Designation: E 222 – 00

Standard Test Methods for Hydroxyl Groups Using Acetic Anhydride Acetylation[1]

This standard is issued under the fixed designation E 222; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These test methods cover the determination of hydroxyl groups attached to primary and secondary carbon atoms in aliphatic and alicyclic compounds and phenols.

1.2 Three test methods are given as follows:

	Sections
Test Method A (Pressure Bottle Method)	8-14
Test Method B (Reflux Method)	15-21
Test Method C (Perchloric Acid Catalyzed Method)	22-28

1.2.1 Test Method A is recommended for general use. Test Method B is included to give a standard procedure for the method that has been used widely. Test Method C is recommended when the results are required in a minimum period of time or where ambient temperature for the reaction is desired.

1.2.2 The results obtained using Test Methods A and B will be essentially the same, but the results obtained using Test Method C will be higher (up to approximately 4 % relative) than those obtained using the other two methods.

1.2.3 Statements on precision are included with each test method. The precision of Test Methods A and C is consistent over a wide range of hydroxyl content (tested over hydroxyl number range of 250 to 1600), whereas Test Method B is less precise at the higher hydroxyl content level than it is at the lower hydroxyl content level. In general, Test Method A is approximately two-fold as precise as Test Method C. Test Method B has approximately the same precision as Test Method C at the lower hydroxyl content level but poorer precision at the higher hydroxyl content level.

1.2.4 The interferences are essentially the same for the three methods. Some compounds can be analyzed using Test Methods A or B but not using Test Method C because of interfering reactions of the strong acid catalyst with the compound being analyzed or the acetate product formed in the determination. However, because of its increased reactivity, Test Method C is applicable for determination of some compounds, particularly sterically hindered secondary alcohols, which react too slowly or not at all in Test Methods A and B.

1.3 The values stated in SI units are to be regarded as the standard.

1.4 Review the current appropriate Material Safety Data Sheets (MSDS) for detailed information concerning toxicity, first aid procedures, and safety precautions.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazards statements are given in Section 7.

NOTE 1—Other methods for determination of hydroxyl groups are given in Test Methods D 1957, D 2195, E 326, E 335, and E 567.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

D 1957 Test Method for Hydroxyl Value of Fatty Oils and Acids[3]

D 2195 Test Methods for Pentaerythritol[3]

E 180 Practice for Determining the Precision of ASTM Methods for Analysis and Testing of Industrial Chemicals[4]

E 200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis[4]

E 203 Test Method for Water Using Karl Fischer Reagent[4]

E 326 Test Method for Hydroxyl Groups by Phthalic Anhydride Esterification[4]

E 335 Test Method for Hydroxyl Groups by Pyromellitic Dianhydride Esterification[4]

E 567 Test Method for Tertiary Hydroxyl Groups with Hydrogen Bromide[4]

3. Terminology

3.1 *Definition*:

3.1.1 *hydroxyl number*—the milligrams of potassium hydroxide equivalent to the hydroxyl content of 1 g of material. In the case of a pure compound, the hydroxyl number is inversely proportional to the hydroxyl equivalent weight:

$$\text{equivalent weight (g/equivalent)} = \frac{56100}{\text{hydroxyl number}} \quad (1)$$

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[1] These test methods are under the jurisdiction of ASTM Committee E15 on Industrial and Specialty Chemicals and are the direct responsibility of Subcommittee E15.01 on General Standards..

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 06.03.

[4] *Annual Book of ASTM Standards*, Vol 15.05.

4. Significance and Use

4.1 Hydroxyl is an important functional group, and knowledge of its content is required in many intermediate and end use applications. The test methods described herein are for the determination of primary and secondary hydroxyl groups and can be used for the assay of compounds containing them.

5. Interferences

5.1 Unless stated otherwise, the following interferences apply to all three test methods:

5.1.1 Pentavalent nitrogen compounds, amides, some ethers, and some carbonyl compounds may interfere with the accuracy of the test method.

5.1.2 Tertiary alcohols, cyanohydrins, some hydroxylated fatty acids, certain substituted phenols, and some polyhydroxyl compounds will react in a nonstoichiometric manner.

5.1.3 Primary and secondary amines and mercaptans usually will react quantitatively along with the hydroxyl group.

5.1.4 Excessive amounts of water in the sample will interfere by consuming the reagent. Provisions are made to accommodate a small amount of water by adjustment of the sample size used for the analysis.

5.1.5 Free acids interfere by consuming the standard alkali solution, and strong bases interfere by consuming an equivalent amount of acetic acid; provisions for determining and applying corrections for these interferences are included in the test methods. Some of the higher fatty acids may be converted to anhydrides, releasing water which will consume acetylation reagent.

5.1.6 In Test Method C, epoxy, poly(oxyethylene), poly(oxypropylene), and furan rings interfere. Enols, imides, hydrazides, and some oximes will react in a nonstoichiometric manner.

5.1.7 Phenol (in contrast to other phenolics) gives low results with Test Methods A and B.

5.1.8 With Test Methods A and B, epoxy compounds will give erroneously high results.

NOTE 2—In a study performed by the American Oil Chemists' Society, satisfactory results were obtained with epoxidized soybean oil, epoxidized tall oil, and epoxidized castor oil when the acetylation was carried out at room temperature for 24 h.

5.1.9 Presence of an olefinic or acetylenic unsaturation in the hydroxyl-containing compound should have no effect on the hydroxyl content result obtained with Test Methods A and B, but may give a positive interference with Test Method C.

5.1.10 Test Methods A and B as written (using a visual indicator) may not be applicable to samples containing heat-sensitive impurities, leading to high color in the reacted solution.

6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[5] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 Unless otherwise indicated, references to water shall be understood to mean Type II or Type III reagent water conforming to Specification D 1193.

7. Hazards

7.1 Acetic anhydride, pyridine, and 1,2-dichloroethane are eye, skin, and respiratory irritants. Avoid bodily contact with these reagents and use only in a well-ventilated area.

7.2 Perchloric acid is commonly available in 60 to 72 % concentrations. These solutions may form explosive mixtures with certain organic materials. Dehydrating agents may cause the formation of the anhydrous acid which is unstable at ambient temperature and explodes on contact with most organic materials. The acid is an acute irritant to the eyes, skin, and mucous membranes. Avoid bodily contact. Wash all spills with copious amounts of water.

TEST METHOD A
(Pressure Bottle Method)

8. Summary of Test Method

8.1 The sample is acetylated with a solution of acetic anhydride in pyridine in a pressure bottle at 98°C. The excess reagent is hydrolyzed with water, and the acetic acid is titrated with standard sodium hydroxide solution. The hydroxyl content is calculated from the difference in titration of the blank and sample solutions.

9. Apparatus

9.1 *Bag*, heavy fabric, with draw string, to hold bottle (9.2). As an alternative a stainless steel mesh jacket fitted to cover the bottle may be used.

9.2 *Bottle*, pressure, heat-resistant, approximately 350 mL.[6]

9.3 *Buret*, 100-mL total capacity, range of graduated portion 50 mL, 0.1-mL graduations, preferably equipped with PTFE stopcock (see Note 6).

9.4 *Steam Bath*, 98± 2°C, containing enough water to cover the liquid in the sample bottles. It is critical that the water level be as prescribed and that the temperature be within the prescribed range and uniform throughout the bath.

10. Reagents

10.1 *Acetic Anhydride*. (**Caution:** see 7.1)

10.2 *Acetylation Reagent*—Mix 127 mL of acetic anhydride with 1000 mL of pyridine (10.5). The reagent shall be prepared

[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[6] A suitable bottle is available from B. Preiser Co. Inc., Catalog No. 10-5485, Chemical Rubber Co., Catalog No. 33052A; and Scientific Glass Co., Catalog No. B5317.

fresh daily and kept in a dark bottle. It should not be used if darker than a pale yellow color.

10.3 *Hydrochloric Acid, Standard Solution* (*0.5 N*)—Prepare and standardize in accordance with the appropriate sections of Practice E 200. Determine and record the temperature at which the standardization was performed. The concentration of the solution shall be corrected to the temperature at which the determination is performed as described in 10.6. The factor for the thermal expansion of this solution is 0.00014. This solution is required only if a correction is to be applied for the presence of strong base in the sample being analyzed.

10.4 *Phenolphthalein Indicator Solution*—Dissolve 1 g of phenolphthalein in 100 mL of pyridine.

10.5 *Pyridine*, containing 0.30 to 0.45 % water. Determine the water content of the pyridine using Test Method E 203, and add the required amount of water. The volume of water to add per litre of pyridine may be calculated as follows:

$$\text{Water to add, mL} = 4.0 - 9A \tag{2}$$

where:

A = percent water in pyridine.

10.6 *Sodium Hydroxide, Standard Solution* (*0.5 N*) (**Caution**: See 7.1)—Prepare and standardize in accordance with the appropriate sections of Practice E 200. Determine and record the temperature at which the standardization was performed. The factor for thermal expansion of this solution is 0.00014. For calculation of the hydroxyl content, the normality of the solution shall be corrected to the temperature at which the determination is performed by the following:

$$N_{t2} = N_{t1} + (t_1 - t_2)\,(F) \tag{3}$$

where:

N_{t_1} = normality when standardized,
N_{t_2} = normality during analysis of samples,
t_1 = temperature of solution (°C) during standardization,
t_2 = temperature of solution (°C) during analysis of samples, and
F = factor to correct for thermal expansion of the solution (see each solution for appropriate factor).

11. Procedure

11.1 To each of a sufficient number of pressure bottles to make all blank and sample determinations in duplicate, pipet 20.0 mL of the acetylation reagent. A uniform drainage time must be used for all aliquots.

11.2 Reserve two of the bottles for the blank determination. Into the other bottles introduce an appropriate weight of sample (Note 3, Note 4, and Note 7).

NOTE 3—The sample size is based on a maximum of 9.8 meq of hydroxyl being present. Determine the sample weight using one of the following equations:

$$\text{Sample weight, g} = (561 \times 0.98)/\text{approximate hydroxyl number} \tag{4}$$

$$\text{Sample weight, g} = 0.0098 \times MW/n \tag{5}$$

where:

MW = molecular weight of the hydroxyl-containing compound, and
n = number of hydroxyl groups present in the molecule.

Since the calculated sample weight will be near the maximum permitted by the test method, adhere closely to the indicated weight. The sample should not exceed 10 g.

NOTE 4—If the sample contains an appreciable amount of water, the sample weight must be adjusted to accommodate this interference. In this case, determine the sample weight using one of the following equations:

$$\text{Sample weight, g} = \frac{0.1701 \times 0.98}{0.0094R + [0.01S \times (n \times 17.01)/MW]} \tag{6}$$

$$\text{Sample weight, g} = \frac{550}{\text{approximate hydroxyl number} + (31.2 \times R)} \tag{7}$$

where:

R = water in the sample, %,
S = purity of the sample, %,
MW = molecular weight of the hydroxyl-containing compound, and
n = number of hydroxyl groups present in the molecule.

Precision and accuracy are decreased when appreciable amounts of water are present because of the required decrease in sample size.

11.3 Stopper the bottle and swirl until the sample is completely dissolved. Enclose each bottle in a fabric bag and place all bottles as close together as possible in the steam bath at 98 ± 2°C for 2 h (Note 5). Maintain sufficient water in the bath to cover the level of liquid in the bottles.

NOTE 5—A reaction time of 2 h is satisfactory for most primary alcohols. Secondary alcohols react more slowly, and a general reaction time of 4 h is recommended. For some compounds a shorter or a longer reaction period may be required.

11.4 Remove the bottles from the bath and allow them to cool to room temperature. Untie the bags, uncap the bottles to release any pressure, and then remove the bags.

11.5 Carefully rinse any liquid on the stopper into the bottle and rinse the walls of the flask, using 20 to 30 mL of water. To each of the bottles add clean crushed ice until about one half full.

11.6 Add 1 mL of the phenolphthalein indicator solution and titrate (Note 6) immediately with the 0.5 *N* NaOH solution to the first faint pink end point permanent for 15 s. The solution should be swirled during the titration, with vigorous swirling as the end point is reached. Record the volume of titrant to 0.02 mL (Note 7). Record the temperature of the NaOH solution.

NOTE 6—As a substitute, if the 100-mL buret is not available, the first 50 mL of titrant may be added by pipet (uniform drainage time for all aliquots) and the titration completed with a 50-mL buret.

NOTE 7—If the volume of 0.5 *N* NaOH solution required for the sample is less than 80 % of that required for the blank, the sample was too large and the analysis should be repeated with a smaller weight of sample. In some cases, a smaller excess of acetylation reagent may be adequate, but this must be validated for the particular type compound; in no case should the sample titration be less than 65 % of that required for the blank.

12. Calculation

12.1 Calculate the hydroxyl content in terms of either hydroxyl number or percentage of the hydroxyl-containing compound as follows (Note 8):

$$\text{Hydroxyl number} = \frac{(A - B) \times N_t \times 56.1}{W} \tag{8}$$

$$\text{Hydroxyl-containing compound, weight \%} = \frac{(A - B) \times N_t \times MW \times 100}{W \times n \times 1000} \tag{9}$$

where:

A = NaOH solution, mL, required for titration of the blank,
B = NaOH solution, mL, required for titration of the sample,
N_t = normality of the solution at the temperature during analysis (see 10.6),
n = number of hydroxyl groups in the compound,
MW = molecular weight of the hydroxyl-containing compound, and,
W = sample used, g.

NOTE 8—If the sample contains free acid or strong base, a correction should be applied. This correction may be determined as follows: To 25 mL of pyridine and 25 mL of water in a flask add 1 mL of phenolphthalein indicator solution and titrate with the 0.5 *N* NaOH solution to the first faint pink color which persists for 15 s. To the solution add an accurately weighed sample of approximately the same weight as that used in 10.2, mix by swirling, and titrate to the original end point using 0.5 *N* NaOH solution or 0.5 *N* HCl as required. Use the volume, normality of the titrant, and the weight of sample as "$(A - B)$," "N_t" and "W", respectively, to calculate the correction for hydroxyl number or percent hydroxyl-containing compound in accordance with Section 12. If sodium hydroxide was required to neutralize the sample, add the correction. If hydrochloric acid was required to neutralize the sample, subtract the correction.

13. Report

13.1 Report the percentage of the hydroxyl-containing compound to the nearest 0.1 unit. Report the hydroxyl number to the nearest 0.1 unit if the value is below 100 and to the nearest 1 unit if the value is above 100.

14. Precision and Bias

14.1 *Precision*—The following criteria should be used for judging the acceptability of results (Note 9):

14.1.1 *Repeatability (Single Analyst)*—The coefficient of variation for a single determination has been estimated to be 0.53 % relative at 52 DF. The 95 % limit for the difference between two such runs is 1.5 % relative.

14.1.2 *Laboratory Precision (Between Days Variability)*—The coefficient of variation of results (each the average of duplicates), obtained by the same analyst on different days, has been estimated to be 0.47 % relative at 26 DF. The 95 % limit for the difference between two such averages is 1.3 % relative.

14.1.3 *Reproducibility (Multilaboratory)*—The coefficient of variation of results (each the average of duplicates), obtained by analysts in different laboratories, has been estimated to be 0.62 % relative at 6 DF. The 95 % limit for the difference between two such averages is 1.7 % relative.

14.2 *Bias*—The bias of this test method has not been determined due to the unavailability of suitable reference materials.

NOTE 9—The precision estimates are based on an interlaboratory study performed in 1963[7] on one sample each of dodecanol, 1,6-hexanediol, nonylphenol, and pentaerythritol. Seven laboratories analyzed each sample in duplicate and replicated the analyses on another day for a total of 112 determinations. Practice E 180 was used in developing these precision estimates.

[7] Supporting data are available from ASTM Headquarters. Request RR: E15-1016.

TEST METHOD B
(Reflux Method)

15. Summary of Test Method

15.1 The sample is acetylated with a solution of acetic anhydride in pyridine at the reflux temperature. The excess reagent is hydrolyzed with water, and the acetic acid is titrated with standard sodium hydroxide solution. The hydroxyl content is calculated from the difference in titration of the blank and sample solutions.

16. Apparatus

16.1 *Flasks*, Erlenmeyer, 300-mL, with standard-taper 24/40 joint.

16.2 *Condenser*, West, 400-mm, drip tip, standard-taper 24/40 joint with cooling extending into the joint.

16.3 *Hot Plates*, with variable resistance for temperature control.

16.4 *Buret*, 100-mL total capacity, range of graduated portion 50 mL, 0.1-mL graduations, preferably equipped with PTFE stopcock (see Note 6).

17. Reagents

17.1 See 10.1, 10.3, 10.5 and 10.6.

17.2 *Acetylation Reagent*—Mix 105 mL of acetic anhydride with 1000 mL of pyridine (10.5). The reagent shall be prepared fresh daily and kept in a dark bottle. It should not be used if darker than a pale yellow color. (**Caution**—See 7.1.)

17.3 *Phenolphthalein Indicator Solution*—Dissolve 1 g of phenolphthalein in 100 mL of 1 + 1 aqueous pyridine.

18. Procedure

18.1 Weigh an appropriate size sample into a clean, dry, Erlenmeyer flask. (See Note 3, Note 10, and Note 11.)

NOTE 10—If the volume of 0.5 *N* NaOH solution required for the titration of the sample is less than 80 % of that required for the blank, the sample was too large and the analysis must be repeated with a smaller weight of sample.

NOTE 11—If the sample contains an appreciable amount of water, the sample weight must be adjusted to accommodate this interference. In this case, determine the sample weight using one of the equations given in Note 4.

18.2 Pipet 25.0 mL of the acetylation reagent into the flask; use a uniform drainage time for all aliquots. Connect the flask to the condenser (Note 12), sealing the joint with 1 or 2 drops of pyridine, and place on a hot plate; if necessary, swirl the flask to dissolve the sample. Heat at the reflux temperature for 1½ h (Note 13); the temperature should be regulated so that the vapors condense in the condenser.

NOTE 12—If the surrounding atmosphere is humid, connect the condenser to a drying trap containing a mixture of 2-mesh calcium chloride ($CaCl_2$) with an indicator[8] dispersed throughout.

NOTE 13—For some compounds a shorter or a longer period of reflux may be required.

18.3 Allow the flask to cool somewhat, and then rinse the condenser with 25 mL of water (Note 14). Remove the

[8] Indicating Drierite has been found satisfactory for this purpose.

condenser and rinse the joint of the condenser and the flask with water (Note 14), collecting the rinsing in the flask.

NOTE 14—For easily hydrolyzed materials or materials of unknown stability, substitute pyridine for water in rinsing the condenser. Cool the flask in an ice-water bath before titration. At the end of the titration the temperature of the solution should be no higher than 5°C.

18.4 When the solution is at room temperature (Note 14), add 0.5 to 1.0 mL of phenolphthalein indicator solution and titrate with the 0.5 *N* NaOH solution to the first faint pink end point permanent for 15 s (Note 6). The solution should be swirled or magnetically stirred during the titration, and the solution should be vigorously swirled by hand as the end point is approached. Read the volume of the titrant to 0.02 mL (see Note 10). Record the temperature of the 0.5 *N* NaOH solution.

18.5 Perform a blank determination in parallel using the procedure given in 18.1-18.4, omitting only the addition of sample.

19. Calculation

19.1 Calculate the hydroxyl number or hydroxyl content as described in Section 12.

19.2 If the sample contains free acid or strong base, determine and apply the correction as described in Note 8.

20. Report

20.1 Report the percentage of the hydroxyl-containing compound to the nearest 0.1 unit. Report the hydroxyl number to the nearest 0.1 unit if the value is below 100 and to the nearest 1 unit if the value is above 100.

21. Precision and Bias

21.1 *Precision*—The following criteria should be used for judging the acceptability of results (Note 15):

21.1.1 *Repeatability (Single Analyst)*—At the 250 to 300 hydroxyl number level, the coefficient of variation for a single determination has been estimated to be 0.49 % relative at 44 DF. The 95 % limit for the difference between two such runs is 1.5 % relative. At the 1500 to 1800 hydroxyl number level, the coefficient of variation for a single determination has been estimated to be 0.90 % relative at 41 DF. The 95 % limit for the difference between two such runs is 2.5 % relative.

21.1.2 *Laboratory Precision (Between Days Variability)*—The coefficient of variation of results (each the average of duplicates), obtained by the same analyst on different days, has been estimated to be 0.52 % relative at 38 DF. The 95 % limit for the difference between two such averages is 1.5 relative.

21.1.3 *Reproducibility (Multilaboratory)*:

21.1.3.1 At the 250 to 300 hydroxyl number level, the coefficient of variation of results (each the average of duplicates), obtained by analysts in different laboratories, has been estimated to be 1.03 % absolute at 9 DF. The 95 % limit for the difference between two such averages is 2.9 % absolute.

21.1.3.2 At the 1500 to 1800 hydroxyl number level, the coefficient of variation of results (each the average of duplicates), obtained by analysts in different laboratories, has been estimated to be 1.69 % absolute at 8 DF. The 95 % limit for the difference between two such averages is 4.7 % absolute.

21.2 *Bias*—The bias of this test method has not been determined due to the unavailability of suitable reference materials.

NOTE 15—The precision estimates are based on an interlaboratory study[9] performed in 1961 on one sample each of dodecanol, ethylene glycol, nonylphenol, and pentaerythritol. Eleven laboratories analyzed each sample in duplicate and replicated the analyses on another day for a total of 176 determinations. Practice E 180 was used in developing these precision estimates.

TEST METHOD C
(Perchloric Acid Catalyzed Method)

22. Summary of Test Method

22.1 The sample is acetylated at room temperature with a solution of acetic anhydride and perchloric acid in 1,2-dichloroethane. The excess reagent is hydrolyzed with aqueous dimethylformamide-pyridine, and the resulting acetic acid is titrated with standard sodium hydroxide solution. The hydroxyl content is calculated from the difference in titration of the blank and sample solutions.

23. Apparatus

23.1 *Flask*, Erlenmeyer, 250-mL, with glass stopper.

23.2 *Buret*, 100-mL total capacity, range of graduated portion 50 mL, 0.1-mL graduations, preferably equipped with a PTFE stopcock (see Note 6).

24. Reagents

24.1 *Acetic Anhydride*. (**Caution**—See 7.1)

24.2 *Acetylating Reagent with Perchloric Acid Catalyst*—Acetic anhydride (1 *M*) and perchloric acid (0.15 *N*) in 1,2-dichloroethane. Pour 800 mL of 1,2-dichloroethane into a 1-L glass-stoppered flask and add 12 mL of 72 % perchloric acid and, slowly, while stirring, add 110 mL of acetic anhydride. Dilute with 1,2-dichloroethane to a litre. Allow to come to room temperature. The cooling may be speeded up if necessary. It is recommended that the acetylating reagent not be used unless it is colorless or possesses, at most, a faint yellow color. This reagent is stable for at least 2 months.

NOTE 16—All glassware that has been in contact with perchloric acid or the catalyzed reagent should be rinsed with water before being set aside. Mixtures of perchloric acid and organic matter must not be permitted to concentrate by evaporation because of the possible formation of explosive products.

24.3 *1,2-Dichloroethane*—Spectro-grade is preferred but reagent grade may be used. (**Caution**—See 7.1.)

24.4 *Hydrochloric Acid, Standard Solution* (*0.5 N*)—See 10.3.

24.5 *Indicator Solution*—Titrate 0.1 g of cresol red powder with 13.1 mL of 0.02 *N* sodium hydroxide (NaOH) solution prepared in accordance with the appropriate sections of Practice E 200. Dilute to 100 mL with water to give a 0.1 % neutralized aqueous solution. Similarly prepare a 0.1 % neutralized aqueous solution of thymol blue, using 10.75 mL of 0.02 *N* NaOH solution for 0.1 g of thymol blue powder, and

[9] Supporting data are available from ASTM Headquarters. Request RR: E15-0013. These data have also been used as Example A in Part E2 on Statistical Analysis of Collaborative Data of Practice E 180.

dilute to 100 mL with water. Mix one part of the cresol red solution with 3 parts of the thymol blue solution.

24.6 *Perchloric Acid (72 %)*—Concentrated perchloric acid ($HClO_4$). (**Caution**—See 7.2.)

24.7 *Pyridine*.

24.8 *Dimethylformamide*.

24.9 *Dimethylformamide-Pyridine-Water Solution (6 + 3 + 1)*—Mix 1 volume of water and 3 volumes of pyridine with 6 volumes of dimethylformamide.

24.10 *Sodium Hydroxide, Standard Solution* (*0.5 N*), *Methanolic, Carbonate-Free*—Prepare and standardize in accordance with the appropriate sections of Practice E 200. For calculation of the hydroxyl content, the concentration of the solution shall be corrected to the temperature at which the determination is performed as described in Section 10. The factor for thermal expansion of this solution is 0.00045.

25. Procedure

25.1 To each of a sufficient number of 250-mL glass-stoppered Erlenmeyer flasks to make all blank and sample determinations in duplicate, pipet 20.0 mL of the acetylation reagent. A uniform drainage time must be used for all aliquots.

25.2 Reserve two of the flasks for the blank determination. Into each of the other flasks introduce an appropriate weight of sample (see Note 3 and Note 4).

25.3 Stopper the flask and swirl until solution of the sample is complete. Allow the reaction to proceed at room temperature for 30 min.

NOTE 17—For some compounds a shorter period (often as little as 5 min) or a longer period may be required.

25.4 To each sample and blank add 35 mL of dimethylformamide-pyridine-water solution (6 + 3 + 1), swirl, and allow the flask to stand at least 10 min.

25.5 Add 0.5 to 1 mL of indicator (Note 18) and titrate immediately with 0.5 *N* methanolic sodium hydroxide solution until the color changes from yellow to violet (see Note 6 and Note 19). The solution in the flask should be swirled constantly during the titration. Read the volume of the titrant to 0.02 mL (see Note 10). Determine and record the temperature of the titrant.

NOTE 18—For easily hydrolyzed materials, cool the flask in an ice-water bath before titration. At the end of the titration the temperature of the solution should be no higher than 5°C.

NOTE 19—If the color of the solution prevents accurate observation of the end point, transfer the solution to a 250-mL beaker using a minimum of rinse water and titrate potentiometrically using a glass-calomel electrode pair.

26. Calculation

26.1 Calculate the hydroxyl content in terms of either hydroxyl number or percentage of the hydroxyl-containing compound as follows (Note 20):

$$\text{Hydroxyl number} = \frac{(A - B) \times N_t \times 56.1}{W} \quad (10)$$

$$\text{Hydroxyl-containing compound, wt\%} = \frac{(A - B) \times N_t \times MW \times 100}{W \times n \times 1000} \quad (11)$$

where:

A = NaOH solution, mL, required for titration of the blank,
B = NaOH solution, mL, required for titration of the sample,
N_t = normality of the NaOH solution at the temperature during analysis (see 24.10),
n = number of hydroxyl groups in the compound,
MW = molecular weight of the hydroxyl-containing compound, and
W = sample used, g.

NOTE 20—If the sample contains free acid or strong base, a correction should be applied. This correction may be determined as follows: to 20 mL of 1,2-dichloroethane and 35 mL of dimethylformamide-pyridine-water solution (6 + 3 + 1) add 1 mL of indicator and titrate with the 0.5 *N* NaOH solution to the blue-violet end point. To the solution add an accurately weighed sample of approximately the same weight as that used in 25.2, mix by swirling, and titrate to the original end point using 0.5 *N* NaOH solution or 0.5 *N* HCl as required. Use the volume, normality of the titrant, and the weight of sample as "($A - B$)," "N_t" and "W," respectively, to calculate the correction for hydroxyl number or percent hydroxyl-containing compound in accordance with Section 26. If sodium hydroxide was required to neutralize the sample, add the correction. If hydrochloric acid was required to neutralize the sample, subtract the correction.

27. Report

27.1 Report the percentage of the hydroxyl-containing compound to the nearest 0.1 unit. Report the hydroxyl number to the nearest 0.1 unit if the value is below 100 and to the nearest 1 unit if the value is above 100.

28. Precision and Bias

28.1 *Precision*—The following criteria should be used for judging the acceptability of results (Note 21):

28.1.1 *Repeatability (Single Analyst)*—The coefficient of variation for a single determination has been estimated to be 0.55 % relative at 58 DF. The 95 % limit for the difference between two such runs is 1.5 % relative.

28.1.2 *Laboratory Precision (Between Days Variability)*—The coefficient of variation of results (each the average of duplicates), obtained by the same analyst on different days, has been estimated to be 0.64 % relative at 29 DF. The 95 % limit for the difference between two such averages is 1.8 % relative.

28.1.3 *Reproducibility (Multilaboratory)*—The coefficient of variation of results (each the average of duplicates), obtained by analysts in different laboratories, has been estimated to be 1.3 % relative at 7 DF. The 95 % limit for the difference between two such averages is 3.6 % relative.

28.2 *Bias*—The bias of this test method has not been determined due to the unavailability of suitable reference materials.

NOTE 21—The precision estimates are based on an interlaboratory study[10] performed in 1971 on one sample each of dodecanol, 1,6-hexanediol, nonylphenol, and pentaerythritol. Nine laboratories analyzed each sample in duplicate and replicated the analyses on another day for a total of 144 determinations. Practice E 180 was used in developing these precision estimates.

[10] Supporting data are available from ASTM Headquarters. Request RR: E15-1016.

29. Keywords

29.1 acetic anhydride; acetylation; hydroxyl; hydroxyl groups; hydroxyl number; primary; secondary; volumetric