BIOSPECTRA
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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, maintenanceand 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. REFERENCES:

4.1. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration SOP
4.2. BSI-SOP-0098, Balance SOP
4.3. BSI-SOP-0126, Laboratory Notebooks
4.4. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
4.5. BSI-SOP-0134, Pipette SOP
4.6. BSI-SOP-0135, Laboratory Chemicals
4.7. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration SOP
4.8. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration SOP
4.9. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.10. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.11. ACS, Reagent Chemicals, current edition

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Anton Paar MCP 5300 Polarimeter
5.3. Blue M Convection Oven
5.4. Calibrated Pipette
5.5. Calibrated Timer
5.6. Hot Plate
5.7. Lambda 25 UV/Vis Spectrophotometer
5.8. Metrohm 914 pH Conductometer
5.9. pH Probe
5.10. VWR Gravity Convection Oven
5.11. XL200 pH/mV/Conductivity Meter

6. REAGENTS:

6.1. **Anthrone Solution:** Prepare immediately before use. Weigh 90 – 100mg of anthrone powder into a beaker, add 50mL of concentrated sulfuric acid, dissolve, and mix thoroughly.
6.2. **Anthrone Powder:** Purchased Commercially.
6.3. **Glacial Acetic Acid:** Purchased Commercially.
6.4. **Purified Water:** In-House or Purchased Commercially.
6.5. **Sulfuric Acid, concentrated:** Purchased Commercially.

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7. ANALYTICAL PROCEDURES:

Note: Specific rotation is based on the dried basis sample weight and requires the Loss on Drying result to perform the test correctly.

7.1. APPEARANCE — Refer to Summary Sheet:

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. If the sample does not conform to these specifications, notify the QC Manager immediately.

7.2. COLD WATER SOLUBILITY — Refer to Summary Sheet:

7.2.1. Cool purified water to 2-8°C, a minimum of 100mL is required per test.
  7.2.1.1. Prepare a 1% solution of the specified sample.
  7.2.1.2. Accurately weigh 1.0 grams of sample to a beaker.
  7.2.1.3. Add stir bar.
  7.2.1.4. Add 100mL of the cooled water to the beaker.
  7.2.1.5. Stir to dissolve completely.
7.2.2. Solubility in the cooled water should be clear and complete to report as soluble in cold water.

7.3. COLOR OF SOLUTION 360nm, 10% — Refer to Summary Sheet:

7.3.1. Prepare a 10% solution of the specified sample.
  7.3.1.1. Accurately weigh 5.0 grams of sample.
  7.3.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 50mL with purified water.
  7.3.1.3. Swirl to dissolve completely.
7.3.2. Refer to Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1cm pathlength at 360nm.

7.4. IDENTIFICATION TEST — Refer to Summary Sheet:

7.4.1. **Dextran Identification**
7.4.2. Sample Preparation (~1% Dextran Sample Solution):
  7.4.2.1. Add 1g of sample to a 100mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.
7.4.3. Anthrone Solution Preparation:
  7.4.3.1. Note: Prepare immediately before use.
  7.4.3.2. Weigh 90 – 100mg of Anthrone Powder into a 100mL beaker. Add 50mL of concentrated sulfuric acid, dissolve, and mix thoroughly.
7.4.4. Procedure:
  7.4.4.1. Into a test tube, pipette 1.0mL of 1% Dextran Solution and 5.0mL of Anthrone Solution and mix well.
  7.4.4.2. Heat the tube in a boiling water bath for 10 minutes.
  7.4.4.3. The solution should turn green then a blue-green color.
  7.4.4.4. To the test tube add a few drops of Glacial Acetic Acid.
  7.4.4.5. The blue-green color does not change with the addition of Glacial Acetic acid to report as passes test.

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7.5. **LOSS ON DRYING** **Refer to Summary Sheet:**

7.5.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.
7.5.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
7.5.3. Transfer ~2 grams of the sample to the LOD vial and accurately weigh the vial and contents.
7.5.4. Place the LOD vial containing the sample into the oven and dry at 105 ± 2°C for 5 hours.
7.5.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.5.6. Reweigh the LOD vial and sample.
7.5.7. Calculate the %LOD as follows:

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

7.6. **pH (10%)** **Refer to Summary Sheet:**

7.6.1. Transfer 5 grams of neat sample solution to a 50mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.
7.6.2. Transfer solution to a beaker and add stir bar.
7.6.3. Follow the appropriate SOP for pH calibration and measurement.

7.7. **SPECIFIC ROTATION $[\alpha]_D^{20}$** **Refer to Summary Sheet:**

7.7.1. Sample Preparation (2% Dextran Solution):
7.7.1.1. Transfer 2 grams of sample to a 100mL volumetric flask, dissolve, and dilute to volume with purified water. Mix thoroughly. Solution may be scaled as needed.
7.7.2. Refer to the MCP 5300 Polarimeter SOP for instrument analysis, concentration is calculated on the dried basis.
7.7.3. Analysis: Perform at 20°C.

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