 40-mesh screen.

Procedure

Place the sample to be extracted in the Soxhlet apparatus being certain that the sample does not extend above the level of the top of the siphon tube. Extract at a rate of not less than four siphonings per hour for 4 hours with the alcohol-benzene mixture. After 4 hours, transfer the crucible to a suction flask and wash with alcohol to remove the benzene.

The alcohol wash consists of transferring the sample to a Büchner funnel[2] and washing with alcohol three times under suction, then covering the sample with alcohol and letting it stand for 10 minutes with the suction off. Pull off the alcohol with suction and wash three times again. The sequence of soaking and washing is then repeated two more times.

If an alcohol extraction is necessary,[3] return the sample to the extraction thimble and extract in the Soxhlet with 95 percent alcohol for 4 hours. This is followed by a water wash to remove the alcohol. If the alcohol extraction is not necessary, the alcohol is removed directly by a water wash. The procedure for washing with water (not hot) is the same as the alcohol wash. After the water wash, the sample is air dried.

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[2] If a large amount of sample is being prepared, it is extracted in a thimble and washed on a Büchner; if a small amount is being prepared, it can be contained in the Soxhlet in a porous crucible and washed in the same porous crucible.

[3] An alcohol extraction is necessary for woods high in tannins. Oaks, Chestnut, and redwood require the alcohol Soxhlet extraction; the various species of spruce, pine, fir, hemlock, poplar, beech, and maple usually do not.

MINIMUM SAMPLE SIZES REQUIRED FOR ANALYSES

(Single Determinations by Methods in Use at FPL)

Analysis	Minimum Sample Requirements
Acetyl groups (alkaline hydrolysis)	20 µg. of acetyl; 25 mg. wood (opt.)
Alcohol-benzene solubility	2 g. (wood)
Alkoxyl groups	20 µg. methoxyl; usually 2 to 5 mg. sample
Alpha, beta, gamma cellulose	3 g. (pulp)
Ash	10 mg. ash, can be estimated on a microscale (.05 mg.)
C-methyl	20 µg. of C-methyl
Carbon-hydrogen	3 mg. (min.), 5 mg. (opt.)
Charcoal	1 g.
Ether solubility	2 g. (wood)
Ethyl alcohol (by diffusion)	1 ml. containing from 0.1 to 0.4 mg. ethanol per ml.
Extractives	0.5 g. wood (using small extractors)
Formaldehyde (colorimetric)	1 ml. containing 1 to 6 µg. formaldehyde
Furfural (by UV)	3 ml. containing 6 µg. of furfural (0.1 mg. furfural is needed if a distillation step is necessary)
Glycerol (colorimetric)	0.5 ml. sample containing from 0.4 to 1.2 mg. glycerol per ml.
Halides (gravimetric)	1 to 5 g. wood (depending on halide content)
Holocellulose	2 g. (wood)

Analysis	Minimum Sample Requirements
Hydroxyl groups (by acetylation)	3 mg.
Hydroxymethylfurfural (by UV)	3 ml. containing 6 μg. HMF
Infrared spectrum	1 mg. (50 μg. using ultramicrotechniques)
Ion exchange capacity (carboxyl content) of pulps	5 g. (pulp)
Itaconic acid (colorimetric)	1 ml. containing from 1 to 100 μg.
Lactic acid (colorimetric)	0.2 to 2.0 mg. lactic acid
Levulinic acid (titration)	1 mg. per ml. (min.), 3 mg. per ml. (opt.)
Lignin in pulp	2 g. pulp by TAPPI method, 0.5 g. by FPL method
Lignin in wood	1 g. wood by TAPPI method, 0.3 g. by FPL method (can be scaled down to 50 mg.), 2 mg. by spectrophotometry
Lignin oxidation by nitrobenzene	Sample to yield 0.1 mg. product
Moisture	2.0 g. wood or pulp
Neutralization equivalent	0.01 meq. (usually 5 to 10 mg. sample) gives a 1.0 ml. titration
Nickel	0.1 g. nickel
Nitrogen (Dumas)	30 to 50 μg. nitrogen
Nitrogen (Kjeldahl)	
(a) total	10 mg. nitrogen
(b) ammoniacal	10 mg. nitrogen
(c) urea	0.1 g. urea
(d) qualitative	0.02 to 0.03 μg. nitrogen

Analysis	Minimum Sample Requirements
Pentosans (colorimetric) in pulp	
(a) 1 pct. pentosan	4 g. sample
(b) 10 pct. pentosan	1 g. sample
pH of wood	1 g.
Polyethylene glycol (in wood)	50 mg. polyethylene glycol
Propylene glycol (colorimetric)	1 ml. containing 2 or more µg. propylene glycol
Pyroligneous acid	600 ml. crude pyroligneous acid
Sieve analysis	100 g. of sample
Silica (by HF)	Sample containing 0.1 mg. silica
Sodium (by flame)	5 ml. of a 5 p.p.m. solution
Solids in water	500 ml. sample
Sugars (see "wood sugars")	
Sugar alcohols and glycols (by periodate oxidation)	0.5 ml. containing from 0.5 to 10 pct. of polyol to be measured
Sulfate (in cellulose nitrate)	0.4 mg. sulfate
Tannins (hide powder reaction)	Sample to contain 4 g. tannins
Total organic (dichromate oxidation)	2 ml. containing 0.5 mg. organics
Ultraviolet spectrum	1 ml. of solution (concentration requirements vary widely)
Uronic acids (gravimetric)	Sample to contain 25 to 100 mg. carboxyl carbon dioxide (2.0 g. wood or pulp)

Analysis	Minimum Sample Requirements
Wood Sugars	
(a) Total sugars	
(1) in solution	8 μg. in 3/4 ml. (colorimetric); 400 μg. in 1 ml. (titrimetric)
(2) in wood	0.3 g. wood
(3) in pulp	0.1 g. (can be scaled down to 25 mg.) pulp
(b) specific sugars (chromatographic)	
(1) ratios in solution	0.25 ml. containing 100 μg. of each sugar (single streaked) 0.50 ml. containing 50 μg. of each sugar (double streaked)
(2) ratios in wood (3 or 5 sugars)	0.3 g.
(3) ratios in pulp (3 or 5 sugars)	0.1 g.
(4) absolute concentration in solutions	0.25 ml. containing 100 μg. of each sugar (single streaked) 0.50 ml. containing 50 μg. of each sugar (double streaked)
(5) absolute concentration in wood	Not done chromatographically (do total sugars and then ratios using 0.3 g.)
(6) absolute concentration in pulp	0.3 g.

SPECTROMETRY NOMENCLATURE[1]

The following list was compiled by Analytical Chemistry. The sources used were: ASTM Committee E-13 on Absorption Spectroscopy (1959 revision of tentative definitions); H. K. Hughes et al. (Anal. Chem. 24(1349), 1952; Chemical Abstracts; and the advisory board of Analytical Chemistry.

Absorbance, A. (Not optical density, absorbancy, or extinction.) Logarithm to the base 10 of the reciprocal of the transmittance $A = \log_{10} (1/T)$.

Absorptivity, a. (Not k.) (Not absorbancy index, specific extinction, or extinction coefficient.) Absorbance divided by the product of the concentration of the substance (in g./l.) and the sample path length (in cm.),

$$a = \frac{A}{bc}$$

Absorptivity, Molar, ϵ. (Not molar absorbancy index, molar extinction coefficient, or molar absorption coefficient.) Product of absorptivity, a, and the molecular weight of the substance.

Angstrom, Å. Unit of length equal to 1/6438.4696 of wavelength of redline of Cd. For practical purposes, it is considered equal to 10^{-8} cm.

Beer's Law. (Representing Beer-Lambert law.) Absorptivity of a substance is a constant with respect to changes in concentration.

Concentration, c. Quantity of the substance contained in a unit quantity of sample. (In absorption spectrometry it is usually expressed in grams or moles per liter.)

Frequency. Number of cycles per unit time.

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[1]Taken from "Spectrometry Nomenclature," Anal. Chem. 38: 201. 1966.

Infrared. The region of the electromagnetic spectrum extending from approximately 0.78 to 300 microns.

Micron, μ. Unit of length equal to 10^{-6} meter.

Millimicron, mμ. Unit of length equal to one thousandth of a micron. Almost but not exactly equal to 10 angstroms.

Sample Path Length, b. (Not ℓ or d.) Internal cell or sample length, usually given in centimeters.

Spectrograph. Instrument with an entrance slit and dispersing device that uses photography to obtain a record of spectral range. The radiant power passing through the optical system is integrated over time, and the quantity recorded is a function of radiant energy.

Spectrometer, Optical. Instrument with an entrance slit, a dispersing device, and with one or more exit slits, with which measurements are made at selected wavelengths within the spectral range, or by scanning over the range. The quantity detected is a function of radiant power.

Spectrometry. Branch of physical science treating the measurement of spectra.

Spectrophotometer. Spectrometer with associated equipment, so that it furnishes the ratio, or a function of the ratio, of the radiant power of two beams as a function of spectral wavelength. These two beams may be separated in time, space, or both.

Transmittance, T. (Not transmittancy or transmission.) The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample.

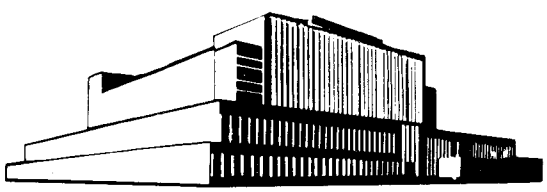
Ultraviolet. The region of the electromagnetic spectrum from approximately 10 to 380 mμ. The term without further qualification usually refers to the region from 200 to 380 mμ.

Visible. Pertaining to radiant energy in the electromagnetic spectral range visible to the human eye (approximately 380 to 780 mμ).

Wavelength. (One word.) The distance, measured along the line of propagation, between two points that are in phase on adjacent waves--units Å., mμ, and μ

Wavenumber. (One word.) Number of waves per unit length. The usual unit of wavenumber is the reciprocal centimeter, $cm.^{-1}$. In terms of this unit, the wavenumber is the reciprocal of the wavelength when the latter is in centimeters in vacuo.

SUBJECT INDEX



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