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Perflutren Protein-Type A Microspheres Injectable Suspension

» Perflutren Protein-Type A Microspheres Injectable Suspension is a sterile, nonpyrogenic suspension of microspheres produced by dispersing perflutren (octafluoropropane) gas in an aqueous solution of diluted sterile Albumin Human. It contains not less than 0.8 percent and not more than 1.2 percent protein. It may contain stabilizers, but contains no preservatives.

Packaging and storage—Preserve in single-dose, tight containers that contain perflutren gas in the headspace, and store in a refrigerator.

Labeling—Label it to indicate that perflutren gas is contained within the microspheres. The labeling also provides the following warnings: "Do not use if lower layer is cloudy or turbid, contains visible foreign matter, or if the contents do not appear as a homogeneous, opaque, milky-white suspension after mixing. Do not use if the upper white layer of product is absent. Do not inject air into the vial. Invert the vial, and gently rotate to resuspend the microspheres. Do not use if, after resuspension, the solution appears to be clear rather than opaque milky-white."

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mL of Perflutren Protein-Type A Microspheres Injectable Suspension.

Safety—It meets the requirements for biologics as set forth for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* ⟨88⟩.

Sterility Tests ⟨71⟩ : meets the requirements.

pH ⟨791⟩: between 6.4 and 7.4.

Microsphere size and concentration—

Electrolyte solution—Use filtered and buffered saline electrolyte solution.[1]

Diluent—Prepare a solution that contains, in each L of water, 1.5 g of sodium lauryl sulfate and 0.1 g of thimerosal. Prior to use, pass the solution through a 0.2-µm nylon filter. [Note—The *Diluent* is to be used exclusively to prepare the *Reference stock solution* described below; it must not be used to prepare the *Test solution.*]

Reference stock solution—Transfer a quantity of NIST traceable microspheres suspension containing about 0.5 g of microspheres directly into a tared centrifuge tube equipped with a cap, and weigh.[2] Using the density and concentration of the microspheres obtained from the Certificate of Analysis, calculate the volume occupied by the microspheres and the number of microspheres in the portion taken. Calculate the target total volume, in mL, by dividing the number of microspheres in the portion taken by the target concentration of 2.0×10^8 microspheres per mL. Calculate the target *Diluent* volume by subtracting the volume occupied by the microspheres from the target total volume. Transfer the target volume of *Diluent* to the centrifuge tube containing the portion of microspheres taken, and mix the tube vigorously for 1 hour. The prepared *Reference stock solution*, which contains a suspension of microspheres with a target mean particle diameter of 5.2 µm and a concentration of 2.0×10^8 microspheres per mL, is divided into smaller containers and stored at 5°.

Reference solution—Equilibrate the *Reference stock solution* to room temperature, and mix thoroughly. Immediately transfer 20 µL of the *Reference stock solution* to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Blank solution—Use 200 mL of *Electrolyte solution.*

Test solution—Allow the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [Note—After resuspension, the contents should appear as a homogeneous, opaque, milky-white suspension.] Immediately withdraw a 20-µL aliquot, transfer to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Test apparatus—Use a multichannel particle analyzer that operates on the electrical zone-sensing principle.[3] The analyzer is fitted and calibrated with an aperture tube having a 50-µm orifice. The multichannel particle analyzer is equipped with software capable of data-smoothing, data extrapolation, distribution graphing, and data conversion. Analyze the *Blank solution*, the *Reference solution*, and the *Test solution* as directed for *Procedure:* the total count in the *Blank solution* is not more than 500; the mean particle diameter of microspheres in the *Reference solution* is within 5% of the mean particle diameter of microspheres in the *Reference stock solution;* the concentration of the *Reference solution* is within 10% of the concentration of the *Reference stock solution;* and the coincidence effect in the analysis of the *Test solution* is not more than 5%.

Procedure—Rinse the orifice of the aperture tube with *Electrolyte solution* before and after analyzing each preparation. Place the *Blank solution* in the apparatus, and adjust the vacuum on the sample stand so that the counting begins about 12 seconds after the analyzer is set to the counting position. Set the data acquisition to stop when one of the following conditions is met: preset length of time, preset volume, preset

number of counts in any channel, or total counts. Collect the count versus the channel data for the *Blank solution*, and analyze using the data-smoothing, data extrapolation, distribution graphing, and data conversion features of the system software. In the same manner, analyze the *Reference solution* and the *Test solution.* The *Test solution* data are normalized and expressed as the number of microspheres per mL: the concentration of microspheres is between 5.0×10^8 and 8.0×10^8 per mL. Calculate the percentage of microspheres less than 10 µm in size in the portion of Injectable Suspension taken by the formula:

$$100(P_A/P_B)$$

in which P_A is the number of microspheres in the 1- to 10-µm size range, and P_B is the number of microsphere particles in the 1- to 32-µm size range. Not fewer than 93% of microsphere particles is smaller than 10 µm.

Container headspace content—

Reference solutions—Use 99% perflutren reference standard[4] (electronic grade perflutren gas of at least 99 molar % purity) and 60% perflutren reference standard[4] (an electronic grade gas in air mixture containing 60 molar % perflutren gas).

Blank solution—Use ambient air.

Test solution—Use gas from the container headspace.

Chromatographic system (see *Chromatography ⟨621⟩*)—The gas chromatograph is equipped with a thermal conductivity detector and a 0.53-mm × 25-m fused-silica (porous layer open tubular) column coated with Al_2O_3/KCl (aluminum oxide deactivated with potassium chloride).[5] The carrier gas is helium with a flow rate adjusted to obtain a retention time of about 1.5 to 1.8 minutes for perflutren. The column temperature is maintained at about 65°, the injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the *Reference solutions* as directed for *Procedure:* the resolution, *R,* between perflutren and air is not less than 2; and the relative standard deviation for replicate injections is not more than 5%. The measured value for the 99% perflutren reference standard is within 5% of the nominal value.

Procedure—Using a gas-tight syringe, separately inject 10 µL of the *Blank solution*, the *Reference solutions*, and the *Test solution* into the chromatograph. Record the chromatograms, and measure the responses for the major peaks. The percentages of perflutren in the 99% perflutren reference standard and in the *Test solution* are calculated by comparing the peak areas in each with the peak areas obtained from the 60% perflutren reference standard. The container headspace of Injectable Suspension contains not less than 60% of perflutren gas.

Microsphere perflutren content—

Reference stock solutions—The 97% decafluorobutane reference standard is decafluorobutane gas of at least 97 molar % purity.[6] The 5% decafluorobutane–5% perflutren reference standard is a mixture containing 5 molar % decafluorobutane gas and 5 molar % perflutren gas in air.[7] The 99% perflutren reference standard is electronic grade perflutren gas of at least 99 molar % purity.[4]

Analysis vial—Transfer 100 µL of 97% decafluorobutane reference standard gas and 100 µL of glacial acetic acid to a 2-mL vial equipped with a septum cap.

Reference solution—Transfer 100 µL of 99% perflutren reference standard and 0.75 mL of 1% Albumin Human to an *Analysis vial*, and incubate by mixing for at least 3 hours.

Test solution—Allow a vial of the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [Note—After resuspension, the contents of the vial should appear as a homogeneous, opaque, milky-white suspension.] Withdraw 0.75 mL of Injectable Suspension, and transfer to another *Analysis vial.* Incubate the *Test solution* by mixing for at least 3 hours.

Chromatographic system (see *Chromatography ⟨621⟩*)—Prepare as directed for *Container headspace content.* The carrier gas is helium with a flow rate adjusted to obtain the following retention times: 1.0 to 1.1 minutes for air, 1.3 to 1.5 minutes for perflutren, and 1.5 to 2.5 minutes for decafluorobutane. The column temperature is maintained at about 85°, and then after elution of the perflutren the temperature is increased at a rate of 50° per minute to 120°, and maintained at 120° for 2 minutes. The injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the 5% decafluorobutane–5% perflutren reference standard and the *Reference solution* as directed for *Procedure:* the resolution, *R,* between air and perflutren is not less than 2; the resolution, *R,* between perflutren and decafluorobutane is not less than 5; the relative standard deviation determined from the perflutren peak response for the *Reference solution* is not more than 5%; and the relative standard deviation determined from the response ratios for replicate injections of the 5% decafluorobutane–5% perflutren reference standard is not more than 5%.

Procedure—Inject 20 µL of the headspace gas from the vials containing the *Reference solution* and the *Test solution* into the gas chromatograph. Record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg per mL, of perflutren in the portion of Injectable Suspension taken by the formula:

$$(0.188M/V)(R_S/R_U)$$

in which M is the number of µmols of decafluorobutane in an *Analysis vial* after addition of the Injectable Suspension; V is the volume, in mL, of Injectable Suspension added to the *Analysis vial;* and R_S and R_U are the peak area ratios of decafluorobutane to perflutren obtained from the

Reference solution and the *Test solution*, respectively. The quantity of perflutren in the Injectable Suspension is between 0.11 mg per mL and 0.33 mg per mL.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests ⟨1⟩*; with the exception of *Foreign and Particulate Matter*.

Assay for protein—

Diluted antifoam reagent—Transfer 100 µL of antifoam reagent[8] to a suitable container, and dilute with water to 10 mL.

Blank preparation—Transfer 500 µL of Sodium Chloride Injection to a culture tube. Dilute the contents of the tube with water to 2 mL, and add 10 µL of *Diluted antifoam reagent*.

Standard preparations—Transfer 25-, 50-, 62.5-, 75-, and 100-µL aliquots of protein standard solution[9] containing 8 g per dL into separate tubes. Dilute the contents of each tube with water to 2.00 mL, and add 10 µL of the *Diluted antifoam reagent* to each tube. During the *Procedure,* the addition of 3.0 mL of biuret reagent TS to each of the tubes produces *Standard preparations* with protein concentrations of 0.4, 0.8, 1.0, 1.2, and 1.6 mg per mL.

Assay preparation—Equilibrate each container of Injectable Suspension to room temperature, and mix each for at least 5 minutes to ensure a homogeneous suspension. Vent the container, and transfer 500-µL aliquots into separate tubes. Dilute the contents of each tube with water to 2 mL, and add 10 µL of *Diluted antifoam reagent*.

Procedure—To each of the tubes containing the *Blank preparation, Standard preparations,* and *Assay preparation,* add 3.0 mL of biuret reagent TS, mix, and allow to stand for 30 minutes, accurately timed, for maximum color development. The *Blank preparation, Standard preparations,* and *Assay preparation* are treated identically. Using the *Blank preparation,* set the absorbance equal to zero. Determine the absorbance of each of the *Standard preparations* and the *Assay preparation* in 1-cm cells with a suitable spectrophotometer at a wavelength of 540 nm. Using linear regression, analyze the data obtained for each of the *Standard preparations.* Calculate the correlation coefficient, slope, and *y*-intercept values: the correlation coefficient is not less than 0.995. Calculate the quantity, in mg, of protein in each mL of the Injectable Suspension by the formula:

$$10[(A_U - y\text{-intercept})/\text{slope}]$$

in which 10 is the dilution factor; and A_U is the absorbance of the *Assay preparation:* the calculated quantity of protein in the Injectable Suspension is between 8 and 12 mg per mL.

[1] Filtered and buffered saline electrolyte solution is available as ISOTON(®) II from Beckman Coulter, Inc., Fullerton, CA.

[2] Microspheres with a mean particle diameter of 5 µm are available as NIST traceable Dynospheres from Bangs Laboratories, Inc., Fishers, IN.

[3] A suitable multichannel particle analyzer is available as the Multisizer Model IIe from Beckman Coulter, Inc., Fullerton, CA.

[4] A suitable grade of perflutren (octafluoropropane) is available from Air Products and Chemicals, Inc., Allentown, PA.

[5] The column is available from Varian U.S.A. Chrompack, Walnut Creek, CA, catalog number CP7517, as an Al_2O_3/KCl PLOT column (0.53-mm ID, 25-m length).

[6] The gas is available under product code 03047567SR-LD from Scott Medical Products, Plumsteadville, PA.

[7] The mixture of the two gases in air is available under product code 03047566SR-LD from Scott Medical Products, Plumsteadville, PA.

[8] Available as Antifoam Reagent, catalog number 2210, from Dow Corning Corporation, Midland, MI.

[9] Available as Bovine Serum Albumin, SRM 927c, Standard Reference Materials, National Institute of Standards and Technology, Gaithersburg, MD.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
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