



100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

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. 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 $105 \pm 2^\circ\text{C}$ 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 $105 \pm 2^\circ\text{C}$ 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:

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

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 .