and filter with a mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If a heavy precipitate has formed in the cylinder, centrifugalize at low speed, decant the clear liquid, and wash 3 times with 20 cc. portions of the alcohol-ether mixture, shaking the mixture thoroughly each time and separating the precipitate by means of the centrifuge. Wash the paper with the alcohol-ether mixture and evaporate the filtrate and washings on the water bath to about 5 cc., add 20 cc. of H₂O, and again evaporate to 5 cc.; again add 20 cc. of H₂O and evaporate to 5 cc.; finally add 10 cc. of H₂O and evaporate to 5 cc.

These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if the concentration of the latter is less than 50%.

Transfer the residue with hot H₂O to a 50 cc. volumetric flask, cool, add the Ag₂CO₃ prepared from 0.1 g. of Ag₂SO₄, shake, and allow to stand 10 minutes. Then add 0.5 cc. of basic Pb-acetate solution, shake occasionally, and allow to stand 10 minutes. Make up to the mark, shake well, filter, rejecting the first portion of the filtrate, and pipet 25 cc. of the clear filtrate into a 250 cc. volumetric flask.

Add 1 cc. of H₂SO₄ to precipitate the excess of Pb and then 30 cc. of Reagent (a). Add carefully 24 cc. of H₂SO₄, rotating the flask gently to mix the contents and avoid violent ebullition, and then place in a boiling water bath for exactly 20 minutes. Remove the flask from the bath, dilute, cool, and make up to the mark at room temperature. The quantity of strong dichromate solution used must be sufficient to leave an excess of about 12.5 cc. at the end of the oxidation, the quantity given above (30 cc.) being sufficient for ordinary vinegar containing about 0.35 g. or less of glycerol per 100 cc.

Standardize the ferrous ammonium sulfate solution against Reagent (b) by introducing from the respective burets approximately 20 cc. of each of these solutions into a beaker containing 100 cc. of H₂O. Complete the titration, using Reagent (d) as an outside indicator. From this titration calculate the volume (F) of the ferrous ammonium sulfate solution equivalent to 20 cc. of the dilute and therefore, to 1 cc. of Reagent (a).
In place of Reagent (b) solution substitute a buret containing the oxidized glycerol with an excess of Reagent (a) and ascertain how many cc. are equivalent to \((F)\) cc. of the ferrous ammonium sulfate solution and, therefore, to 1 cc. of Reagent (a). Then 250, divided by this last equivalent, \(=\) the number of cc. of Reagent (a) present in excess in the 250 cc. flask after oxidation of the glycerol.

The number of cc. of Reagent (a) added, minus the excess found after oxidation, multiplied by 0.02, gives the grams of glycerol per 100 cc. of vinegar.

9. GLYCEROL IN SWEET WINES—OFFICIAL

With wines in which the extract exceeds 5 g. per 100 cc., heat 100 cc. to boiling in a flask and treat with successive small portions of milk of lime until the wine becomes first darker and then lighter in color. Cool, add 200 cc. of 95% alcohol, allow the precipitate to subside, filter, and wash with 95% alcohol. Treat the combined filtrate and washings as directed under 5 or 6.

10. GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express this ratio as \(X:100\), in which \(X\) is obtained by multiplying the percentage weight of glycerol by 100 and dividing the result by the percentage of alcohol by weight.

EXTRACT

11. I. From the Specific Gravity of the Dealcoholized Wine—Official

Calculate the specific gravity of the dealcoholized wine by the following formula:

\[
S = G + 1 - A, \quad \text{in which}
\]

- \(S\) = specific gravity of the dealcoholized wine;
- \(G\) = specific gravity of the wine, 3; and
- \(A\) = specific gravity of the distillate obtained in the determination of alcohol, 4 (a).

From Table A2, ascertain the percentage by weight of extract in the dealcoholized wine corresponding to the value of \(S\). Mul-
tiply the figure thus obtained by the value of 5 to obtain the g. of extract per 100 cc. of wine.

12. II. By Evaporation—Official

(a) In dry wines, having an extract content of less than 3 grams per 100 cc.—Evaporate 50 cc. of the sample on a water bath to a sirupy consistency in a 75 cc. flat-bottomed Pt dish, approximately 85 mm. in diameter. Heat the residue for 2–5 hours in a drying oven at the temperature of boiling H₂O, cool in a desiccator, and weigh as soon as the dish and contents reach room temperature.

(b) In sweet wines.—If the extract content is between 3 and 6 g. per 100 cc., treat 25 cc. of the sample as directed under (a). If the extract exceeds 6 g. per 100 cc., however, the result, obtained as directed under 11, is accepted, and no gravimetric determination is attempted because of the inaccurate results obtained by drying levulose at a high temperature.

13. NON-SUGAR SOLIDS—OFFICIAL

Determine the non-sugar solids (sugar-free extract) by subtracting the quantity of reducing sugars before inversion, 14, from the extract, 11 or 12. If sucrose is present in the wine, determine the nonsugar solids by subtracting the sum of reducing sugars before inversion and the sucrose from the extract.

14. REDUCING SUGARS—OFFICIAL

(a) Dry wines.—Place 200 cc. of the wine in a porcelain dish; exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity, 44; and evaporate to about ¾ the original volume. Transfer to a 200 cc. flask, add sufficient neutral Pb-acetate solution to clarify, dilute to the mark with H₂O, shake, and pass through a folded filter. Remove the Pb with dry K-oxalate and determine, reducing sugars as directed under 15–18.

(b) Sweet wines.—With sweet wines, approximate the sugar content by subtracting 2 from the result in the determination of
the extract and employ such a quantity of the sample that the aliquot taken for the Cu reduction shall not exceed 240 mg. of invert sugar. Proceed as directed under (a) except to take this smaller quantity of the sample for the determination.

15. *Munson and Walker General Method—Official*

**REAGENTS**

(a) *Asbestos.*—Digest the asbestos, which should be the amphibole variety, with HCl \( (1 + 3) \) for 2–3 days. Wash free from acid, digest for a similar period with 10% NaOH solution, and then treat for a few hours with hot alkaline tartrate solution (old alkaline tartrate solutions that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash the asbestos free from alkali; digest for several hours with HNO\(_3\) \( (1 + 3) \); and, after washing free from acid, shake with H\(_2\)O into a fine pulp. In preparing the Gooch crucible, make a film of asbestos \( \frac{1}{4} \) inch thick and wash thoroughly with H\(_2\)O to remove fine particles of asbestos. If the precipitated Cu\(_2\)O is to be weighed as such, wash the crucible with 10 cc. of alcohol, then with 10 cc. of ether; dry for 30 minutes at 100°; cool in a desiccator; and weigh.

*Soxhlet's modification of Fehling's solution.*—Prepared by mixing immediately before use, equal volumes of (a) and (b).

(a) *Copper sulfate solution.*—Dissolve 34.639 g. of CuSO\(_4\).5H\(_2\)O in H\(_2\)O, dilute to 500 cc., and filter through prepared asbestos.

(b) *Alkaline tartrate solution.*—Dissolve 173 g. of Rochelle salts and 50 g. of NaOH in H\(_2\)O, dilute to 500 cc., allow to stand for 2 days, and filter through prepared asbestos.

16. **PRECIPITATION OF CUPROUS OXIDE**

Transfer 25 cc. of each of the CuSO\(_4\) and alkaline tartrate solutions to a 400 cc. beaker of alkali-resistant glass and add 50 cc. of the reducing sugar solution, or if a smaller volume of sugar solution is used, add H\(_2\)O to make the final volume 100 cc.
Heat the beaker on an asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 minutes, and continue the boiling for exactly 2 minutes. (It is important that these directions be strictly observed. To regulate the burner for this purpose it is advisable to make preliminary tests, using 50 cc. of the reagent and 50 cc. of H₂O before proceeding with the actual determination.) Keep the beaker covered with a watch-glass during the heating. Filter the hot solution at once through an asbestos mat in a porcelain Gooch crucible, using suction. Wash the precipitate of Cu₂O thoroughly with H₂O at a temperature of about 60° and either weigh directly as Cu₂O as directed under 17, or determine the quantity of reduced Cu as described under 18. Conduct a blank determination, using 50 cc. of the reagent and 50 cc. of H₂O, and if the weight of Cu₂O obtained exceeds 0.5 mg., correct the result of the reducing sugar determination accordingly. The alkaline tartrate solution deteriorates on standing, and the quantity of Cu₂O obtained in the blank increases.

17. Determination of Reduced Copper

Direct Weighing of Cuprous Oxide

(This method should be used only for determinations in solutions of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities, including sucrose, determine the Cu of the Cu₂O by one of the methods described under 18, since the Cu₂O is very likely to be contaminated with foreign matter.)

Prepare a Gooch crucible as directed under 15. Collect the precipitated Cu₂O on the mat as directed under 16 and wash thoroughly with hot H₂O, then with 10 cc. of alcohol, and finally with 10 cc. of ether. Dry the precipitate for 30 minutes in a water oven at the temp. of boiling H₂O, cool, and weigh. Calculate the weight of metallic Cu, using the factor 0.8882. Obtain from Table A7 the weight of invert sugar equivalent to the weight of Cu.

The number of mg. of Cu reduced by a given quantity of reducing sugar varies, depending upon whether or not sucrose is
present. In the tables the absence of sucrose is assumed except in the entries under invert sugar, where, in addition to the column for invert sugar alone, there are given one column for mixtures of invert sugar and sucrose containing 0.4 g. of total sugar in 50 cc. of solution and one column for invert sugar and sucrose when the 50 cc. of solution contains 2 g. of total sugar. Two entries are also given under lactose and sucrose mixtures, showing proportions of 1 part lactose to 4 and 12 parts of sucrose, respectively.

18. REAGENTS

Volumetric Thiosulfate Method

Standard thiosulfate solution.—Prepare a solution of Na$_2$S$_2$O$_3$ containing 19 g. of pure crystals in 1 liter. Weigh accurately about 0.2 g. of pure Cu and place in a flask of 250 cc. capacity. Dissolve by warming with 5 cc. of a mixture of equal volumes of strong HNO$_3$ and H$_2$O. Dilute to 50 cc., boil to expel the red fumes, add a slight excess of strong Br water, and boil until the Br is completely driven off. Cool, and add a strong NaOH solution with agitation until a faint turbidity of Cu(OH)$_2$ appears (about 7 cc. of a 25% NaOH solution is required). Discharge the turbidity with a few drops of 80% acetic acid and add 2 drops in excess. (The solution should now occupy a volume of 50–70 cc.) Add 10 cc. of 30% KI solution. Titrate at once with the thiosulfate solution until the brown tinge becomes weak and add sufficient starch indicator to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. (If at this point the thiosulfate is added dropwise and a little time is allowed for complete reaction after each addition, no difficulty is experienced in determining the end point within a single drop.) 1 cc. of the thiosulfate solution = about 0.005 g. of Cu.

18a. DETERMINATION

After washing the precipitated Cu$_2$O, cover the Gooch with a watch-glass and dissolve the oxide by means of 5 cc. of warm HNO$_3$ (1 + 1) poured under the watch-glass with a pipet. Col-
SUCROSE

lect the filtrate in a 250 cc. flask and wash the watch-glass and the Gooch free from Cu, using about 50 cc. of H₂O. Boil to expel red fumes; add a slight excess of Br water; boil off the Br completely; and proceed as directed under 18, beginning with "Cool and add a strong NaOH solution. . . ."

SUCROSE

19. I. By Reducing Sugars Before and After Inversion—Official

Determine the reducing sugars (clarification having been effected with neutral Pb-acetate, never with basic Pb-acetate) as directed under 15 and calculate to invert sugar from A7. Invert the solution as directed under 20 (b) or (c), or 22 (b) or (c); exactly neutralize the acid; and again determine the reducing sugars, but calculate them to invert sugar from the table referred to above, using the invert sugar column alone. Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion and multiply the difference by 0.95 to obtain the percentage of sucrose. The solutions should be diluted in both determinations so that not more than 240 mg. of invert sugar is present in the quantity taken for reduction. It is important that all lead be removed from the solution with anhydrous powdered K-oxalate or Na₂CO₃ before reduction.

20. II. By Polarization—Official

Polarize before and after inversion in a 200 mm. tube, as directed under 20 or 22, a portion of the filtrate obtained under 14. In calculating the percentage of sucrose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc. to the normal weight for the instrument.

(a) Direct reading.—Pipet one 50 cc. portion of the Pb-free filtrate into a 100 cc. flask, dilute with H₂O to the mark, mix well, and polarize in a 200 mm. tube. The result, multiplied by 2, is the direct reading (P of formula given below) or polarization before inversion. (If a 400 mm. tube is used, the reading equals P.)
(b) Invert reading.—First determine the quantity of acetic acid necessary to render 50 cc. of the Pb-free filtrate distinctly acid to methyl red indicator; then to another 50 cc. of the lead-free solution in a 100 cc. volumetric flask, add the requisite quantity of acid and 5 cc. of the invertase preparation, fill the flask with H₂O nearly to 100 cc., and let stand overnight (preferably at a temperature not less than 20°). Cool, and dilute to 100 cc. at 20°. Mix well and polarize at 20° in a 200 mm. tube. If the analyst is in doubt as to the completion of the hydrolysis, allow a portion of the solution to remain for several hours and again polarize. If there is no change from the previous reading, the inversion is complete, and the reading and temperature of the solution should be carefully noted. If it is necessary to work at a temperature other than 20°, which is permissible within narrow limits, the volumes must be completed and both direct and invert readings must be made at the same temperature. Correct the invert reading for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the following formula:

\[ S = \frac{100 (P - I)}{142.1 + 0.073 (m - 13) - t/2}, \]

in which

- \( S \) = percentage of sucrose;
- \( P \) = direct reading, normal soln.;
- \( I \) = invert reading, normal soln.;
- \( t \) = temp. at which readings are made; and
- \( m \) = g. of total solids in 100 cc. of the invert soln. read in the polariscope.

Determine the total solids as directed under 11.

(c) Rapid inversion at 55–60°.—If more rapid inversion is desired, proceed as follows: Prepare the sample as directed under (a) and to 50 cc. of the Pb-free filtrate in a 100 cc. volumetric flask add glacial acetic acid in sufficient quantity to render the solution distinctly acid to methyl red. The quantity of acetic acid required should be determined before pipetting the 50 cc. portion. Then add 10 cc. of invertase solution, mix thoroughly, place the flask in a water bath at 55–60°, and allow to stand at that temperature for 15 minutes with occasional shaking. Cool, add Na₂CO₃ solution until dis-
tinctly alkaline to litmus paper, dilute to 100 cc. at 20°, mix well, and determine the polarization at 20° in a 200 mm. tube. Allow the solution to remain in the tube for 10 minutes and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. Carefully note the reading and the temperature of the solution. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the formula given under (b).

(If the solution has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposition of fructose ordinarily is more rapid than that of the other sugars present. If the solution has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 minutes if he has satisfied himself that he is adding Na₂CO₃ in sufficient amount to complete mutarotation at once without causing any destruction of sugar during the period intervening before polarization.)

21. II. By Polarization Before and After Inversion With Hydrochloric Acid—Official

(In the presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, the optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

22. (a) Direct reading.—Proceed as directed under 20 (a).

(b) Invert reading.—Pipet a 50 cc. portion of the Pb-free filtrate into a 100 cc. flask and add 25 cc. of H₂O. Then add, little by little, while rotating the flask, 10 cc. of HCl (sp. gr. 1.1029 at 20/4° (or 24.85° Brix at 20°)). Heat a water bath to 70° and regulate the burner so that the temperature of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates 67°. This preliminary heating period should require from 2½-2¾ minutes.
ANALYSIS OF ALCOHOLIC BEVERAGES

From the moment the thermometer in the flask indicates 67°, leave the flask in the bath for exactly 5 minutes longer, during which time the temperature should gradually rise to about 69.5°. Plunge the flask as once into H₂O at 20°. When the contents have cooled to about 35°, remove the thermometer from the flask, rinse it, and fill almost to the mark. Leave the flask in the bath at 20° for at least 30 minutes longer and finally make up exactly to volume. Mix well and polarize the solution in a 200 mm. tube provided with a lateral branch and a water jacket, maintaining a temperature of 20°. This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temperature other than 20°, which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temp.

Calculate sucrose by the following formula:

\[ S = \frac{100 (P - I)}{143 + 0.0676 (m - 13) - t/2}, \]

in which

- \( S \) = percentage of sucrose;
- \( P \) = direct reading, normal soln.;
- \( I \) = invert reading, normal soln.;
- \( t \) = temp. at which readings are made; and
- \( m \) = g. of total solids in 100 cc. of the invert soln. read in the polariscope.

Determine the total solids as directed under 11.

(c) Inversion at room temperature.—The inversion may also be accomplished as follows: (1) To 50 cc. of the clarified soln., freed from Pb, add 10 cc. of HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°) and set aside for 24 hours at a temp. not below 20°; or, (2) if the temp. is above 25°, set aside for 10 hours. Make up to 100 cc. at 20° and polarize as directed under (b). Under these conditions the formula must be changed to the following:

\[ S = \frac{100 (P - I)}{143.2 + 0.0676 (m - 13) - t/2}. \]

23. COMMERCIAL GLUCOSE—OFFICIAL

Polarize a portion of the clarified filtrate after inversion in a 200 mm. jacketed tube at 87°, as directed under 24. In calcu-
lating the percentage of glucose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc. to the normal weight for the instrument.

24. Commercial glucose cannot be determined accurately owing to the varying quantities of dextrin, maltose, and dextrose present in the product. However, in sirups in which the quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by the following formula:

\[ G = \frac{(a - S) \times 100}{211}, \text{ in which} \]

- \( G \) = percentage of commercial glucose solids;
- \( a \) = direct polarization, normal soln.; and
- \( S \) = percentage of cane sugar.

Express the results in terms of commercial glucose solids polarizing \(+211^\circ\text{V}\). (This result may be recalculated in terms of commercial glucose of any Baume reading desired.)

25. ASH—OFFICIAL

Proceed as directed below using the residue from 50 cc. of the wine.

Weigh a quantity of the substance representing about 2 g. of dry material and burn at a low heat, not exceeding dull redness, until free from C. If a C-free ash cannot be obtained in this manner, exhaust the charred mass with hot \( \text{H}_2\text{O} \), collect the insoluble residue on an ashless filter, and burn the filter and contents to a white or nearly white ash. Add the filtrate, evaporate to dryness, and heat at dull redness until the ash is white or grayish white. Cool in a desiccator and weigh.

26. ASH EXTRACT RATIO—OFFICIAL

Express results as \( 1 : X \), in which \( X \) is the quotient obtained by dividing the g. of extract per 100 cc. by the g. of ash per 100 cc.
276  ANALYSIS OF ALCOHOLIC BEVERAGES

27.  ALKALINITY OF THE WATER-SOLUBLE ASH—OFFICIAL

Extract the ash obtained as directed under 25 with successive small portions of hot H₂O until the filtrate amounts to about 60 cc.

Cool the filtrate and titrate with 0.1 N HCl, using methyl orange indicator. Express the alkalinity in terms of the number of cc. of 0.1 N acid per 100 cc. of the wine.

28.  ALKALINITY OF THE WATER-INSOLUBLE ASH—OFFICIAL

Ignite the filter and residue from 27 in the Pt. dish in which the wine was ashed and proceed as directed below. Express the alkalinity in terms of the number of cc. of 0.1 N acid required to neutralize the water-insoluble ash from 100 cc. of the wine.

Add an excess of 0.1 N HCl (usually 10-15 cc.) to the ignited insoluble ash in the Pt. dish, heat to boiling on an asbestos plate, cool, and titrate the excess of HCl with 0.1 N NaOH, using methyl orange indicator.

29.  PHOSPHORIC ACID—OFFICIAL

Dissolve the ash obtained as directed under 25 in 50 cc. of boiling HNO₃ (1 + 9), filter, wash the filter, and determine P₂O₅ in the combined filtrate and washings as directed below. If the ash ignites without difficulty, no free phosphoric acid need be suspected. Should there be any free acid, the ash remains black even after repeated leaching. In such cases, add Ca-acetate or a mixture containing 3 parts of Na₂CO₃ and 1 part of NaNO₃ to avoid loss of P₂O₅ before attempting to ash. Add NH₄OH in slight excess; and barely dissolve the precipitate formed with a few drops of HNO₃, stirring vigorously. If HCl or H₂SO₄ has been used as a solvent, add about 15 g. of crystalline NH₄NO₃ or a soln. containing that quantity. To the hot soln. add 70 cc. of molybdate soln. for every decigram of P₂O₅ present. Digest at about 65° for 1 hour, and determine whether or not the P₂O₅ has been completely precipitated by the addition of more molybdate soln. to the clear supernatant liquid. Filter,
and wash with cold H₂O or preferably with the NH₄NO₃ soln. Dissolve the precipitate on the filter with NH₄OH (1 + 1) and hot H₂O and wash into a beaker to a volume of not more than 100 cc. Neutralize with HCl, using litmus paper or bromthymol blue as an indicator; cool; and from a buret add slowly (about 1 drop per second), stirring vigorously, 15 cc. of magnesia mixture for each decigram of P₂O₅ present. After 15 min. add 12 cc. of NH₄OH. Let stand until the supernatant liquid is clear (usually 2 hours), filter, wash the precipitate with the dilute NH₄OH until the washings are practically free from chlorides, dry, burn first at a low heat and ignite to constant weight, preferably in an electric furnace, at 950-1,000°; cool in a desiccator, and weigh as Mg₂P₂O₇. Calculate and report the result as percentage of P₂O₅.

30. SULFURIC ACID—OFFICIAL

Precipitate directly the H₂SO₄ in 50 cc. of the wine by means of 10% BaCl₂ soln. after acidifying with a small excess of HCl, and determine the resulting BaSO₄ as directed below. Allow the precipitate to stand for at least 6 hours before filtering. Report as SO₃, using the factor 0.3430.

Heat to boiling and add slowly in small quantities a 10% BaCl₂ soln. until no further precipitate is formed. Continue the boiling for about 5 min. and allow to stand for 5 hours or longer in a warm place. Decant the liquid through an ashless filter or an ignited and weighed Gooch crucible, treat the precipitate with 15-20 cc. of boiling H₂O, transfer to the filter, and wash with boiling H₂O until the filtrate is free from chlorides. Dry the precipitate and filter, ignite, and weigh as BaSO₄.

31. CHLORIDES—OFFICIAL

To 100 cc. of dry wine or 50 cc. of sweet wine, add sufficient Na₂CO₃ to make distinctly alkaline. Evaporate to dryness, ignite at a heat not above low redness, cool, extract the residue with hot H₂O, acidify the water extract with HNO₃ (1 + 4), and determine chlorides as directed under 32 or 34.
32. **Gravimetric Method**

To the soln. prepared as directed in 31 add a 10% AgNO₃ soln., avoiding more than a slight excess. Heat to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a weighed Gooch crucible, previously heated to 140-150°, and wash with hot H₂O, testing the filtrate to prove excess of AgNO₃. Dry the AgCl at 140-150°, cool, and weigh. Report as percentage of Cl.

33. **Volumetric Method**

**REAGENTS**

(a) *Silver nitrate.*—Adjust to exact 0.1 N strength by standardizing against a 0.1 N NaCl soln. containing 5.846 g. of pure NaCl per liter.

(b) *Ammonium or potassium thiocyanate.*—0.1 N. Adjust by titrating against the 0.1 N AgNO₃.

(c) *Ferric indicator.*—A saturated soln. of ferric ammonium alum.

(d) *Nitric acid.*—Free from lower oxides of N by diluting the usual pure acid with about ¼ volume of H₂O, and boiling until perfectly colorless.

34. **DETERMINATION**

To the soln. prepared as directed under 31, add a known volume of the 0.1 N AgNO₃ in slight excess. Stir well, filter, and wash the AgCl precipitate thoroughly. To the combined filtrate and washings add 5 cc. of the ferric indicator and a few cc. of the HNO₃ and titrate the excess of Ag with the 0.1 N thiocyanate until a permanent light brown color appears. From the number of cc. of 0.1 N AgNO₃ used, calculate the quantity of Cl. 1 cc. of 0.1 N AgNO₃ = 0.00355 g. of Cl.

35. **TOTAL ACIDS—OFFICIAL**

Measure 20 cc. of the wine into a 250 cc. beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1 N NaOH
Determine the end point with neutral 0.05% azolitmin soln. as an outside indicator. Place the indicator in the cavities of a spot plate and spot the wine into the azolitmin soln. The end point is reached when the color of the indicator remains unchanged by the addition to the wine of a few drops of 0.1 N alkali.

In the case of wines that are artificially colored and therefore cannot be titrated satisfactorily in the above manner, it will be found helpful to use phenolphthalein powder (one part of phenolphthalein mixed with 100 parts of dry, powdered K₂SO₄) as an indicator. Place this indicator in the cavities of a spot plate and spot the wine into the powder. The end of the titration is indicated when the powder acquires a pink tint.

Express the result in terms of tartaric acid. 1 cc. of 0.1 N NaOH soln. = 0.0075 g. of tartaric acid.

VOLATILE ACIDS

36. Method I—Official

Heat rapidly to incipient boiling 50 cc. of the wine in a 500 cc. distillation flask and pass steam through until 15 cc. of the distillate requires only 2 drops of 0.1 N NaOH soln. for neutralization. Boil the H₂O used to generate the steam several minutes before connecting the steam generator with the distillation flask in order to expel CO₂. Titrate rapidly with 0.1 N NaOH soln., using phenolphthalein indicator. The color should remain about 10 seconds. Express the result as acetic acid. 1 cc. of 0.1 N NaOH soln. = 0.0060 g. of acetic acid.

37. Method II—Official

Introduce 10 cc. of the wine, previously freed from CO₂, into the inner tube of a modified Sellier distillation apparatus (Fig. 50); add a small piece of paraffin to prevent foaming; and adjust the tube and its contents in place within the larger flask, which contains 100 cc. of recently boiled H₂O. Connect with a condenser as illustrated in the figure and distil by heating the outer
When 50 cc. of the distillate has been collected, empty the receiver into a beaker and titrate with 0.1 N NaOH soln., using phenolphthalein indicator. Continue the distillation and titrate each succeeding 10 cc. of distillate until not more than 1 drop of standard alkali is required to reach the neutral point. Usually 80 cc. of distillate will contain all the volatile acids.

**Fig. 50.**

38. FIXED ACIDS—OFFICIAL

To obtain the quantity of fixed acids, expressed as tartaric acid, multiply the quantity of volatile acids by 1.25 and subtract this product from the total acids.

39. TOTAL TARTARIC ACID—OFFICIAL

Neutralize 100 cc. of the wine with N NaOH soln., calculating from the acidity, 44, the number of cc. of N alkali necessary for the neutralization. If the volume of the soln. is increased more than 10% by the addition of the alkali, evaporate to approximately 100 cc. Add to the neutralized soln. 0.075 g. of tartaric acid for each cc. of N alkali added and after the tartaric acid has dissolved add 2 cc. of glacial acetic acid and 15 g. of KCl. After the KCl has dissolved, add 15 cc. of 95% alcohol; stir vigorously until the K-bitartrate begins to precipitate; and let stand in an ice-box at 15-18° for at least 15 hours. Decant the liquid from the separated K-bitartrate on a Gooch crucible prepared with a very thin film of asbestos, or on filter paper in a
Büchner funnel. Wash the precipitate and filter 3 times with a few cc. of a mixture of 15 g. of KCl, 20 cc. of 95% alcohol, and 100 cc. of H₂O, using not more than 20 cc. of the wash soln. in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made; wash the Gooch crucible or Büchner funnel with hot H₂O, using about 50 cc. in all; heat to boiling; and titrate the hot soln. with 0.1 N NaOH soln., using phenolphthalein indicator. Increase the number of cc. of 0.1 N alkali required by 1.5 cc. to allow for the solubility of the precipitate. 1 cc. of 0.1 N alkali is equivalent, under these conditions, to 0.015 g. of tartaric acid. To obtain the g. of total tartaric acid per 100 cc. of the wine, subtract the quantity of tartaric acid added from this result.

40. FREE TARTARIC ACID AND CREAM OF TARTAR—OFFICIAL

Calculate the free tartaric acid and cream of tartar in the following manner:

Let \( A = \) total tartaric acid in 100 cc. of wine, divided by 0.015;
\( B = \) total alkalinity of the ash (sum of \( C \) and \( D \));
\( C = \) alkalinity of water-soluble ash; and
\( D = \) alkalinity of water-insoluble ash.

Then

(1) If \( A \) is greater than \( B \),
    Cream of tartar \( = 0.0188 \times C \), and
    Free tartaric acid \( = 0.015 \times (A - B) \);
(2) If \( A \) equals \( B \) or is smaller than \( B \) but greater than \( C \)
    Cream of tartar \( = 0.0188 \times C \), and
    Free tartaric acid \( = 0 \); and
(3) If \( A \) is smaller than \( C \),
    Cream of tartar \( = 0.0188 \times A \), and
    Free tartaric acid \( = 0 \).

41. TANNIN AND COLORING MATTER—OFFICIAL

REAGENTS

(a) Oxalic acid.—0.1 \( N \). 1 cc. = 0.00416 g. of tannin.
(b) Standard potassium permanganate soln. — Dissolve 1.333 g. of KMnO₄ in 1 liter of H₂O and standardize the soln. against (a).
ANALYSIS OF ALCOHOLIC BEVERAGES

(c) Indigo soln.—Dissolve 6 g. of Na-sulfindigotate in 500 cc. of H₂O by heating, cool, add 50 cc. of H₂SO₄, make up to 1 liter, and filter.

(d) Purified boneblack.—Boil 100 g. of finely powdered boneblack with successive portions of HCl (± 3), filter, and wash with boiling H₂O until free from chlorides. Keep covered with H₂O.

42. DETERMINATION

Dealcoholize 100 cc. of the wine by evaporation and dilute with H₂O to the original volume. Transfer 10 cc. to a 2-liter porcelain dish and add about 1 liter of H₂O and exactly 20 cc. of the indigo soln. Add the standard KMnO₄ soln., 1 cc. at a time, until the blue color changes to green; then add a few drops at a time until the color becomes golden yellow. Designate the number of cc. of KMnO₄ soln. used as "a."

Treat 10 cc. of the dealcoholized wine, prepared as above, for 15 min. with boneblack; filter; and wash thoroughly with H₂O. Add 1 liter of H₂O and 20 cc. of the indigo soln. and titrate with KMnO₄, as above. Designate the number of cc. of KMnO₄ used as "b."

Then a — b = c, the number of cc. of the KMnO₄ soln. required for the oxidation of the tannin and coloring matter in 10 cc. of the wine.

43. CRUDE PROTEIN—OFFICIAL

Determine N in 50 cc. of the wine as directed below, and multiply the result by 6.25.

Place the sample in a digestion flask. Add approximately 0.7 g. of HgO, or its equivalent in metallic Hg, and 20-30 cc. of H₂SO₄ (0.1-0.3 g. of crystallized CuSO₄ may also be used in addition to the Hg, or in many cases, in place of it). Place the flask in an inclined position and heat below the boiling point of the acid until frothing has ceased. (A small piece of paraffin may be added to prevent extreme foaming.) Increase the heat until the acid boils briskly and digest for a time after the mixture...
is colorless or nearly so, or until oxidation is complete. (The digestion usually requires at least 2 hours.)

After cooling, dilute with about 200 cc. of H₂O, and add a few pieces of granulated Zn or pumice stone to prevent bumping, and 25 cc. of K₂S or Na₂S₂O₃ soln. with shaking. (If Na₂S₂O₃ is to be used, it should first be mixed with the NaOH so that they may be added together. When no Hg or HgO is used the addition of K₂S or Na₂S or Na₂S₂O₃ soln. is unnecessary.) Next add sufficient NaOH soln. to make the reaction strongly alkaline (50 cc. is usually sufficient), pouring it down the side of the flask so that it does not mix at once with the acid soln. Connect the flask to the condenser by means of a Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the standard acid in the receiver; mix the contents by shaking; and distil until all NH₃ has passed over into a measured quantity of the standard acid. (The first 150 cc. of the distillate will generally contain all the NH₃.) Titrate with standard alkali soln., using the methyl red or cochineal indicator.

44. PENTOSANS—OFFICIAL

Proceed as directed under 45, 46, except to use 100 cc. of the wine and 43 cc. of HCl in beginning the distillation. Owing to the interference of sugars this determination can be made in dry wines only.

45. REAGENTS

(a) Hydrochloric acid.—Contains 12% by weight HCl. To 1 volume of HCl add 2 volumes of H₂O. Determine the percentage of acid by titration against standard alkali and adjust to proper strength by dilution or addition of more strong acid, as may be necessary.

(b) Phloroglucin.—Dissolve a small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of H₂SO₄. A violet color indicates the presence of diresorcin. A phloroglucin which gives more than a faint coloration may be purified by the following method:
Heat in a beaker about 300 cc. of the dilute HCl (a) and 11 g. of commercial phloroglucin, added in small quantities at a time, stirring constantly until it is nearly dissolved. Pour the hot soln. into a sufficient quantity of the same HCl (cold) to make the volume 1500 cc. Allow to stand at least over night, preferably several days, to permit the diresorcin to crystallize. Filter immediately before using. A yellow tint does not interfere with its usefulness. In using, add the volume containing the required quantity of phloroglucin to the distillate.

46. DETERMINATION

Place the sample in a 300 cc. distillation flask, together with 100 cc. of the dilute HCl and several pieces of recently ignited pumice stone. Place the flask on a wire gauze; connect with a condenser; and heat, rather gently at first, but then regulating so as to distil over 30 cc. in about 10 min. Pass the distillate through a small filter paper. Replace the 30 cc. distilled by a like quantity of the dilute acid, added by means of a separatory funnel in such a manner as to wash down the particles adhering to the sides of the flask, and continue the process until the distillate amounts to 360 cc. To the total distillate add gradually a quantity of phloroglucin dissolved in the dilute HCl and thoroughly stir the resulting mixture. The quantity of phloroglucin used should be about double that of the furfural expected. The soln. turns yellow, then green, and very soon there appears an amorphous greenish precipitate that grows darker rapidly, till it becomes almost black. Make the soln. up to 400 cc. with the dilute HCl and allow to stand over night.

Collect the amorphous black precipitate in a weighed Gooch crucible having an asbestos mat, wash carefully with 150 cc. of H₂O so that the H₂O is not entirely removed from the crucible until the very last, then dry for 4 hours at the temp. of boiling H₂O, cool, and weigh in a weighing bottle. The increase in weight is taken to be furfural phloroglucide. To calculate the furfural, pentose, or pentosan from the phloroglucide, use the following formulas given by Kröber: