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DEXTRAN POWDER TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for analyzing Dextran powders.

2. SCOPE:

2.1. Applies to the testing of Dextran powders in the Laboratory at all BioSpectra Facilities. Methods include testing for all types of Dextran powders; only the specific tests required for the desired type must be tested.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing or qualified designee is responsible for the control, training, maintenance and implementation of this procedure.

3.2. The Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Laboratory Manager if any analyses fail to meet their respective specifications.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Calibrated Timer
4.3. Anton Paar Density Meter, or equivalent
4.4. ICP-MS
4.5. Calibrated Oven
4.6. Calibrated Pipette
4.7. Micro-Viscometer
4.8. Milli-Q IQ 7005 with IQ-Element and Q-Pod Water Purification System
4.9. Muffle Furnace
4.10. OPI-180 OD Handheld Colorimeter
4.11. XL200 pH Meter, or equivalent
4.12. Polarimeter
4.13. Ubbelohde Viscometer
4.14. UV/Vis Spectrophotometer

5. REAGENTS:

5.1. **1N Hydrochloric Acid:** Purchased Commercially.
5.2. **0.1N Silver Nitrate:** Purchased Commercially.
5.3. **Anthrone Powder:** Purchased Commercially.
5.4. **Glacial Acetic Acid:** Purchased Commercially.
5.5. **Nitric Acid, concentrated:** Purchased Commercially.
5.6. **Purified Water:** In-House or Purchased Commercially.
5.7. **Sulfuric Acid, concentrated:** Purchased Commercially.

6. REFERENCES:

6.1. BSI-ATM-0093, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3, & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
6.2. BSI-PRL-0521, Analytical Method Validation Protocol: Determination of ICH Q3D Elemental Impurities and Iron by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
6.3. BSI-RPT-0988, Analytical Method Validation Report: Determination of ICH Q3D Elemental Impurities and Iron by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
6.4. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration SOP

6.5. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.6. BSI-SOP-0098, Balance SOP
6.7. BSI-SOP-0126, Laboratory Notebooks
6.8. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
6.9. BSI-SOP-0134, Pipette SOP
6.10. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration SOP
6.11. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration SOP
6.12. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.13. BSI-SOP-0259, Fisher Scientific Isotemp Water Bath Operation and Calibration SOP
6.14. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
6.15. BSI-SOP-0386, Operation and Maintenance of the Milli-Q IQ 7005 with IQ-Element and Q-Pod Water Purification Systems
6.16. BSI-SOP-0486, Viscometer SOP
6.17. BSI-SOP-0490, MCP 5300 Polarimeter SOP
6.18. BSI-SOP-0574, Anton Paar Lovis 2000M Microviscometer SOP
6.19. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
6.20. ACS, Reagent Chemicals, current edition

7. ANALYTICAL PROCEDURES:

7.1. APPEARANCE AND COLOR :

7.1.1. Place 10 grams of sample in a clean, dry, glass beaker.
7.1.2. In an area with sufficient lighting, view the sample from all sides.
7.1.3. The sample should be white to slightly off white in color and characteristic of a powder.
7.1.4. If the appearance and color result is unable to be definitively determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP.
7.1.5. If the sample does not conform to these specifications, notify the Laboratory Manager immediately.

7.2. CHLORIDE CONTENT :

7.2.1. Thoroughly rinse 50mL Nessler Color Comparison Tubes using purified water prior to use.
7.2.2. Standard Preparation:
7.2.2.1. Pipette 0.705mL of 1N Hydrochloric Acid into a 50mL Nessler Color Comparison Tube and dilute to approximately 40mL with Purified Water.
7.2.3. Sample Preparation:
7.2.3.1. Weigh 2.50 grams of sample and quantitatively transfer to a 50mL Nessler Color Comparison Tube.
7.2.3.2. Dilute to approximately 40mL with Purified Water and dissolve sample.
7.2.3.3. If necessary, acidify the solution with nitric acid to litmus.
7.2.4. Analysis:
7.2.4.1. Add to each solution, 1mL of Concentrated Nitric Acid and 1mL of 0.1N Silver Nitrate.
7.2.4.2. Dilute to 50mL with Purified Water. Cover with parafilm and mix by inversion.
7.2.4.3. After 5 minutes, view the solutions against a dark background. If the turbidity of the sample preparation does not exceed that produced by the 10000ppm Chloride Standard, report the result as <10000ppm.

7.3. COLD WATER SOLUBILITY (1% SOLUTION) :

7.3.1. Cool purified water to 2-8°C, a minimum of 100mL is required per test.
7.3.2. 1% Sample Solution Preparation:
7.3.2.1. Accurately weigh 1.0 grams of sample to a beaker.
7.3.2.2. Add stir bar.
7.3.2.3. Add 100mL of the cooled water to the beaker.
7.3.2.4. Stir to dissolve completely.
7.3.3. Solubility in the cooled water should be clear and complete to report as “Passes Test.”

7.4. COLOR OF SOLUTION (10% SOLUTION) :

7.4.1. 10% Sample Solution Preparation:
7.4.1.1. Accurately weigh 5.0 grams of sample.
7.4.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 50mL with purified water.
7.4.1.3. Swirl to dissolve completely.
7.4.2. Refer to Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1cm pathlength at 360nm and 375nm.

7.5. **HEAVY METALS (AS Pb) :**

7.5.1. Refer to BSI-ATM-0093, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3, & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate for sample preparation and analysis.

7.6. **IDENTIFICATION TEST :**

7.6.1. **Dextran Identification**

7.6.2. 1% Sample Solution Preparation:

7.6.2.1. Add 1g of sample to a 100mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.

7.6.3. Anthrone Solution Preparation:

7.6.3.1. **Note:** Prepare immediately before use.

7.6.3.2. Weigh 90 – 100mg of Anthrone Powder into a 100mL beaker. Add 50mL of concentrated Sulfuric Acid, dissolve, and mix thoroughly.

7.6.4. Analysis:

7.6.4.1. Into a test tube, pipette 1.0mL of *1% Sample Solution* and 5.0mL of *Anthrone Solution* and mix well.

7.6.4.2. Heat the tube in a boiling water bath for 10 minutes.

7.6.4.3. The solution should turn green then a blue-green color.

7.6.4.4. To the test tube add a few drops of Glacial Acetic Acid.

7.6.4.5. The blue-green color does not change with the addition of Glacial Acetic acid to report as "Passes Test."

7.7. **INTRINSIC VISCOSITY (1% SOLUTION) @ 37°C :**

7.7.1. **Primary Method**

7.7.1.1. 1% Sample Solution Preparation:

7.7.1.1.1. Weigh and transfer 1.0 grams of sample into a 100mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

7.7.1.2. Analysis:

7.7.1.2.1. Perform analysis at 37°C.

7.7.1.2.2. Refer to BSI-SOP-0574, Anton Paar Lovis 2000M Microviscometer SOP for instrument operation and analysis.

7.7.1.2.3. Calculate the Intrinsic Viscosity using the following equation:

$$Intrinsic\ Viscosity = \frac{\eta - \eta_0}{\eta_0}$$

7.7.1.2.4. η = Kinematic Viscosity of the Sample (mm^2/s).

7.7.1.2.5. η_0 = Kinematic Viscosity of Purified Water (mm^2/s).

7.8. **LOSS ON DRYING :**

7.8.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.

7.8.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record the weight.

7.8.3. Transfer ~2 grams of the sample to the LOD vial and accurately weigh the vial and contents, record the weight.

7.8.4. Place the LOD vial containing the sample into the oven and dry at 105 ± 2°C for 5 hours.

7.8.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

7.8.6. Reweigh the LOD vial and sample.

7.8.7. Calculate the %LOD as follows:

$$\%LOD = \frac{Inital\ Sample\ Weight\ (g) - Final\ Sample\ Weight\ (g)}{Initial\ Sample\ Weight\ (g)} \times 100$$

7.9. **pH (10% SOLUTION) :**

7.9.1. Accurately weigh 5.0 grams of sample and transfer to a suitable beaker.
7.9.2. Add 50mL of Purified Water and dissolve the sample.
7.9.3. Follow the appropriate SOP for pH calibration and measurement.

7.10. **RESIDUE ON IGNITION (ASH CONTENT) :**

7.10.1. Turn on the Muffle Furnace and allow it to stabilize at 600°C. Follow muffle furnace calibration procedure for operation of furnace.
7.10.2. Inspect a quartz crucible for cracks, chips, and discoloration.
7.10.3. Utilize forceps to insert and remove the crucible from the furnace.
7.10.4. Ignite a quartz crucible at 600 ± 50°C for 30 minutes. Cool in a desiccator for 1.5 hours and weigh using an analytical balance.
7.10.5. Weight 1.0 g of sample in the previously ignited quartz crucible. Moisten the sample with a small amount of concentrated Sulfuric Acid (between 0.2-1.0mL).
7.10.6. Volatilize the sample until the sample is thoroughly charred and white fumes are no longer evolved. Heat the sample slowly, so that the sample does not boil over and the sample is not lost.
7.10.7. Ignite in the muffle furnace at 600 ± 50°C for 15 minutes, or until all the carbon has been removed.
7.10.8. Cool in a desiccator for 1.5 hours and weigh on an analytical balance.
7.10.9. Calculate the %ROI as follows:

$$\%ROI = \frac{Residue\ Weight\ (g)}{Sample\ Weight\ (g)} \times 100$$

7.10.10. If the amount of residue exceeds the limit specified, repeat the moistening with concentrated Sulfuric Acid, using up to 1mL, heat to char, then ignite at 600 ± 50°C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.5mg, or until the specification is met.

7.11. **SPECIFIC ROTATION $[\alpha]_D^{20}$ (2% SOLUTION) @ 20°C :**

7.11.1. **Note:** Loss on Drying result required prior to analysis.
7.11.2. 2% Sample Solution Preparation:
7.11.2.1. Weigh and transfer 2 grams of sample to a 100mL volumetric flask, dissolve, dilute to volume with purified water, and mix thoroughly.
7.11.3. Analysis:
7.11.3.1. Analysis: Perform at 20°C.
7.11.3.2. Optical Zero Reference: Purified Water
7.11.3.3. Refer to BSI-SOP-0490, MCP 5300 Polarimeter SOP for instrument operation and sample analysis.