that the colorimetric reference solution and that of the specimen under test are treated alike in all respects. The comparison of colors is best made in layers of equal depth, and viewed transversely against a white background (see also *Visual Comparison* under *Spectrophotometry and Light-Scattering* ⟨851⟩). It is particularly important that the solutions be compared at the same temperature, preferably 25°.

Cobaltous Chloride CS—Dissolve about 65 g of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 5 mL of this solution into a 250-mL iodine flask, add 5 mL of hydrogen peroxide TS and 15 mL of sodium hydroxide solution (1 in 5), boil for 10 minutes, cool, and add 2 g of potassium iodide and 20 mL of dilute sulfuric acid (1 in 4). When the precipitate has dissolved, titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 23.79 mg of $CoCl_2 \cdot 6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 59.5 mg of $CoCl_2 \cdot 6H_2O$.

Cupric Sulfate CS—Dissolve about 65 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask, add 40 mL of water, 4 mL of acetic acid, 3 g of potassium iodide, and 5 mL of hydrochloric acid, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 24.97 mg of $CuSO_4 \cdot 5H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 62.4 mg of $CuSO_4 \cdot 5H_2O$.

Ferric Chloride CS—Dissolve about 55 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask, add 15 mL of water, 3 g of potassium iodide, and 5 mL of hydrochloric acid, and allow the mixture to stand for 15 minutes. Dilute with 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 27.03 mg of $FeCl_3 \cdot 6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 45.0 mg of $FeCl_3 \cdot 6H_2O$.

INDICATOR SOLUTIONS

See *TEST SOLUTIONS.*

TEST SOLUTIONS (TS)

Certain of the following test solutions are intended for use as acid-base indicators in volumetric analyses. Such solutions should be so adjusted that when 0.15 mL of the indicator solution is added to 25 mL of carbon dioxide-free water, 0.25 mL of 0.02 N acid or alkali, respectively, will produce the characteristic color change. Similar solutions are intended for use in pH measurement. Where no special directions for their preparation are given, the same solution is suitable for both purposes.

Where it is directed that a volumetric solution be used as the test solution, standardization of the solution used as TS is not required.

In general, the directive to prepare a solution "fresh" indicates that the solution is of limited stability and must be prepared on the day of use.

For the preparation of Test Solutions, use reagents of the quality described under *Reagents.*

Acetaldehyde TS—Mix 4 mL of acetaldehyde, 3 mL of alcohol, and 1 mL of water. Prepare this solution fresh.

Acetate Buffer TS—Dissolve 320 g of ammonium acetate in 500 mL of water, add 5 mL of glacial acetic acid, dilute with water to 1000.0 mL, and mix. This solution has a pH between 5.9 and 6.0.

Acetic Acid, Glacial, TS—Determine the water content of a specimen of glacial acetic acid by the *Titrimetric Method* (see *Water Determination* ⟨921⟩). If the acid contains more than 0.4% of water, add a few mL of acetic anhydride, mix, allow to stand overnight, and again determine the water content. If the acid contains less than 0.02% of water, add sufficient water to make the final concentration between 0.02% and 0.4%, mix, allow to stand overnight, and again determine the water content. Repeat the adjustment with acetic anhydride or water, as necessary, until the resulting solution shows a water content of not more than 0.4%.

Acetic Acid, Strong, TS—Add 300.0 mL of glacial acetic acid, and dilute with water to 1000 mL. This solution contains about 30% (v/v) of CH_3COOH and has a concentration of about 5 N.

Acetic Acid–Ammonium Acetate Buffer TS—Dissolve 77.1 g of ammonium acetate in water, add 57 mL of glacial acetic acid, and dilute with water to 1000 mL.

Acetone, Buffered, TS—Dissolve 8.15 g of sodium acetate and 42 g of sodium chloride in about 100 mL of water, and add 68 mL of 0.1 N hydrochloric acid and 150 mL of acetone. Mix, and dilute with water to 500 mL.

Acid Ferric Chloride TS—Mix 60 mL of glacial acetic acid with 5 mL of sulfuric acid, add 1 mL of ferric chloride TS, mix, and cool.

Acid Ferrous Sulfate TS—See *Ferrous Sulfate, Acid, TS.*

Acid Stannous Chloride TS—See *Stannous Chloride, Acid, TS.*

Acid Stannous Chloride TS, Stronger—See *Stannous Chloride, Acid, TS.*

Albumen TS—Carefully separate the white from the yolk of a strictly fresh hen's egg. Shake the white with 100 mL of water until mixed and all but the chalaza has undergone solution; then filter. Prepare the solution fresh.

Alcohol–Phenol TS—Dissolve 780 mg of phenol in alcohol to make 100 mL.

Alcoholic TS—It contains 95 parts of specially denaturated alcohol 3A with 5 parts of isopropyl alcohol. The final concentrations are approximately 90% alcohol, 5% methanol, and 5% isopropanol.

[NOTE—A suitable grade is available as Reagent alcohol, catalog number R8382, available at www.sigma-aldrich.com.]

Alcoholic Ammonia TS—See *Ammonia TS, Alcoholic.*

Alcoholic Mercuric Bromide TS—See *Mercuric Bromide TS, Alcoholic.*

Alcoholic Potassium Hydroxide TS—See *Potassium Hydroxide TS, Alcoholic.*

Alkaline Cupric Citrate TS—See *Cupric Citrate TS, Alkaline.*

Alkaline Cupric Citrate TS 2—See *Cupric Citrate TS 2, Alkaline.*

Alkaline Cupric Iodide TS—See *Cupric Iodide TS, Alkaline.*

Alkaline Cupric Tartrate TS *(Fehling's Solution)*—See *Cupric Tartrate TS, Alkaline.*

Alkaline Mercuric–Potassium Iodide TS—See *Mercuric–Potassium Iodide TS, Alkaline.*

Alkaline Picrate TS—See *Picrate TS, Alkaline.*

Alkaline Sodium Hydrosulfite TS—See *Sodium Hydrosulfite TS, Alkaline.*

Amaranth TS—Dissolve 20 mg of amaranth in 10 mL of water.

Aminonaphtholsulfonic Acid TS—Accurately weigh 5 g of sodium sulfite, 94.3 g of sodium bisulfite, and 700 mg of 1,2,4-aminonaphtholsulfonic acid, and mix. Prepare aminonaphtholsulfonic acid TS fresh on the day of use by dissolving 1.5 g of the dry mixture in 10 mL of water.

Ammonia TS—It contains between 9.5% and 10.5% of NH_3. Prepare by diluting 350 mL of *Ammonia Water, Stronger* (see in the section, *Reagents*) with water to make 1000 mL.

Ammonia TS 2—Prepare by diluting 13.5 mL of *Ammonia Water, Stronger* (see *Reagent Specifications* in the section *Reagents*) with water to make 100 mL.

Ammonia TS, Alcoholic—A solution of ammonia gas in alcohol. Clear, colorless liquid having a strong odor of ammonia. Specific gravity: about 0.80. It contains between 9% and 11% of NH_3. Store it in alkali-resistant containers, in a cold place.

Ammonia TS, Stronger—Use *Ammonia Water, Stronger* (see in the section *Reagents*).

Ammonia–Ammonium Chloride Buffer TS—Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonium hydroxide, and dilute with water to 1000 mL.

Ammonia–Cyanide TS—Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

Ammoniacal Potassium Ferricyanide TS—Dissolve 2 g of potassium ferricyanide in 75 mL of water, add 25 mL of ammonium hydroxide, and mix.

Ammoniated Cupric Oxide TS—See *Cupric Oxide, Ammoniated, TS*.

Ammonium Acetate TS—Dissolve 10 g of ammonium acetate in water to make 100 mL.

Ammonium Carbonate TS—Dissolve 20 g of ammonium carbonate and 20 mL of ammonia TS in water to make 100 mL.

Ammonium Carbonate TS 2—Prepare a 158-mg/mL solution of ammonium carbonate in water.

Ammonium Chloride TS—Dissolve 10.5 g of ammonium chloride in water to make 100 mL.

Ammonium Chloride–Ammonium Hydroxide TS—Mix equal volumes of water and ammonium hydroxide, and saturate with ammonium chloride.

Ammonium Molybdate TS—Dissolve 6.5 g of finely powdered molybdic acid in a mixture of 14 mL of water and 14.5 mL of ammonium hydroxide. Cool the solution, and add it slowly, with stirring, to a well-cooled mixture of 32 mL of nitric acid and 40 mL of water. Allow to stand for 48 hours, and filter through a fine-porosity, sintered-glass crucible. This solution deteriorates upon standing and is unsuitable for use if, upon the addition of 2 mL of dibasic sodium phosphate TS to 5 mL of the solution, an abundant yellow precipitate does not form at once or after slight warming. Store it in the dark. If a precipitate forms during storage, use only the clear supernatant.

Ammonium Oxalate TS—Dissolve 3.5 g of ammonium oxalate in water to make 100 mL.

Ammonium Phosphate, Dibasic, TS *(Ammonium Phosphate TS)*—Dissolve 13 g of dibasic ammonium phosphate in water to make 100 mL.

Ammonium Polysulfide TS—Yellow liquid, made by saturating ammonium sulfide TS with sulfur.

Ammonium Pyrrolidinedithiocarbamate, Saturated, TS—Add about 10 g of ammonium pyrrolidinedithiocarbamate to a 1000-mL volumetric flask, and dilute with water to volume.

Ammonium Reineckate TS—Shake about 500 mg of ammonium reineckate with 20 mL of water frequently during 1 hour, and filter. Use within 2 days.

Ammonium Sulfide TS—Saturate ammonia TS with hydrogen sulfide by bubbling hydrogen sulfide gas through the solution for 1 minute. This solution must be freshly prepared. The solution is not rendered turbid either by magnesium sulfate TS or by calcium chloride TS (*carbonate*). This solution is unstable for use if an abundant precipitate of sulfur is present.

Residue on ignition: not more than 0.05%.

Ammonium Thiocyanate TS—Dissolve 8 g of ammonium thiocyanate in water to make 100 mL.

Ammonium Vanadate TS—Dissolve 2.5 g of ammonium vanadate in 500 mL of boiling water, cool, and add 20 mL of nitric acid. Mix, cool, and add water to make 1 L. Store in polyethylene containers.

Anthrone TS—Within 12 hours of use, rapidly dissolve 35 mg of anthrone in a hot mixture of 35 mL of water and 65 mL of sulfuric acid. Immediately cool in an ice bath to room temperature, and filter through glass wool. Allow the solution to stand at room temperature for 30 minutes before use.

Antimony Trichloride TS—Dissolve 20 g of antimony trichloride in chloroform to make 100 mL. Filter if necessary.

Barium Chloride TS—Dissolve 12 g of barium chloride in water to make 100 mL.

Barium Hydroxide TS—A saturated solution of barium hydroxide in recently boiled water. Prepare the solution fresh.

Barium Nitrate TS—Dissolve 6.5 g of barium nitrate in water to make 100 mL.

Betanaphthol TS—See *2-Naphthol TS*.

Biuret Reagent TS—Dissolve 1.5 g of cupric sulfate and 6.0 g of potassium sodium tartrate in 500 mL of water in a 1000-mL volumetric flask. Add 300 mL of carbonate-free sodium hydroxide solution (1 in 10), dilute with carbonate-free sodium hydroxide solution (1 in 10) to 1000 mL, and mix.

Blue Tetrazolium TS—Dissolve 500 mg of blue tetrazolium in alcohol to make 100 mL.

Brilliant Blue G TS—Transfer 25 mg of brilliant blue G to a 100-mL volumetric flask, add 12.5 mL of alcohol and 25 mL of phosphoric acid, dilute with water to volume, and mix.

Bromine TS *(Bromine Water)*—A saturated solution of bromine, prepared by agitating 2 to 3 mL of bromine with 100 mL of cold water in a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum. Store it in a cold place, protected from light.

Bromine–Sodium Acetate TS—Dissolve 100 g of sodium acetate in 1000 mL of glacial acetic acid, add 50 mL of bromine, and mix.

***p*-Bromoaniline TS**—Add 8 g of *p*-bromoaniline to a mixture of 380 mL of thiourea-saturated glacial acetic acid, 10 mL of sodium chloride solution (1 in 5), 5 mL of oxalic acid solution (1 in 20), and 5 mL of dibasic sodium phosphate solution (1 in 10) in a low-actinic glass bottle. Mix, and allow to stand overnight before using. Protect from light, and use within 7 days.

Bromocresol Blue TS—Use *Bromocresol Green TS*.

Bromocresol Green TS—Dissolve 50 mg of bromocresol green in 100 mL of alcohol, and filter if necessary.

Bromocresol Green–Methyl Red TS—Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in 180 mL of alcohol, and dilute with water to 200 mL.

Bromocresol Purple TS—Dissolve 250 mg of bromocresol purple in 20 mL of 0.05 N sodium hydroxide, and dilute with water to 250 mL.

Bromophenol Blue TS—Dissolve 100 mg of bromophenol blue in 100 mL of diluted alcohol, and filter if necessary.

Bromothymol Blue TS—Dissolve 100 mg of bromothymol blue in 100 mL of diluted alcohol, and filter if necessary.

Buffered Acetone TS—See *Acetone, Buffered, TS*.

Calcium Chloride TS—Dissolve 7.5 g of calcium chloride in water to make 100 mL.

Calcium Hydroxide TS—Use *Calcium Hydroxide Topical Solution* (USP monograph).

Calcium Sulfate TS—A saturated solution of calcium sulfate in water.

Ceric Ammonium Nitrate TS—Dissolve 6.25 g of ceric ammonium nitrate in 10 mL of 0.25 N nitric acid. Use within 3 days.

Chloral Hydrate TS—Dissolve 50 g of chloral hydrate in a mixture of 15 mL of water and 10 mL of glycerin.

Chlorine TS *(Chlorine Water)*—A saturated solution of chlorine in water. Place the solution in small, completely

filled, light-resistant containers. Chlorine TS, even when kept from light and air, is apt to deteriorate. Store it in a cold, dark place. For full strength, prepare this solution fresh.

Chromotropic Acid TS—Dissolve 50 mg of chromotropic acid or its disodium salt in 100 mL of 75% sulfuric acid, which may be made by cautiously adding 75 mL of sulfuric acid to 33.3 mL of water.

Cobalt–Uranyl Acetate TS—Dissolve, with warming, 40 g of uranyl acetate in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Similarly, prepare a solution containing 200 g of cobaltous acetate in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Mix the two solutions while still warm, and cool to 20°. Maintain the temperature at 20° for about 2 hours to separate the excess salts from solution, and then pass through a dry filter.

Cobaltous Chloride TS—Dissolve 2 g of cobaltous chloride in 1 mL of hydrochloric acid and sufficient water to make 100 mL.

Congo Red TS—Dissolve 500 mg of congo red in a mixture of 10 mL of alcohol and 90 mL of water.

***m*-Cresol Purple TS**—Dissolve 0.10 g of metacresol purple in 13 mL of 0.01 N sodium hydroxide, dilute with water to 100 mL, and mix.

Cresol Red TS—Triturate 100 mg of cresol red in a mortar with 26.2 mL of 0.01 N sodium hydroxide until solution is complete, then dilute the solution with water to 250 mL.

Cresol Red–Thymol Blue TS—Add 15 mL of thymol blue TS to 5 mL of cresol red TS, and mix.

Crystal Violet TS—Dissolve 100 mg of crystal violet in 10 mL of glacial acetic acid.

Cupric Acetate TS—Dissolve 100 mg of cupric acetate in about 5 mL of water to which a few drops of acetic acid have been added. Dilute to 100 mL, and filter, if necessary.

Cupric Acetate TS, Stronger *(Barfoed's Reagent)*—Dissolve 13.3 g of cupric acetate in a mixture of 195 mL of water and 5 mL of acetic acid.

Cupric-Ammonium Sulfate TS—To cupric sulfate TS add ammonia TS, dropwise, until the precipitate initially formed is nearly but not completely dissolved. Allow to settle, and decant the clear solution. Prepare this solution fresh.

Cupric Citrate TS—Dissolve 25 g of cupric sulfate, 50 g of citric acid, and 144 g of anhydrous sodium carbonate in water, and dilute with water to 1000 mL.

Cupric Citrate TS, Alkaline—With the aid of heat, dissolve 173 g of dihydrated sodium citrate and 117 g of monohydrated sodium carbonate in about 700 mL of water, and filter through paper, if necessary, to obtain a clear solution. In a separate container dissolve 17.3 g of cupric sulfate in about 100 mL of water, and slowly add this solution, with constant stirring, to the first solution. Cool the mixture, add water to make 1000 mL, and mix.

Cupric Citrate TS 2, Alkaline—With the aid of heat, dissolve about 173 g of sodium citrate dihydrate and 117 g of sodium carbonate monohydrate in about 700 mL of water, and filter. In a second flask, dissolve about 27.06 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in about 100 mL of water. Slowly combine the two solutions while stirring, and dilute with water to 1000 mL.

Cupric Iodide TS, Alkaline—Dissolve 7.5 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in about 100 mL of water. In a separate container dissolve 25 g of anhydrous sodium carbonate, 20 g of sodium bicarbonate, and 25 g of potassium sodium tartrate in about 600 mL of water. With constant stirring, add the cupric sulfate solution to the bottom of the alkaline tartrate solution by means of a funnel that touches the bottom of the container. Add 1.5 g of potassium iodide, 200 g of anhydrous sodium sulfate, 50 to 150 mL of 0.02 M potassium iodate, and sufficient water to make 1000 mL.

Cupric Oxide, Ammoniated, TS *(Schweitzer's Reagent)*—Dissolve 10 g of cupric sulfate in 100 mL of water, add sufficient sodium hydroxide solution (1 in 5) to precipitate the copper hydroxide, collect the latter on a filter, and wash free from sulfate with cold water. Dissolve the precipitate, which must be kept wet during the entire process, in the minimum quantity of ammonia TS necessary for complete solution.

Cupric Sulfate TS—Dissolve 12.5 g of cupric sulfate in water to make 100 mL.

Cupric Tartrate TS, Alkaline *(Fehling's Solution)*—

The Copper Solution (A)—Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, showing no trace of efflorescence of adhering moisture, in water to make 500 mL. Store this solution in small, tight containers.

The Alkaline Tartrate Solution (B)—Dissolve 173 g of crystallized potassium sodium tartrate and 50 g of sodium hydroxide in water to make 500 mL. Store this solution in small, alkali-resistant containers.

For use, mix exactly equal volumes of *Solutions A* and *B* at the time required.

Delafield's Hematoxylin TS—Prepare 400 mL of a saturated solution of ammonium alum *(Solution A).* Dissolve 4 g of hematoxylin in 25 mL of alcohol, mix it with *Solution A,* and allow it to stand for 4 days in a flask closed with a pledget of purified cotton and exposed to light and air *(Solution B).* Then filter *Solution B,* and add to it a *Solution C* consisting of a mixture of 100 mL of glycerin and 100 mL of methanol. Mix, and allow the mixture to stand in a warm place, exposed to light, for 6 weeks until it becomes dark-colored. Store in tightly stoppered bottles.

For use in staining endocrine tissue, dilute this test solution with an equal volume of water.

Denaturated Alcoholic TS—A specially denaturated alcohol containing either rubber hydrocarbon solvent of heptane or toluene. [NOTE—A suitable grade is available from www.lyondell.com or from www.sasol.com, as Ethanol SDA 2B HEP 200, or Ethanol SDA 2B TOL 200, or Ethanol SDA 2B TOL 190, or Alcohol SDA 2B-2.]

Denigès' Reagent—See *Mercuric Sulfate TS.*

Diazobenzenesulfonic Acid TS—Place in a beaker 1.57 g of sulfanilic acid, previously dried at 105° for 3 hours, add 80 mL of water and 10 mL of diluted hydrochloric acid, and warm on a steam bath until dissolved. Cool to 15° (some of the sulfanilic acid may separate but will dissolve later), and add slowly, with constant stirring, 6.5 mL of sodium nitrite solution (1 in 10). Then dilute with water to 100 mL.

Dichlorofluorescein TS—Dissolve 100 mg of dichlorofluorescein in 60 mL of alcohol, add 2.5 mL of 0.1 N sodium hydroxide, mix, and dilute with water to 100 mL.

2,7-Dihydroxynaphthalene TS—Dissolve 100 mg of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow the solution to stand until the yellow color disappears. If the solution is very dark, discard it and prepare a new solution from a different supply of sulfuric acid. This solution is stable for approximately 1 month if stored in a dark bottle.

Diiodofluorescein TS—Dissolve 500 mg of diiodofluorescein in a mixture of 75 mL of alcohol and 30 mL of water.

Diluted Lead Subacetate TS—See *Lead Subacetate TS, Diluted.*

***p*-Dimethylaminobenzaldehyde TS**—Dissolve 125 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 65 mL of sulfuric acid and 35 mL of water, and add 0.05 mL of ferric chloride TS. Use within 7 days.

Dinitrophenylhydrazine TS—Carefully mix 10 mL of water and 10 mL of sulfuric acid, and cool. To the mixture, contained in a glass-stoppered flask, add 2 g of 2,4-dinitrophenylhydrazine, and shake until dissolved. To the solution add 35 mL of water, mix, cool, and filter.

Diphenylamine TS—Dissolve 1.0 g of diphenylamine in 100 mL of sulfuric acid. The solution should be colorless.

Diphenylcarbazone TS—Dissolve 1 g of crystalline diphenylcarbazone in 75 mL of alcohol, then add alcohol to make 100 mL. Store in a brown bottle.

Dithizone TS—Dissolve 25.6 mg of dithizone in 100 mL of alcohol. Store in a cold place, and use within 2 months.

Dragendorff's TS—Mix 850 mg of bismuth subnitrate with 40 mL of water and 10 mL of glacial acetic acid *(Solu-*

tion A). Dissolve 8 g of potassium iodide in 20 mL of water *(Solution B).* Mix equal portions of *Solution A* and *Solution B* to obtain a stock solution, which can be stored for several months in a dark bottle. Mix 10 mL of the stock solution with 20 mL of glacial acetic acid, and dilute with water to make 100 mL.

Edetate Disodium TS—Dissolve 1 g of edetate disodium in 950 mL of water, add 50 mL of alcohol, and mix.

Eosin Y TS (adsorption indicator)—Dissolve 50 mg of eosin Y in 10 mL of water.

Eriochrome Black TS—Dissolve 200 mg of eriochrome black T and 2 g of hydroxylamine hydrochloride in methanol to make 50 mL.

Eriochrome Cyanine TS—Dissolve 750 mg of eriochrome cyanine R in 200 mL of water, add 25 g of sodium chloride, 25 g of ammonium nitrate, and 2 mL of nitric acid, and dilute with water to 1000 mL.

Fehling's Solution—See *Cupric Tartrate TS, Alkaline.*

Ferric Ammonium Sulfate TS—Dissolve 8 g of ferric ammonium sulfate in water to make 100 mL.

Ferric Chloride TS—Dissolve 9 g of ferric chloride in water to make 100 mL.

Ferroin TS—Dissolve 0.7 g of ferrous sulfate and 1.76 g of *o*-phenanthroline monohydrochloride monohydrate in water, and dilute with water to 100 mL.

Ferrous Sulfate TS—Dissolve 8 g of clear crystals of ferrous sulfate in about 100 mL of recently boiled and thoroughly cooled water. Prepare this solution fresh.

Ferrous Sulfate, Acid, TS—Dissolve 7 g of ferrous sulfate crystals in 90 mL of recently boiled and thoroughly cooled water, and add sulfuric acid to make 100 mL. Prepare this solution immediately prior to use.

Folin-Ciocalteu Phenol TS—Into a 1500-mL flask introduce 100 g of sodium tungstate, 25 g of sodium molybdate, 700 mL of water, 50 mL of phosphoric acid, and 100 mL of hydrochloric acid. Gently reflux the mixture for about 10 hours, and add 150 g of lithium sulfate, 50 mL of water, and a few drops of bromine. Boil the mixture, without the condenser, for about 15 minutes, or until the excess bromine is expelled. Cool, dilute with water to 1 L, and filter: the filtrate has no greenish tint. Before use, dilute 1 part of the filtrate with 1 part of water. When used for protein determination (i.e., Lowry assay), this reagent must be further diluted (1:5) with water. See *Method 2* in *Total Protein Assay* under *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.

Formaldehyde TS—Use *Formaldehyde Solution* (see in the section *Reagents*).

Fuchsin–Pyrogallol TS—Dissolve 100 mg of basic fuchsin in 50 mL of water that previously has been boiled for 15 minutes and allowed to cool slightly. Cool, add 2 mL of a saturated solution of sodium bisulfite, mix, and allow to stand for not less than 3 hours. Add 0.9 mL of hydrochloric acid, mix, and allow to stand overnight. Add 100 mg of pyrogallol, shake until solution is effected, and dilute with water to 100 mL. Store in an amber-colored glass bottle in a refrigerator.

Fuchsin–Sulfurous Acid TS—Dissolve 200 mg of basic fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution of 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid. Dilute the solution with water to 200 mL, and allow to stand for at least 1 hour. Prepare this solution fresh.

Gastric Fluid, Simulated, TS—Dissolve 2.0 g of sodium chloride and 3.2 g of purified pepsin, that is derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protein, in 7.0 mL of hydrochloric acid and sufficient water to make 1000 mL. [NOTE—Pepsin activity is described in the *Food Chemicals Codex* specifications under *General Tests and Assays*.] This test solution has a pH of about 1.2.

Gelatin TS (for the assay of *Corticotropin Injection*)—Dissolve 340 g of acid-treated precursor gelatin (Type A) in water to make 1000 mL. Heat the solution in an autoclave at 115° for 30 minutes after the exhaust line temperature has reached 115°. Cool the solution, and add 10 g of phenol and 1000 mL of water. Store in tight containers in a refrigerator.

Glacial Acetic Acid TS—See *Acetic Acid, Glacial, TS.*

Glucose Oxidase–Chromogen TS—A solution containing, in each mL, 0.5 µmol of 4-aminoantipyrine, 22.0 µmol of sodium *p*-hydroxybenzoate, not less than 7.0 units of glucose oxidase, and not less than 0.5 units of peroxidase, and buffered to a pH of 7.0 ± 0.1.

Suitability—When used for determining glucose in Inulin, ascertain that no significant color results by reaction with fructose, and that a suitable absorbance-versus-concentration slope is obtained with glucose.

[NOTE—A suitable grade is available, as a concentrate, from Worthington Diagnostics, Division of Millipore Corp., www.millipore.com.]

Glycerin Base TS—To 200 g of glycerin add water to bring the total weight to 235 g. Add 140 mL of 1 N sodium hydroxide and 50 mL of water.

Gold Chloride TS—Dissolve 1 g of gold chloride in 35 mL of water.

Hydrogen Peroxide TS—Use *Hydrogen Peroxide Topical Solution* (USP monograph).

Hydrogen Sulfide TS—A saturated solution of hydrogen sulfide, made by passing H_2S into cold water. Store it in small, dark amber-colored bottles, filled nearly to the top. It is unsuitable unless it possesses a strong odor of H_2S, and unless it produces at once a copious precipitate of sulfur when added to an equal volume of ferric chloride TS. Store in a cold, dark place.

Hydroxylamine Hydrochloride TS—Dissolve 3.5 g of hydroxylamine hydrochloride in 95 mL of 60% alcohol, and add 0.5 mL of bromophenol blue solution (1 in 1000 of alcohol) and 0.5 N alcoholic potassium hydroxide until a greenish tint develops in the solution. Then add 60% alcohol to make 100 mL.

8-Hydroxyquinoline TS—Dissolve 5 g of 8-hydroxyquinoline in alcohol to make 100 mL.

Indigo Carmine TS *(Sodium Indigotindisulfonate TS)*—Dissolve a quantity of sodium indigotindisulfonate, equivalent to 180 mg of $C_{16}H_8N_2O_2(SO_3Na)_2$, in water to make 100 mL. Use within 60 days.

Indophenol–Acetate TS (for the assay of *Corticotropin Injection*)—To 60 mL of standard dichlorophenol-indophenol solution (see in the section *Volumetric Solutions*) add water to make 250 mL. Add to the resulting solution an equal volume of sodium acetate solution freshly prepared by dissolving 13.66 g of anhydrous sodium acetate in water to make 500 mL and adjusting with 0.5 N acetic acid to a pH of 7. Store in a refrigerator, and use within 2 weeks.

Intestinal Fluid, Simulated, TS—Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, and add 77 mL of 0.2 N sodium hydroxide and 500 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with either 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 6.8 ± 0.1. Dilute with water to 1000 mL.

Iodine TS—Use *0.1 N Iodine* (see in the section *Volumetric Solutions*).

Iodine, Diluted TS—Transfer 10.0 mL of 0.1 N iodine VS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Iodine Monochloride TS—Dissolve 10 g of potassium iodide and 6.44 g of potassium iodate in 75 mL of water in a glass-stoppered container. Add 75 mL of hydrochloric acid and 5 mL of chloroform, and adjust to a faint iodine color (in the chloroform) by adding dilute potassium iodide or potassium iodate solution. If much iodine is liberated, use a stronger solution of potassium iodate than 0.01 M at first, making the final adjustment with the 0.01 M potassium iodate. Store in a dark place, and readjust to a faint iodine color as necessary.

Iodine and Potassium Iodide TS 1—Dissolve 500 mg of iodine and 1.5 g of potassium iodide in 25 mL of water.

Iodine and Potassium Iodide TS 2—Dissolve 12.7 g of iodine and 20 g of potassium iodide in water, and dilute with water to 1000.0 mL. To 10.0 mL of this solution, add 0.6 g of potassium iodide, and dilute with water to 100.0 mL. Prepare immediately before use.

Iodine and Potassium Iodide TS 3—Dissolve 0.127 g of iodine and 0.20 g of potassium iodide in water, and dilute with water to 10.0 mL.

Iodobromide TS—Dissolve 20 g of iodine monobromide in glacial acetic acid to make 1000 mL. Store in glass containers, protected from light.

Iodochloride TS—Dissolve 16.5 g of iodine monochloride in 1000 mL of glacial acetic acid.

Iodoplatinate TS—Dissolve 300 mg of platinic chloride in 97 mL of water. Immediately prior to use, add 3.5 mL of potassium iodide TS, and mix.

Iron–Phenol TS *(Iron-Kober Reagent)*—Dissolve 1.054 g of ferrous ammonium sulfate in 20 mL of water, and add 1 mL of sulfuric acid and 1 mL of 30 percent hydrogen peroxide. Mix, heat until effervescence ceases, and dilute with water to 50 mL. To 3 volumes of this solution contained in a volumetric flask add sulfuric acid, with cooling, to make 100 volumes. Purify phenol by distillation, discarding the first 10% and the last 5%, collecting the distillate, with exclusion of moisture, in a dry, tared glass-stoppered flask of about twice the volume of the phenol. Solidify the phenol in an ice bath, breaking the top crust with a glass rod to ensure complete crystallization. Weigh the flask and its contents, add to the phenol 1.13 times its weight of the iron–sulfuric acid solution prepared as directed, insert the stopper in the flask, and allow to stand, without cooling but with occasional mixing, until the phenol is liquefied. Shake the mixture vigorously until mixed, allow to stand in the dark for 16 to 24 hours, and again weigh the flask and its contents. To the mixture add 23.5% of its weight of a solution of 100 volumes of sulfuric acid in 110 volumes of water, mix, transfer to dry glass-stoppered bottles, and store in the dark, protected from atmospheric moisture. Use within 6 months. Dispense the reagent from a small-bore buret, arranged to exclude moisture, capable of delivering 1 mL in 30 seconds or less, and having no lubricant, other than reagent, on its stopcock. Wipe the buret tip with tissue before each addition.

Iron Salicylate TS—Dissolve 500 mg of ferric ammonium sulfate in 250 mL of water containing 10 mL of diluted sulfuric acid, and add water to make 500 mL. To 100 mL of the resulting solution add 50 mL of a 1.15% solution of sodium salicylate, 20 mL of diluted acetic acid, and 80 mL of a 13.6% solution of sodium acetate, then add water to make 500 mL. Store in a well-closed container. Protect from light. Use within 2 weeks.

Lanthanum Nitrate TS—Dissolve 5.0 g of lanthanum nitrate hexahydrate in 100 mL of water.

Lead Acetate TS—Dissolve 9.5 g of clear, transparent crystals of lead acetate in recently boiled water to make 100 mL. Store in well-stoppered bottles.

Lead Acetate TS, Alcoholic—Dissolve 2 g of clear, transparent crystals of lead acetate in alcohol to make 100 mL. Store in tight containers.

Lead Subacetate TS—Dissolve 40.0 g of lead acetate in 90 mL of carbon dioxide-free water. Adjust with 10 M sodium hydroxide to a pH of 7.5, centrifuge, and use the clear supernatant. It contains NLT 16.7% (w/w) and NMT 17.4% (w/w) of Pb in a form corresponding to the formula $C_8H_{14}O_{10}Pb_3$. The solution remains clear when stored in a well-closed container.

Lead Subacetate TS, Diluted—Dilute 3.25 mL of lead subacetate TS with water, recently boiled and cooled, to make 100 mL. Store in small, well-filled, tight containers.

Litmus TS—Digest 25 g of powdered litmus with three successive 100-mL portions of boiling alcohol, continuing each extraction for about 1 hour. Filter, wash with alcohol, and discard the alcohol filtrate. Macerate the residue with about 25 mL of cold water for 4 hours, filter, and discard the filtrate. Finally digest the residue with 125 mL of boiling water for 1 hour, cool, and filter.

Locke-Ringer's Solution—See *Locke-Ringer's TS*.

Locke-Ringer's TS *(Locke-Ringer's Solution)*—

Sodium Chloride	9.0 g
Potassium Chloride	0.42 g
Calcium Chloride	0.24 g
Magnesium Chloride	0.2 g
Sodium Bicarbonate	0.5 g
Dextrose	0.5 g
Water, recently distilled from a hard-glass flask, a sufficient quantity to make	1000 mL

Prepare fresh each day. The constituents (except the dextrose and the sodium bicarbonate) may be made up in stock solutions and diluted as needed.

Magnesia Mixture TS—Dissolve 5.5 g of magnesium chloride and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, set the mixture aside for a few days in a well-stoppered bottle, and filter. If the solution is not perfectly clear, filter it before using.

Magnesium Sulfate TS—Dissolve 12 g of crystals of magnesium sulfate, selected for freedom from efflorescence, in water to make 100 mL.

Malachite Green TS—Dissolve 1 g of malachite green oxalate in 100 mL of glacial acetic acid.

Mallory's Stain—Dissolve 500 mg of water-soluble aniline blue, 2 g of orange G, and 2 g of oxalic acid in 100 mL of water.

Mayer's Reagent—See *Mercuric–Potassium Iodide TS*.

Mercuric Acetate TS—Dissolve 6.0 g of mercuric acetate in glacial acetic acid to make 100 mL. Store in tight containers, protected from direct sunlight.

Mercuric–Ammonium Thiocyanate TS—Dissolve 30 g of ammonium thiocyanate and 27 g of mercuric chloride in water to make 1000 mL.

Mercuric Bromide TS, Alcoholic—Dissolve 5 g of mercuric bromide in 100 mL of alcohol, employing gentle heat to facilitate solution. Store in glass containers, protected from light.

Mercuric Chloride TS—Dissolve 6.5 g of mercuric chloride in water to make 100 mL.

Mercuric Iodide TS *(Valser's Reagent)*—Slowly add potassium iodide solution (1 in 10) to red mercuric iodide until almost all of the latter is dissolved, and filter off the excess. A solution containing 10 g of potassium iodide in 100 mL dissolves approximately 14 g of HgI_2 at 20°.

Mercuric Nitrate TS—Dissolve 40 g of mercuric oxide (red or yellow) in a mixture of 32 mL of nitric acid and 15 mL of water. Store in glass containers, protected from light.

Mercuric–Potassium Iodide TS *(Mayer's Reagent)*—Dissolve 1.358 g of mercuric chloride in 60 mL of water. Dissolve 5 g of potassium iodide in 10 mL of water. Mix the two solutions, and dilute with water to 100 mL.

Mercuric–Potassium Iodide TS, Alkaline *(Nessler's Reagent)*—Dissolve 143 g of sodium hydroxide in 700 mL of water. Dissolve 50 g of red mercuric iodide and 40 g of potassium iodide in 200 mL of water. Pour the iodide solution into the hydroxide solution, and dilute with water to 1000 mL. Allow to settle, and use the clear supernatant.

Mercuric Sulfate TS *(Denigès' Reagent)*—Mix 5 g of yellow mercuric oxide with 40 mL of water, and while stirring slowly add 20 mL of sulfuric acid, then add another 40 mL of water, and stir until completely dissolved.

Mercurous Nitrate TS—Dissolve 15 g of mercurous nitrate in a mixture of 90 mL of water and 10 mL of diluted nitric acid. Store in dark, amber-colored bottles in which a small globule of mercury has been placed.

Metaphenylenediamine Hydrochloride TS—Dissolve 1 g of metaphenylenediamine hydrochloride in 200 mL of water. The solution must be colorless when used. If necessary, decolorize by heating with activated charcoal.

Metaphosphoric–Acetic Acids TS—Dissolve 15 g of metaphosphoric acid in 40 mL of glacial acetic acid and sufficient water to make 500 mL. Store in a cold place, and use within 2 days.

Methoxyphenylacetic TS—Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of *Tetramethylammonium Hydroxide TS*, and add 20 mL of dehydrated alcohol. Store in a polyethylene container.

Methyl Orange TS—Dissolve 100 mg of methyl orange in 100 mL of water, and filter if necessary.

Methyl Purple TS—Use *Methyl Red–Methylene Blue TS*.

Methyl Red TS—Dissolve 100 mg of methyl red in 100 mL of alcohol, and filter if necessary.

Methyl Red TS 2—To 1.86 mL of 0.1 M sodium hydroxide and 50 mL of alcohol, add 50 mg of methyl red, and dilute with water to 100 mL.

Methyl Red TS, Methanolic—Dissolve 1 g of methyl red in 100 mL of methanol, and filter, if necessary. Store protected from light, and use within 21 days.

Methyl Red–Methylene Blue TS—Add 10 mL of methyl red TS to 10 mL of methylene blue TS, and mix.

Methyl Violet TS—Use *Crystal Violet TS*.

Methyl Yellow TS—Prepare a solution containing 0.10 mg per mL in alcohol.

Methyl Yellow–Methylene Blue TS—Dissolve 1 g of methyl yellow and 100 mg of methylene blue in 125 mL of methanol.

3-Methyl-2-benzothiazolinone Hydrazone Hydrochloride TS—Dissolve 0.1 g of 3-methyl-2-benzo thiazolinone hydrazone hydrochloride monohydrate in 10 mL of water, dilute the resulting solution with methanol to 100 mL, and mix.

Methylene Blue TS—Dissolve 125 mg of methylene blue in 100 mL of alcohol, and dilute with alcohol to 250 mL.

Methylthionine Perchlorate TS—To 500 mL of potassium perchlorate solution (1 in 1000) add dropwise, with constant shaking, methylene blue solution (1 in 100) until a slight, permanent turbidity results. Allow the precipitate to settle, decant the supernatant through paper, and use only the clear solution.

Millon's Reagent—To 2 mL of mercury in a conical flask add 20 mL of nitric acid. Shake the flask under a hood to break up the mercury into small globules. After about 10 minutes, add 35 mL of water, and, if a precipitate or crystals appear, add sufficient dilute nitric acid (1 in 5, prepared from nitric acid from which the oxides have been removed by blowing air through it until it is colorless) to dissolve the separated solid. Add sodium hydroxide solution (1 in 10) dropwise, with thorough mixing, until the curdy precipitate that forms after the addition of each drop no longer redissolves but is dispersed to form a suspension. Add 5 mL more of the dilute nitric acid, and mix. Prepare this solution fresh.

Molybdo-phosphotungstate TS *(Folin-Denis Reagent)*—To about 350 mL of water contained in a round-bottom flask add 50 g of sodium tungstate, 12 g of phosphomolybdic acid, and 25 mL of phosphoric acid. Boil the mixture under a reflux condenser for 2 hours, then cool, dilute with water to 500 mL, and mix. Store in tight containers, protected from light, and in a cold place.

1-Naphthol Reagent—Dissolve 1 g of 1-naphthol in 25 mL of methanol. Prepare this solution fresh.

1-Naphthol TS—Use *1-Naphthol Reagent*.

2-Naphthol TS *(Betanaphthol TS)*—Dissolve 1 g of 2-naphthol in 100 mL of sodium hydroxide solution (1 in 100).

***p*-Naphtholbenzein TS**—Dissolve 250 mg of *p*-naphtholbenzein in 100 mL of glacial acetic acid.

***N*-(1-Naphthyl)ethylenediamine Dihydrochloride TS**—Dissolve 100 mg of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 mL of a mixture of 7 parts of acetone and 3 parts of water.

Nessler's Reagent—See *Mercuric–Potassium Iodide TS, Alkaline*.

Neutral Red TS—Dissolve 100 mg of neutral red in 100 mL of 50% alcohol.

Nickel Standard Solution TS—Dissolve 4.78 g of nickel (II) sulfate heptahydrate in water, and dilute with water to 1000 mL. Immediately prior to use, dilute 10.0 mL of the solution so obtained with water to 1000 mL. Suitable nickel standard solutions are also available commercially.

Ninhydrin TS—Use *Triketohydrindene Hydrate TS*.

***p*-Nitroaniline TS**—To 350 mg of *p*-nitroaniline add 1.5 mL of hydrochloric acid, and mix. Dilute with water to 50 mL, mix, and allow to settle. Place 5 mL of the clear supernatant in a 100-mL volumetric flask, and immerse it in an ice bath. While it is in the ice bath, add 1 mL of hydrochloric acid, then add, in small portions, 2 mL of sodium nitrite solution (1 in 100), dilute with water to volume, and mix.

Nitrophenanthroline TS—Dissolve 150 mg of 5-nitro-1,10-phenanthroline in 15 mL of freshly prepared ferrous sulfate solution (1 in 140).

Oracet Blue B TS—A 1 in 200 solution of oracet blue B in glacial acetic acid.

Orthophenanthroline TS—Dissolve 150 mg of orthophenanthroline in 10 mL of a solution of ferrous sulfate, prepared by dissolving 700 mg of clear crystals of ferrous sulfate in 100 mL of water. The ferrous sulfate solution must be prepared immediately before dissolving the orthophenanthroline. Store in well-closed containers.

Oxalic Acid TS—Dissolve 6.3 g of oxalic acid in water to make 100 mL.

Palladium Chloride TS, Buffered—Weigh 500 mg of palladium chloride into a 250-mL beaker, add 5 mL of concentrated hydrochloric acid, and warm the mixture on a steam bath. Add 200 mL of hot water in small increments with continued heating until solution is complete. Transfer the solution to a 250-mL volumetric flask, and dilute with water to volume. Transfer 50 mL to a 100-mL volumetric flask. Add 10 mL of 1 M sodium acetate and 9.6 mL of 1 N hydrochloric acid. Dilute with water to volume.

Perchloric Acid TS—Dilute 8.5 mL of perchloric acid with water to 100 mL.

Phenol TS—Dissolve 1.2 g of phenol in alcohol to make 10 mL. Prepare weekly.

Phenol Red TS *(Phenolsulfonphthalein TS)*—Dissolve 100 mg of phenolsulfonphthalein in 100 mL of alcohol, and filter if necessary.

pH 4.7 Phenol Red TS—Dissolve 33 mg of phenolsulfonphthalein in 1.5 mL of 2 N sodium hydroxide solution, dilute with water to 100 mL, and mix *(Solution A).* Dissolve 25 mg of ammonium sulfate in 235 mL of water, add 105 mL of 2 N sodium hydroxide solution and 135 mL of 2 N acetic acid, and mix *(Solution B).* Add 25 mL of *Solution A* to *Solution B,* and mix. If necessary, adjust the pH of this solution to 4.7.

Phenoldisulfonic Acid TS—Dissolve 2.5 g of phenol in 15 mL of sulfuric acid in a flask of suitable capacity. Add 7.5 mL of fuming sulfuric acid, stir well, and heat at 100° for 2 hours. Transfer the product, while still fluid, to a glass-stoppered bottle, and, when desired for use, warm in a water bath until liquefied.

Phenolphthalein TS—Dissolve 1 g of phenolphthalein in 100 mL of alcohol.

Phenylhydrazine Acetate TS—Dissolve 10 mL of phenylhydrazine and 5 mL of glacial acetic acid in water to make 100 mL.

Phenylhydrazine–Sulfuric Acid TS—Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of a cooled mixture of equal volumes of sulfuric acid and water.

Phloroglucinol TS—Dissolve 500 mg of phloroglucinol in 25 mL of alcohol. Store in tight containers, protected from light.

Phosphatic Enzyme TS—Dissolve 5 g of phosphatic enzyme in water to make 50 mL. Prepare this solution fresh.

Phosphomolybdic Acid TS—Dissolve 20 g of phosphomolybdic acid in alcohol to make 100 mL. Filter the solution, and use only the clear filtrate.

Phosphotungstic Acid TS—Dissolve 1 g of phosphotungstic acid in water to make 100 mL.

Picrate TS, Alkaline—Mix 20 mL of trinitrophenol solution (1 in 100) with 10 mL of sodium hydroxide solution (1 in 20), dilute with water to 100 mL, and mix. Use within 2 days.

Picric Acid TS—See *Trinitrophenol TS*.

Platinic Chloride TS—Dissolve 2.6 g of platinic chloride in water to make 20 mL.

Platinum–Cobalt TS—Dissolve 1.246 g of potassium chloroplatinate (K_2PtCl_6) and 1.000 g of cobalt chloride ($CoCl_2 \cdot 6H_2O$) in water, add 100 mL of hydrochloric acid, and dilute with water to 1 L.

Potassium Acetate TS—Dissolve 10 g of potassium acetate in water to make 100 mL.

Potassium–Bismuth Iodide TS—Dissolve 12.5 g of tartaric acid in 25 mL of water, then dissolve 1.06 g of bismuth subnitrate in this mixture *(Solution A).* Dissolve 20 g of potassium iodide in 25 mL of water *(Solution B).* Dissolve 100 g of tartaric acid in 450 mL of water *(Solution C).* Add *Solutions A* and *B* to *Solution C,* and mix.

Potassium Carbonate TS—Dissolve 7 g of anhydrous potassium carbonate in water to make 100 mL.

Potassium Chromate TS—Dissolve 10 g of potassium chromate in water to make 100 mL.

Potassium Dichromate TS—Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

Potassium Ferricyanide TS—Dissolve 1 g of potassium ferricyanide in 10 mL of water. Prepare this solution fresh.

Potassium Ferrocyanide TS—Dissolve 1 g of potassium ferrocyanide in 10 mL of water. Prepare this solution fresh.

Potassium Hydroxide TS—Dissolve 6.5 g of potassium hydroxide in water to make 100 mL.

Potassium Hydroxide TS, Alcoholic—Use *0.5 N Potassium Hydroxide, Alcoholic* (see in the section *Volumetric Solutions*).

Potassium Hydroxide TS 2, Alcoholic—Dissolve 130 g of potassium hydroxide, with cooling, in 200 mL of water. Add alcohol to 1000 mL. Store in a well-stoppered dark glass bottle.

Potassium Iodide TS—Dissolve 16.5 g of potassium iodide in water to make 100 mL. Store in light-resistant containers.

Potassium Iodide and Starch TS—Dissolve 0.75 g of potassium iodide in 100 mL of water. Heat to boiling, and add, with stirring, a solution of 0.5 g of soluble starch in 35 mL of water. Boil for 2 minutes, and allow to cool.

Sensitivity—Mix 15 mL in 0.05 mL of glacial acetic acid and 0.3 mL of diluted iodine TS: a blue color is produced.

Potassium Iodoplatinate TS—Dissolve 200 mg of platinic chloride in 2 mL of water, mix with 25 mL of potassium iodide solution (1 in 25), and add water to make 50 mL.

Potassium Permanganate TS—Use *0.1 N Potassium Permanganate* (see in the section *Volumetric Solutions*).

Potassium Pyroantimonate TS—Dissolve 2 g of potassium pyroantimonate in 85 mL of hot water. Cool quickly, and add 50 mL of a solution containing 50 mg/mL of potassium hydroxide in water and 1 mL of sodium hydroxide solution (8.5 in 100). Allow to stand for 24 h, filter, and dilute with water to 150 mL.

Potassium Sulfate TS—Dissolve 1 g of potassium sulfate in water to make 100 mL.

Potassium Thiocyanate TS—Dissolve 9.7 g of potassium thiocyanate in water to make 100 mL.

Pyridine–Pyrazolone TS—To 100 mL of a saturated solution of 1-phenyl-3-methyl-2-pyrazoline-5-one add 20 mL of a 1 in 1000 solution of 3,3′-dimethyl-1,1′-diphenyl-[4,4′-bi-2-pyrazoline]-5,5′-dione in pyridine. Store in a dark bottle, and use within 3 days.

Pyrogallol TS, Alkaline—Dissolve 500 mg of pyrogallol in 2 mL of water. Dissolve 12 g of potassium hydroxide in 8 mL of water. The solutions should be freshly prepared and mixed immediately before use.

Quinaldine Red TS—Dissolve 100 mg of quinaldine red in 100 mL of alcohol.

Quinone TS—Dissolve 500 mg of *p*-benzoquinone in 2.5 mL of glacial acetic acid, and dilute with alcohol to 50 mL. Prepare this solution fresh daily.

Resorcinol TS—Dissolve 1 g of resorcinol in hydrochloric acid to make 100 mL.

Ruthenium Red TS—Dissolve 10 g of lead acetate in water, dilute with water to 100 mL, and add 80 mg of ruthenium red. The solution is wine-red in color. [NOTE—If necessary, add additional ruthenium red to obtain a wine-red color.]

Saline TS—Dissolve 9.0 g of sodium chloride in water to make 1000 mL.

[NOTE—Where pyrogen-free saline TS is specified in this Pharmacopeia, saline TS that has met the requirements of the *Pyrogen Test* ⟨151⟩ is to be used.]

Saline TS, Pyrogen-Free—See *Saline TS*.

Schweitzer's Reagent—See *Cupric Oxide, Ammoniated, TS*.

Silver–Ammonia–Nitrate TS—Dissolve 1 g of silver nitrate in 20 mL of water. Add ammonia TS, dropwise, with constant stirring, until the precipitate is almost but not entirely dissolved. Filter, and store in tight, light-resistant containers.

Silver–Ammonium Nitrate TS—See *Silver–Ammonia–Nitrate TS*.

Silver Diethyldithiocarbamate TS—Dissolve 1 g of silver diethyldithiocarbamate in 200 mL of pyridine from a freshly opened bottle or that which has been recently distilled. Store in light-resistant containers, and use within 30 days.

Silver Nitrate TS—Use *0.1 N Silver Nitrate* (see in the section *Volumetric Solutions*).

Simulated Gastric Fluid TS—See *Gastric Fluid, Simulated, TS*.

Simulated Intestinal Fluid TS—See *Intestinal Fluid, Simulated, TS*.

Sodium Acetate TS—Dissolve 13.6 g of sodium acetate in water to make 100 mL.

Sodium Alizarinsulfonate TS—Dissolve 100 mg of sodium alizarinsulfonate in 100 mL of water, and filter.

Sodium Aminoacetate TS *(Sodium Glycinate TS)*—Dissolve 3.75 g of aminoacetic acid in about 500 mL of water, add 2.1 g of sodium hydroxide, and dilute with water to 1000 mL. Mix 9 mL of the resulting solution with 1 mL of dilute glacial acetic acid (1 in 300). This test solution has a pH between 10.4 and 10.5.

Sodium Bisulfite TS—Dissolve 10 g of sodium bisulfite in water to make 30 mL. Prepare this solution fresh.

Sodium Bitartrate TS—Dissolve 1 g of sodium bitartrate in water to make 10 mL. Prepare this solution fresh.

Sodium Carbonate TS—Dissolve 10.6 g of anhydrous sodium carbonate in water to make 100 mL.

Sodium Chloride TS, Alkaline—Dissolve 2 g of sodium hydroxide in 100 mL of water, saturate the solution with sodium chloride, and filter.

Sodium Citrate TS—Dissolve 73.5 g of sodium citrate dihydrate in water to make 250 mL.

Sodium Citrate TS, Alkaline—Dissolve 50 g of sodium citrate dihydrate and 2.5 g of sodium hydroxide in water to make 250 mL.

Sodium Cobaltinitrite TS—Dissolve 10 g of sodium cobaltinitrite in water to make 50 mL, and filter if necessary.

Sodium Fluoride TS—Dry about 500 mg of sodium fluoride at 200° for 4 hours. Accurately weigh 222 mg of the dried material, and dissolve in water to make 100.0 mL. Pipet 10 mL of this solution into a 1-L volumetric flask, and dilute with water to volume. Each mL of this solution corresponds to 0.01 mg of fluorine (F).

Sodium Hydrosulfite TS, Alkaline—Dissolve 25 g of potassium hydroxide in 35 mL of water, and 50 g of sodium hydrosulfite in 250 mL of water. When the test solution is required, mix 40 mL of the hydroxide solution with the 250 mL of the hydrosulfite solution. Prepare this solution fresh.

Sodium Hydroxide TS—Dissolve 4.0 g of sodium hydroxide in water to make 100 mL.

Sodium Hydroxide TS 2—Transfer 8.5 g of sodium hydroxide to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.

Sodium Hydroxide TS 3—Prepare a 420-mg/mL solution of sodium hydroxide in water.

Sodium Hypobromite TS—To a solution of 20 g of sodium hydroxide in 75 mL of water add 5 mL of bromine. After solution has taken place, dilute with water to 100 mL. Prepare this solution fresh.

Sodium Hypochlorite TS—Use *Sodium Hypochlorite Solution* (see in the section *Reagent Specifications*).

Sodium Iodohydroxyquinolinesulfonate TS—Dissolve 8.8 g of iodohydroxyquinoline sulfonic acid in 200 mL of water, and add 6.5 mL of 4 N sodium hydroxide. Dilute with water to 250 mL, mix, and filter.

Sodium Nitroferricyanide TS—Dissolve 1 g of sodium nitroferricyanide in water to make 20 mL. Prepare this solution fresh.

Dibasic Sodium Phosphate TS—Dissolve 12 g of clear crystals of dibasic sodium phosphate in water to make 100 mL.

Sodium Phosphotungstate TS—To a solution of 20 g of sodium tungstate in 100 mL of water add sufficient phosphoric acid to impart a strongly acid reaction to litmus, and filter. When required for use, decant the clear solution from any sediment that may be present. Store in tight, light-resistant containers.

Sodium Sulfide TS—Dissolve 1 g of sodium sulfide in water to make 10 mL. Prepare this solution fresh.

Sodium Tartrate TS—Dissolve 11.5 g of sodium tartrate in water to make 100 mL.

Sodium Tetraphenylboron TS—Dissolve 1.2 g of sodium tetraphenylboron in water to make 200 mL. If necessary, stir for 5 minutes with 1 g of aluminum oxide, and filter to clarify.

Sodium Thioglycolate TS—Dissolve 1.5 g of sodium thioglycolate in 450 mL of water, and add 50 mL of alcohol. Use within 3 days.

Sodium Thiosulfate TS—Use *0.1 N Sodium Thiosulfate* (see in the section *Volumetric Solutions*).

Standard Lead Solution—See under *Heavy Metals* ⟨231⟩.

Stannous Chloride, Acid, TS—Dissolve 8 g of stannous chloride in 500 mL of hydrochloric acid. Store in glass containers, and use within 3 months.

Stannous Chloride, Acid, Stronger, TS—Dissolve 40 g of stannous chloride in 100 mL of hydrochloric acid. Store in glass containers, and use within 3 months.

Starch TS—Mix 1 g of soluble starch with 10 mg of red mercuric iodide and sufficient cold water to make a thin paste. Add 200 mL of boiling water, and boil for 1 minute with continuous stirring. Cool, and use only the clear solution. [NOTE—Commercially available, stabilized starch indicator solutions may be used, including mercury-free solutions preserved with other compounds such as salicylic acid.]

Starch, Iodide-Free, TS—Mix 1 g of soluble starch with sufficient cold water to make a thin paste. While stirring, add 100 mL of boiling water, and allow to cool. Prepare this solution immediately before use. Iodide-free starch TS shows a blue color when 20 mL of potassium iodide solution (1 in 400) and 0.05 mL of an iodine–potassium iodide solution (prepared by dissolving 127 mg of iodine and 800 mg of potassium iodide in water and diluting with water to 100 mL) are added to 1 mL of the iodide-free starch TS.

Starch Iodide Paste TS—Heat 100 mL of water in a 250-mL beaker to boiling, add a solution of 0.75 g of potassium iodide in 5 mL of water, then add 2 g of zinc chloride dissolved in 10 mL of water, and, while the solution is boiling, add, with stirring, a smooth suspension of 5 g of soluble starch in 30 mL of cold water. Continue to boil for 2 minutes, then cool. Store in well-closed containers in a cold place.

Starch iodide paste TS must show a definite blue streak when a glass rod, dipped in a mixture of 1 mL of 0.1 *M* sodium nitrite, 500 mL of water, and 10 mL of hydrochloric acid, is streaked on a smear of the paste.

Starch–Potassium Iodide TS—Dissolve 500 mg of potassium iodide in 100 mL of freshly prepared starch TS. Prepare this solution fresh.

Stronger Cupric Acetate TS—See *Cupric Acetate TS, Stronger*.

Sudan III TS—Dissolve 0.05 g of Sudan III in 25 mL of alcohol, with warming if necessary. Cool, add 25 mL of glycerin, and mix. Filter if undissolved material persists.

Sudan IV TS—Dissolve 0.5 g of Sudan IV in chloroform to make 100 mL.

Sulfanilic Acid TS—Dissolve 800 mg of sulfanilic acid in 100 mL of acetic acid. Store in tight containers.

Diazotized Sulfanilic Acid TS—Dissolve 0.9 g of sulfanilic acid in 9 mL of hydrochloric acid with warming, and dilute with water to 100 mL. Cool 10 mL of this solution in iced water, and add 10 mL of a sodium nitrite solution (4.5 in 100) previously cooled in iced water. Allow to stand at 0° for at least 15 minutes (the solution may be kept for 3 days at this temperature). Immediately before use, add 20 mL of sodium carbonate solution (1 in 10).

Sulfanilic-1-Naphthylamine TS—Dissolve 500 mg of sulfanilic acid in 150 mL of acetic acid. Dissolve 100 mg of 1-naphthylamine hydrochloride in 150 mL of acetic acid, and mix the two solutions. The pink color that may develop on standing can be removed by treatment with zinc.

Sulfanilic-α-Naphthylamine TS—See *Sulfanilic-1-Naphthylamine TS*.

Sulfomolybdic Acid TS—Dissolve, with the aid of heat, 2.5 g of ammonium molybdate in 20 mL of water, add 50 mL of 12 N sulfuric acid, and dilute with water to 100 mL. Store this solution in a polyethylene container.

Sulfuric Acid TS—Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% (w/w) of H_2SO_4.

[NOTE—Since the acid concentration may change upon standing or upon intermittent use, the concentration should be checked frequently and solutions assaying more than 95.5% or less than 94.5% discarded.]

Sulfuric Acid–Formaldehyde TS—Add 1 drop of formaldehyde TS to each mL of sulfuric acid, and mix. Prepare this solution fresh.

Tannic Acid TS—Dissolve 1 g of tannic acid in 1 mL of alcohol, and dilute with water to 10 mL. Prepare this solution fresh.

Tartaric Acid TS—Dissolve 3 g of tartaric acid in water to make 10 mL. Prepare this solution fresh.

Tetrabromophenolphthalein Ethyl Ester TS—Dissolve 100 mg of tetrabromophenolphthalein ethyl ester in 90 mL of glacial acetic acid, and dilute with glacial acetic acid to 100 mL. Prepare this solution fresh.

Tetramethylammonium Hydroxide TS—Use an aqueous solution containing, in each 100 mL, the equivalent of 10 g of anhydrous tetramethylammonium hydroxide.

Thioacetamide TS—Dissolve 4 g of thioacetamide in 100 mL of water.

Thioacetamide–Glycerin Base TS—Mix 0.2 mL of thioacetamide TS and 1 mL of glycerin base TS, and heat in a boiling water bath for 20 seconds. Use the mixture immediately.

Thorium Nitrate TS—Dissolve 1 g of thorium nitrate in water to make 100 mL. Filter, if necessary.

Thymol Blue TS—Dissolve 100 mg of thymol blue in 100 mL of alcohol, and filter if necessary.

Thymolphthalein TS—Dissolve 100 mg of thymolphthalein in 100 mL of alcohol, and filter if necessary.

Titanium Trichloride TS—Dissolve 15 g of titanium trichloride in 100 mL of 10% hydrochloric acid solution.

Titanium Trichloride–Sulfuric Acid TS—Mix carefully 20 mL of titanium trichloride TS in 13 mL of sulfuric acid. Add sufficient 30% hydrogen peroxide to produce a yellow color. Heat until white fumes are evolved, allow to cool, and dilute with water. Repeat the evaporation and addition of

water until a colorless solution is obtained. Dilute with water to 100 mL.

***p*-Toluenesulfonic Acid TS**—Dissolve 2 g of *p*-toluenesulfonic acid in 10 mL of a mixture of 7 parts of acetone and 3 parts of water.

Triketohydrindene Hydrate TS *(Ninhydrin TS)*—Dissolve 200 mg of triketohydrindene hydrate in water to make 10 mL. Prepare this solution fresh.

Trinitrophenol TS *(Picric Acid TS)*—Dissolve the equivalent of 1 g of anhydrous trinitrophenol in 100 mL of hot water. Cool the solution, and filter if necessary.

Triphenyltetrazolium Chloride TS—Dissolve 500 mg of triphenyltetrazolium chloride in dehydrated alcohol to make 100 mL.

Xylenol Orange TS—Dissolve 100 mg of xylenol orange in 100 mL of alcohol.

Zinc Uranyl Acetate TS—Dissolve 50 g of uranyl acetate in a mixture of 15 mL of glacial acetic acid and water to make 500 mL. Then dissolve 150 g of zinc acetate in a mixture of 15 mL of glacial acetic acid and water to make 500 mL. Mix the two solutions, allow to stand overnight, and pass through a dry filter, if necessary.

VOLUMETRIC SOLUTIONS

Normal Solutions—Normal solutions are solutions that contain 1 gram equivalent weight of the active substance in each 1000 mL of solution; that is, an amount equivalent to 1.0079 g of hydrogen or 7.9997 g of oxygen. Normal solutions and solutions bearing a specific relationship to normal solutions, and used in volumetric determinations, are designated as follows: normal, 1 N; double-normal, 2 N; half-normal, 0.5 N; tenth-normal, 0.1 N; fiftieth-normal, 0.02 N; hundredth-normal, 0.01 N; thousandth-normal, 0.001 N.

Molar Solutions—Molar solutions are solutions that contain, in 1000 mL, 1 gram-molecule of the reagent. Thus, each liter of a molar solution of sulfuric acid contains 98.07 g of H_2SO_4 and each liter of a molar solution of potassium ferricyanide contains 329.25 g of $K_3Fe(CN)_6$. Solutions containing, in 1000 mL, one-tenth of a gram-molecule of the reagent are designated "tenth-molar," 0.1 M; and other molarities are similarly indicated.

Empirical Solutions—It is frequently difficult to prepare standard solutions of a desired theoretical normality, and this is not essential. A solution of approximately the desired normality is prepared and standardized by titration against a primary standard solution. The normality factor so obtained is used in all calculations where such empirical solutions are employed. If desired, an empirically prepared solution may be adjusted downward to a given normality provided it is strong enough to permit dilution.

All volumetric solutions, whether made by direct solution or by dilution of a stronger solution, must be thoroughly mixed by shaking before standardization. As the strength of a standard solution may change upon standing, the factor should be redetermined frequently.

When solutions of a reagent are used in several normalities, the details of the preparation and standardization are usually given for the normality most frequently required. Stronger or weaker solutions are prepared and standardized in the same general manner as described, using proportionate amounts of the reagent. It is possible in many instances to prepare lower normalities accurately by making an exact dilution of a stronger solution. Volumetric solutions prepared by dilution should be restandardized either as directed for the stronger solution or by comparison with another volumetric solution having a known ratio to the stronger solution.

Dilute solutions that are not stable, as, for instance, potassium permanganate 0.01 N and more dilute sodium thiosulfate, are preferably prepared by exactly diluting the higher normality with thoroughly boiled and cooled water on the same day they are required for use.

Blank Determinations—Where it is directed that "any necessary correction" be made by a blank determination, the determination is to be conducted with the use of the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted. Appropriate blank corrections are to be made for all Pharmacopeial titrimetric assays (see *Titrimetry* ⟨541⟩).

All Pharmacopeial assays that are volumetric in nature indicate the weight of the substance being assayed to which each mL of the primary volumetric solution is equivalent. In general, these equivalents may be derived by simple calculation from the data given under *Molecular Formulas and Weights,* in the *Reference Tables.*

Preparation and Methods of Standardization of Volumetric Solutions

The following directions give only one method for standardization, but other methods of standardization, capable of yielding at least the same degree of accuracy, may be used. The values obtained in the standardization of volumetric solutions are valid for all Pharmacopeial uses of these solutions, regardless of the instrumental or chemical indicators employed in the individual monographs. Where the apparent normality or molarity of a titrant depends upon the special conditions of its use, the individual monograph sets forth the directions for standardizing the reagent in the specified context. For those salts that usually are available as certified primary standards, or that are available as highly purified salts of primary standard quality, it is permissible to prepare solutions by accurately weighing a suitable quantity of the salt and dissolving it to produce a specific volume of solution of known concentration. Acetic, hydrochloric, and sulfuric acids may be standardized against a sodium hydroxide solution that recently has been standardized against a certified primary standard.

All volumetric solutions, if practicable, are to be prepared, standardized, and used at the standard temperature of 25°. If a titration is carried out with the volumetric solution at a markedly different temperature, standardize the volumetric solution used as the titrant at that different temperature, or make a suitable temperature correction.

Acetic Acid, Double-Normal (2 N)
$C_2H_4O_2$, **60.05**
120.10 g in 1000 mL

Add 116 mL of glacial acetic acid to sufficient water to make 1000 mL after cooling to room temperature.

Ammonium Thiocyanate, Tenth-Normal (0.1 N)
NH_4SCN, **76.12**
7.612 g in 1000 mL

Dissolve about 8 g of ammonium thiocyanate in 1000 mL of water, and standardize the solution as follows.

Accurately measure about 30 mL of 0.1 N silver nitrate VS into a glass-stoppered flask. Dilute with 50 mL of water, then add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate TS, and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown color.

$$N = \frac{\text{mL } AgNO_3 \text{ x N } AgNO_3}{\text{mL } NH_4SCN \text{ Solution}}$$

If desirable, 0.1 N ammonium thiocyanate may be replaced by 0.1 N potassium thiocyanate where the former is directed in various tests and assays.

Bismuth Nitrate, 0.01 M
$Bi(NO_3)_3 \cdot 5H_2O$, **485.07**
1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate