BIOSPECTRA
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DEXTRAN M.W. 10,000 POWDER
TESTING METHODS

TABLE OF CONTENTS

1. PURPOSE: 3
2. SCOPE: 3
3. RESPONSIBILITIES: 3
4. REFERENCES: 3
5. EQUIPMENT: 3
6. REAGENTS: 4
7. ANALYTICAL PROCEDURES: 5

1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for analyzing Dextran (10,000 m.w.) powders.

2. SCOPE:

2.1. Applies to the testing of Dextran (10,000 m.w.) 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 Laboratory Manager or qualified designee is responsible for the control, training, maintenance and implementation of this procedure.
3.2. The Laboratory Technicians 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-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
4.2. BSI-ATM-0145, Analytical Method for Phenol Content in Dextran via GC-FID.
4.3. BSI-ATM-0146, Dextran Low Molecular Weight Distribution via Gel Permeation Chromatography (GPC) with RI Detection.
4.4. 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
4.5. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration SOP
4.6. BSI-SOP-0094, Muffle Furnace SOP and Calibration
4.7. BSI-SOP-0098, Balance SOP
4.8. BSI-SOP-0126, Laboratory Notebooks
4.9. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
4.10. BSI-SOP-0134, Pipette SOP
4.11. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration SOP
4.12. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration SOP
4.13. BSI-SOP-0254, Spectrum Two UATR SOP
4.14. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.15. BSI-SOP-0259, Fisher Scientific Isotemp Water Bath Operation and Calibration SOP
4.16. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP
4.17. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
4.18. BSI-SOP-0386, Operation and Maintenance of the Milli-Q IQ 7005 with IQ-Element and Q-Pod Water Purification Systems
4.19. BSI-SOP-0486, Viscometer SOP
4.20. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.21. BSI-SOP-0574, Anton Paar Lovis 2000M Microviscometer SOP
4.22. ACS, Reagent Chemicals, current edition

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Beakers, Various Sizes

5.3. Bunsen Burner
5.4. Calibrated Timer
5.5. Class A Volumetric Flasks, Various Sizes
5.6. Density Meter
5.7. Desiccator
5.8. Endosafe PTS Reader
5.9. Graduated Cylinders, Various Sizes
5.10. Hot Plate
5.11. HPLC with RI Detection
5.12. Ice Bath
5.13. ICP-MS
5.14. Litmus Paper
5.15. Micropipettes
5.16. Micro-Viscometer
5.17. Milli-Q IQ 7005 with IQ-Element and Q-Pod Water Purification System
5.18. Muffle Furnace
5.19. Nessler Color Comparison Tubes
5.20. Oven
5.21. Parafilm
5.22. pH Meter
5.23. pH Probe
5.24. Polarimeter
5.25. Stir Bar
5.26. Stir Plate
5.27. Test Tubes
5.28. Transfer Pipettes
5.29. UATR IR Spectrometer
5.30. UV/Vis Spectrophotometer
5.31. Water Bath

6. REAGENTS:

6.1. **1N Hydrochloric Acid:** Purchased Commercially.
6.2. **0.1N Silver Nitrate:** Purchased Commercially.
6.3. **0.01N Sulfuric Acid (H2SO4) VS:** Slowly add 10 mL of 0.1N sulfuric acid to a small amount of purified water. Dilute to 100 mL with purified water.
6.4. **1-0.01 EU/mL Endotoxin Cartridge:** Purchased Commercially.
6.5. **Anthrone Powder:** Purchased Commercially.
6.6. **Boric Acid:** Purchased commercially
6.7. **Boric Acid Solution (1 in 25):** Weigh 4 g of boric acid. Transfer to a 100-mL volumetric flask. Dissolve and dilute to volume with purified water.
6.8. **Cupric Sulfate, powder:** Purchased commercially.
6.9. **Dextran 4 Analytical Reference Standard**: Purchased commercially.
6.10. **Dextran 10 Analytical Reference Standard**: Purchased commercially.
6.11. **Dextran 40 Analytical Reference Standard**: Purchased commercially.
6.12. **Dextran 70 Analytical Reference Standard**: Purchased commercially.
6.13. **Dextran 250 Analytical Reference Standard**: Purchased commercially.
6.14. **Glacial Acetic Acid:** Purchased Commercially.
6.15. **Hydrogen Peroxide (H2O2):** Purchased commercially

6.16. **Methyl Orange:** Dissolve 0.10 g of methyl orange in 100 mL of Purified water. Filter if necessary.
6.17. **Methyl Red TS:** Dissolve 100 mg of methyl red in 100 mL of alcohol and filter if necessary.
6.18. **Methylene Blue:** Purchased Commercially.
6.19. **Nitric Acid, concentrated:** Purchased Commercially.
6.20. **NIST Tris, or equivalent:** Purchased Commercially.
6.21. **LAL Reagent Water:** Purchased Commercially.
6.22. **Phenol Reference Standard**: Purchased Commercially.
6.23. **Potassium Sulfate, powder:** Purchased Commercially.
6.24. **Purified Water:** In-House or Purchased Commercially.
6.25. **Sodium Hydroxide Solution (2 in 5):** Dissolve 200 g of sodium hydroxide in 500 mL of purified water.
6.26. **Sodium Sulfate, Anhydrous**: Purchased Commercially.
6.27. **Sulfuric Acid, concentrated:** Purchased Commercially.

7. ANALYTICAL PROCEDURES:

7.1. ACIDITY / ALKALINITY :

7.1.1. Refer to pH (10% solution), Section 7.14.
7.1.2. The pH must be NMT 7.0 and NLT 4.5 to pass test.

7.2. APPEARANCE AND COLOR :

7.2.1. Place 10 grams of 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 slightly off white in color and characteristic of a powder. If the sample does not conform to these specifications, notify the Laboratory Manager immediately.

7.3. CHLORIDE CONTENT :

7.3.1. Thoroughly rinse 50 mL Nessler Color Comparison Tubes using purified water prior to use.
7.3.2. Standard Preparation:
7.3.2.1. Pipette 0.705 mL of 1N Hydrochloric Acid into a 50 mL Nessler Color Comparison Tube and dilute to approximately 40 mL with Purified Water.
7.3.3. Sample Preparation:
7.3.3.1. Weigh 2.50 grams of sample and quantitatively transfer to a 50 mL Nessler Color Comparison Tube.
7.3.3.2. Dilute to approximately 40 mL with Purified Water and dissolve sample.
7.3.3.3. If necessary, acidify the solution with nitric acid to litmus.
7.3.4. Analysis:
7.3.4.1. Add to each solution, 1 mL of Concentrated Nitric Acid and 1 mL of 0.1N Silver Nitrate.
7.3.4.2. Dilute to 50 mL with Purified Water. Cover with parafilm and mix by inversion.
7.3.4.3. After 5 minutes, view the solutions against a dark background. If the turbidity of the sample preparation does not exceed that produced by the 10,000 ppm Chloride Standard, report the result as <10,000 ppm or <1%.

7.4. **COLD WATER SOLUBILITY (1% SOLUTION) :**

7.4.1. Cool purified water to 2-8°C, a minimum of 100 mL is required per test.

7.4.2. 1% Sample Solution Preparation:

7.4.2.1. Accurately weigh 1.0 grams of sample and transfer to a suitable beaker.

7.4.2.2. Add stir bar.

7.4.2.3. Add 100 mL of the cooled water to the beaker.

7.4.2.4. Stir to dissolve completely.

7.4.3. Solubility in the cooled water should be clear and complete to report as "Passes Test."

7.5. **COLOR OF SOLUTION (10% SOLUTION) :**

7.5.1. 10% Sample Solution Preparation:

7.5.1.1. Accurately weigh 5.0 grams of sample.

7.5.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 50 mL with purified water.

7.5.1.3. Swirl to dissolve completely.

7.5.2. Refer to Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1 cm pathlength at 375nm.

7.6. **ENDOTOXIN :**

7.6.1. Accurately weighed 140mg ± 5mg into a sterile tube and dissolve with 9mL of LAL Reagent Water.

7.6.2. Check the pH and, if necessary, adjust the pH to 6 – 8 using appropriate buffer.

7.6.3. Dilute to 10mL with LAL Reagent Water and mix thoroughly.

7.6.4. The final sample concentration is 13.5 mg/mL to 14.5 mg/mL.

7.6.5. Refer to the Endosafe NexGen-PTS Endotoxin Reader SOP for instrument operation and sample analysis.

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

7.7.1. 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.

7.8. **IDENTIFICATION (IR) :**

7.8.1. Follow the Spectrum Two UATR SOP for sample preparation and analysis.

7.9. **IDENTIFICATION TEST :**

7.9.1. **Dextran Identification**

7.9.2. 1% Sample Solution Preparation:

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

7.9.3. Anthrone Solution Preparation:

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

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

7.9.4. Analysis:

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

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

7.9.4.2.1. **Note:** The test tube should not make direct contact with the bottom of the beaker used as a water batch because it can overheat the Anthrone solution and turn it brown, which will result in not being able to determine the final color change. Suspend the test tube using a clamp and ring stand so that the sample is only exposed to the temperature of the boiling water bath.

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

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

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

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

7.10.1. **Primary Method**

7.10.1.1. 1% Sample Solution Preparation:

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

7.10.1.2. Analysis:

7.10.1.2.1. Perform analysis at 37°C, allow viscometer approximately 1-2 hours to reach 37°C and stabilize prior to analysis.

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

7.10.1.2.3. Calculate the Intrinsic Viscosity using the following equation:

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

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

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

7.10.2. **Alternative Method**

7.10.2.1. **Note:** This is a manual test and due to the split-second determination window, if the efflux time is missed, no checklist is required. Document in the notebook that the efflux time could not be determined. Proceed again with Step 6.1.6 in BSI-SOP-0486, "Charge (fill) the viscometer by pouring enough sample though the fill tube to fill the lower reservoir until the meniscus is between the minimum and the maximum fill lines marked on the reservoir."

7.10.2.2. **Note:** All samples and blanks are to be analyzed five (5) times and the average efflux time used in calculating the specific viscosity.

7.10.2.3. 1% Sample Solution Preparation:

7.10.2.3.1. 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.

7.10.2.4. Analysis: Refer to BSI-SOP-0486

7.10.2.4.1. Perform analysis at 37°C.

7.10.2.4.2. Calculate the Intrinsic Viscosity using the following equation:

$$Intrinsic\ Viscosity = \frac{\left(\left(\frac{Average\ Efflux\ Time\ of\ Sample\ (sec)}{Average\ Efflux\ Time\ of\ Blank\ (sec)}\right) - 1\right)}{Sample\ Weight\ (g)\ \times \left(\frac{100 - LOD\ (\%)}{100}\right)}$$

7.11. **LOSS ON DRYING :**

7.11.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.
7.11.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record the weight.
7.11.3. Transfer ~2 grams of the sample to the LOD vial and accurately weigh the vial and contents, record the weight.
7.11.4. Place the LOD vial containing the sample into the oven and dry at 105 ± 2°C for 5 hours.
7.11.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.11.6. Reweigh the LOD vial and sample.
7.11.7. Calculate the %LOD as follows:

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

7.12. **MICROBIAL CONTENT :**

7.12.1. Package and send no less than 65g of sample solution to Mary Paul Laboratories with a properly filled out Analysis Request Form.
7.12.2. Analysis required:
7.12.2.1. Total Aerobic Microbial Count (TAMC)
7.12.2.2. Total Yeast and Mold Count (TYMC)
7.12.2.3. Escherichia Coli (E.Coli)

7.13. **NITROGEN CONTENT :**

7.13.1. Select appropriately-sized glassware, from which the nitrogen is first liberated by acid digestion and then transferred quantitatively to the titration vessel by steam distillation.
7.13.2. Preparation Procedure:
7.13.2.1. Accurately weigh and transfer 2.5 grams of sample to the digestion flask of the apparatus.
7.13.2.2. To the flask, add 1 gram of a powdered mixture of potassium sulfate and cupric sulfate (10:1), and wash down any adhering material from the neck of the flask with Purified Water.
7.13.2.3. Add 30mL of concentrated Sulfuric Acid, allowing it to rinse down the wall of the flask.
7.13.2.4. While swirling the flask, carefully add 1mL of 30% Hydrogen Peroxide down the inside of the flask.
7.13.2.4.1. **NOTE:** Do NOT add hydrogen peroxide while the flask is being heated, or reaction may be violent.
7.13.3. Heating:
7.13.3.1. Place the flask in the bowl of the electric heater and loosely place thermal beads around the edges, as proper insulation will aid in speeding up the digestion process. **NOTE:** Avoid allowing the beads beneath the flask. A Bunsen Burner may also be used in lieu of an electric heater, without the thermal beads.

7.13.3.2. At the beginning of the digestion, monitor and adjust heat as needed. The solution should be bubbling but should remain below the neck of the flask. Heat the flask until the solution has a clear blue color and the sides of the flask are free from carbonaceous material.

7.13.3.3. Once the color and absence of carbonaceous material has been achieved, carefully add 70mL of Purified Water to the digestion mixture, cool the solution, and arrange for steam distillation.

7.13.4. Steam Distillation:

7.13.4.1. Using a funnel, add 45mL of Sodium Hydroxide Solution (2 in 5) in such manner as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution.

7.13.4.2. Rinse the funnel with 10mL of Purified Water, tightly close the apparatus, and begin the distillation with steam immediately.

7.13.4.3. Receive the distillate in 15mL of Boric Acid Solution (1 in 25), to which has been added 3 drops of Methyl Red-Methylene Blue TS and sufficient water to cover the end of the condensing tube.

7.13.4.4. Continue the distillation until the distillate measures 80 to 100mL.

7.13.4.5. Remove the absorption flask and rinse the end of the condensing tube with a small quantity of water.

7.13.5. Titrate the distillate with 0.01N Sulfuric Acid VS.

7.13.5.1. Perform a blank determination, and make any necessary correction. Each mL of 0.01N Sulfuric Acid VS is equivalent to 140.1 µg of nitrogen.

7.13.6. Sulfuric Acid 0.01N reagent value: Run once to verify 0.01N sulfuric acid.

7.13.7. Accurately weigh about 0.036 g of Tromethamine, previously dried at 105°C for 3 hours. Dissolve sample in 50 mL of purified water. Add 3 drops of methyl orange. Titrate with 0.01N H_2SO_4 to a colorimetric endpoint.

7.13.7.1. Each 1.2114 mg of NIST Tromethamine is equivalent to 1 mL of 0.01N H_2SO_4.

N H_2SO_4 = (g Tromethamine) / (0.12114 x mL x 0.01 N H_2SO_4)

$$\% \, Nitrogen = \frac{(EP_1 - EP_{Blank}) \times 1.41 \times N \; of \; H_2SO_4}{Sample \; Weight \; (g)}$$

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

7.14.1. Accurately weigh 5.0 grams of sample and transfer to a suitable beaker.

7.14.2. Add 50 mL of Purified Water and dissolve the sample.

7.14.3. Follow the appropriate SOP for pH calibration and measurement @ 25°C ±2°C.

7.15. **PHENOL CONTENT:**

7.15.1. Refer to DCN: BSI-ATM-0145, Analytical Method for Phenol Content in Dextran via GC-FID.

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

7.16.1. Turn on the Muffle Furnace and allow it to stabilize at 600°C. Follow muffle furnace calibration procedure for operation of furnace.

7.16.2. Inspect a quartz crucible for cracks, chips, and discoloration.

7.16.3. Utilize forceps to insert and remove the crucible from the furnace.

7.16.4. 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.

7.16.5. 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).
7.16.6. 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.
7.16.7. Ignite in the muffle furnace at 600 ± 50°C for 15 minutes, or until all the carbon has been removed.
7.16.8. Cool in a desiccator for 1.5 hours and weigh on an analytical balance.
7.16.9. Calculate the %ROI as follows:

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

7.16.10. 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.

7.17. **SPECIFIC ROTATION $[\alpha]_D^{15}$ (2% SOLUTION) @ 15°C :**

7.17.1. **Note:** Loss on Drying and Phenol Content result required prior to analysis.
7.17.2. 2% Sample Solution Preparation:
7.17.2.1. Weigh and transfer 2 grams of sample to a 100 mL volumetric flask, dissolve, dilute to volume with purified water, and mix thoroughly.
7.17.3. Analysis:
7.17.3.1. Analysis: Perform at 15°C.
7.17.3.2. **Input the drying loss to be "0.0000%"**
7.17.3.3. Correct the measured sample weight for purity, with the Loss on Drying (%) and Phenol Content (%), using the equation below:

$$Corrected\ Weight\ (g) = Measured\ Weight\ (g) \times \left(\frac{100 - LOD\% - Phenol\ Content\ \%}{100}\right)$$

7.17.3.3.1. Input the concentration corrected for purity.
7.17.3.4. Optical Zero Reference: Purified Water
7.17.3.5. Refer to BSI-SOP-0490, MCP 5300 Polarimeter SOP for instrument operation and sample analysis.

7.18. **WEIGHT AVERAGE MOLECULAR MASS ($\bar{M}_W$) :**

7.18.1. Refer to DCN: BSI-ATM-0146, Dextran Low Molecular Weight Distribution via Gel Permeation Chromatography (GPC) with RI Detection.