

1995

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NF 18

THE UNITED STATES PHARMACOPEIA

THE NATIONAL FORMULARY

By authority of the United States Pharmacopeial Convention, Inc., meeting at Washington, D.C., March 8–10, 1990. Prepared by the Committee of Revision and published by the Board of Trustees

Official from January 1, 1995



UNITED STATES PHARMACOPEIAL CONVENTION, INC.
12601 Twinbrook Parkway, Rockville, MD 20852

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12601 Twinbrook Parkway, Rockville, MD 20852.
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ISSN 0195-7996
ISBN 0-913595-76-4 (cloth)
0-913595-81-0 (leather)



Printed by Rand McNally, 1133 County Street, Taunton, MA 02780-3795

$$(330.32/308.34)(900C)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Warfarin RS in the *Standard stock solution*, 330.32 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively, and R_U and R_S are the ratios of the peak responses of warfarin to those of propylparaben obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{19}H_{15}NaO_4$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

pH 7.4 buffer and Chromatographic system—Proceed as directed in the *Assay* under *Warfarin Sodium*.

Solvent mixture—Prepare a mixture of pH 7.4 buffer and acetonitrile (85:15).

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (68:32:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Prepare a solution of propylparaben in acetonitrile having a concentration of 1 mg per mL.

Diluted internal standard solution—Dilute a volume of *Internal standard solution* with *Solvent mixture* to obtain a solution having a concentration of 0.1 mg of propylparaben per mL.

Standard preparation—Transfer about 62.5 mg of USP Warfarin RS, accurately weighed, to a 200-mL volumetric flask, and dissolve in 78 mL of 0.1 N sodium hydroxide. Add 50 mL of 0.2 M monobasic potassium phosphate, dilute with water to volume, and mix. Transfer 15.0 mL of this solution to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Solvent mixture* to volume.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of warfarin sodium, to a 50-mL volumetric flask, and add 5 mL of *Internal standard solution* and about 30 mL of *Solvent mixture*. Sonicate for 10 minutes, and then shake by mechanical means for 60 minutes. Dilute with solvent mixture to volume, and filter.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{19}H_{15}NaO_4$ in the portion of Tablets taken by the formula:

$$(330.32/308.34)C(R_U/R_S)$$

in which 330.32 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively, C is the concentration, in mg per mL, of USP Warfarin RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak responses of warfarin to those of propylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Water for Injection

» Water for Injection is water purified by distillation or by reverse osmosis. It contains no added substance.

NOTE—Water for Injection is intended for use as a solvent for the preparation of parenteral solutions. Where used for the preparation of parenteral solutions subject to final sterilization, use suitable means to minimize microbial growth, or first render the Water for Injection sterile and thereafter protect it from microbial contamination. For parenteral solutions that are prepared under aseptic conditions and are not sterilized by appropriate filtration or in the final container, first render the Water for Injection sterile and, thereafter, protect it from microbial contamination.

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measure the quantity, in mg, of warfarin taken by the

sights of warfarin concentration, in mg, of warfarin to propylparaben and the Standard

Warfarin Sodium Tablets

Warfarin Sodium Tablets contain not less than 90 percent and not more than 105.0 percent of the labeled amount of $C_{19}H_{15}NaO_4$.

Packaging and storage—Preserve in tight, light-resistant containers.

Reference standards (11)—USP Warfarin RS.

Identification—

A—The retention time of the major peak obtained from the *Assay preparation* corresponds to that obtained from the *Standard solution*, both relative to the internal standard, obtained as directed in the *Assay*.

B—Triturate a quantity of finely powdered Tablets, equivalent to about 200 mg of warfarin sodium, with 50 mL of water, centrifuge, and filter the supernatant liquid. Extract with 50 mL of ether, transfer the aqueous layer to a second separator, and discard the ether. Adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper, and extract with 50 mL of chloroform. Transfer the chloroform layer to another separator, extract with 50 mL of sodium hydroxide solution (1 in 250), and discard the chloroform. Transfer the aqueous layer to a beaker, and adjust with hydrochloric acid to a pH of less than 3 (using the pH indicator paper) to precipitate the warfarin. Stir the mixture and allow the precipitate to coagulate. Filter, wash the precipitate with four 5-mL portions of water. If the precipitate is not white or practically white, dissolve it in a minimum volume of sodium hydroxide solution (1 in 250), dilute with water to 50 mL, and repeat the foregoing procedure, beginning with "Extract with 50 mL of ether." Dry the precipitate in a vacuum over phosphorus pentoxide for 4 hours: the infrared absorption spectrum of the warfarin so obtained exhibits maxima at the same wavelengths as that of a similar preparation of USP Warfarin RS.

C—Dissolve a quantity of finely powdered Tablets, equivalent to about 100 mg of warfarin sodium, in 25 mL of water, and filter, if necessary: a 5-mL portion of the filtrate responds to the tests for Sodium (191).

Dissolution (711)—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.

Mobile phase and Chromatographic system—Proceed as directed in the *Assay*.

Internal standard solution—Prepare a solution of propylparaben in water containing, in each mL, an amount of propylparaben equivalent to 0.0025 times the labeled amount, in mg, of warfarin sodium in each Tablet. [NOTE—A small amount of methanol may be used, if necessary, to dissolve the propylparaben.]

Standard stock solution—Dissolve an accurately weighed quantity of USP Warfarin RS in water to obtain a solution having a known concentration of about 0.0011 L mg per mL, L being the labeled amount, in mg, of warfarin sodium in the Tablets. [NOTE—Use a small amount of 0.1 N sodium hydroxide to aid in dissolution.]

Standard solution—To 3.0 mL of *Standard stock solution*, add 1.0 mL of *Internal standard solution*, and mix.

Test solution—To a filtered 3.0-mL aliquot of the solution under test, add 1.0 mL of *Internal standard solution*, and mix.

Procedure—Separately inject equal volumes (about 40 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of warfarin sodium dissolved by the formula:

Packaging and storage—Where packaged, preserve in tight containers. Where packaged, it may be stored at a temperature below or above the range in which microbial growth occurs.

USP Reference standards (11)—*USP Endotoxin RS*.

Bacterial endotoxins (85)—It contains not more than 0.25 USP Endotoxin Unit per mL.

Bacteriostatic Water for Injection

» Bacteriostatic Water for Injection is Sterile Water for Injection containing one or more suitable antimicrobial agents.

NOTE—Use Bacteriostatic Water for Injection with due regard for the compatibility of the antimicrobial agent or agents it contains with the particular medicinal substance that is to be dissolved or diluted.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass, of not larger than 30-mL size.

Labeling—Label it to indicate the name(s) and proportion(s) of the added antimicrobial agent(s). Label it also to include the statement "NOT FOR USE IN NEWBORNS" in boldface capital letters on the label immediately under the official name, printed in a contrasting color, preferably red. Alternatively, the statement may be placed prominently elsewhere on the label if the statement is enclosed within a box.

USP Reference standards (11)—*USP Endotoxin RS*.

Antimicrobial agent(s)—It meets the requirements under *Antimicrobial Preservatives—Effectiveness* (51), and meets the labeled claim for content of the antimicrobial agent(s), as determined by the method set forth under *Antimicrobial Agents—Content* (341).

Bacterial endotoxins (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

Sterility—It meets the requirements under *Sterility Tests* (71). **pH** (791): between 4.5 and 7.0, determined potentiometrically in a solution prepared by the addition of 0.30 mL of saturated potassium chloride solution to 100 mL of test specimen.

Particulate matter (788): meets the requirements under *Small-volume Injections*.

Other requirements—It meets the requirements of the tests for *Sulfate, Calcium, Carbon dioxide, and Heavy metals* under *Sterile Water for Injection*.

Sterile Water for Inhalation

» Sterile Water for Inhalation is water purified by distillation or by reverse osmosis and rendered sterile. It contains no antimicrobial agents, except where used in humidifiers or other similar devices and where liable to contamination over a period of time, or other added substances.

NOTE—Do not use Sterile Water for Inhalation for parenteral administration or for other sterile compendial dosage forms.

Packaging and storage—Preserve in single-dose containers.

Labeling—Label it to indicate that it is for inhalation therapy only and that it is not for parenteral administration.

USP Reference standards (11)—*USP Endotoxin RS*.

Bacterial endotoxins (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

Sterility—It meets the requirements under *Sterility Tests* (71). **pH** (791): between 4.5 and 7.5, in a solution containing 0.30 mL of saturated potassium chloride solution per 100 mL of test specimen.

Chloride—To 20 mL in a color-comparison tube add 5 drops of nitric acid and 1 mL of silver nitrate TS, and gently mix; any turbidity formed within 10 minutes is not greater than that produced in a similarly treated control consisting of 20 mL of *High-purity Water* (see under *Chemical Resistance—Glass Containers* (661)), containing 10 µg of Cl, viewed downward over a dark surface with light entering the tubes from the sides (0.5 ppm).

Other requirements—It meets the requirements of the tests for *Sulfate, Calcium, Carbon dioxide, and Heavy metals* under *Purified Water* and of the tests for *Ammonia, Oxidizable substances, and Total solids* under *Sterile Water for Injection*.

Sterile Water for Injection

» Sterile Water for Injection is Water for Injection sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

Packaging and storage—Preserve in single-dose glass or plastic containers, of not larger than 1-liter size. Glass containers are preferably of Type I or Type II glass.

Labeling—Label it to indicate that no antimicrobial or other substance has been added, and that it is not suitable for intravascular injection without its first having been made approximately isotonic by the addition of a suitable solute.

USP Reference standards (11)—*USP Endotoxin RS*.

Bacterial endotoxins (85)—It contains not more than 0.25 USP Endotoxin Unit per mL.

Sterility—It meets the requirements under *Sterility Tests* (71). **Particulate matter** (788): meets the requirements under *Small-volume Injections*.

Ammonia—For Sterile Water for Injection in containers having a fill volume of less than 50 mL, dilute 50 mL with 50 mL of *High-purity Water* (see *Reagents under Containers* (661)), and use this dilution as the test solution; where the fill volume is 50 mL or more, use 100 mL of Sterile Water for Injection as the test solution. To 100 mL of the test solution add 2 mL of alkaline mercuric-potassium iodide TS; any yellow color produced immediately is not darker than that of a control containing 30 µg of added NH₃ in *High-purity Water* (see *Reagents under Containers* (661)) (0.6 ppm for Sterile Water for Injection in containers having a fill volume of less than 50 mL; 0.3 ppm where the fill volume is 50 mL or more).

Chloride—To 20 mL in a color-comparison tube add 5 drops of nitric acid and 1 mL of silver nitrate TS, and gently mix; any turbidity formed within 10 minutes is not greater than that produced in a similarly treated control consisting of 20 mL of *High-purity Water* (see under *Reagents in Containers* (661)) containing 10 µg of Cl (0.5 ppm), viewed downward over a dark surface with light entering the tubes from the sides.

Oxidizable substances—To 100 mL add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Injection in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.1 N potassium permanganate, and boil for 5 minutes; where the fill volume is 50 mL or more, add 0.2 mL of 0.1 N potassium permanganate, and boil for 5 minutes. If a precipitate forms, cool in an ice bath to room temperature, and filter through a sintered-glass filter: the pink color does not completely disappear.

Total solids—Proceed as directed in the test for *Total solids* under *Purified Water*. The following limits apply for Sterile Water for Injection in containers having a fill volume of less than 30 mL, 0.004%; where the fill volume is 30 mL or more, but less than 100 mL, 0.003%; and for a fill volume of 100 mL or more, 0.002%.

Other requirements—It meets the requirements of the tests for pH, Sulfate, Calcium, Carbon dioxide, and Heavy metals under Purified Water.

Sterile Water for Irrigation

» Sterile Water for Irrigation is Water for Injection sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may contain a volume of more than 1 liter, and may be designed to empty rapidly.

Labeling—Label it to indicate that no antimicrobial or other substance has been added. The designations "For irrigation only" and "Not for injection" appear prominently on the label.

USP Reference standards (11)—USP Endotoxin RS.

Other requirements—It meets the requirements of all of the tests under Sterile Water for Injection except the test for Particulate matter (788).

Purified Water

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» Purified Water is water obtained by distillation, ion-exchange treatment, reverse osmosis, or other suitable process. It is prepared from water complying with the regulations of the federal Environmental Protection Agency with respect to drinking water. It contains no added substance.

NOTE—Purified Water is intended for use as an ingredient in the preparation of compendial dosage forms. Where used for sterile dosage forms, other than for parenteral administration, process the article to meet the requirements under Sterility Tests (71), or first render the Purified Water sterile and thereafter protect it from microbial contamination. Do not use Purified Water in preparations intended for parenteral administration. For such purposes use Water for Injection, Bacteriostatic Water for Injection, or Sterile Water for Injection.

Packaging and storage—Where packaged, preserve in tight containers.

Labeling—Where packaged, label it to indicate the method of preparation.

pH (791): between 5.0 and 7.0, determined potentiometrically in a solution prepared by the addition of 0.30 mL of saturated potassium chloride solution to 100 mL of test specimen.

Chloride—To 100 mL add 5 drops of nitric acid and 1 mL of silver nitrate TS: no opalescence is produced.

Sulfate—To 100 mL add 1 mL of barium chloride TS: no turbidity is produced.

Limit of ammonia—To 100 mL add 2 mL of alkaline mercuric-potassium iodide TS: any yellow color produced immediately is not darker than that of a control containing 30 µg of added NH₃ in High-purity Water (see under Reagents in Containers (661)) (0.3 ppm).

Calcium—To 100 mL add 2 mL of ammonium oxalate TS: no turbidity is produced.

Carbon dioxide—To 25 mL add 25 mL of calcium hydroxide TS: the mixture remains clear.

Heavy metals—Adjust 40 mL of Purified Water with 1 N acetic acid to a pH of 3.0 to 4.0 (using short-range pH indicator paper), add 10 mL of freshly prepared hydrogen sulfide TS, and allow the liquid to stand for 10 minutes: the color of the liquid, when viewed downward over a white surface, is not darker than the color of a mixture of 50 mL of the same Purified Water with the same amount of 1 N acetic acid as was added to the test specimen, matched color-comparison tubes being used for the comparison.

Oxidizable substances—To 100 mL add 10 mL of 2 N sulfuric acid, and heat to boiling. Add 0.1 mL of 0.1 N potassium permanganate, and boil for 10 minutes: the pink color does not completely disappear.

Total solids—Evaporate 100 mL on a steam bath to dryness, and dry the residue at 105° for 1 hour: not more than 1 mg of residue remains (0.001%).

Wax, Carnauba—see Wax, Carnauba NF

Wax, Cetyl Esters—see Cetyl Esters Wax NF

Wax, Emulsifying—see Wax, Emulsifying NF

Wax, Microcrystalline—see Wax, Microcrystalline NF

Wax, White—see Wax, White NF

Wax, Yellow—see Wax, Yellow NF

White Lotion

» Prepare White Lotion as follows:

Zinc Sulfate	40 g
Sulfurated Potash.....	40 g
Purified Water, a sufficient quantity, to make.....	1000 mL

Dissolve the Zinc Sulfate and the Sulfurated Potash separately, each in 450 mL of Purified Water, and filter each solution. Add the sulfurated potash solution slowly to the zinc sulfate solution with constant stirring. Then add the required amount of purified water, and mix.

NOTE—Prepare the Lotion fresh, and shake it thoroughly before dispensing.

Packaging and storage—Dispense in tight containers.

Witch Hazel

» Witch Hazel is a clear, colorless distillate prepared from recently cut and partially dried dormant twigs of *Hamamelis virginiana* Linné.

Prepare Witch Hazel as follows. Macerate a weighed amount of the twigs for about 24 hours in about twice their weight of water, then distil until not less than 800 mL and not more than 850 mL of clear, colorless distillate is obtained from each 1000 g of the twigs taken. Add 150 mL of Alcohol to each 850 mL of distillate, and mix thoroughly.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

General Chapters

General Tests and Assays

General Requirements for Tests and Assays

(1) INJECTIONS

INTRODUCTION

Parenteral articles are preparations intended for injection through the skin or other external boundary tissue, rather than through the alimentary canal, so that the active substances they contain are administered, using gravity or force, directly into a blood vessel, organ, tissue, or lesion. Parenteral articles are prepared scrupulously by methods designed to ensure that they meet Pharmacopeial requirements for sterility, pyrogens, particulate matter, and other contaminants, and, where appropriate, contain inhibitors of the growth of microorganisms. An Injection is a preparation intended for parenteral administration and/or for constituting or diluting a parenteral article prior to administration.

NOMENCLATURE AND DEFINITIONS

Nomenclature

The following nomenclature pertains to five general types of preparations, all of which are suitable for, and intended for, parenteral administration. They may contain buffers, preservatives, or other added substances.

1. [DRUG] *Injection*—Liquid preparations that are drug substances or solutions thereof.
2. [DRUG] *for Injection*—Dry solids that, upon the addition of suitable vehicles, yield solutions conforming in all respects to the requirements for *Injections*.
3. [DRUG] *Injectable Emulsion*—Liquid preparations of drug substances dissolved or dispersed in a suitable emulsion medium.
4. [DRUG] *Injectable Suspension*—Liquid preparations of solids suspended in a suitable liquid medium.
5. [DRUG] *for Injectable Suspension*—Dry solids that, upon the addition of suitable vehicles, yield preparations conforming in all respects to the requirements for *Injectable Suspensions*.

Definitions

PHARMACY BULK PACKAGE

A *Pharmacy bulk package* is a container of a sterile preparation for parenteral use that contains many single doses. The

contents are intended for use in a pharmacy admixture program and are restricted to the preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes.

The closure shall be penetrated only one time after constitution with a suitable sterile transfer device or dispensing set which allows measured dispensing of the contents. The *Pharmacy bulk package* is to be used only in a suitable work area such as a laminar flow hood (or an equivalent clean air compounding area).

Designation as a *Pharmacy bulk package* is limited to preparations from *Nomenclature* categories 1, 2, or 3 as defined above. *Pharmacy bulk packages*, although containing more than one single dose, are exempt from the multiple-dose container volume limit of 30 mL and the requirement that they contain a substance or suitable mixture of substances to prevent the growth of microorganisms.

Where a container is offered as a *Pharmacy bulk package*, the label shall (a) state prominently "Pharmacy Bulk Package—Not for direct infusion," (b) contain or refer to information on proper techniques to help assure safe use of the product, and (c) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under the labeled storage conditions.

LARGE- AND SMALL-VOLUME INJECTIONS

Where used in this Pharmacopeia, the designation *Large-volume intravenous solution* applies to a single-dose injection that is intended for intravenous use and is packaged in containers labeled as containing more than 100 mL. The designation *Small-volume Injection* applies to an Injection that is packaged in containers labeled as containing 100 mL or less.

BIOLOGICS

The Pharmacopeial definitions for sterile preparations for parenteral use generally do not apply in the case of the biologics because of their special nature and licensing requirements (see *Biologics* (1041)).

INGREDIENTS

Vehicles and Added Substances

Aqueous Vehicles—The vehicles for aqueous Injections meet the requirements of the *Pyrogen Test* (151) or the *Bacterial Endotoxins Test* (85), whichever is specified. *Water for Injection* generally is used as the vehicle, unless otherwise specified in the individual monograph. Sodium chloride may be added in amounts sufficient to render the resulting solution isotonic; and *Sodium Chloride Injection*, or *Ringer's Injection*, may be used in whole or in part instead of *Water for Injection* unless otherwise specified in the individual monograph. For conditions applying to other adjuvants, see *Added Substances* in this chapter.

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Other Vehicles—Fixed oils used as vehicles for nonaqueous injections are of vegetable origin, are odorless or nearly so, and have no odor suggesting rancidity. They meet the requirements of the test for *Solid paraffin* under *Mineral Oil*, the cooling bath being maintained at 10°, have a *Saponification* value of between 185 and 200 (see *Fats and Fixed Oils* (401)), have an *Iodine* value of between 79 and 128 (see *Fats and Fixed Oils* (401)), and meet the requirements of the following tests.

Unsaponifiable Matter—Reflux on a steam bath 10 mL of the oil with 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of alcohol, with occasional shaking until the mixture becomes clear. Transfer the solution to a shallow dish, evaporate the alcohol on a steam bath, and mix the residue with 100 mL of water; a clear solution results.

Free Fatty Acids—The free fatty acids in 10 g of oil require for neutralization not more than 2.0 mL of 0.020 *N* sodium hydroxide (see *Fats and Fixed Oils* (401)).

Synthetic mono- or diglycerides of fatty acids may be used as vehicles, provided they are liquid and remain clear when cooled to 10° and have an *Iodine* value of not more than 140 (see *Fats and Fixed Oils* (401)).

These and other nonaqueous vehicles may be used, provided they are safe in the volume of injection administered, and also provided they do not interfere with the therapeutic efficacy of the preparation or with its response to prescribed assays and tests.

Added Substances—Suitable substances may be added to preparations intended for injection to increase stability or usefulness, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No coloring agent may be added, solely for the purpose of coloring the finished preparation, to a solution intended for parenteral administration (see also *Added Substances* under *General Notices* and *Antimicrobial Preservatives—Effectiveness* (51)).

Observe special care in the choice and use of added substances in preparations for injection that are administered in a volume exceeding 5 mL. The following maximum limits prevail unless otherwise directed: for agents containing mercury and the cationic, surface-active compounds, 0.01%; for those of the types of chlorobutanol, cresol, and phenol, 0.5%; and for sulfur dioxide, or an equivalent amount of the sulfite, bisulfite, or metabisulfite of potassium or sodium, 0.2%.

A suitable substance or mixture of substances to prevent the growth of microorganisms must be added to preparations intended for injection that are packaged in multiple-dose containers, regardless of the method of sterilization employed, unless otherwise directed in the individual monograph, or unless the active ingredients are themselves antimicrobial. Such substances are used in concentrations that will prevent the growth of or kill microorganisms in the preparations for injection. Such substances also meet the requirements of *Antimicrobial Preservatives—Effectiveness* (51) and *Antimicrobial Agents—Content* (341). Sterilization processes are employed even though such substances are used (see also *Parenteral and Topical Preparations* in the section *Added Substances* under *General Notices* and *Sterilization and Sterility Assurance of Compensial Articles* (1211)). The air in the container may be evacuated or be displaced by a chemically inert gas. Where specified in a monograph, information regarding sensitivity of the article to oxygen is to be provided in the labeling.

LABELS AND LABELING

Labeling—[NOTE—See definitions of “label” and “labeling” under *Labeling* in the section *Preservation, Packaging, Storage, and Labeling* of the *General Notices*.]

The label states the name of the preparation; in the case of a liquid preparation, the percentage content of drug or amount of drug in a specified volume; in the case of a dry preparation, the amount of active ingredient; the route of administration; a statement of storage conditions and an expiration date; the name of the manufacturer and distributor; and an identifying lot number. The lot number is capable of yielding the complete manufacturing history of the specific package, including all manufacturing, filling, sterilizing, and labeling operations.

Where the individual monograph permits varying concentrations of active ingredients in the large-volume parenteral, the

concentration of each ingredient named in the official title is stated as if part of the official title, e.g., Dextrose Injection 5%, or Dextrose (5%) and Sodium Chloride (0.2%) Injection.

The labeling includes the following information if the complete formula is not specified in the individual monograph: (1) In the case of a liquid preparation, the percentage content of each ingredient or the amount of each ingredient in a specified volume, except that ingredients added to adjust to a given pH or to make the solution isotonic may be declared by name and a statement of their effect; and (2) in the case of a dry preparation or other preparation to which a diluent is intended to be added before use, the amount of each ingredient, the composition of recommended diluent(s) [the name(s) alone, if the formula is specified in the individual monograph], the amount to be used to attain a specific concentration of active ingredient and the final volume of solution so obtained, a brief description of the physical appearance of the constituted solution, directions for proper storage of the constituted solution, and an expiration date limiting the period during which the constituted solution may be expected to have the required or labeled potency if it has been stored as directed.

Containers for injections that are intended for use as dialysis, hemofiltration, or irrigation solutions and that contain a volume of more than 1 liter are labeled to indicate that the contents are not intended for use by intravenous infusion.

Injections intended for veterinary use are labeled to that effect.

The container is so labeled that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents.

PACKAGING

Containers for Injections

Containers, including the closures, for preparations for injections do not interact physically or chemically with the preparations in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use. The container is made of material that permits inspection of the contents. The type of glass preferable for each parenteral preparation is usually stated in the individual monograph.

For definitions of single-dose and multiple-dose containers, see *Containers* under *General Notices*. Containers meet the requirements under *Containers* (661).

Containers are closed by fusion, or by application of suitable closures, in such manner as to prevent contamination or loss of contents. Closures for multiple-dose containers permit the withdrawal of the contents without removal or destruction of the closure. The closure permits penetration by a needle, and, upon withdrawal of the needle, at once recloses the container against contamination.

The use of a black closure system on a vial (e.g., a black flip-off button and a black ferrule to hold the elastomeric closure), or the use of a black band or series of bands above the constriction on an ampul, is prohibited except for *Potassium Chloride for Injection Concentrate*.

Containers for Sterile Solids

Containers, including the closures, for dry solids intended for parenteral use do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use.

A container for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained.

Where the *Assay* in a monograph provides a procedure for *Assay preparation* in which the total withdrawable contents are to be withdrawn from a single-dose container with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle not less than 2.5 cm (1 inch) in length, care being taken to expel any air bubbles, and discharged into a container for dilution and assay.

Volume in Container—Each container of an Injection is filled with a volume in slight excess of the labeled "size" or that volume which is to be withdrawn. The excess volumes recommended in the accompanying table are usually sufficient to permit withdrawal and administration of the labeled volumes.

DETERMINATION OF VOLUME OF INJECTION IN CONTAINERS

Select 1 or more containers if the volume is 10 mL or more, 3 or more if the volume is more than 3 mL and less than 10 mL, or 5 or more if the volume is 3 mL or less. Take up individually the contents of each container selected into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2.5 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its rated volume. Alternatively, the contents of the syringe may be discharged into a dry, tared beaker, the volume, in mL, being calculated as the weight, in g, of Injection taken divided by its density. The contents of two or three 1-mL or 2-mL containers may be pooled for the measurement, provided that a separate, dry syringe assembly is used for each container. The content of containers holding 10 mL or more may be determined by means of opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the labeled volume in the case of containers examined individually or, in the case of 1-mL and 2-mL containers, is not less than the sum of the labeled volumes of the containers taken collectively.

For Injections in multiple-dose containers labeled to yield a specific number of doses of a stated volume, proceed as directed in the foregoing, using the same number of separate syringes as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

For Injections containing oil, warm the containers, if necessary, and thoroughly shake them immediately before removing the contents. Cool to 25° before measuring the volume.

Packaging and Storage

The volume of Injection in single-dose containers provides the amount specified for parenteral administration at one time and in no case is more than sufficient to permit the withdrawal and administration of 1 liter.

Preparations intended for intraspinal, intracisternal, or peridural administration are packaged only in single-dose containers.

Unless otherwise specified in the individual monograph, no multiple-dose container contains a volume of Injection more than sufficient to permit the withdrawal of 30 mL.

Injections packaged for use as irrigation solutions or for hemofiltration or dialysis or for parenteral nutrition are exempt from the 1-liter restriction of the foregoing requirements relating to packaging. Containers for Injections packaged for use as hemofiltration or irrigation solutions may be designed to empty rapidly and may contain a volume of more than 1 liter.

Injections labeled for veterinary use are exempt from packaging and storage requirements concerning the limitation to single-dose containers and the limitation on the volume of multiple-dose containers.

FOREIGN MATTER AND PARTICLES

Foreign Matter

Every care should be exercised in the preparation of all products intended for injection, to prevent contamination with microorganisms and foreign material. Good pharmaceutical practice requires also that each final container of Injection be subjected individually to a physical inspection, whenever the nature of the container permits, and that every container whose contents show evidence of contamination with visible foreign material be rejected.

Particulate Matter

All large-volume Injections for single-dose infusion, and those small-volume Injections for which the monographs specify such requirements, are subject to the particulate matter limits set forth under *Particulate Matter in Injections* (788). An article packaged as both a large-volume and a small-volume Injection must be labeled as containing 100 mL or less if the individual monograph includes a test for *Particulate matter*; it meets the requirements set forth for *Large-volume Injections for Single-dose Infusion* where the container is labeled as containing more than 100 mL. Injections packaged and labeled for use as irrigating solutions are exempt from requirements for *Particulate matter*.

STERILITY

Sterility Tests—Preparations for injection meet the requirements under *Sterility Tests* (71).

CONSTITUTED SOLUTIONS

Dry solids from which constituted solutions are prepared for injection bear titles of the form [DRUG] for Injection. Since these dosage forms are constituted at the time of use by the health care practitioner, tests and standards pertaining to the solution as constituted for administration are not included in the individual monographs on sterile dry solids or liquid concentrates. However, in the interest of assuring the quality of injection preparations as they are actually administered, the following nondestructive tests are provided for demonstrating the suitability of constituted solutions when they are prepared just prior to use.

Completeness and Clarity of Solution—Constitute the solution as directed in the labeling supplied by the manufacturer for the sterile dry dosage form.

A: The solid dissolves completely, leaving no visible residue as undissolved matter.

B: The constituted solution is not significantly less clear than an equal volume of the diluent or of Purified Water contained in a similar vessel and examined similarly.

Particulate Matter—Constitute the solution as directed in the labeling supplied by the manufacturer for the sterile dry dosage form: the solution is essentially free from particles of foreign matter that can be observed on visual inspection.

Labeled Size	Recommended Excess Volume	
	For Mobile Liquids	For Viscous Liquids
0.5 mL	0.10 mL	0.12 mL
1.0 mL	0.10 mL	0.15 mL
2.0 mL	0.15 mL	0.25 mL
5.0 mL	0.30 mL	0.50 mL
10.0 mL	0.50 mL	0.70 mL
20.0 mL	0.60 mL	0.90 mL
30.0 mL	0.80 mL	1.20 mL
50.0 mL		
or more	2%	3%

(11) USP REFERENCE STANDARDS

USP Reference Standards are established and released under the authority of the USPC Board of Trustees upon recommendation of the USP Reference Standards Committee, which passes on the selection and suitability of each lot. The critical characteristics of each lot of specimen selected for the standard are usually determined independently in three or more laboratories. The USP Drug Research and Testing Laboratory (see Preface) and the FDA laboratories participate in testing almost all new Standards and replacements for existing Standards. In addition, laboratories throughout the nation, both academic and industrial, participate in the testing.

(785) OSMOLARITY

Osmotic pressure is fundamentally related to all biological processes that involve diffusion of solutes or transfer of fluids through membranes. Thus, knowledge of the osmolar concentrations of fluids is essential. The labels of Pharmacopeial solutions that provide intravenous replenishment of fluid, nutrients, or electrolyte(s), as well as of the osmotic diuretic Mannitol Injection, are required to state the osmolar concentration. The declaration of osmolar concentration on the label of a solution serves primarily to inform the practitioner whether the solution is hypo-osmotic, iso-osmotic, or hyper-osmotic. A quantitative statement facilitates calculation of the dilution required to render a hyper-osmotic solution iso-osmotic. It simplifies many calculations involved in peritoneal dialysis and hemodialysis procedures. The osmolar concentration of an intravenously compounded intravenous solution prepared in pharmacy (e.g., a hyperalimentation solution) from osmolar solutions also can be obtained simply by summing the osmoles contributed by each constituent.

The units of osmolar concentration are usually expressed as milliosmoles (abbreviation: mOsmol) of solute per liter of solution. In general terms, the weight of an osmole is the gram molecular weight of a substance divided by the number of ions or chemical species (n) formed upon dissolution. In ideal solutions, for example, $n = 1$ for glucose, $n = 2$ for sodium chloride, $n = 3$ for calcium chloride, and $n = 4$ for sodium citrate. The ideal osmolar concentration may be determined according to the formula:

$$\text{Osmolar concentration (mOsmol/liter)} = \text{mOsm} \\ = \frac{\text{wt. of substance (g/liter)}}{\text{mol. wt. (g)}} \times \text{number of species} \times 1000.$$

As the concentration of the solute increases, interaction among solute particles increases, and actual osmolar values decrease when compared to ideal values. Deviation from ideal conditions is usually slight in solutions within the physiologic range and for more dilute solutions, but for highly concentrated solutions the actual osmolarities may be appreciably lower than ideal values. For example, the ideal osmolality of 0.9% Sodium Chloride Injection is $3.84 \times 2 \times 1000 = 308$ milliosmoles per liter. In fact, however, n is slightly less than 2 for solutions of sodium chloride at this concentration, and the actual measured osmolality of 0.9% Sodium Chloride Injection is about 286 milliosmoles per liter.

The theoretical osmolality of a complex mixture, such as Protein Hydrolysate Injection, cannot be readily calculated. In such instances, actual values of osmolar concentration are to be used to meet the labeling requirement set forth in the individual monograph. They are determined by calculating the osmolality from measured values of osmolal concentration and water content. Each mole of solute added to 1 kg of water lowers the freezing point approximately 1.86° and lowers the vapor pressure approximately 1.3 mm of mercury (at 25°). These physical changes are measurable, and they permit accurate estimations of osmolal concentrations.

Where osmometers that measure the freezing-point depression are employed, a measured volume of solution (usually 2 mL) is placed in a glass tube immersed in a temperature-controlled bath. A thermistor and a vibrator are lowered into the mixture, and the temperature of the bath is decreased until the mixture is super-cooled. The vibrator is activated to induce crystallization of the water in the test solution, and the released heat of fusion raises the temperature of the mixture to its freezing point. By means of a Wheatstone bridge, the recorded freezing point is converted to a measurement in terms of milliosmolality, or its equivalent for dilute solutions, milliosmolarity. The instru-

ment is calibrated by using two standard solutions of sodium chloride that span the expected range of osmolarities.

Osmometers that measure the vapor pressures of solutions are less frequently employed. They require a smaller volume of specimen (generally about 5 μ L), but the accuracy and precision of the resulting osmolality determination are comparable to those obtained by the use of osmometers that depend upon the observed freezing points of solutions.

Labeling—Where an osmolality declaration is required in the individual monograph, the label states the total osmolar concentration in milliosmoles per liter. Where the contents are less than 100 mL, or where the label states that the article is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in milliosmoles per milliliter.

(788) PARTICULATE MATTER IN INJECTIONS

Particulate matter consists of mobile, randomly-sourced, extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis due to the small amount of material that it represents and to its heterogeneous composition. Injectable solutions, including solutions constituted from sterile solids intended for parenteral use, should be essentially free from particles that can be observed on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating subvisible extraneous particles within specific size ranges.

Microscopic and light obscuration procedures for the determination of particulate matter are given herein. Unless otherwise specified in the individual monograph, the article is expected to conform to the limits for the *Light Obscuration Particle Count Test*.

All large-volume injections for single-dose infusion and those small-volume injections for which the monographs specify such requirements are subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph.

Not all injection formulations can be examined for particles by one or both of these tests. Any product that is not a pure solution having a clarity and a viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Such materials may be analyzed by the microscopic method. Emulsions, colloids, and liposomal preparations are examples. Refer to the specific monographs when a question of test applicability occurs. Higher limits are appropriate for certain articles and are specified in the individual monographs.

In the tests described below for large-volume and small-volume injections, the results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans based upon known operational factors must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units. Sampling plans should be based on consideration of product volume, numbers of particles historically found to be present in comparison to limits, particle size distribution of particles present, and variability of particle counts between units.

LIGHT OBSCURATION PARTICLE COUNT TEST

USP Reference Standards (11)—*USP Particle Count RS*.

The test applies to large-volume injections labeled as containing more than 100 mL, unless otherwise specified in the individual monograph. It counts suspended particles that are solid or liquid. This test applies also to single-dose or multiple-dose small-volume injections labeled as containing 100 mL or less that are either in solution or in solution constituted from sterile solids, where a test for particulate matter is specified in the individual monograph. Injections packaged in prefilled syringes and cartridges are exempt from these requirements, as are products for which the

individual monograph specifies that the label states that the product is to be used with a final filter.

Test Apparatus

The apparatus is an electronic, liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample-feeding device. A variety of suitable devices of this type are commercially available. It is the responsibility of those performing the test to ensure that the operating parameters of the instrument are appropriate to the required accuracy and precision of the test result, and that adequate training is provided for those responsible for the technical performance of the test.

It is important to note that for Pharmacopeial applications the ultimate goal is that the particle counter reproducibly size and count particles present in the injectable material under investigation. The instruments available range from systems where calibration and other components of standardization must be carried out by manual procedures to sophisticated systems incorporating hardware- or software-based functions for the standardization procedures. Thus, it is not possible to specify exact methods to be followed for standardization of the instrument, and it is necessary to emphasize the required end result of a standardization procedure rather than a specific method for obtaining this result. This section is intended to emphasize the criteria that must be met by a system rather than specific methods to be used in their determination. It is the responsibility of user to apply the various methods of standardization applicable to a specific instrument. Critical operational criteria consist of the following.

Sensor Concentration Limits—Use an instrument that has a concentration limit (the maximum number of particles per mL) identified by the manufacturer that is greater than the concentration of particles in the test specimen to be counted. The vendor-certified concentration limit for a sensor is specified as that count level at which coincidence counts due to simultaneous presence of two or more particles in the sensor view volume comprise less than 10% of the counts collected for 10- μ m particles.

Sensor Dynamic Range—The dynamic range of the instrument used (range of sizes of particles that can be accurately sized and counted) must include the smallest particle size to be enumerated in the test articles.

Instrument Standardization

The following discussion of instrument standardization emphasizes performance criteria rather than specific methods for calibrating or standardizing a given instrument system. This approach is particularly evident in the description of calibration, where allowance must be made for manual methods as well as those based on firmware, software, or the use of electronic testing instruments. Appropriate user validation of software and firmware systems is essential to performance of the test according to requirements. Since different brands of instruments may be used in the test, the user is responsible for ensuring that the counter used is operated according to the manufacturer's specific instructions; the principles to be followed to ensure that instruments operate within acceptable ranges are defined below.

The following information for instrument standardization helps ensure that the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and count accuracy are appropriate to performance of the test. Conduct these procedures at intervals of not more than six months.

SAMPLE VOLUME ACCURACY

Since the particle count from a sample aliquot varies directly with the volume of fluid sampled, it is important that the sampling accuracy is known to be within a certain range. For a sample volume determination, determine the dead (tare) volume in the sample feeder with *Water for Injection* or distilled water that has been passed through a filter having a porosity of 1.2 μ m or finer. Transfer a volume of *Water for Injection* that is greater than the sample volume to a container, and weigh. Withdraw through the sample feeding device a volume that is appropriate for the specific sampler, and again weigh the container. Determine the sample volume by subtracting the tare volume from the combined sample plus tare volumes. Verify that the value obtained is within $\pm 5\%$ of the appropriate sample volume for the test. Alternatively, the sample volume may be determined using a suitable Class A grad-

uated cylinder (see *Volumetric Apparatus* (31)). [Note: The amount of sample withdrawn prior to counting. This volume may be determined for syringe-operated samplers by setting the sample volume to zero and initiating sampling, so that the only volume of solution drawn is the tare. Subtract the tare volume from the total volume of solution drawn in the sampling cycle to determine the sample volume.]

SAMPLE FLOW RATE

Verify that the flow rate is within the manufacturer's specifications for the sensor used. This may be accomplished by using a calibrated stop watch to measure the time required by using the instrument to withdraw and count a specific sample volume. The time between beginning and ending of the count cycle is denoted by instrument indicator lights or other means. Sensors may be operated accurately over a range of flow rates. Perform the *Test Procedure* at the same flow rate as that selected for calibration of the instrument.

CALIBRATION

Use one of the following methods.

Manual Method—Calibrate the instrument with a minimum of three calibrators, each consisting of near-monosize polystyrene spheres drawing diameters of about 10, 15, and 25 μ m, in an aqueous vehicle.* The calibrator spheres must have a mean diameter of within 5% of the 10-, 15-, and 25- μ m nominal diameters and be standardized against materials traceable to NIST standard reference materials. The number of spheres counted must be within the sensor's concentration limit. Prepare suspensions of the calibrator spheres in water at a concentration of 1000 to 5000 particles per mL, determine the channel setting that corresponds to the highest count setting for the sphere distribution. This is determined by using the highest count threshold setting to split the distribution into two bins containing equal numbers of counts, with the instrument set in the differential count mode (moving window half-count method). Use only the central portion of the distribution in this calculation to avoid including asymmetrical portions of the peak. The portion of the distribution, which must be divided equally, is the count window. The window is bounded by threshold settings that will define a threshold voltage window of $\pm 20\%$ around the mean diameter of the test spheres. The window is intended to include all single spheres, taking into account the standard deviation of the spheres and the sensor resolution, while excluding noise and aggregates of spheres. The value of 20% was chosen based on the worst-case sensor resolution of 10% and the worst-case standard deviation of the spheres of 10%. Since the thresholds are proportional to the area of the spheres rather than the diameter, the lower and upper voltage settings are determined by the equations:

$$V_L = 0.64V_p$$

in which V_L is the lower voltage setting and V_p is the voltage at the peak center, and

$$V_U = 1.44V_p$$

in which V_U is the upper voltage setting.

Once the center peak thresholds are determined, use these thresholds for the standards to create a regression of log voltage versus log particle size, from which the instrument settings for the 10- and 25- μ m sizes can be determined.

Automated Method—The calibration (size response) curve may be determined for the instrument-sensor system by the use of validated software routines offered by instrument vendors; these may be included as part of the instrument software or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a response curve equivalent to that attained by the manual method and if the automated calibration is validated as necessary by the user.

Electronic Method—Using a multichannel peak height analyzer, determine the center channel of the particle counter pulse

* ASTM standard F658-87 provides useful discussions pertaining to calibration procedures applying near-monosize latex spheres.

response for each standard suspension. This peak voltage setting becomes the threshold used for calculation of the voltage response curve for the instrument. The standard suspensions to be used for the calibration are run in order, and median pulse voltages for each are determined. These thresholds are then used to generate the size response curve manually or via software routines. The thresholds determined from the multichannel analyzer data are then transferred to the counter to complete the calibration. If this procedure is used with a comparator-based instrument, the comparators of the counter must be adjusted accurately beforehand.

SENSOR RESOLUTION

The particle size resolution of the instrumental particle counter is dependent upon the sensor used and may vary with individual sensors of the same model. Determine the resolution of the particle counter for 10- μ m particles using the monosized 10- μ m calibrator spheres. The relative standard deviation of the size distribution of the standard particles used is not more than 5%. Acceptable methods of determining particle size resolution are (1) manual response; (2) using an electronic method of measuring and sorting particle sensor voltage output with a multichannel analyzer; and (3) automated methods.

Manual Method—Adjust the particle counter to operate in the cumulative mode or total count mode. Refer to the calibration curve obtained earlier, and determine the threshold voltage for the 10- μ m monosize spheres. Adjust 3 channels of the counter to be used in the calibration procedure as follows:

Channel 1 is set for 90% of the threshold voltage.

Channel 2 is set for the threshold voltage.

Channel 3 is set for 110% of the threshold voltage.

Draw a sample through the sensor, observing the count in Channel 2. When the particle count in that channel has reached approximately 1000, stop counting, and observe the counts in Channels 1 and 3. Check to see if the Channel 1 count and the Channel 3 count are $168 \pm 10\%$ and $32 \pm 10\%$, respectively, of the count in Channel 2. If not, adjust Channel 1 and Channel 3 thresholds to meet these criteria. When these criteria have been satisfied, draw a sample of suspension through the counter until the counts in Channel 2 have reached approximately 10,000, or until an appropriate volume (e.g., 10 mL) of the sphere suspension has been counted. Verify that Channel 1 and Channel 3 counts are $168 \pm 3\%$ and $32 \pm 3\%$, respectively, of the count in Channel 2. Record the particle size for the thresholds just determined for Channels 1, 2, and 3. Subtract the particle size for Channel 2 from the size for Channel 3. Subtract the particle size for Channel 1 from the size for Channel 2. The values so determined are the observed standard deviations on the positive and negative side of the mean count for the 10- μ m standard. Calculate the percentage of resolution of the sensor by the formula:

$$100 \left(\frac{\sqrt{S_o^2 - S_s^2}}{D} \right),$$

in which S_o is the highest observed standard deviation determined for the sphere, S_s is the supplier's reported standard deviation for the spheres, and D is the diameter, in μ m, of the spheres as specified by the supplier. The resolution is not more than 10%.

Automated Method—Software is available for some counters that allows for the automated determination of sensor resolution. This software may be included in the instrument or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a resolution determination equivalent to the manual method and if the automated resolution determination is validated as necessary by the user.

Electronic Method—Record the voltage output distribution of the particle sensor, using a multichannel analyzer while sampling suspension of the 10- μ m particle size standard. To determine resolution, move the cursor of the multichannel analyzer up and down the electric potential scale from the median pulse voltage to identify a channel on each side of the 10- μ m peak that has approximately 61% of the counts observed in the center channel. Use the counter size response curve to convert the mV values of these two channels to particle sizes provides the particle size

at within 1 standard deviation of the 10- μ m standard. Use these values to calculate the resolution as described under *Manual Method*.

PARTICLE COUNTING ACCURACY

Determine the particle counting accuracy of the instrument, using *Method 1* (for small-volume injections) or *Method 2* (for large-volume injections).

Method 1—

Procedure—Prepare the suspension and blank using the USP Particle Count RS. Set the instrument to count at 10 and 15 μ m. Mix the blank by inverting 25 times within 10 seconds, and degas the mixture by sonicating for 30 seconds or by allowing to stand. Remove the closure from the container, and gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis. Withdraw directly from the container, three consecutive volumes of not less than 5 mL each, obtain the particle counts, and discard the data from the first portion. [NOTE—Complete the procedure within five minutes.] Repeat the procedure, using the suspension in place of the blank. From the averages of the counts resulting from the analysis of the two portions of the suspension at 10 μ m and from the analysis of the two portions of the blank at 10 μ m, calculate the number of particles in each mL taken by the formula:

$$(P_s - P_b)/V,$$

in which P_s is the average particle count obtained from the suspension, P_b is the average particle count obtained from the blank, and V is the average volume, in mL, of the 4 portions tested. Repeat the calculations, using the results obtained at 15 μ m.

Interpretation—The instrument meets the requirements for *Particle Counting Accuracy* if the count obtained at 10 μ m and the ratio of the counts obtained at 10 μ m to those obtained at 15 μ m conform to the values that accompany the USP Particle Count RS. If the instrument does not meet the requirements for *Particle Counting Accuracy*, recalibrate with the remaining suspension and blank. If the results of the second test are within the limits given above, the instrument meets the requirements of the *Particle Counting Accuracy* test. If the results of the second test are within the limits given above, the instrument meets the requirements of the test for *Particle Counting Accuracy*. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures, and retest the instrument.

Method II—

Procedure—Using standard calibrator spheres having a nominal diameter of 15 to 30 μ m, prepare a suspension containing between 50 and 200 particles per mL. Degas the suspension by mild sonication (at 8 mW or less) for 30 seconds or by allowing to stand. Properly suspend the particles by stirring gently, and perform five counts on 5-mL volumes of the suspension, using the particle counter 10- μ m size threshold. Obtain the mean cumulative particle count per mL. Pipet a volume of this suspension containing 250 to 500 particles into a filter funnel prepared as described for *Filtration Apparatus* under *Microscopic Particle Count*. After drying the membrane, count the total number of standard spheres collected on the membrane filter. This count should be within 20% of the mean instrumental count per mL for the suspension.

Test Environment

Perform the test in an environment that does not contribute any significant amount of particulate matter. Specimens must be cleaned to the extent that any level of extraneous particulate added has a negligible effect on the outcome of the test. Test specimen, glassware, closures, and other required equipment preferably are prepared in an environment protected by high efficiency particulate air (HEPA) filters. Nonscedding garments and powder-free gloves preferably are worn throughout the preparation of samples.

Cleanse glassware, closures, and other required equipment preferably by immersing and scrubbing in warm, nonionic detergent solution. Rinse in flowing tap water, and then rinse in flowing filtered water. Organic solvents may also be used.

facilitate cleaning. [NOTE—These steps describe one way to clean equipment; alternatively, particulate-free equipment may be obtained from a suitable vendor.] Finally, rinse the equipment in filtered water, using a hand-held pressure nozzle with final filter or other appropriate filtered water source, such as distilled water passed through a capsule filter. The filter used should have a porosity of 1.2 μm or finer.

To collect background counts, use a cleaned vessel of the type and volume representative of that to be used in the test. Place a 20-mL volume of filtered water in the vessel, and agitate the sample in the cleaned glassware by inversion or swirling. Degas by sonicating for 30 seconds or by allowing to stand. Swirl the vessel containing the water sample by hand or agitate by mechanical means to suspend particles. Withdraw and obtain the particle counts for three consecutive samples of not less than 5 mL each, disregarding the first count. If more than 10 particles of 10 μm or greater size, or more than 2 particles of 25 μm or greater size are observed in the combined 10-mL sample, the environment is not suitable for particulate analysis: the filtered water and glassware have not been properly prepared or the counter is generating spurious counts. In this case, repeat the preparatory steps until conditions of analysis are suitable for the test.

Test Procedure

TEST PREPARATION

For containers having volumes of less than 25 mL, test a solution pool of 10 or more units. Single units of small-volume injections may be tested individually if the individual unit volume is 25 mL or greater.

Prepare the test specimens in the following sequence. Remove outer closures, sealing bands, and any loose or shedding paper labels. Rinse the exterior of containers with filtered distilled water as described under *Test Environment*, and dry, taking care to protect the containers from environmental contamination. Withdraw the contents of the containers in the normal or customary manner of use, or as instructed in the package labeling, except that containers with removable stoppers may be sampled directly by removing the closure, or if test specimens are being pooled, by removing the closure and emptying the contents into a clean container.

DETERMINATION

Liquid Fill (where the contents of each unit are less than 25 mL)—Mix each unit by inverting it 20 times to resuspend any particles. [NOTE—Because of the small volume of some products, it may be necessary to agitate the solution more vigorously in order to suspend the particles completely and homogeneously.] Open and combine, in a cleaned container, the contents of 10 or more units to obtain a volume of not less than 20 mL. Degas by sonicating for 30 seconds or by allowing to stand until the solution is free from air bubbles. Gently stir the contents of the container by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Withdraw not less than 3 aliquot portions, each not less than 5 mL in volume, into the light obscuration counter sensor. Obtain the particle counts, and discard the data from the first portion.

Liquid Fill (where the contents of each unit are 25 mL or more, and where the option of testing individual units is selected)—Mix 1 unit by inverting it 20 times. Degas the solution by sonicating or by allowing it to stand until the solution is free from air bubbles. Remove the closure, and insert the counter probe into the center of the solution. Gently agitate the contents of the unit by hand-swirling or by mechanical means. Withdraw not less than 3 aliquot portions, each not less than 5 mL in volume, into the light obscuration counter sensor. Obtain the particle counts, and discard the data from the first portion.

Dry or Lyophilized Fill—Open the container, taking care not to contaminate the opening or cover. Constitute with a suitable volume of filtered water, or with the appropriate filtered diluent if water is not suitable. Replace the closure, and manually agitate the container to dissolve the drug. Allow to stand until the drug is completely dissolved. Prior to analysis, gently stir the contents of the containers by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Pool or test individually the appropriate number of units, and withdraw not

less than 3 aliquot portions, each not less than 5 mL in volume, into the light obscuration counter sensor. Obtain the particle counts, and discard the data from the first aliquot.

Solid Drugs Packaged with Diluents—For products packaged in containers that are constructed to hold the drug product and a solvent in separate compartments, mix each unit as directed in the labeling, activating and agitating each unit so as to ensure thorough mixing of the separate components. Analyze the solutions as described under *Liquid Fill*.

Multiple-dose Containers—For products labeled *Pharmacy Bulk Packages*, proceed for each unit as directed under *Test Preparation*, calculating the results on the basis of a sample volume that is equal to the maximum dose stated in the labeling. For the calculations below, consider a maximum-dose volume to be the equivalent of the contents of one full container.

Calculations

Pooled Samples (Small-volume Injections)—Average the counts from the 2 or more aliquot portions analyzed. Calculate the number of particles in each container by the formula:

$$PV/V_a n,$$

in which P is the average particle count obtained from the portions analyzed, V_t is the volume of pooled sample, in mL, V_a is the volume, in mL, of each portion analyzed, and n is the number of containers pooled.

Individual Samples (Small-volume Injections)—Average the counts obtained for the 5-mL or greater aliquot portions from each separate unit analyzed, and calculate the number of particles in each container by the formula:

$$PV/V_a,$$

in which P is the average particle count obtained from the portions analyzed, V is the volume, in mL, of the tested unit, and V_a is the volume, in mL, of each portion analyzed.

Individual Unit Samples (Large-volume Injections)—Average the counts obtained for the two or more 5-mL aliquot portions taken from the solution unit. Calculate the number of particles in each mL taken by the formula:

$$P/V,$$

in which P is the average particle count for an individual 5 mL or greater sample volume, and V is the volume, in mL, of the portion taken.

Interpretation

The injection meets the requirements of the test if the average numbers of particles present in the units tested do not exceed the appropriate value listed in Table 1. If the average number of particles exceeds the limit, test the article by the *Microscopic Particle Count Test*.

Table 1. Light Obscuration Test Particle Count.

	$\geq 10 \mu\text{m}$	$\geq 25 \mu\text{m}$
Small-volume Injections:	6000	600 per container
Large-volume Injections:	25	3 per mL

MICROSCOPIC PARTICLE COUNT TEST

The microscopic particulate matter test may be applied to both large-volume and small-volume injections. This test enumerates subvisible, essentially solid, particulate matter in these products on a per-volume or per-container basis, after collection on a microporous membrane filter. Some articles cannot be tested meaningfully by light obscuration. In such cases, individual monographs specify only this microscopic assay. Solutions exempted from analysis using the microscopic assay are identified on a monograph basis. Examples are solutions of viscosity too high to filter readily (e.g., concentrated dextrose, starch solutions, or emulsions). Similarly, products known to contain amorphous, semi-

USP 23
liquid or of
not be teste
little or no
appearance
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liquid or otherwise morphologically indistinct materials should not be tested by the microscopic method. These materials show little or no surface relief and present a gelatinous or film-like appearance. Since in solution this material consists of units on the order of 1 μm or less, which may be counted only after aggregation or deformation on an analytical membrane, interpretation of enumeration may be aided by testing a sample of the solution by the light obscuration particle count method.

Test Apparatus

Microscope—Use a compound binocular microscope that corrects for changes in interpupillary distance by maintaining a constant tube length. The objective and eyepiece combination of lenses must give a magnification of $100 \pm 10\times$. The objective must be of $10\times$ nominal magnification, a planar achromat or better in quality, with a minimum numerical aperture of 0.25. In addition, the objective must be compatible with an episcopic illuminator attachment. The eyepieces must be of $10\times$ magnification, with a field number of ≥ 15 (widefield). In addition, one eyepiece must be designed to accept and focus an eyepiece graticule. The microscope must have a mechanical stage capable of holding and traversing the entire filtration area of a 25-mm or 47-mm membrane filter.

Illuminators—Two illuminators are required. One is an external, focusable auxiliary illuminator adjustable to give incident oblique illumination at an angle of 10° to 20° . The other is an episcopic brightfield illuminator internal to the microscope. Both illuminators must be of a wattage sufficient to provide a bright, even source of illumination and may be equipped with blue daylight filters to decrease operator fatigue during use.

Circular Diameter Graticule—Use a circular diameter graticule (see Figure 1) matched to the microscope model objective and eyepiece such that the sizing circles are within 2% of the stated size at the plane of the stage.

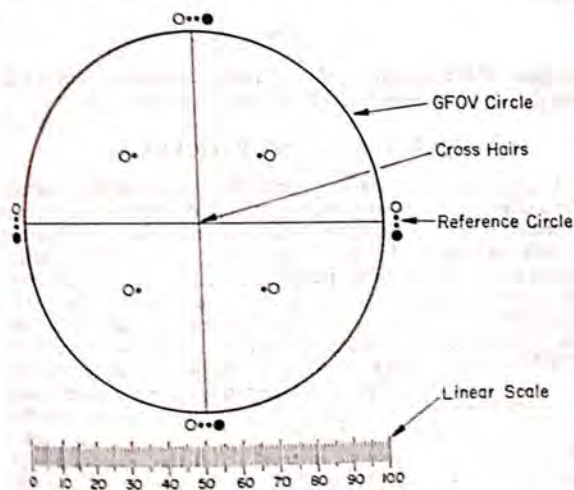


Fig. 1. Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10- μm and 25- μm diameters at $100\times$ are provided as comparison scales for particle sizing.

Micrometer—Use a stage micrometer, graduated in 10- μm increments, that is certified by NIST.

Filtration Apparatus—Use a filter funnel suitable for the volume to be tested, having a minimum diameter of about 21 mm. The funnel is made of plastic, glass, or stainless steel. Use a filter support made of stainless steel screen or sintered glass as the filtration diffuser. The filtration apparatus is equipped with a vacuum source, a solvent dispenser capable of delivering solvents filtered at 1.2 μm or finer retention rating at a range of pressures from 10 psi to 80 psi, and membrane filters (25 mm or 47 mm nongridded or gridded, black or dark gray, of mixed cellulose ester, with a porosity of 1.0 μm or finer). Use blunt forceps to handle membrane filters.

Test Environment

A laminar flow hood or other laminar airflow enclosure, having a capacity sufficient to envelope the area in which the analysis is prepared, with HEPA-filtered air having not more than 100 particles (0.5 μm or smaller) per cubic foot. For the blank determination, deliver from the pressure dispenser a volume of filtered water equal to the sample volume into the *Filtration Apparatus*. Apply vacuum and draw the entire volume of water through the membrane filter. Remove the membrane from the filter funnel base, and place atop a strip of double-sided tape in a petri slide or petri dish. After allowing the membrane to dry, examine it microscopically at a magnification of $100\times$. If not more than 10 particles 10 μm or larger in size and no particles 25 μm or larger are present within the filtration area, the background particle level is sufficiently low for performance of the microscopic assay.

Preparation of Test Apparatus

Throughout this procedure, preferably use suitable powder-free gloves, and thoroughly clean glassware and equipment that have been rinsed successively with a warm, residue-free solution of detergent, hot water, filtered distilled or deionized water, and isopropyl alcohol. [NOTE—Filter the distilled or deionized water and the isopropyl alcohol prior to use, using filters having a porosity of 1.2 μm or finer.] Perform the rinsing under the laminar flow enclosure equipped with HEPA filters. Allow the glassware and filtration apparatus to dry under the hood, upstream of all other operations. Preferably, the hood is located in a separate room that is supplied with filtered air-conditioned air and maintained under positive pressure with respect to the surrounding areas.

Prior to conducting the test, clean the work surfaces of the laminar flow enclosure with an appropriate solvent. Prepare the test specimens in the following sequence. Remove outer closures, sealing bands, and any loose or shedding paper labels. Rinse the exterior of containers with filtered Purified Water. Withdraw the contents of the containers in the normal or customary manner of use or as instructed in the package labeling, except that containers with removable stoppers may be sampled by removing the closure and emptying the contents into a clean container or into the filtration funnel.

The number of samples chosen must be adequate to provide a statistically sound assessment of whether a batch or other large group of units represented by the samples meets or exceeds the limit. Test a solution pool of 10 or more units, or test individual units. For large-volume injections, individual units are tested.

MICROSCOPE PREPARATION

Place the auxiliary illuminator close to the microscope stage, focusing the illuminator to give a concentrated area of illumination on a filter membrane positioned on the microscope stage. Adjust the illuminator height so that the angle of incidence of the light is 10° to 20° with the horizontal. Using the internal episcopic brightfield illuminator, fully open the field and aperture diaphragms. Center the lamp filament, and focus the microscope on a filter containing particles. Adjust the intensity of reflected illumination until particles are clearly visible and show pronounced shadows. Adjust the intensity of episcopic illumination to the lowest setting, then increase the intensity of episcopic illumination until shadows cast by particles show the least perceptible decrease in contrast.

OPERATION OF CIRCULAR DIAMETER GRATICULE

The relative error of the graticule used must initially be measured with an NIST-certified stage micrometer. To accomplish this, align the graticule micrometer scale with the stage micrometer so that they are parallel. (Compare the scales, using as large a number of graduations on each as possible). Read the number of graticule scale divisions, *GSD*, compared to stage micrometer divisions, *SMD*. Calculate the relative error by the formula:

$$(GSD - SMD)/100SMD.$$

A relative error of $\pm 2\%$ is acceptable. The basic technique of measurement applied with the use of the circular diameter gra-

ticle is to transform mentally the image of each particle into a circle and then compare it to the 10- and 25- μ m graticule reference circles. The sizing process is carried out without superimposing the particle on the reference circles; particles are not moved from their locations within the graticule field of view (the large circle) for comparison to the reference circles. Use the inner diameter of the clear graticule reference circles to size white and transparent particles. Use the outer diameter of the black opaque graticule reference circles to size dark particles.

Rotate the graticule in the right microscope eyepiece so that the linear scale is located at the bottom of the field of view, bringing the graticule into sharp focus by adjusting the right eyepiece diopter ring while viewing an out-of-focus specimen. Focus the microscope on a specimen, looking through the right eyepiece only. Then, looking through the left eyepiece, adjust the left eyepiece diopter to bring the specimen into sharp focus.

PREPARATION OF FILTRATION APPARATUS

Preferably, wash the filtration funnel, base, and diffuser in a solution of liquid detergent and hot water. Rinse with hot water. Following the hot water rinse, apply a second rinse with filtered Purified Water, using a pressurized jet of water over the entire exterior and interior surfaces of the filtration apparatus. Repeat the pressurized rinse procedure using filtered isopropyl alcohol. Finally, using the pressurized rinser, rinse the apparatus with filtered Purified Water.

Assemble the cleaned filtration apparatus with the diffuser on top of the filtration base, placing the clean membrane filter on top of the diffuser. Remove a membrane filter from its container using ultracleaned blunt forceps. Use a low pressurized stream of filtered Purified Water to wash both sides of the filter thoroughly by starting at the top and sweeping back and forth to the bottom. Place the funnel assembly on top of the filtration base, and lock it into place.

TEST PREPARATIONS

Liquid Fill (Large or Small-volume Injections)—Thoroughly mix the units to be tested by inverting 20 times. Clean the outer surface of the solution container thoroughly with pressurized filtered Purified Water. Open the units in a manner consistent with generation of lowest possible numbers of background particles. In a cleaned container, open and combine the contents of not less than 10 containers, or filter the contents of individual containers.

Dry Powder Vials—Constitute the material with an appropriate diluent using the method least likely to introduce extraneous contamination. Add the constituted solution to the filter funnel atop a sufficient volume of filtered water to bring the total volume to be filtered to about 100 mL, or use a smaller funnel. Pool the desired number of units, and proceed as directed under *Test Procedure*.

Drug-Diluent Products—For products packaged in containers that are constructed to hold the drug product and a solvent in separate compartments, mix each unit as directed in the labeling, activating and agitating each unit in order to ensure thorough mixing of the separate components. Proceed as directed under *Test Procedure*.

Multiple-dose Containers—For products labeled as *Pharmacy Bulk Packages*, proceed as directed under *Liquid Fill*, and calculate the results on a sample volume that is equal to the maximum dose given in the labeling. For the calculations below, consider this portion to be the equivalent of the contents of one full container.

Test Procedure—Fill the filtration funnel with test solution and apply vacuum. [NOTE—For large-volume injections, do not allow the fluid volume in the filtration funnel to drop below 100 mL between refills.] After the last addition of solution, begin rinsing the walls of the funnel by directing a low pressure stream of filtered Purified Water in a circular pattern along the walls of the funnel, and stop rinsing the funnel before the volume falls below about one-fourth of the fill level if a partial count procedure is to be performed. Maintain the vacuum until all the liquid in the funnel is gone. Turn the vacuum off, and remove the filtration funnel from the filtration base, removing the filter with blunt forceps to a petri slide. Secure in place with double stick tape, and label with sample identification. Allow the filter to air-dry in the clean bench with the cover ajar.

NOTE—If a small-volume injection unit of 25 mL or a solution pool of less than 25 mL, total volume is to be tested and the partial count procedure is to be used, a volume of diluent sufficient to bring the total volume to about 100 mL may be added, or, alternatively, a smaller funnel may be used. After adding the pooled material, apply vacuum and proceed with filtration as specified above for a liquid solution unit.

Enumeration of Particles

The microscopic test described in this section is flexible in that it can count, in particles per mL, specimens containing 1 particle per mL as well as those containing significantly higher numbers of particles per mL. This method may be used where all particles on an analysis membrane surface are counted or where only those particles on some fractional area of a membrane surface are counted.

TOTAL COUNT PROCEDURE

In performance of a total count, the graticule field of view (GFOV) defined by the large circle of the graticule is ignored, and the vertical crosshair is used. Scan the entire membrane from right to left in a path that adjoins but does not overlap the first scan path. Repeat this procedure, moving from left to right, to left until all particles on the membrane are counted. Record the total number of particles that are 10 μ m or larger and the number that are 25 μ m or larger. For large-volume injections, calculate the particle count, in particles per mL, for the units tested by the formula:

$$P/V,$$

where P is the total number of particles counted, and V is the volume, in mL, of the solution. For small-volume injections, calculate the particle count, in particles per container, by the formula:

$$P/n,$$

in which P is the total number of particles counted, and n is the number of units pooled (1 in the case of a single unit).

PARTIAL COUNT PROCEDURE

If a partial count of particles on a membrane is to be performed, the analyst must first ensure that an even distribution of particles is present on the membrane. This is assessed by rapid scanning in order to look for clumps of particles. None should be present. Count the 10- μ m or larger particles in one GFOV at the edge of the filtration area as well as those in the center of the GFOV. The number of ≥ 10 - μ m or larger particles in the GFOV with the highest total particle count is not more than twice that of the GFOV with the lowest particle count. Reject a filter failing these criteria, and prepare another if a partial count procedure is used, or, alternatively, analyze this membrane by the total count method.

The normal number of GFOV counted for a partial count is 20. If a smaller confidence interval about the result is desired, a larger number of fields and particles may be counted. Count all particles that have a circular area diameter of 10 μ m or larger and 25 μ m or larger within the GFOV and those that are in contact with the right side of the GFOV circle. Do not count particles outside of the GFOV. Ignore those that touch the left side of the GFOV circle. The dividing line between right and left sides of the GFOV circle is the vertical cross hair. [NOTE—Make the best possible judgment on particle size without changing the microscope magnification or illumination.]

The analyst may increase or decrease the number of fields counted to achieve a desired confidence interval about the count obtained. Calculate the confidence interval about the number of 10- μ m or larger and 25- μ m or larger particles per mL by the formula:

$$\sqrt{P},$$

in which P is the number of particles counted. In the event that filtration of a solution results in a particle count too high to enumerate accurately in a GFOV, a single quadrant of the GFOV may be counted with a 47-mm filter and a fractional aliquot of the unit.

To perform a partial count of the particles on a membrane, start at the right center edge of the filtration area and begin counting adjacent GFOVs. When the left edge of the filtration area is reached, move one GFOV toward the top of the filter and continue counting GFOVs by moving in the opposite direction. Moving from one GFOV to the next can be accomplished by one of two methods. One method is to define a landmark (particle or surface irregularity in the filter) and move over one GFOV in relation to the landmark. A second method is to use the vernier on the microscope stage to move one millimeter between GFOVs. To facilitate the latter, adjust the microscope x and y stage positioning controls to a whole number at the starting position at the center right edge of the filtration area, then each GFOV will be one whole division of movement of the x stage positioning control. If the top of the filtration area is reached before the desired number of GFOVs is reached, begin again at the right center edge of the filtration area one GFOV lower than the first time. This time move downward on the membrane when the end of a row of GFOVs is reached. Continue as before until the number of GFOVs is complete.

For large-volume injections, if a partial count procedure for the $\geq 10\text{-}\mu\text{m}$ and $\geq 25\text{-}\mu\text{m}$ size ranges is used, calculate the particles per mL by the formula:

$$PA/A_pV,$$

in which P is the number of particles counted, A_p is the filtration area, in mm^2 , of the membrane, A_f is the partial area counted, in mm^2 , based on the number of graticule fields counted, a V is the volume, in mL, of solution filtered. For a solution pool (for small-volume injection units containing less than 25 mL) or for a single unit of a small-volume injection, calculate the number of particles per unit by the formula:

$$PA/A_p n,$$

in which n is the number of units counted (1 in the case of a single unit), and the other terms are as defined above.

Interpretation

The injection meets the requirements of the test if the average number of particles present in the units tested does not exceed the values listed in Table 2.

Table 2. Microscopic Method Particle Count.

	$\geq 10\text{ }\mu\text{m}$	$\geq 25\text{ }\mu\text{m}$
Large-volume Injections:	12	2 per mL

(791) pH

For compendial purposes, pH is defined as the value given by a suitable, properly standardized, potentiometric instrument (pH meter) capable of reproducing pH values to 0.02 pH unit using an indicator electrode sensitive to hydrogen-ion activity, the glass electrode, and a suitable reference electrode such as calomel or silver-silver chloride. The instrument should be capable of sensing the potential across the electrode pair and, for pH standardization purposes, applying an adjustable potential to the circuit by manipulation of "standardization," "zero," "asymmetry," or "calibration" control, and should be able to control the change in millivolts per unit change in pH reading through a "temperature" and/or "slope" control. Measurements are made at $25 \pm 2^\circ$, unless otherwise specified in the individual monograph or herein.

The pH scale is defined by the equation:

$$\text{pH} = \text{pH}_s + (E - E_s)/k,$$

in which E and E_s are the measured potentials where the galvanic cell contains the solution under test, represented by pH, and the cell contains the solution for standardization, represented by appropriate Buffer Solution for Standardization, respectively. The value of k is the change in potential per pH unit change in pH and is theoretically $[0.05916 + 0.000198(t - 25^\circ)]$ volts at any temperature t . This operational pH scale

is established by assigning rounded pH values to the Buffer Solutions for Standardization from the corresponding National Institute of Standards and Technology molal solutions.

It should be emphasized that the definitions of pH, the pH scale, and the values assigned to the Buffer Solutions for Standardization are for the purpose of establishing a practical, operational system so that results may be compared between laboratories. The pH values thus measured do not correspond exactly to those obtained by the classical definition, $\text{pH} = -\log [\text{H}^+(\text{aq})]$. So long as the solution being measured is sufficiently similar in composition to the buffer used for standardization, the operational pH corresponds fairly closely to the theoretical pH. Although no claim is made with respect to the suitability of the system for measuring hydrogen-ion activity or concentration, the values obtained are closely related to the activity of the hydrogen ion in aqueous solutions.

Where a pH meter is standardized by use of an aqueous buffer and then used to measure the "pH" of a nonaqueous solution or suspension, the ionization constant of the acid or base, the dielectric constant of the medium, the liquid-junction potential (which may give rise to errors of approximately 1 pH unit), and the hydrogen-ion response of the glass electrode are all changed. For these reasons, the values so obtained with solutions that are only partially aqueous in character can be regarded only as apparent pH values. However, acidity may be accurately measured with the proper use of electrodes and instrument standardization.

Buffer Solutions for Standardization of the pH Meter—

Buffer Solutions for Standardization are to be prepared as directed in the accompanying table.* Buffer salts of requisite purity can be obtained from the National Institute of Science and Technology. Solutions may be stored in chemically resistant, tight containers, such as Type I glass bottles. Fresh solutions should be prepared at intervals not to exceed 3 months. The table indicates the pH of the buffer solutions as a function of temperature. The instructions presented here are for the preparation of solutions having the designated molal (m) concentrations. For convenience, and to facilitate their preparation, however, instructions are given in terms of dilution to a 1000-mL volume rather than specifying the use of 1000 g of solvent, which is the basis of the molality system of solution concentration. The indicated quantities cannot be computed simply without additional information.

Potassium Tetraoxalate, 0.05 m —Dissolve 12.61 g of $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$ in water to make 1000 mL.

Potassium Biphthalate, 0.05 m —Dissolve 10.12 g of $\text{KHC}_8\text{H}_4\text{O}_4$, previously dried at 110° for 1 hour, in water to make 1000 mL.

Equimolal Phosphate, 0.05 m —Dissolve 3.53 g of Na_2HPO_4 and 3.39 g of KH_2PO_4 , each previously dried at 120° for 2 hours, in water to make 1000 mL.

Sodium Tetraborate, 0.01 m —Dissolve 3.80 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in water to make 1000 mL. Protect from absorption of carbon dioxide.

Calcium Hydroxide, saturated at 25° —Shake an excess of calcium hydroxide with water, and decant at 25° before use. Protect from absorption of carbon dioxide.

Because of variations in the nature and operation of the available pH meters, it is not practicable to give universally applicable directions for the potentiometric determinations of pH. The general principles to be followed in carrying out the instructions provided for each instrument by its manufacturer are set forth in the following paragraphs. Examine the electrodes and, if present, the salt bridge prior to use. If necessary, replenish the salt bridge solution, and observe other precautions indicated by the instrument or electrode manufacturer.

To standardize the pH meter, select two Buffer Solutions for Standardization whose difference in pH does not exceed 4 units

* Commercially available buffer solutions for pH meter standardization, standardized by methods traceable to the National Institute of Standards and Technology (NIST), labeled with a pH value accurate to 0.01 pH unit, and provided with a table showing the pH values at various temperatures, may be used. Solutions prepared from ACS reagent grade materials or other suitable materials, in the stated quantities, may be used provided the pH of the resultant solution is the same as that of the solution prepared from the NIST certified material.