BIOSPECTRA

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DEXTRAN SULFATE 8000MW (DS8) SOLUTIONS TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory personnel with a procedure for analyzing Dextran Sulfate 8000MW solutions (DS8).

2. SCOPE:

2.1. Applies to the testing of Dextran Sulfate 8000MW (DS8) solutions in the Laboratory at all BioSpectra Facilities. Methods include testing for all types of Dextran Sulfate 8000MW (DS8) solutions; 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-ATM-0094, Analytical Method for the Quantification of Sulfur by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in Dextran Sulfate
4.2. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration SOP
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0126, Laboratory Notebooks
4.5. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
4.6. BSI-SOP-0134, Pipette SOP
4.7. BSI-SOP-0135, Laboratory Chemicals
4.8. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration SOP
4.9. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration SOP
4.10. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.11. BSI-SOP-0259, Fisher Scientific Water Bath Operation and Calibration SOP
4.12. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
4.13. BSI-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES
4.14. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.15. ACS, Reagent Chemicals, current edition

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Anton Paar MCP 5300 Polarimeter or Equivalent
5.3. Anton Paar DMA 35 Portable Density Meter Operation and Calibration
5.4. Blue M Convection Oven
5.5. Calibrated Pipette
5.6. Calibrated Timer
5.7. Endosafe NexGen PTS Endotoxin Reader
5.8. Hot Plate
5.9. Lambda 25 UV/Vis Spectrophotometer
5.10. Litmus Paper
5.11. Metrohm 914 pH Conductometer
5.12. Muffle Furnace
5.13. Perkin Elmer Avio 500 ICP-OES
5.14. pH Probe
5.15. Thermometer
5.16. VWR Gravity Convection Oven

5.17. XL200 pH/mV/Conductivity Meter
5.18. Water Bath, Heated
5.19. Refrigerator/Freezer
5.20. Volumetric Flasks, Various Sizes

6. REAGENTS:

6.1. 0.02N Hydrochloric Acid: Slowly add 20mL of 0.1N hydrochloric acid to 80mL of purified water to make a total volume of 100mL or purchased commercially.
6.2. 0.1M Barium Chloride: Dissolve 2.4g of barium chloride dihydrate in purified water and dilute with purified water to make 100mL.
6.3. 0.1N Hydrochloric Acid: Purchased Commercially.
6.4. 0.1N Silver Nitrate: Purchased Commercially.
6.5. 1% Acrinol: Dissolve 1.0 grams of Acrinol Monohydrate in purified water and dilute with purified water to 100mL.
6.6. 1.0M Sodium Chloride: Transfer 58.44g of Sodium Chloride to a 1000mL volumetric flask, dissolve, and dilute to volume with purified water.
6.7. 2N Sodium Hydroxide: Dissolve 8g of Sodium Hydroxide in purified water to make 100mL. Preserve in polyethylene bottles.
6.8. Acrinol Monohydrate: Purchased Commercially.
6.9. Anhydrous Sodium Sulfate: Purchased Commercially.
6.10. 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.
6.11. Anthrone Powder: Purchased Commercially.
6.12. Barium Chloride Dihydrate: Purchased Commercially.
6.13. Barium Chloride TS (~0.5M): Dissolve 30g of barium chloride dihydrate in water to make 250mL.
6.14. Dextrose (D-Glucose) Certified Reference Standard (CRS): Purchased Commercially.
6.15. Glacial Acetic Acid: Purchased Commercially.
6.16. Hydrochloric Acid, concentrated: Purchased Commercially.
6.17. Hydrochloric Acid, Dilute (~10%): Dilute 23.6mL of concentrated hydrochloric acid with water to make 100mL.
6.18. LAL Reagent Water: Purchased Commercially.
6.19. Nitric Acid, concentrated: Purchased Commercially.
6.20. Purified Water: In-House or Purchased Commercially.
6.21. Sodium Chloride: Purchased Commercially.
6.22. Sulfate Standard Solution (0.2% SO_4^{2-} Solution): Dissolve 0.296g of anhydrous sodium sulfate in purified water and dilute with purified water to 100mL.
6.23. Sulfuric Acid, **concentrated:** Purchased Commercially.

7. ANALYTICAL PROCEDURES:

7.1. **Note: The following tests require reporting on the dried basis and require a loss on drying result for report; Glucose, Specific Rotation, and Total Sulfur Content.**

7.2. **APPEARANCE** **Refer to Summary Sheet:**

7.2.1. Place ~50mL sample in a clean, dry, glass beaker.
7.2.2. In an area with sufficient lighting, view the sample from all sides.
7.2.3. The sample should be white to light yellow to orange in color and characteristic of a liquid.

7.3. **CLARITY (20% SOLUTION AT 360nm)** **Refer to Summary Sheet:**

7.3.1. Analyze the sample neat.
7.3.2. Refer to Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1cm path length at 360nm.

7.4. **CHLORIDE CONTENT** **Refer to Summary Sheet:**

7.4.1. Thoroughly rinse 50mL Nessler Color Comparison Tubes using purified water prior to use.
7.4.2. Standard Preparation:
7.4.2.1. Pipette 0.705mL of 0.02N Hydrochloric Acid into a 50mL Nessler Color Comparison Tube and dilute to approximately 40mL with purified water.
7.4.3. Sample Preparation:
7.4.3.1. Weigh 2.50 grams of sample and quantitatively transfer to a 50mL Nessler Color Comparison Tube.
7.4.3.2. Dilute to approximately 40mL with purified water and dissolve sample.
7.4.3.3. If necessary, acidify the solution with nitric acid to litmus.
7.4.4. Procedure:
7.4.4.1. Add to each solution, 1mL of concentrated nitric acid and 1mL of 0.1N Silver Nitrate.
7.4.4.2. Dilute to 50mL with purified water. Cover with parafilm and mix by inversion.
7.4.4.3. After 5 minutes, view the solutions against a dark background. If the turbidity of the sample preparation does not exceed that produced by the 1000ppm Chloride Standard, report the result as <1000ppm.

7.5. **DENSITY** **Refer to Summary Sheet:**

7.5.1. Refer to the DMA 35 Portable Density Meter Operation and Calibration SOP, analyze sample neat.

7.6. **FREE INORGANIC SULFATE** **Refer to Summary Sheet:**

7.6.1. Standard Preparation:
7.6.1.1. Pipette 5mL of Sulfate Standard Solution (0.2% SO_4^{2-} Solution) into a test tube.
7.6.2. Sample Preparation:
7.6.2.1. 1% Dextran Sulfate Sample Stock Solution
7.6.2.2. Weigh out 5.0 grams of sample, transfer to a 100mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.
7.6.2.3. Note: A 1% Dextran Sulfate Sample Solution is also used in the pH test and the dextran sulfate identification tests. A stock solution may be prepared and used in any of these tests as long as it is treated as expiring in 48 hours.
7.6.2.4. Pipette 5mL of 1% Dextran Sulfate Sample Stock Solution into a test tube.
7.6.3. Procedure:
7.6.3.1. To the standard and samples, add 0.5mL of hydrochloric acid, dilute (~10%) and 1mL of 0.1M Barium Chloride.
7.6.3.2. Mix well and allow to stand at room temperature for 15 minutes.
7.6.3.3. If the turbidity of the sample preparation does not exceed that produced by the standard, the sample passes test.

7.7. GLUCOSE CONTENT — Refer to Summary Sheet:

7.7.1. Sample Preparation:

7.7.1.1. Sample Stock Solution (~5mg/mL Dextran Sulfate): Weigh out 5.0 grams of Dextran Sulfate Solution into a 200mL volumetric flask. Fill ~3/4 full with purified water and swirl to dissolve. Fill to volume with purified water and mix by inversion. Scale if required.

7.7.1.2. Sample Test Solution (0.05mg/mL Dextran Sulfate): Pipette 1.0mL of Sample Stock Solution into a 100mL volumetric flask, fill to volume with purified water, and mix by inversion. Scale if required.

7.7.2. Standard Preparation:

7.7.2.1. Glucose Standard Stock Solution (440µg/mL Glucose): Weigh out 110mg equivalent of Dextrose (D-Glucose) CRS into a 250mL volumetric flask. Fill ~3/4 full with purified water and swirl to dissolve. Fill to volume with purified water and mix by inversion. Refer to the Dextrose (D-Glucose) CRS Certificate of Analysis values for purity corrections:

$$Dextrose\ CRS\ Weight\ (mg) = \frac{\left(\frac{0.440mg}{mL}\right) \times \left(Final\ Volume\ (mL)\right)}{Dextrose\ CRS\ Purity\ \left(\frac{mg}{mg}\right)}$$

7.7.2.2. Calibration Standards: Per the "Glucose Calibration Standard Preparations" Table, pipette Glucose Standard Stock Solution into a 100mL volumetric flask, fill to volume with purified water, and mix by inversion.

Glucose Calibration Standard Preparations

Standard ID	Glucose Concentration (µg/mL)	Glucose Standard Stock Solution Amount (mL)	Final Volume (mL)
1	13.2µg/mL	3.0mL	100mL
2	33.0µg/mL	7.5mL	100mL
3	52.8µg/mL	12.0mL	100mL

7.7.3. Procedure:

7.7.3.1. Pipette 0.50mL of each glucose calibration standard, sample test solution, and a blank (purified water) into microcentrifuge.

7.7.3.2. Anthrone Solution Preparation:

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

7.7.3.2.2. Weigh 90 – 100mg of Anthrone Powder into a beaker. Add 50mL of concentrated sulfuric acid, dissolve, and mix thoroughly.

7.7.3.3. Carefully and uniformly add 1.0mL of Anthrone Solution into each of the microcentrifuge tubes, mix, and immediately place the microcentrifuge tubes in a hot bath at 80°C minimum for 9 minutes.

7.7.3.3.1. **Note:** Mixing of the samples and anthrone solution is extremely exothermic.

7.7.3.4. After 9 minutes, remove the microcentrifuge tubes from the hot bath and place in a temperature monitored refrigerator for 5 minutes.

7.7.3.5. After 5 minutes remove the microcentrifuge tubes from ice or a temperature monitored refrigerator and allow to come to room temperature.

7.7.4. Quantitative Reporting:

7.7.4.1. Calibrate the UV/Vis Spectrophotometer by ensuring that the Blank is assigned as "Blank", the Calibration Standard IDs 1 through 3 are assigned as "Standard", and all samples are assigned as "Sample" in the "Type" column on the "Sample Info" window of the PerkinElmer UV WinLab software.

7.7.4.2. Input the Calibration Standards concentrations in parts per million (ppm) into the "Concentration" column on the "Sample Info" window of the PerkinElmer UV WinLab software.

7.7.4.2.1. **Note:** This should be automatically populated through the method.

7.7.4.3. Measure the absorbance of the standards and samples at 625nm as per the Lambda 25 UV/Vis Operation and Calibration SOP.

7.7.5. Result Reporting:

7.7.5.1. System Suitability

7.7.5.1.1. The Correlation Coefficient (r^2) of the calibration curve must be NLT 0.99.

7.7.5.2. The Glucose Content is determined using the following equations:

7.7.5.2.1. Dextran Sulfate Concentration (ppm) on the Dried Basis:

$$Dextran\ Sulfate\ Concentration\ (Dried\ Basis)(ppm) = \frac{Sample\ Weight\ (g) \times \left(100 - LOD\ (\%)\right)}{2}$$

7.7.5.2.2. Glucose Content (%w/w)

$$Glucose\ Content\ \left(\%\frac{w}{w}\right) = \frac{Glucose\ Concentration\ (ppm)}{Dextran\ Sulfate\ Concentration\ (Dried\ Basis)(ppm)} \times 100$$

7.8. **IDENTIFICATION TEST** **Refer to Summary Sheet:**

7.8.1. **Note:** The Identification Test consists of three separate tests, Acrinol, Sulfate, and Dextran Identification. All three separate identification tests must pass to report the Identification Test as passes.

7.8.2. **Acrinol Identification**

7.8.2.1. Sample Preparation (~5% Dextran Sulfate Sample Solution):

7.8.2.1.1. Pipette 12.5mL of sample solution to a 50mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.

7.8.2.2. Procedure:

7.8.2.2.1. Into two (2) clean test tubes, per sample, add 1.0mL of 1% Acrinol, 5.0mL of 5% Dextran Sulfate Sample Solution, and mix well.

7.8.2.2.2. A yellow flocculent precipitate should form in both test tubes.

7.8.2.2.3. To one test tube add a few drops of hydrochloric acid, dilute (~10%) and mix well.

7.8.2.2.4. To the other test tube, add a few drops of 2N sodium hydroxide and mix well.

7.8.2.2.5. The yellow flocculent precipitate should be almost insoluble in either acid or alkali to report as passes test.

7.8.3. **Dextran Identification**

7.8.3.1. Sample Preparation (~1% Dextran Sulfate Sample Solution):

7.8.3.1.1. Add 5mL of sample to a 100mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.

7.8.3.2. Anthrone Solution Preparation:
7.8.3.2.1. **Note:** Prepare immediately before use.
7.8.3.2.2. Weigh 90 – 100mg of Anthrone Powder into a 100mL beaker. Add 50mL of concentrated sulfuric acid, dissolve, and mix thoroughly.
7.8.3.3. Procedure:
7.8.3.3.1. Into a test tube, pipette 1.0mL of 1% Dextran Sulfate Solution and 5.0mL of Anthrone Solution and mix well.
7.8.3.3.2. Heat the tube in a boiling water bath for 10 minutes.
7.8.3.3.3. The solution should turn green then a blue-green color.
7.8.3.3.4. To the test tube add a few drops of Glacial Acetic Acid.
7.8.3.3.5. The blue-green color does not change with the addition of Glacial Acetic acid to report as passes test.

7.8.4. **Sulfate Identification**
7.8.4.1. Pipette 10mL of purified water into a beaker containing a stir bar.
7.8.4.2. Slowly and with caution add 10mL of concentrated hydrochloric acid to the beaker.
7.8.4.3. Place the beaker on a hot plate and stir using the magnetic stir bar.
7.8.4.4. Add 5mL of sample and transfer to the beaker.
7.8.4.5. Heat the beaker to boiling with continuous mixing for two (2) minutes then allow to cool to room temperature.
7.8.4.6. Add a few drops of barium chloride TS (~0.5M).
7.8.4.7. A heavy precipitate of barium sulfate should form to report as passes test.

7.9. **LOSS ON DRYING (Total Dissolved Solids)** **Refer to Summary Sheet:**

NOTE: For In process testing required for Dextran Sulfate 8000, LOD is to be performed in duplicate due to the length of the analysis. This is to be prepared in an event that one test is not reportable. Report average if both tests run to completion.

7.9.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.
7.9.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
7.9.3. Transfer ~2 grams of the sample to the LOD vial and accurately weigh the vial and contents.
7.9.4. Place the LOD vial containing the sample into the oven and dry at 105 ± 2°C for 5 hours.
7.9.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.9.6. Reweigh the LOD vial and sample.
7.9.7. Place back in oven for an additional hour, remove and allow to cool in desiccator for 15 minutes and reweigh.
7.9.8. If necessary, continue to dry until constant weight; NMT 0.5mg difference between weighings.
7.9.9. Calculate the %LOD and % Total Dissolved Solids (TDS) as follows:

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

$$\%TDS = 100\% - LOD\%$$

7.10. **pH (1 in 20 Dilution)** **Refer to Summary Sheet:**

7.10.1. Transfer 5 grams of neat sample solution to a 100mL volumetric flask and dissolve in purified water. Dilute to volume with purified water and mix well to prepare a 1% solution.
7.10.2. Follow the appropriate SOP for pH calibration and measurement.

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

7.11.1. Sample Preparation (~5% Dextran Sulfate Solution):

7.11.1.1. Transfer 12.5 grams of sample to a 50mL volumetric flask, dissolve, and dilute to volume with purified water. Mix thoroughly. Solution may be scaled as needed.

7.11.2. Refer to the MCP 5300 Polarimeter SOP for instrument analysis, concentration is calculated on the dried basis.

7.11.3. Analysis: Perform at 20°C.

7.12. **TOTAL SULFUR CONTENT** **Refer to Summary Sheet:**

7.12.1. Refer to Analytical Method for the Quantification of Sulfur by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in Dextran Sulfate (DCN: BSI-ATM-0094), for sample preparation and analysis.