Reagents, Indicators and Solutions

This section deals with the reagents and solutions required in conducting the Pharmacopeial and the National Formulary tests and assays.

As is stated in the *General Notices*, listing of reagents, indicators, and solutions in the Pharmacopeia in no way implies that they have therapeutic utility; thus, any reference to the USP in their labeling is to include the term "reagent" or "reagent grade."

Reagents required in the tests and assays for the Pharmacopeial and National Formulary articles are listed in this section, generally with specifications appropriate to their intended uses. Exceptions to the latter include those reagents for which corresponding specifications are presented in the current edition of *Reagent Chemicals*, published by the American Chemical Society, and reagents for which specifications could not be drafted in time for inclusion here. Thus, where it is directed to "Use ACS reagent grade," it is intended that a grade meeting the corresponding specifications of the current edition of ACS *Reagent Chemicals* shall be used. Where no such specifications exist, and where it is directed to "Use a suitable grade," the intent is that a suitable reagent grade available commercially shall be used. Occasionally, additional test(s) augment the designation "suitable grade," as indicated in the text. Listed also are some, but not all, reagents that are required only in determining the quality of other reagents. For those reagents that are not listed, satisfactory specifications are available in standard reference works.

In those instances in which a reagent required in a Pharmacopeial or National Formulary test or assay need not be of analytical reagent quality, it suffices to refer to the monograph for that article appearing in this Pharmacopeia or the National Formulary or the current edition of the Food Chemicals Codex (FCC). In such cases it is to be understood that the specifications are minimum requirements and that any substance meeting more rigid specifications for chemical purity is suitable.

Where the name of a reagent specified in a test or assay is the same as the title of a *USP* or *NF* article, and it does not appear among the following *Reagent Specifications*, a substance meeting the requirements of the *USP* or *NF* monograph is to be used (e.g., *Benzocaine*, USP; or *Propylparaben*, NF). However, reference is specifically made, under *Reagent Specifications*, to a reagent bearing the name of a *USP* or *NF* article: (1) where there are requirements for a reagent in addition to the *USP* or *NF* monograph requirements (e.g., *Sodium Salicylate*, USP; or *Isopropyl Myristate*, NF), (2) where a source other than the *USP* or *NF* monograph is specified (e.g., *Lactose*, ACS reagent; or *Hydrochloric Acid*, ACS reagent), (3) where complete reagent specifications differ from the *USP* or *NF* monograph standards (e.g., *Calcium Lactate*; or *Thymol*), or (4) where a standard material is included among the reagent specifications (e.g., *Calcium Carbonate*, primary standard; or *Sodium Carbonate*, primary standard).

Reagents and solutions should be preserved in tight containers made of resistant glass or other suitable material. Directions for storage in light-resistant containers should be carefully observed.

Stoppers and stopcocks brought into contact with substances capable of attacking or penetrating their surfaces may be given a protective coating of a thin film of a suitable lubricant unless specifically interdicted.

Where a particular brand or source of a material or piece of equipment, or the name and address of a manufacturer, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

Atomic absorption and flame photometry require the use of a number of metal-ion standard solutions. While the individual monographs usually provide directions for preparation of these solutions, use of commercially prepared standardized solutions of the appropriate ions is permissible, provided that the analyst confirms the suitability of the solutions and has data to support their use.

Reagents are substances used either as such or as constituents of solutions.

Indicators are reagents used to determine the specified endpoint in a chemical reaction, to measure hydrogen-ion concentration (pH), or to indicate that a desired change in pH has been effected. They are listed together with indicator test papers.

Buffer Solutions are referred to separately.

Colorimetric Solutions, abbreviated "CS," are solutions used in the preparation of colorimetric standards for comparison purposes.

Test Solutions, abbreviated "TS," are solutions of reagents in such solvents and of such definite concentrations as to be suitable for the specified purposes.

Volumetric Solutions, abbreviated "VS" and known also as **Standard Solutions**, are solutions of reagents of known concentration intended primarily for use in quantitative determinations. Concentrations are usually expressed in terms of normality.

Water—As elsewhere in the Pharmacopeia, where "water", without qualification, is mentioned in the tests for reagents or in directions for preparing test solutions, etc., Purified Water (USP monograph) is always to be used. *Carbon dioxide-free water* is Purified Water that has been boiled vigorously for 5 minutes or more and allowed to cool while protected from absorption of carbon dioxide from the atmosphere, or Purified Water that has a resistivity of not less than 18 Mohm-cm. *Deaerated water*, for purposes other than dissolution and drug release testing, is Purified Water that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 minutes and cooling or by the application of ultrasonic vibration. *Particle-free water* is water that has been passed through a 0.22-µm filter.

Organic-free water is Purified Water that produces no significantly interfering peaks when chromatographed as it is indicated in *Identification, Control, and Quantification of Residual Solvents* under *Residual Solvents* ⟨467⟩

Chromatographic Solvents and Carrier Gases—The chromatographic procedures set forth in the Pharmacopeia may require use of solvents and gases that have been especially purified for such use. The purpose may be (a) to exclude certain impurities that interfere with the proper conduct of the test procedure, or (b) to extend the life of a column by reducing the build-up of impurities on the column. Where solvents and gases are called for in chromatographic procedures, it is the responsibility of the analyst to ensure the suitability of the solvent or gas for the specific use. Solvents and gases suitable for specific high-pressure or other chromatographic uses are available as specialty products from various reagent supply houses, although there is no assurance that similar products from different suppliers are of equivalent suitability in any given procedure. The reagent specifications provided herein are for general analytical uses of the solvents and gases and not for chromatographic uses for which the especially purified specialty products may be required.

Reagents

For the purposes of the following specifications, these definitions apply: A *blank* consists of the same quantities of the same reagents treated in the same manner as the specimen under test. A *control* is a blank to which has been added the limiting quantity of the substance being tested for, or is a specified comparison solution prepared as directed in the particular test.

The values given in boldface type following chemical symbols and formulas represent, respectively, atomic and molecular weights of the substances concerned.

Color and turbidity comparisons are to be made in color-comparison tubes that are matched as closely as possible in internal diameter and in all other respects, as directed for *Visual Comparison* under *Spectrophotometry and Light-scattering* 〈851〉. Such tubes frequently are called “Nessler tubes.”

In making visual comparisons of the densities of turbid fluids, compensate for differences in color, if necessary, by viewing the turbidity through a column of water, the depth of which is determined by the volume specified in the individual reagent specification. Place the water in color-comparison tubes, and hold one of the tubes above the control tube and the other below the specimen tube.

Where an expression such as “Retain the filtrate” appears it is to be understood, unless otherwise indicated, that the washings of the residue are not to be added to the filtrate obtained. In the test heading, *Calcium, magnesium, and* R_2O_3 *precipitate*, the expression R_2O_3 is intended to indicate the residue on ignition from compounds precipitated upon the addition of ammonium hydroxide, such as Fe_2O_3 and Al_2O_3.

GENERAL TESTS FOR REAGENTS

The following general test methods are provided for the examination of reagents to determine their compliance with the specifications of the individual reagents and are to be used unless it is otherwise directed in such specifications.

Boiling or Distilling Range for Reagents

Use the following procedure for determining the boiling or distilling range of reagents, unless otherwise directed in the individual specifications:

APPARATUS—Use apparatus similar to that specified for *Distilling Range—Method I* 〈721〉, except that the distilling flask is to be of 250-mL capacity, to have a short neck, and to be connected to the condenser by means of a three-way connecting tube fitted with standard-taper ground joints.

PROCEDURE—Place the distilling flask in an upright position in the perforation in the asbestos board, and connect it to the condenser.

Measure 100 mL of the liquid to be tested in a graduated cylinder, and transfer to the boiling flask together with some device to prevent bumping. Use the cylinder as the receiver for the distillate. Insert the thermometer, and heat so as to distill at the rate of 3 mL to 5 mL per minute. Make a preliminary trial, if necessary, to determine the adjustment for the proper rate of heating. Read the thermometer when about 20 drops have distilled and thereafter at volumes of distillate of 5, 10, 40, 50, 60, 90, and 95 mL. Continue the distillation until the dry point is reached.

The *Boiling or Distilling Range* is the interval between the temperatures when 1 mL and 95 mL, respectively, have distilled.

Arsenic in Reagents

Select reagents for this test for a low arsenic content, so that a blank test results in either no stain or one that is barely perceptible.

APPARATUS—Prepare a generator by fitting a 1-hole rubber stopper into a wide-mouth bottle of about 60-mL capacity. Through the perforation insert a vertical exit tube about 12 cm in total length and 1 cm in diameter along the entire upper portion (for about 8 cm) and constricted at its lower extremity to a tube about 4 cm in length and about 5 mm in diameter. The smaller portion of the tube should extend to just slightly below the stopper. Place washed sand or a pledget of purified cotton in the upper portion to about 3 cm from the top of the tube. Moisten the sand or cotton uniformly with lead acetate TS, and remove any excess or adhering droplets of the latter from the walls of the tube. Into the upper end of this tube fit a second glass tube 12 cm in length, having an internal diameter of 2.5 to 3 mm, by means of a rubber stopper. Just before running the test, place a strip of mercuric bromide test paper (see under *Indicator and Test Papers*) in this tube, crimping the upper end of the strip so that it will remain in position about 2 cm above the rubber stopper. Clean and dry the tube thoroughly each time it is used.

STANDARD ARSENIC SOLUTION—Use *Standard Preparation* prepared as directed under *Arsenic* 〈211〉.

TEST PREPARATION—Add 1 mL of sulfuric acid to 5 mL of a solution of the chemical substance (1 in 25), unless another quantity is directed in the individual reagent specification. Omit its addition entirely in the case of inorganic acids. Unless especially directed otherwise, add 10 mL of sulfurous acid. Evaporate the liquid in a small beaker, on a steam bath, until it is free from sulfurous acid and has been reduced to about 2 mL in volume. Dilute with water to 5 mL to obtain the *Test Preparation.* Substances subjected to special treatments specified in the individual reagent specification may be used directly as the *Test Preparation.*

[NOTE—Solutions prepared by the dissolving of the chemical substances in dilute acids are not considered to have undergone special treatment.]

STANDARD STAIN—Place in the generator bottle 5 mL of potassium iodide TS, 2.0 mL of *Standard Arsenic Solution*, 5 mL of acid stannous chloride TS, and 28 mL of water. Add 1.5 g of granulated zinc (in No. 20 powder), and immediately insert the stopper containing the exit tube. Keep the generator bottle immersed in water at 25° during the period of the test to moderate the reaction so that the stain will take the form of a distinctive band to facilitate the comparison of color intensity. When evolution of hydrogen has continued for 1 hour, remove the mercuric bromide test paper for comparison. This stain represents 2 µg of arsenic.

PROCEDURE—Pipet into the generator bottle 5 mL of potassium iodide TS and 5 mL of the *Test Preparation*, and add 5 mL of acid stannous chloride TS. Set the apparatus aside at room temperature for a period of 10 minutes, then add 25 mL of water and 1.5 g of granulated zinc (in No. 20 powder), and proceed as directed under *Standard Stain.* Remove the mercuric bromide test paper, and compare the stain upon it with the *Standard Stain:* the stain produced by the chemical tested does not exceed the standard stain in length or in intensity of color, indicating not more than 10 parts of arsenic per million parts of the substance being tested. Since light, heat, and moisture cause the stain to