Octyl Sulfate, Sodium Salt, $C_8H_{17}O_4SNa$—**232.27**—White powder.

Solubility—A 2-g portion dissolves in 100 mL of water.

Melting range ⟨741⟩: between 195° and 197°, with decomposition.

Odorless Absorbent Paper—See *Filter Paper, Quantitative*.

Olefin Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator in a stabilized form of permanganate.

Measuring range: 0.06 to 3.2 Vol.–% Propylene, 0.04 to 2.4 Vol.–% Butylene.

[NOTE—Available from Draeger Safety, Inc., www.draeger.com.]

Oligo-deoxythymidine—Polymeric length: 18. Use a suitable grade.

[NOTE—A suitable grade is available from BD Biosciences, www.bdbiosciences.com.]

Orange G *(the sodium salt of azobenzene-betanaphthol disulfonic acid),* $C_6H_5N{:}NC_{10}H_4(OH)(SO_3Na)_2$-2,6,8—**452.37** [1936-15-8]—Orange to brick-red powder or dark red crystals. Readily soluble in water, yielding an orange-yellow solution; slightly soluble in alcohol; insoluble in ether and in chloroform. The addition of tannic acid TS to its 1 in 500 solution causes no precipitation *(acid color)*. The addition of hydrochloric acid to a mixture of 500 mg of zinc dust and 10 mL of its 1 in 500 solution produces decolorization. When filtered, the colorless filtrate, on standing exposed to air, does not regain its original color *(presence of azo-group)*. When heated, orange G does not deflagrate (distinction from *nitro colors*). The addition of barium or calcium chloride TS to a concentrated solution of orange G produces a colored, crystalline precipitate. The addition of hydrochloric acid to its 1 in 500 solution produces no change; the addition of sodium hydroxide TS to a similar solution produces a yellowish red to a Bordeaux color but no precipitation. Orange G dissolves in sulfuric acid with an orange to yellowish-red color. No change in color results upon diluting the solution cautiously with water.

Orcinol *(5-Methylresorcinol),* $C_7H_8O_2 \cdot H_2O$—**142.15** [6153-39-5]—White to light tan crystals.

Assay—Transfer about 60 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask and dilute with methanol to volume, and mix. Using a suitable spectrophotometer, 1-cm cells, and methanol as the blank, record the absorbance of the solution at the wavelength of maximum absorbance at about 273 nm. From the observed absorbance, calculate the absorptivity (see *Spectrophotometry and Light-scattering* ⟨851⟩): the absorptivity is not less than 13.2, corresponding to not less than 98% of $C_7H_8O_2 \cdot H_2O$.

Melting range ⟨741⟩: between 58° and 61°.

Orthophenanthroline—See *1,10-Phenanthroline*.

Osmium Tetroxide *(Osmic Acid; Perosmic Anhydride),* OsO_4—**254.23** [20816-12-0]—Use ACS reagent grade.

Oxalic Acid, $H_2C_2O_4 \cdot 2H_2O$—**126.07** [6153-56-6]—Use ACS reagent grade.

3,3′-Oxydipropionitrile, $O(CH_2CH_2CN)_2$—**124.14** [1656-48-0]—Clear, colorless to slightly yellow liquid. Refractive index: about 1.446 at 20°.

Boiling range: between 174° and 176° at 10 mm of mercury.

Oxygen-Helium Certified Standard—A mixture of 1.0% oxygen in industrial grade helium. It is available from most suppliers of specialty gases.

Packings for High-Pressure Liquid Chromatography—See packings for high-pressure liquid chromatography in the *Chromatographic Reagents* section under *Chromatography* ⟨621⟩.

Palladium Catalyst—Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Palladium Catalyst, Type I (5% Palladium on Calcium Carbonate)," from Engelhard Industries, Inc., fax number (864) 885-1375.]

Palladium Chloride, $PdCl_2$—**177.33** [7647-10-1]—Brown, crystalline powder. Soluble in water, in alcohol, in acetone, and in diluted hydrochloric acid.

Assay—Dissolve 80 mg, accurately weighed, in 10 mL of diluted hydrochloric acid, dilute with water to 50 mL, and add 25 mL of a 1 in 100 solution of dimethylglyoxime in alcohol. Allow to stand for 1 hour, and filter. Check for complete precipitation with the dimethylglyoxime solution. Ignite the precipitate in a tared platinum crucible at 850° for 2 hours, cool, and weigh the palladium. The weight of the residue is not less than 59.0% of the weight of the test specimen.

Palladous Chloride—See *Palladium Chloride.*

Change to read:

Pancreatic Digest of Casein (a bacteriological peptone; *Tryptone*)—A grayish-yellow powder, having a characteristic, but not putrescent, odor. Freely soluble in water; insoluble in alcohol and in ether.■1S *(USP35)*

Nitrogen content (Reagent test)—Determine by the Kjeldahl method: ■9.0%–14.0% is found.■1S *(USP35)*

Loss on drying ⟨731⟩—Dry it at 100° to constant weight: it loses not more than 7.0% of its weight.

Residue on ignition ⟨281⟩—Ignite 500 mg with 1 mL of sulfuric acid: the residue weighs not more than 75 mg (15%).

■1S *(USP35)*

Microbial content—■NMT 10,000 cfu/g.■1S *(USP35)*

Bacteriological test—■1S *(USP35)* Prepare ■medium■1S *(USP35)* of the following composition:

■2% of digest, 0.5% of sodium chloride, and 1.5% of agar in purified water.■1S *(USP35)*

Adjust ■with diluted hydrochloric acid or diluted sodium hydroxide to a pH of 7.2–7.4. Autoclave at 121° for 15 min.■1S *(USP35)*

■1S *(USP35)*

Growth-supporting properties—■Slants of the above medium, inoculated with *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048, *Salmonella enterica* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228, show characteristic growth after incubation for 24 h.

The above medium, to which 5% of sheep blood or rabbit blood has been added, and which has been inoculated and poured into Petri dishes, shows characteristic alpha or beta zones around colonies of *Streptococcus pneumoniae* ATCC 6305 and *Streptococcus pyogenes* ATCC 49117, recognizable within 24 h and fully developed after 48 h of incubation.

The above medium, to which 10% of sheep blood or rabbit blood has been added, and which then has been heated to 80°–90° until the blood has turned chocolate-brown, permits the growth of *Neisseria gonorrhoeae* ATCC 19424 colonies within 48 h when incubated in an atmosphere containing 10% of carbon dioxide.■1S *(USP35)*

Pancreatin [8049-47-6]—Use a grade of pancreatin which meets the USP requirements for amylase, lipase, and protease activities specified for the official substance.

Papaic Digest of Soybean Meal—A soluble nutrient material prepared by the action of the enzyme papain on soybean meal followed by suitable purification and concentration. It meets the specifications under *Pancreatic Digest of Casein*, except with respect to *Nitrogen content* and except that it shows substantial amounts of reducing sugars. It contains fermentable carbohydrates and gives positive tests for indole, acetylmethylcarbinol, and sulfide upon inoculation and incubation with the specified organisms.

Nitrogen content (Reagent test)—Determine by the Kjeldahl method, using a test specimen previously dried at 105° to constant weight: not less than 8.5% is found.

Paper, Odorless Absorbent—See *Filter Paper, Quantitative*.

***Para*-aminobenzoic Acid** *(p-Aminobenzoic Acid),* $H_2NC_6H_4COOH$—**137.14** [150-13-0]—White or slightly yellow crystals or crystalline powder, becoming discolored on exposure to air or light. One g dissolves in 170 mL of water, in 9 mL of boiling water, in 8 mL of alcohol, and in 50 mL of ether. Freely soluble in solutions of alkali hydroxides and carbonates; soluble in warm glycerin; sparingly soluble in diluted hydrochloric acid; slightly soluble in chloroform. Store in tight, light-resistant containers.

Assay—Accurately weigh about 300 mg, previously dried at 105° for 2 hours, and transfer to a beaker or casserole. Add 5 mL of hydrochloric acid and 50 mL of water, and stir until dissolved. Cool to about 15°, add about 25 g of crushed ice, and slowly titrate with 0.1 M sodium nitrite VS until a glass rod dipped into the titrated solution produces an immediate blue ring when touched to starch iodide paper. When the titration is complete, the endpoint is reproducible after the mixture has been allowed to stand for 1 minute. Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of $C_7H_7NO_2$. Not less than 98.5% is found.

Melting range ⟨741⟩: between 186° and 189°.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.2% of its weight.

Residue on ignition (Reagent test): not more than 0.1%.

Paraformaldehyde, $(CH_2O)_n$ [30525-89-4]—Fine, white powder.

Assay—Transfer about 1 g, accurately weighed, to a 250-mL conical flask containing 50.0 mL of 1 N sodium hydroxide VS, and mix by swirling. Immediately, and slowly, add 50 mL of hydrogen peroxide TS, previously neutralized to bromothymol blue, through a small funnel placed in the neck of the flask. After the reaction moderates, rinse the funnel and inner wall of the flask with water, allow the solution to stand for 30 minutes, add bromothymol blue TS, and titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 30.03 mg of HCHO: not less than 95% is found.

Residue on ignition: not more than 0.1%.

Solubility in ammonia—Dissolve 5 g in 50 mL of ammonia TS: a practically clear, colorless solution results.

Reaction—Shake 1 g with 20 mL of water for about 1 minute, and filter: the filtrate is neutral to litmus.

Pectate Lyase [9015-75-2]—An enzyme obtained from *Aspergillus sp.* Light brown, viscous liquid. Specific gravity is about 1.5. It is readily soluble in water. It is supplied at approximately 14 units per mL (at pH 8.0 in Tris-HCl buffer [50 mM of Tris(hydroxymethyl)aminomethane containing 1 mM of $CaCl_2$, pH 8.0] in a solution of 50% glycerol and 0.02% sodium azide. One unit is defined as the enzyme activity that produces 1 μmol of unsaturated product per minute.

Activity

Pectin solution—Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a 100-mL volumetric flask. [NOTE—Pectin has a molecular weight of 103,000 Da; its degree of esterification (percentage of galacturonic acid groups substituted with methyl) is 12.] Moisten with 0.1 mL of 2-propanol. Add 50 mL of water to the flask, and mix the solution with a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the solution to a pH of 12. Stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 minutes. Adjust the solution with 0.5 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Tris buffer solution—Transfer 6.055 g of Tris(hydroxymethyl)aminomethane and 0.147 g of calcium chloride ($CaCl_2 \cdot H_2O$) to a 1000-mL volumetric flask containing 950 mL of water, and mix. Adjust the solution with 1 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Diluted pectate lyase—Transfer 0.5 mL of Pectate Lyase to a 50-mL volumetric flask, dilute with *Tris buffer solution* to volume, and mix.

Procedure—Add the solutions set forth in the table below to quartz cuvettes.

Label	Tris buffer solution (mL)	Pectin solution (mL)	Diluted pectate lyase (mL)	Water (mL)
Enzyme blank	0.5	1.0	0	1.0
Test blank	0.5	0	0.5	1.5
Test solution	0.5	1.0	0.5	0.5

Perform the test on the solutions so obtained, using a suitable UV-Vis spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩) and using water as the blank. Mix the solutions well at time 0, and immediately measure the absorbances at 235 nm. Record the value for the *Enzyme blank*, A_{0-EB}; for the *Test blank*, A_{0-TB}; and for the *Test solution*, A_{0-TS}. After incubation at room temperature for 30 minutes, determine the absorbance again at 235 nm for the *Enzyme blank*, A_{30-EB}; for the *Test blank*, A_{30-TB}; and for the *Test solution*, A_{30-TS}. One unit is defined as the enzymatic activity that produces 1 μmol of unsaturated product from pectin per minute. Calculate the Pectate Lyase activity, in units per mL, using the following formula:

$$\frac{50(10^3)[(A_{30-TS} - A_{30-EB} - A_{30-TB}) - (A_{0-TS} - A_{0-EB} - A_{0-TB})]}{30\varepsilon_{235}L}$$

in which 50 is the volume, in mL, of *Diluted pectate lyase*; 10^3 is the unit conversion factor; 30 is the time, in minutes, of the reaction; ε_{235} is the molar extinction coefficient, in $M^{-1}cm^{-1}$, of the reaction product (4600 M^{-1} cm^{-1}); and L is the path length, in cm, of the reaction cuvette (1 cm). Alternatively, these solutions, after being mixed in the cuvettes, can be immediately measured at 235 nm continuously in a recording UV-Vis spectrophotometer set up for kinetic assays. The result is obtained by correcting the blank determination, using the *Enzyme blank* and the *Test blank*.

Penicillinase—See *Beta-lactamase*.

Pentadecane, $C_{15}H_{32}$—**212.41** [629-62-9]—Colorless liquid.

Assay—Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-μm layer of phase G2; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; and the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{15}H_{32}$ peak is not less than 99% of the total peak area.

Refractive index ⟨831⟩: between 1.430 and 1.434 at 20°.

Pentane *(n-Pentane),* C_5H_{12}—**72.15** [109-66-0]—Clear, colorless, *flammable* liquid. Very slightly soluble in water. Miscible with alcohol, with ether, and with many organic solvents. Specific gravity: about 0.62.

Boiling range (Reagent test)—Not less than 95% distils between 34° and 36°.

1-Pentanesulfonic Acid Sodium Salt—See *Sodium 1-Pentanesulfonate*.

2-Pentanone, $C_5H_{10}O$—**86.13** [107-87-9]—Use a suitable grade.

Pepsin [9001-75-6]—Use *Pepsin* (*Enzyme Preparations*) FCC, having an activity of 1.0 to 1.17 Pepsin units per mg. Pepsin of higher activity may be reduced to this activity by admixture with pepsin of lower activity or with lactose.

Pepsin, Purified—A white or yellowish-white powder, spongy mass, or translucent scales or granules. Freely soluble in water, producing more or less opalescence; practically insoluble in alcohol, in chloroform, and in ether. Purified Pepsin used in the second tier of the *Dissolution* test has an activity that is determined by the following method.

Activity—

PEPSIN SOLUTION—Transfer about 2.5 mg of Purified Pepsin, accurately measured, to a 100-mL volumetric flask, dilute with 10 mM hydrochloric acid to volume, and mix. [NOTE—Prepare immediately before use.]

2.0% HEMOGLOBIN SOLUTION—Dissolve and dilute 2.5 g of bovine hemoglobin with water to 100 mL. Dilute 80 mL of this solution with 0.3 M hydrochloric acid to a volume of 100 mL.

TRICHLOROACETIC ACID SOLUTION—Dilute 5 g of trichloroacetic acid with water to 100 mL.

TEST SOLUTION—Transfer 5.0 mL of *2.0% Hemoglobin solution* to a suitable container equilibrated at 37°. Add 1.0 mL of *Pepsin solution*, mix by swirling, and incubate at 37° for 10 minutes. Immediately add 10.0 mL of *Trichloroacetic acid solution*, mix by swirling, and incubate at 37° for 5 minutes. Pass through a filter having a 0.8-µm or finer porosity.

CONTROL SOLUTION—Transfer 5.0 mL of *2.0% Hemoglobin solution* to a suitable container equilibrated at 37°. Mix by swirling, and incubate at 37° for 10 minutes. Immediately add 10.0 mL of *Trichloroacetic acid solution* and 1.0 mL of *Pepsin solution*, mix by swirling, and incubate at 37° for 5 minutes. Pass through a filter having a 0.8-µm or finer porosity.

PROCEDURE—Determine the absorbances of the *Test solution* and *Control solution*, in 1-cm cells, at a wavelength of about 280 nm, using water as the reference. Calculate the activity of the portion of Purified Pepsin taken by the formula:

$$10{,}000(A_U - A_C)$$

in which A_U and A_C are the absorbances of the *Test solution* and the *Control solution*, respectively.

Peptic Digest of Animal Tissue (a bacteriological peptone)—Tan powder, having a characteristic, but not putrescent, odor. Soluble in water; insoluble in alcohol and in ether. An autoclaved solution (2 in 100) is clear and is neutral or nearly so in its reaction.

Degree of digestion—Dissolve 1 g in 10 mL of water, and use this solution for the following tests:

(*a*) Overlay 1 mL of the digest solution with 0.5 mL of a solution of 1 mL of glacial acetic acid in 10 mL of diluted alcohol: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results, indicating the absence of undigested protein.

(*b*) Mix 1 mL of the digest solution with 4 mL of saturated zinc sulfate: a small amount of precipitate is formed, indicating the presence of proteoses. Filter, and retain the filtrate.

(*c*) To 1 mL of the filtrate from the preceding test add 1 drop of bromine TS: the light yellow color changes to a red-brown, indicating the presence of tryptophane.

Nitrogen content, *Loss on drying*, *Residue on ignition*, and *Nitrite*—Proceed as directed under *Pancreatic Digest of Casein*.

Microbial content—Dissolve 1 g in 10 mL of water. Spread 0.01 mL on one square centimeter of a glass slide. Stain by the Gram method, and examine with an oil-immersion lens: not more than a total of 50 microorganisms, or clumps, are visible in 10 consecutive fields.

Bacteriologic test—It meets the following tests for bacteria-nutrient properties. Prepare media of the following compositions:

(*a*) 2% of digest and sufficient phenol red TS to give a perceptible color in water;

(*b*) 0.1% of digest in water;

(*c*) 0.1% of digest and 0.5% of dextrose in water;

(*d*) 1% of digest in water.

Adjust all media to a final pH of 7.2 to 7.4. Place 5 mL of (*a*) in Durham fermentation tubes, and 5 mL each of (*b*), (*c*), and (*d*) in ordinary test tubes. Autoclave the media at 121° for 15 minutes. After autoclaving, and after standing for 24 hours, all media are clear.

Presence of fermentable carbohydrate—Inoculate medium (*a*) with *Escherichia coli* and with *Streptococcus liquefaciens:* acid is produced by *E. coli* but not by *S. liquefaciens* during incubation for 24 hours.

Production of indole—Inoculate medium (*b*) with *Escherichia coli* and with *Aerobacter aerogenes*, and incubate for 24 hours. Test by adding about 0.5 mL of *p*-dimethylaminobenzaldehyde TS: the appearance of a pink or red color (soluble in chloroform) indicates the production of indole by *E. coli*. The *A. aerogenes* culture gives a negative test.

Production of acetylmethylcarbinol—Inoculate medium (*c*) with *Escherichia coli* and with *Aerobacter aerogenes*, and incubate for 24 hours. Test by adding to the culture an equal volume of sodium hydroxide solution (1 in 10), shaking well, and allowing to stand at room temperature for several hours: the appearance of a pink color indicates the production of acetylmethylcarbinol by *A. aerogenes*. The *E. coli* culture gives a negative test.

Production of hydrogen sulfide—Inoculate medium (*d*) with *Salmonella typhosa*. Hold a strip or loop of lead acetate test paper between the cotton plug and the mouth of the test tube so that it hangs about 5 cm above the medium. Then incubate for 24 hours: the lower part of the lead acetate test paper shows an appreciable amount of brownish blackening (*lead sulfide*).

Peptone, Dried *(Meat Peptone)*—Reddish-yellow to brown powder, having a characteristic, but not putrescent, odor. Soluble in water, forming a yellowish-brown solution having a slight acid reaction; insoluble in alcohol and in ether.

Nitrogen content (Reagent test)—Determine by the Kjeldahl method, using a test specimen previously dried at 105° to constant weight: between 14.2% and 15.5% of nitrogen is found, corresponding to not less than 89% of protein.

Residue on ignition (Reagent test)—Ignite 500 mg with 1 mL of sulfuric acid: the residue weighs not more than 25 mg (5.0%).

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 7.0% of its weight.

Coagulable protein—Heat a filtered solution (1 in 20) to boiling: no precipitate forms.

Proteoses—Mix 5 mL of a filtered solution (1 in 10) with 20 mL of a filtered solution of zinc sulfate (made by dissolving 50 g of the salt in 35 mL of water): not more than a slight, flocculent precipitate is formed.

Perchloric Acid *(70 Percent Perchloric Acid),* $HClO_4$—**100.46** [7601-90-3]—Use ACS reagent grade (containing between 69.0% and 72.0% of $HClO_4$).

Periodic Acid, H_5IO_6—**227.94** [10450-60-9]—White to pale yellow crystals. Very soluble in water. Undergoes slow decomposition to iodic acid. Use ACS reagent grade.

Petroleum Benzin—See *Hexane, Solvent*.

Phases for Gas Chromatography—See phases for gas chromatography in the *Chromatographic Columns* section under *Chromatography* ⟨621⟩.

Phenacetin [62-44-2]—Use a suitable grade.

1,10-Phenanthroline *(Orthophenanthroline),* $C_{12}H_8N_2 \cdot H_2O$—**198.22** [5144-89-8]—Use ACS reagent grade.

***o*-Phenanthroline Monohydrochloride Monohydrate,** $C_{12}H_8N_2 \cdot HCl \cdot H_2O$—**234.69** [3829-86-5]—Use a suitable grade.

Phenol [108-95-2]—Use ACS reagent grade.

Phenol Red, Sodium, $C_{19}H_{13}O_5SNa$—**376.4** [34487-61-1]—Red to brown powder. Use ACS reagent grade.

Phenolsulfonphthalein—Use *Phenol Red* (see *Indicators* under *Indicators and Indicator Test Papers*).

Phenoxybenzamine Hydrochloride *[N-(2-Chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine Hydrochloride],* $C_{18}H_{22}ClNO \cdot HCl$—**340.29** [63-92-3]—White, crystalline powder.

Melting range ⟨741⟩: between 137° and 140°.

Absorptivity—Its absorptivity, 1%, 1 cm, in the range of 272 nm to 290 nm, in chloroform solution is about 178.

3-Phenoxybenzoic Acid, $C_{13}H_{10}O_3$—**214.22** [3739-38-6]—Use a suitable grade.

Melting range ⟨741⟩: between 149° and 150°.

2-Phenoxyethanol, $C_6H_5OCH_2CH_2OH$—**138.16** [122-99-6]—Colorless, slightly viscous liquid. Soluble in water. Miscible with alcohol, with acetone, and with glycerin. Density: about 1.107.

Assay—To 2 g, accurately weighed, add 10 mL of a freshly prepared solution made by dissolving 25 g of acetic anhydride in 100 g of anhydrous pyridine. Swirl to mix the liquids, heat on a steam bath for 45 minutes, add 10 mL of water, heat for 2 additional minutes, and cool. Add 10 mL of normal butyl alcohol, shake vigorously, add phenolphthalein TS, and titrate with 1 N sodium hydroxide VS. Perform a blank test using the same quantities of the same reagents, and in the same manner, and make any necessary correction. Each mL of 1 N sodium hydroxide is equivalent to 138.2 mg of $C_8H_{10}O_2$. Not less than 99% is found.

Phenol—Add 0.2 mL of it to 20 mL of water, mix, and to 5 mL of the mixture add 0.2 mL of Millon's reagent. Warm the solution at 60° for 90 seconds, and allow to stand: no pink or red color is produced within 1 minute.

Phenyl Ether—See *Diphenyl Ether*.

Phenyl Isocyanate, C_6H_5NCO—**119.12** [103-71-9]—Clear, colorless to straw-yellow liquid of medium volatility. [**CAUTION**—*Phenyl Isocyanate is a violent lacrimator, and the vapor is highly toxic. Handle with care.*]

Assay—Transfer 250 mg, accurately weighed, to a glass-stoppered, 250-mL flask. Exercise care to avoid loss by volatilization, and avoid breathing the vapor. Add 20 mL of butylamine solution (25 g of butylamine diluted to 1000 mL with dioxane previously dried over potassium hydroxide pellets), insert the stopper in the flask, and allow to stand for 15 minutes. Add a few drops of methyl red TS and 25 mL of water, and titrate the excess amine with 0.1 N sulfuric acid VS. Perform a blank titration on 20 mL of the butylamine solution (see *Residual Titrations* ⟨541⟩). Subtract the volume of 0.1 N sulfuric acid consumed in the test specimen titration from that consumed in the blank titration. Each mL of 0.1 N sulfuric acid, representing this difference, is equivalent to 11.91 mg of C_6H_5NCO: not less than 97.0% of C_6H_5NCO is found.

2-Phenylacetamide *(α-Phenylacetamide),* C_8H_9NO—**135.16** [103-81-1]—Bimorphous plates or leaflets. Slightly soluble in water. Use a suitable grade.

Melting range ⟨741⟩: between 156° and 158°.

***dl*-Phenylalanine**, $C_9H_{11}NO_2$—**165.19** [150-30-1]—Use a suitable grade.

***o*-Phenylenediamine Dihydrochloride**, $C_6H_8N_2 \cdot 2HCl$—**181.1**—White powder.

Assay—When tested by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of butyl alcohol, water, and acetic acid (12:5:3), and examined under short-wavelength UV light, a single spot is exhibited, with trace impurities.

***p*-Phenylenediamine Dihydrochloride**—See *p-Phenylenediamine Hydrochloride*.

***p*-Phenylenediamine Hydrochloride** *(1,4-Diaminobenzene Dihydrochloride),* $C_6H_8N_2 \cdot 2HCl$—**181.06**—White to pale tan crystals or crystalline powder, turning red on exposure to air. Freely soluble in water; slightly soluble in alcohol and in ether. Preserve in well-closed containers, protected from light.

Insoluble matter—Dissolve 1 g in 10 mL of water: the solution is clear and complete.

Molar absorptivity (see *Spectrophotometry and Light-scattering* ⟨851⟩)—Dissolve 60 mg in 100.0 mL of water, and mix. Pipet 2 mL of this solution into a 50-mL volumetric flask, dilute with pH 7 buffer solution to volume, and mix. The molar absorptivity of this solution, at 239 nm, is not less than 9000.

Phenylglycine (D(–)-*2-Phenylglycine*), $(C_6H_5CH(NH_2)COOH)$—**151.17** [875-74-1]—Use a suitable grade.

Phenylhydrazine, $C_6H_5NHNH_2$—**108.14** [100-63-0]—A colorless, or slightly yellowish, highly refractive liquid. [NOTE—Protect from light, and distill under reduced pressure shortly prior to use.]

Congealing temperature ⟨651⟩: not below 16°.

Insoluble matter—Shake 1 mL with 20 mL of diluted acetic acid: the resulting solution is clear or practically so.

Residue on ignition (Reagent test)—Ignite 1 mL with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

Phenylhydrazine Hydrochloride, $C_6H_5NHNH_2 \cdot HCl$—**144.60** [59-88-1]—White or yellowish crystals or powder. Soluble in water and in alcohol. Store in tight containers, protected from light. Use a suitable grade with a content of not less than 99%.

Phenylmethylsulfonyl Fluoride, $C_7H_7FO_2S$—**174.2** [329-98-6]—White to faint yellow powder. Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

3-Phenylphenol *(m-Phenylphenol),* $C_6H_5C_6H_4OH$—**170.21** [580-51-8]—White to off-white, crystalline powder.

Assay—Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G1; the injection port temperature is maintained at 250°; the column temperature is maintained at 150° and programmed to rise 15° per minute to 250°; and the detector temperature is maintained at 310°. The area of the 3-phenylphenol peak is not less than 98% of the total peak area.

Melting range ⟨741⟩: between 76° and 79°.

Phloroglucinol, $C_6H_3(OH)_3 \cdot 2H_2O$—**162.14** [6099-90-7]—White or yellowish-white crystals or a crystalline powder. Soluble in alcohol and in ether; slightly soluble in water.

Insoluble in alcohol—Dissolve 1 g in 20 mL of alcohol: a clear and complete solution results.

Melting range, Class Ia ⟨741⟩: between 215° and 219°.

Residue on ignition (Reagent test)—Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

Diresorcinol—Heat to boiling a solution of 100 mg in 10 mL of acetic anhydride, cool the solution, and superimpose it upon 10 mL of sulfuric acid: no violet color appears at the zone of contact of the liquids.

Phloxine B *(Acid red 92; Eosin 10B; 2′,4′,5′,7′-tetrabromo-4,5,6,7-tetrachlorofluorescein disodium salt)* $C_{20}H_2Br_4Cl_4Na_2O_5$—**829.63** [18472-87-2]—Use a suitable grade with a dye content of not less than 80%, certified by the Biological Stain Commission.

Phosphatase Enzyme, Alkaline—Use a suitable grade from intestinal origin.

[NOTE—A suitable grade is available from Worthington Biochemical Corp., www.worthington-biochem.com.]

Phosphatic Enzyme—An enzyme preparation of microbial origin, high in both phosphatase and amylase activity, the former being the property that renders it suitable for use in the liberation of thiamine from its orthophosphate and pyrophosphate esters. Light cream-colored or slightly gray

powder. Freely soluble in water. It hydrolyzes 300 times its weight of starch in 30 minutes.

Amylase activity—Place in a test tube 5 mL of a 1 in 50 solution of soluble starch in 0.2 M, pH 5 sodium acetate buffer (containing 1.6 g of anhydrous sodium acetate in each L and sufficient glacial acetic acid to adjust to a pH of 5), and add 4 mL of water. Mix, and place in a water bath at 40°. Add 1 mL of a solution containing 0.3 mg of the phosphatic enzyme, mix, and note the exact time. After 30 minutes remove 1.0 mL of the mixture, and add it to 5.0 mL of 0.0005 N iodine in a 20- × 150-mm test tube: a clear, red color results.

Phosphomolybdic Acid, approximately $20MoO_3 \cdot P_2O_5 \cdot 51H_2O$—**3939.49** [11104-88-4]—Use ACS reagent grade.

Phosphoric Acid, H_3PO_4—**98.00** [7664-38-2]—Use ACS reagent grade.

Phosphorous Acid *(Phosphonic Acid),* H_3O_3P—**82.00** [13598-36-2]—Use a suitable grade with a content of not less than 99%.

Phosphorus, Red, P—**At. Wt. 30.97376**—A dark red powder. Insoluble in water and in dilute acids; soluble in dehydrated alcohol.

Yellow phosphorus—Shake 20 g with 75 mL of carbon disulfide in a glass-stoppered vessel, and allow to stand in the dark overnight. Filter, and wash the residue with carbon disulfide until the filtrate, collected in a graduated cylinder, measures 100 mL. Evaporate the solvent to 10 mL by immersing the cylinder in hot water. Dip a strip of cupric sulfate test paper in the remaining solvent: no more color is produced than in a similar strip dipped into 10 mL of solution in carbon disulfide containing 3 mg of yellow phosphorus (0.015% as P).

Soluble substances—Digest 2 g with 30 mL of acetic acid on a steam bath for 15 minutes. Cool, dilute with water to 40 mL, and filter. Evaporate 20 mL of the filtrate on a steam bath, and dry at 105° for 2 hours: the residue weighs not more than 6 mg (0.6%).

Phosphorus Pentoxide *(Phosphoric Anhydride),* P_2O_5—**141.94** [1314-56-3]—Use ACS reagent grade.

Phosphotungstic Acid, approximately $24WO_3 \cdot P_2O_5 \cdot 51H_2O$—**6624.84**—White or yellowish-green crystals or a crystalline powder. Soluble in water, in alcohol, and in ether.

Insoluble matter (Reagent test): not more than 1 mg, from 5 g (0.02%).

Chloride (Reagent test)—One g shows not more than 0.3 mg of Cl (0.03%).

Nitrate—Dissolve 500 mg in 10 mL of water, and add about 10 mg of sodium chloride, 0.1 mL of indigo carmine TS, and 10 mL of sulfuric acid: the blue color does not disappear within 1 minute (about 0.01%).

Sulfate (Reagent test, *Method I*)—A 500-mg portion shows not more than 0.1 mg of SO_4 (0.02%).

***o*-Phthalaldehyde** *(Phthalic Dicarboxaldehyde),* $C_6H_4(CHO)_2$—**134.13** [643-79-8]—Use a suitable grade.

Phthalazine, $C_8H_6N_2$—**130.15** [253-52-1]—Yellow to tan crystals.

Melting range ⟨741⟩: between 89° and 92°.

Phthalic Acid, $C_8H_6O_4$—**166.13** [88-99-3]—Use ACS reagent grade.

Phthalic Anhydride, $C_8H_4O_3$—**148.12** [85-44-9]—Use ACS reagent grade.

Phthalimide, $C_8H_5NO_2$—**147.13** [85-41-6]—White powder.

Assay—

MOBILE PHASE—Prepare a mixture of isooctane and methyl-*tert*-butyl ether (88:12).

PROCEDURE—Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains packing L3. The flow rate is about 2 mL per minute. The area of the $C_8H_5NO_2$ peak is not less than 99% of the total peak area.

Melting range ⟨741⟩: between 233° and 235°, with decomposition.

2-Picoline, C_6H_7N—**93.13** [109-06-8]—Colorless to yellowish liquid.

Assay—Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2-mm × 2-m glass column packed with 20% liquid phase G16 on 80- to 100-mesh support S1C; the injection port temperature is maintained at 140°; the detector temperature is maintained at 300°; the column temperature is maintained at 90° and programmed to rise 3° per minute to 140°. The area of the C_6H_7N peak is not less than 98% of the total peak area.

Refractive index ⟨831⟩: 1.500 ± 0.002 at 20°.

Picric Acid *(2,4,6-Trinitrophenol; Trinitrophenol),* $C_6H_2(OH)(NO_2)_3$-1,2,4,6—**229.10** [88-89-1]—Use ACS reagent grade.

Picrolonic Acid *(3-Methyl-4-nitro-1-(p-nitrophenyl)-5-pyrazolone),* $C_{10}H_8N_4O_5$—**264.19** [550-74-3]—Yellow to brownish-yellow, crystalline powder. Slightly soluble in water; soluble in alcohol, in chloroform, in ether, in benzene, and in solutions of alkali hydroxides.

Melting range ⟨741⟩: between 115° and 117°.

Residue on ignition (Reagent test): negligible, from 200 mg.

Sensitiveness—Dissolve 25 mg in 10 mL of warm water containing 0.1 mL of glacial acetic acid, and filter the solution, if necessary. Dissolve 100 mg of calcium chloride in 250 mL of water, and mix. Heat 1 mL of the calcium chloride solution in a test tube to about 60°, then add to it 1 mL of the picrolonic acid solution: a bulky precipitate forms in 5 minutes or less.

Pipemidic Acid *(8-Ethyl-3,8-dihydro-5-oxo-2-(1-piperazinyl)pyrido[2,3-d]-pyrimidine-6-carboxylic acid),* $C_{14}H_{17}N_5O_3$—**303.3** [51940-44-4]—Use a suitable grade.

Piperazine *(Diethylenediamine),* $C_4H_{10}N_2 \cdot 6H_2O$—**194.23**—Use a suitable grade.

Piperidine, $C_5H_{11}N$—**85.15** [110-89-4]—Colorless liquid. Miscible with water and with alcohol. Specific gravity: about 0.860.

Congealing range ⟨651⟩: between 12° and 15°.

Boiling range (Reagent test)—Not less than 95% distills between 104° and 106°.

Refractive index: about 1.454.

Change to read:

Platinic Chloride *(Chloroplatinic Acid),* $H_2PtCl_6 \cdot 6H_2O$—**517.90** ■[18497-13-7]■1S *(USP35)*—Use ACS reagent grade Chloroplatinic Acid.

Polydimethylsiloxane, viscosity 0.65 centistokes *(Hexamethyldisiloxane),* $(CH_3)_3SiOSi(CH_3)_3$—**162.38** [107-46-0]—Liquid. Freezes at about 0°.

Refractive index ⟨831⟩: about 1.3770.

Specific gravity ⟨841⟩: about 0.760.

Polyethylene Glycol 200, $H(OCH_2CH_2)_nOH$ in which the average value of *n* is 4—average molecular weight 200 [25322-68-3]—Clear, colorless or almost colorless, viscous, hygroscopic liquid. Very soluble in acetone and in alcohol; practically insoluble in ether and in fatty oils. Use a suitable grade.

Refractive index ⟨831⟩: 1.4590 at 20°.

Density: 1.127 at 25°.

Viscosity: 4.3 centistokes at 98.9°.

Polyethylene Glycol 600 [25322-68-3]—A clear, practically colorless, viscous liquid condensation polymer repre-

sented by $H(OCH_2CH_2)_nOH$, in which *n* varies from 12 to 14. Its average molecular weight is about 600.

It meets the requirements of all of the tests under *Polyethylene Glycol* (*NF* monograph) except *Limit of ethylene glycol and diethylene glycol.*

Polyethylene Glycol 20,000 [25322-68-3]—Molecular weight range: 15,000–20,000. Hard, white, waxy solid, usually supplied in flake form. Soluble in water with subsequent gel formation.

Viscosity of 25% solution ⟨911⟩—Add 50.0 g of test specimen to a 250-mL wide-mouth, screw-cap jar containing 150.0 g of water. Attach the cap securely to the jar, and roll on a mechanical roller until the test specimen is completely dissolved, in 2 to 4 hours. Allow the solution to stand until all air bubbles have disappeared. Another 2 to 4 hours may be required. Adjust the temperature of the solution to 37.8 ± 0.1°, and determine the kinematic viscosity on a suitable viscosimeter of the Ubbelohde type. The viscosity is not less than 100 centistokes.

pH ⟨791⟩: between 6.5 and 8.0 in a solution (1 in 20). [NOTE—A five-fold dilution of the test solution prepared for the *Viscosity of 25% solution* test may be used.]

Residue on ignition ⟨281⟩: not more than 0.7%, the use of sulfuric acid being omitted.

Polyoxyethylene (23) Lauryl Ether *(Brij-35)*—Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Brij-35."]

Polyoxyethylene (20) Sorbitan Monolaurate [9005-64-5]—Viscous, light yellow to yellow-green liquid. Use a suitable grade.

Polysaccharide Molecular Weight Standards—Polymaltotriose polymers of different weight-average molecular weight, M_w, values ranging from 5,000 to 400,000 Da.

[NOTE—A suitable set is available from Shodex (www.shodex.com) as Kit P-82.]

Polystyrene Cation–Exchange Resin—See *Cation–Exchange Resin, Polystyrene*.

Polytef—Use *Poly(tetrafluoroethylene)*.

Polyvinyl Alcohol, $(C_2H_4O)_n$ [9002-89-5]—White powder. Soluble in water; insoluble in organic solvents.

pH ⟨791⟩: between 5.0 and 8.0, in a solution (1 in 25).

Loss on drying—Dry it at 110° to constant weight: it loses not more than 5% of its weight.

Residue on ignition: not more than 0.75%.

[NOTE—Suitable grades are available as catalog number U 232, from J.T. Baker Chemical Co., www.jtbaker.com.]

Potassium Acetate, $KC_2H_3O_2$—**98.14** [127-08-2]—Use ACS reagent grade.

Potassium Alum—Use *Potassium Alum* [see *Potassium Alum* (USP monograph)].

Potassium Arsenate Monobasic, KH_2AsO_4—**180.03** [7784-41-0]—Use a suitable grade with a content of NLT 98%.

Potassium Bicarbonate, $KHCO_3$—**100.12** [298-14-6]—Use ACS reagent grade.

Potassium Biphosphate—See *Potassium Phosphate, Monobasic*.

Potassium Biphthalate *(Acid Potassium Phthalate; Phthalic Acid Monopotassium Salt; Potassium Hydrogen Phthalate Acidimetric Standard),* $KHC_6H_4(COO)_2$—**204.22** [877-24-7]—Use ACS reagent grade Potassium Hydrogen Phthalate, Acidimetric Standard.

Potassium Bisulfate, $KHSO_4$—**136.17** [7646-93-7]—Fused, white, deliquescent masses or granules. Very soluble in water. When ignited, it evolves SO_3 and H_2O, changing first to potassium pyrosulfate, then to sulfate.

Acidity—Dissolve 4 g, accurately weighed, in 50 mL of water, add phenolphthalein TS, and titrate with 1 N alkali: it contains between 34% and 36%, calculated as H_2SO_4.

Insoluble matter and *ammonium hydroxide precipitate*—Dissolve 10 g in 100 mL of water, add methyl red TS, render slightly alkaline with ammonia TS, boil for 1 minute, and digest on a steam bath for 1 hour. Pass through a tared filtering crucible, wash thoroughly, and dry at 105° for 2 hours: the precipitate weighs not more than 1 mg (0.01%).

For the following tests, prepare a *Test solution* as follows. Dissolve 6 g in 45 mL of water, add 2 mL of hydrochloric acid, boil gently for 10 minutes, cool, and dilute with water to 60 mL.

Heavy metals (Reagent test)—To 30 mL of *Test solution* add phenolphthalein TS, and neutralize with ammonia TS. Add 0.5 mL of glacial acetic acid, dilute with water to 40 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 10 mL of *Test solution* and 0.02 mg of added Pb (0.001%).

Iron ⟨241⟩—To 5 mL of *Test solution* add 2 mL of hydrochloric acid, and dilute with water to 47 mL: the solution shows not more than 0.01 mg of Fe (0.002%).

Potassium Bromate, $KBrO_3$—**167.00** [7758-01-2]—Use ACS reagent grade.

Potassium Bromide, KBr—**119.00** [7758-02-3]—Use ACS reagent grade.

Potassium Carbonate—See *Potassium Carbonate, Anhydrous*.

Potassium Carbonate, Anhydrous, K_2CO_3—**138.21** [584-08-7]—Use ACS reagent grade.

Potassium Chlorate, $KClO_3$—**122.55** [3811-04-9]—Use ACS reagent grade.

Potassium Chloride, KCl—**74.55** [7447-40-7]—Use ACS reagent grade.

Potassium Chloroplatinate, K_2PtCl_6—**485.99**—Heavy, yellow powder. Soluble in hydrochloric acid and in nitric acid.

Assay—Accurately weigh about 300 mg, transfer to a 600-mL beaker, add 20 mL of hydrochloric acid, and heat gently if necessary to achieve complete solution. Add zinc granules, slowly, until no more dissolves, then add 2 mL of hydrochloric acid, and digest for 1 hour on a steam bath to coagulate the reduced platinum. Add more acid, if necessary, to ensure that all of the zinc has dissolved. Filter through paper, rinsing the beaker with diluted hydrochloric acid until all of the precipitate is transferred to the filter, then wash with several small portions of water. Ignite the filter in a tared crucible at 800 ± 25° to constant weight. Each mg of residue is equivalent to 1.0 mg of platinum. Not less than 40% is found.

Potassium Chromate, K_2CrO_4—**194.19** [7789-00-6]—Use ACS reagent grade.

Potassium Cyanide, KCN—**65.12** [151-50-8]—Use ACS reagent grade.

Potassium Dichromate, $K_2Cr_2O_7$—**294.18** [7778-50-9]—Use ACS reagent grade.

[NOTE—Potassium dichromate of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 136.]

Potassium Ferricyanide, $K_3Fe(CN)_6$—**329.24** [13746-66-2]—Use ACS reagent grade.

Potassium Ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$—**422.39** [14459-95-1]—Use ACS reagent grade.

Potassium Hyaluronate—White to cream-colored powder. Freely soluble in water. Store in a tight container, in a refrigerator.

Inhibitor content—Prepare as directed in the *Assay* under *Hyaluronidase for Injection* (USP monograph) a quantity of *Standard solution* containing 1 USP Hyaluronidase Unit in each mL, and a similar quantity of acetate-buffered *Standard solution* using as the solvent 0.1 M, pH 6 sodium acetate buffer (prepared by diluting the 0.2 M buffer prepared as directed below with an equal volume of water). Prepare from the potassium hyaluronate under test 10 mL of *Potassium hyaluronate stock solution*, and dilute 2 mL of it with the specified *Phosphate buffer solution* to make a *Hyaluronate solution*. In the same way, and concurrently, dilute a second

2-mL portion of the stock solution with 0.2 M, pH 6 sodium acetate buffer (containing 16.4 g of anhydrous sodium acetate and 0.45 mL of glacial acetic acid in each 1000 mL).

Place 0.50-mL portions of the *Hyaluronate solution* in each of four 16- × 100-mm test tubes, and place 0.50-mL portions of the acetate-buffered *Hyaluronate solution* in two similar tubes. To two of the four tubes containing *Hyaluronate solution* add 0.50 mL of *Diluent for hyaluronidase solutions*, prepared as directed in the *Assay* under *Hyaluronidase for Injection* (USP monograph). To the remaining two tubes, on a rigid schedule, at 30-second intervals, add 0.50 mL of *Standard solution*. Similarly, to the two tubes containing acetate-buffered *Hyaluronate solution* add at 30-second intervals 0.50-mL portions of acetate-buffered *Standard solution*. Then proceed as directed in the second paragraph for *Procedure*, beginning with "Mix the contents," as far as "Plot the average." The reduction in absorbance of acetate-buffered *Hyaluronate solution* is not less than 25% of that observed in the *Hyaluronate solution*.

Turbidity production—The average absorbance of the solutions in the two tubes containing *Hyaluronate solution* and *Diluent for hyaluronidase solutions* prepared in the test for *Inhibitor content* is not less than 0.26 at a wavelength of 640 nm in a suitable spectrophotometer using a 1-cm cell.

Potassium Hydrogen Sulfate, $KHSO_4$—**136.17**—White crystals. Soluble in water.

Melting point ⟨741⟩: about 197°.

Potassium Hydroxide, KOH—**56.11** [1310-58-3]—Use ACS reagent grade.

Potassium Iodate, KIO_3—**214.00** [7758-05-6]—Use ACS reagent grade.

Potassium Iodide, KI—**166.00** [7681-11-0]—Use ACS reagent grade.

Potassium Metabisulfite *(Potassium Disulfite; Potassium Pyrosulfite),* $K_2S_2O_5$—**222.32** [16731-55-8]—Use a suitable grade with a content of not less than 98%.

Potassium Nitrate, KNO_3—**101.10** [7757-79-1]—Use ACS reagent grade.

Potassium Nitrite, KNO_2—**85.10** [7758-09-0]—Use ACS reagent grade.

Potassium Perchlorate, $KClO_4$—**138.55** [7778-74-7]—Use ACS reagent grade.

Potassium Periodate *(Potassium meta-Periodate),* KIO_4—**230.00** [7790-21-8]—Use ACS reagent grade.

Potassium Permanganate, $KMnO_4$—**158.03** [7722-64-7]—Use ACS reagent grade.

Potassium Persulfate, $K_2S_2O_8$—**270.32** [7727-21-1]—Use ACS reagent grade Potassium Peroxydisulfate.

Potassium Phosphate, Dibasic *(Dipotassium Hydrogen Phosphate; Dipotassium Phosphate),* K_2HPO_4—**174.18** [7758-11-4]—Use ACS reagent grade.

Add the following:

▲**Potassium Phosphate, Dibasic, Trihydrate** *(Dipotassium Hydrogen Phosphate Trihydrate; Dipotassium Phosphate),* $K_2HPO_4 \cdot 3H_2O$—**228.22** [16788-57-1]—Use a suitable grade with a content of NLT 99.0%.▲*USP35*

Potassium Phosphate, Monobasic *(Potassium Biphosphate; Potassium Dihydrogen Phosphate),* KH_2PO_4—**136.09** [7778-77-0]—Use ACS reagent grade.

[NOTE—Certified Potassium Dihydrogen Phosphate is available from the National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 186.]

Potassium Phosphate, Tribasic, K_3PO_4—**212.27** [7778-53-2]—Deliquescent, orthorhombic crystals. Use ACS reagent grade.

Potassium Pyroantimonate *(Potassium hexahydroxyantimonate), $KSb(OH)_6$*—**262.90** [12208-13-8]—White crystals or a white, crystalline powder. Sparingly soluble in water. Use a suitable grade.

Potassium Pyrophosphate, $K_4P_2O_7$—**330.34** [7320-34-5]—Colorless, deliquescent granules. Freely soluble in water; insoluble in alcohol.

Potassium Pyrosulfate [7790-62-7]—Usually available as a mixture of potassium pyrosulfate ($K_2S_2O_7$) and potassium bisulfate ($KHSO_4$). Use ACS reagent grade.

Potassium Sodium Tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$—**282.22** [6381-59-5]—Use ACS reagent grade.

Potassium Sulfate, K_2SO_4—**174.26** [7778-80-5]—Use ACS reagent grade.

Potassium Tellurite *(Potassium Tellurate IV),* K_2TeO_3—**253.79** [7790-58-1]—White, granular powder. Soluble in water. Its solution is alkaline.

Assay—Weigh accurately about 120 mg, transfer to a beaker, and dissolve in a mixture of 10 mL of nitric acid, 10 mL of sulfuric acid, and 25 mL of water. Heat to boiling, and boil until copious fumes of sulfur trioxide are evolved. Cool, cautiously add 100 mL of water, heat to boiling, add 6 g of sodium fluoride, and titrate the hot solution with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate is equivalent to 12.69 mg of K_2TeO_3. Not less than 98% is found.

Chloride (Reagent test)—One g shows not more than 0.1 mg of Cl (0.01%).

Potassium Thiocyanate, KSCN—**97.18** [333-20-0]—Use ACS reagent grade.

Potato Starch—See *Starch, Potato*.

Propionaldehyde, C_3H_6O—**58.08** [123-38-6]—Use a suitable grade.

Propionic Anhydride, $C_6H_{10}O_3$—**130.14** [123-62-6]—Colorless liquid. Is decomposed by water. Soluble in methanol, in alcohol, in ether, and in chloroform.

Assay—Accurately weigh about 350 mg into a tared, glass-stoppered flask containing 50 mL of dimethylformamide previously neutralized to the thymol blue endpoint with 0.1 N sodium methoxide in methanol VS. Titrate with 0.1 N sodium methoxide in methanol VS to the thymol blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 13.014 mg of $C_6H_{10}O_3$. Not less than 97.0% is found.

Refractive index ⟨831⟩: between 1.4035 and 1.4045 at 20°.

Propiophenone, $C_9H_{10}O$—**134.18** [93-55-0]—Use a suitable grade.

***iso*-Propyl Alcohol**—See *Isopropyl Alcohol*.

***n*-Propyl Alcohol** *(1-Propanol),* $CH_3CH_2CH_2OH$—**60.10** [71-23-8]—Use ACS reagent grade.

Propylamine Hydrochloride *(1-Propanamine hydrochloride; n-Propylamine hydrochloride),* $C_3H_9N \cdot HCl$—**95.57** [556-53-6]—Use a suitable grade with a content of not less than 99%.

Protein Molecular Weight Standard—Also known as protein molecular weight markers (for SDS-PAGE) and consists of a mixture of several proteins of well-defined molecular weights. The products are generally available in a suitable buffer containing a suitable reducing agent (generally, 100 mM DTT), a preservative (for example, sodium azide), and 50% glycerol to prevent freezing. Use a suitable grade. Store at –20°.

Protein Standard Solution (8 g/dL)—A solution containing 5 g of Albumin Human and 3 g of human gamma globulin per dL.

[NOTE—A suitable grade is available as Protein Standard Solution, catalog number 540-10, from Sigma-Aldrich, www.sigma-aldrich.com.]

Protocatechuic Acid (*3,4-Dihydroxybenzoic acid*), $C_7H_6O_4$—**154.12** [99-50-3]—Use a suitable grade.

Pullulanase *(Amylopectin-6-gluconohydrolase)* [9075-68-7]—An enzyme obtained from *Kleibsiella pneumoniae*. It contains not less than 30 units per mg of protein. One unit defined for enzymatic activity will liberate 1.0 µmol of maltotriose (measured as glucose) from pullulan per minute

at pH 5.0 at 30°. It can be suspended in 3.2 M ammonium sulfate solution, pH 6.2.

Measurement of relative pullulanase activity—

DETERMINATION OF PULLULANASE ACTIVITY—

Substrate—Dissolve pullulan[1] in water to make a 1.25% (w/v) solution. [NOTE—Add pullulan to the water. Clumping will occur if water is added to pullulan.]

Buffer solution A, pH 5.0—Add 0.1 M disodium phosphate (27 g of dibasic sodium phosphate in each L of the solution) to 0.1 M citric acid (21 g of citric acid in each L of the solution) to adjust pH to 5.0.

Buffer solution B, pH 6.0—Add diluted acetic acid to 1 M sodium acetate (136 g of sodium acetate in each L of solution) to adjust the pH to 6.0. Dilute with water to prepare the final buffer solution as 0.01 M acetic acid buffer, pH 6.0.

Somogyi reagent—Add 54 g of disodium phosphate heptahydrate or 28 g of anhydrous disodium hydrogen phosphate and 40 g of potassium sodium tartrate to about 650 mL of water or about 700 mL for anhydrous disodium hydrogen phosphate. Add 100 mL of 1 N sodium hydroxide to this solution and mix. Add 80 mL of 10% cupric sulfate to the solution, and mix. Heat until any solid is completely dissolved. Add 180 g of anhydrous sodium sulfate to the solution and adjust the volume to 1 L. Allow the solution to stand at room temperature for 1 or 2 days to let insoluble matter precipitate. Filter the solution with standard filter paper, and keep the solution in a brown bottle with a ground-glass stopper.

Nelson reagent—Dissolve 50 g of ammonium molybdate in 900 mL of water. Add 42 g of sulfuric acid, and mix. Dissolve 6 g of sodium arsenate or 3.6 g of monobasic potassium arsenate in 50 g of water. Allow the solution to stand in a brown bottle with a ground-glass stopper at 37° for 1 or 2 days.

Glucose standard solution—Dry anhydrous glucose crystals under less than 50-mm Hg at 60° for 5 hours, and calculate the water content. Transfer 10.00 g of dried glucose to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL to a 1-L volumetric flask and dilute to volume with water. Each mL contains 100 µg of glucose.

Pullulanase diluent—Dilute pullulanase with *Buffer solution B, pH 6.0* to prepare a solution having the enzyme activity of about 0.2 units per mL. [NOTE—The measurement range is between 0.1 and 0.4 units per mL.] Record the dilution factor (F_{PD}). This diluent is used as a diluted enzyme solution.

Procedure—Transfer 4 mL of *Substrate* to a test tube and add 0.5 mL of *Buffer solution A, pH 5.0,*mix, and incubate at 30°. Add 0.5 mL of *Pullulanase diluent,* and mix thoroughly. After 30 seconds, transfer 1 mL of this solution to a test tube labeled as "Pullulan test solution 1", and add 2 mL of *Somogyi reagent,* and mix. After 30 minutes and 30 seconds, transfer 1 mL of the mixture of *Substrate* and *Pullulanase diluent* to a second test tube labeled as "Pullulan test solution 2", add 2 mL of *Somogyi reagent,* and mix. In a third test tube labeled as "Standard blank", mix 2 mL of *Somogyi reagent* and 1 mL of water. In a fourth test tube labeled as "Glucose standard solution", mix 2 mL of *Somogyi reagent* and 1 mL of *Glucose standard solution,* and add 1 mL of water. Incubate the fourth test tube in a boiling water bath for exactly 10 minutes. Remove the tube and allow it to cool in cold running water. Add 2 mL of *Nelson reagent,* mix well, and allow the solution to stand for at least 15 minutes. Add 5 mL of water to each of the four test tubes, and mix thoroughly. Determine the absorbance at 520 nm of the Standard blank, A_{blank}, of the *Glucose standard solution,* A_{Std}, of the Pullulan test solution 1, A_0, and of the Pullulan test solution 2, A_{30}, using water as the blank. One unit is defined as the enzymatic activity that produces 1 µmol of maltotriose (measured as glucose) from pullulan per minute. Calculate the pullulanase activity, PA, in units per mL, using the formula:

$$PA = [(A_{30} - A_0)/(A_{Std} - A_{blank})] \times 0.185 \times F_{PD}$$

MEASUREMENT OF PROTEIN AMOUNT (MEASURED AS ALBUMINOID AMOUNT) FOR THE CALCULATION OF SPECIFIC ACTIVITY—

Reagent A—Prepare a solution having known concentrations of about 0.1 N sodium hydroxide and about 0.2 M sodium carbonate.

Reagent B—Transfer 0.5 g of cupric sulfate and 1.0 g of sodium citrate dihydrate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Lowry solution—Mix *Reagent A* and *Reagent B* at the proportion of 50:1.

Diluted Folin-Ciocalteu's phenol reagent (for albuminoid quantification)—Prepare a two-fold dilution of 2 N Folin-Ciocalteu's phenol reagent commercially available or prepare a solution by making an appropriate dilution from Folin-Ciocalteu Phenol TS (see *Method 2* in *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩).

Bovine serum albumin standard stock solution—Transfer 0.05 g of bovine serum albumin to a 500-mL volumetric flask, dissolve in and dilute with water to volume, and mix. It contains 100 µg of bovine serum albumin per mL.

Standard solutions—Using appropriate dilutions of *Bovine serum albumin standard stock solution* in water, prepare five *Standard solutions* having concentration equally spaced between 5 and 100 µg of bovine serum albumin per mL.

Test solution—Dilute pullulanase with *Buffer solution B, pH 6.0* in order to obtain a solution having a concentration between 60 and 70 µg of albuminoid per mL. [NOTE—Water can be used as diluent.] Record the dilution factor, F_{TS}.

Blank solution—Use water.

Procedure—To 0.3 mL in separate tubes of the *Standard solutions,* the *Test solution,* and the *Blank solution,* add 3 mL of *Lowry solution,* and mix. Allow to incubate at room temperature for 10 minutes. Add 0.3 mL of *Diluted Folin-Ciocalteu's phenol reagent* to each tube, mix immediately, and allow to stand at room temperature for 60 minutes. Determine the absorbances of the *Standard solutions* and the *Test solution* at the wavelength of maximum absorbance at about 750 nm, using the *Blank solution* as the blank.

Calculation—[NOTE—The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using linear regression method, plot the absorbances of the *Standard solutions* versus the protein (bovine serum albumin) concentrations, in µg per mL, and determine the best fit curve. Using the plot, determine the concentration, $C_{albuminoid}$, in µg per mL, of protein (albuminoid amount) in the *Test solution*. Calculate the albuminoid concentration, in mg per mL, in the pullulanase taken by the formula:

$$C_{protein} = (C_{albuminoid} \times F_{TS})/1000$$

Calculate the specific activity, SA, in units per mg, of pullulanase using the formula:

$$SA = PA/C_{protein}$$

Pumice—A substance of volcanic origin consisting chiefly of complex silicates of aluminum and alkali metals. Occurs as very light, hard, rough, porous, gray masses, or as a gray-colored powder. Is insoluble in water and is not attacked by diluted acids.

Acid- and water-soluble substances—Boil 2.0 g of powdered pumice with 50 mL of diluted hydrochloric acid under a reflux condenser for 30 minutes. Cool, and filter. To half of the filtrate add 5 drops of sulfuric acid, evaporate to

[1] A suitable supplier for pullulan is www.hayashibara-intl.com.

dryness, ignite, and weigh: the residue weighs not more than 60 mg (6.0%).

Purine, $C_5H_4N_4$—**120.11** [120-73-0]—White to off-white powder.

Melting range ⟨741⟩: between 214° and 217°.

A single spot is exhibited when it is examined by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of butyl alcohol, water, and glacial acetic acid (60:25:15).

Putrescine Dihydrochloride, $C_4H_{12}N_2 \cdot 2HCl$—**161.07** [333-93-7]—White, crystalline powder. Use a suitable grade.

Pyrazole, $C_3H_4N_2$ [288-13-1]—White to pale yellow crystals or crystalline powder. Soluble in water, in alcohol, and in ether.

Melting range ⟨741⟩: between 67° and 71°.

Pyrene, $C_{16}H_{10}$—**202.25** [129-00-0]—White to light yellow crystals.

Assay—Transfer about 9 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Using a suitable spectrophotometer, 1-cm cells, and methanol as the blank, record the absorbance of the solution at the wavelength of maximum absorbance at about 238 nm. From the observed absorbance, calculate the absorptivity (see *Spectrophotometry and Light-scattering* ⟨851⟩): the absorptivity is not less than 432.9, corresponding to not less than 98% of $C_{16}H_{10}$.

Melting range ⟨741⟩: between 149° and 153° over a 2° range.

Pyridine, C_5H_5N—**79.10** [110-86-1]—Use ACS reagent grade.

Pyridine, Dried [110-86-1]—Use ACS reagent grade.

Pyridoxal Hydrochloride, $C_8H_9NO_3 \cdot HCl$—**203.62** [65-22-5]—White to slightly yellow crystals or crystalline powder. Gradually darkens on exposure to air or sunlight. One g dissolves in about 2 mL of water and in about 25 mL of alcohol. Insoluble in acetone, in chloroform, and in ether. Its solutions are acid (pH about 3).

Melting range ⟨741⟩: between 171° and 175° with some decomposition.

Residue on ignition (Reagent test): not more than 0.1%.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Nitrogen content (Reagent test)—Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 6.7% and 7.1% of N is found.

Chloride content—Accurately weigh about 500 mg, previously dried at 105° for 2 hours, and dissolve in 50 mL of water. Add 3 mL of nitric acid and 50.0 mL of 0.1 N silver nitrate VS, then add 5 mL of nitrobenzene, shake for about 2 minutes, add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS: each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 17.2% and 17.7% is found.

Pyridoxal 5-Phosphate, $4\text{-}CHOC_5HN\text{-}2\text{-}CH_3$, 3-OH, $5\text{-}CH_2PO_4H_2 \cdot H_2O$—**265.16** [41468-25-1]—Light yellow powder. Use a suitable grade.

Pyridoxamine Dihydrochloride, $C_8H_{12}N_2O_2 \cdot 2HCl$—**241.11** [524-36-7]—White to slightly yellow crystals or crystalline powder. Gradually darkens on exposure to air or sunlight. One g dissolves in about 1 mL of water and in about 60 mL of alcohol. Insoluble in chloroform and in ether. Its solutions are acid.

Melting range ⟨741⟩: between 225° and 230°, with some decomposition.

Residue on ignition (Reagent test): not more than 0.15%.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Nitrogen content (Reagent test)—Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 11.3% and 11.8% of N is found.

Chloride content—Determine as directed in the test for *Chloride content* under *Pyridoxal Hydrochloride:* between 29.1% and 29.6% of Cl is found.

1-(2-Pyridylazo)-2-naphthol, $C_{15}H_{11}N_3O$—**249.27** [85-85-8]—Stable, orange-red crystals. Soluble in alcohol and in hot solutions of dilute alkalies; slightly soluble in water.

Melting range ⟨741⟩: between 140° and 142°.

Sensitiveness—Add 0.1 mL of a 1 in 1000 solution of it in alcohol to a mixture of 10 mL of water and 1 mL of a buffer solution prepared by mixing 80 mL of 0.2 M acetic acid and 20 mL of sodium acetate solution (8.2 in 100), and mix. To this solution add 1 mL of a mixture of 1 mL of cupric sulfate TS and 2 mL of water, and mix: the color changes from yellow to red.

4-(2-Pyridylazo)resorcinol (*PAR*), $C_{11}H_9N_3O_2$, free acid; $C_{11}H_8N_3NaO_2$, monosodium salt—**215.21,** free acid—**237.21,** monosodium salt [1141-59-9,free acid; 16593-81-0,monosodium salt]—Use ACS reagent grade.

3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic Acid, Disodium Salt *(3-(2-Pyridyl)-5,6-bis(5-sulfo-2-furyl)-1,2,4-triazine, Disodium Salt Hydrate),* $C_{16}H_8N_4Na_2O_8S_2$—**494.37** [79551-14-7]—Use a suitable grade.

[NOTE—A suitable grade is available as product number P4272 from Sigma-Aldrich, 1-800-558-9160; www.sigma-aldrich.com.]

Pyrogallol, $C_6H_3(OH)_3$—**126.11** [87-66-1]—Use ACS reagent grade.

Pyrrole, C_4H_5N—**67.09** [109-97-7]—Clear liquid, colorless when freshly distilled, becoming yellow in a few days. Specific gravity: about 0.94. Insoluble in water; soluble in alcohol, in benzene, and in ether.

Boiling range (Reagent test)—Not less than 90% distills between 128° and 132°.

Pyruvic Acid, $CH_3COCOOH$—**88.06** [127-17-3]—Colorless to light yellow liquid. Miscible with water, with alcohol, and with ether.

Refractive index ⟨831⟩: about 1.43 at 20°.

Assay—Accurately weigh about 1 g, transfer to a suitable container, and add 100 mL of water. Mix, add phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS. Each mL of 0.5 N sodium hydroxide is equivalent to 44.03 mg of $CH_3COCOOH$: not less than 98% of $CH_3COCOOH$ is found.

Quantitative Filter Paper—See *Filter Paper, Quantitative*.

Quinhydrone, $C_6H_4(OH)_2 \cdot C_6H_4O_2$—**218.21** [106-34-3]—Green crystals having a metallic luster. Slightly soluble in cold water; soluble in hot water, in alcohol, and in ether.

Assay—Transfer about 450 mg, accurately weighed, to a glass-stoppered flask, add 50 mL of 1 N sulfuric acid and 3 g of potassium iodide, insert the stopper in the flask, and shake until dissolved. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.405 mg of quinone ($C_6H_4O_2$). Between 49.0% and 51.0% is found.

Alcohol-insoluble matter—Dissolve 10 g in 100 mL of hot alcohol, filter through a suitable tared crucible of fine porosity, and wash with hot alcohol until the last washing is colorless. Dry at 105°, cool in a desiccator, and weigh: the residue weighs not more than 1.0 mg (0.010%).

Residue on ignition (Reagent test): not more than 0.050%, a 2.0-g test specimen being used. Save the residue.

Sulfate—Transfer 1 g to a platinum crucible, add 10 mL of hot water and 0.5 g of sodium carbonate, evaporate to dryness, and ignite, protected from the sulfur in the flame, until the residue is nearly white. Cool, add 20 mL of water