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DEXTRAN SULFATE 8000MW (DS8)
TESTING METHODS

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1. PURPOSE:

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

2. SCOPE:

2.1. Applies to the testing of Dextran Sulfate 8000MW (DS8) in the Laboratory at all BioSpectra Facilities. Methods include testing for all types of Dextran Sulfate 8000MW (DS8) sold by BioSpectra; 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 includesnotifying the Laboratory Manager or qualified designee if any analyses fail to meet their respective specifications.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Anton Paar MCP 5300 Polarimeter
4.3. Calibrated Oven
4.4. Calibrated Pipette
4.5. Calibrated Timer
4.6. Endosafe NexGen PTS Endotoxin Reader
4.7. Lambda 25 UV/Vis Spectrophotometer
4.8. Muffle Furnace
4.9. MCP 300 Polarimeter
4.10. OPI-180 OD Handheld Colorimeter
4.11. Perkin Elmer Avio 500 ICP-OES
4.12. Perkin Elmer NexION 350X ICP-MS
4.13. Ubbelohde Viscometer
4.14. XL200 pH/mV/Conductivity Meter, or equivalent

5. REAGENTS:

5.1. **0.02N Hydrochloric Acid:** Slowly add 20 mL of 0.1N hydrochloric acid to 80 mL of purified water to make a total volume of 100 mL. Can also be purchased commercially.
5.2. **0.1M Barium Chloride:** Dissolve 2.4 g of barium chloride dihydrate in purified water and dilute with purified water to make 100 mL.
5.3. **0.1N Hydrochloric Acid:** Purchased Commercially.
5.4. **0.1N Silver Nitrate:** Purchased Commercially.
5.5. **1% Acrinol:** Dissolve 1.0 g of Acrinol Monohydrate in purified water and dilute with purified water to 100 mL.
5.6. **1 – 0.01 EU/mL LAL Test Cartridges:** Purchased Commercially.
5.7. **1.0M Sodium Chloride:** Transfer 58.44 g of Sodium Chloride to a 1000 mL volumetric flask, dissolve, and dilute to volume with purified water.
5.8. **2N Sodium Hydroxide:** Dissolve 8 g of Sodium Hydroxide in purified water to make 100 mL. Preserve in polyethylene bottles.
5.9. **Acrinol Monohydrate:** Purchased Commercially.
5.10. **Anhydrous Sodium Sulfate:** Purchased Commercially.
5.11. **Anthrone Solution:** Prepare immediately before use. Weigh 90 – 100 mg of anthrone powder into a beaker, add 50 mL of concentrated sulfuric acid, dissolve, and mix thoroughly.

5.12. **Anthrone Powder:** Purchased Commercially.
5.13. **Barium Chloride Dihydrate:** Purchased Commercially.
5.14. **Barium Chloride TS (~0.5M):** Dissolve 30 g of barium chloride dihydrate in water to make 250 mL.
5.15. **Dextrose (D-Glucose) Certified Reference Standard (CRS):** Purchased Commercially.
5.16. **Glacial Acetic Acid:** Purchased Commercially.
5.17. **Hydrochloric Acid, concentrated:** Purchased Commercially.
5.18. **Hydrochloric Acid, Dilute (~10%):** Dilute 23.6 mL of concentrated hydrochloric acid with water to make 100 mL.
5.19. **LAL Reagent Water or equivalent:** Purchased Commercially.
5.20. **Nitric Acid, concentrated:** Purchased Commercially.
5.21. **Purified Water:** In-House or Purchased Commercially.
5.22. **Sodium Chloride:** Purchased Commercially.
5.23. **Sulfate Standard Solution (0.2% SO_4^{2-} Solution):** Dissolve 0.296 g of anhydrous sodium sulfate in purified water and dilute with purified water to 100 mL.
5.24. **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-ATM-0094, Analytical Method: Quantification of Sulfur by ICP-OES in Dextran Sulfate
6.3. BSI-ATM-0100, Analytical Method for the Determination of Manganese by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
6.4. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products
6.5. BSI-RPT-1296, Analytical Method Validation Report: Dextran Sulfate Glucose Content via UV/Vis Spectroscopy
6.6. BSI-RPT-1339, Analytical Method Verification Report: Limit of Pyridine in Dextran Sulfate
6.7. BSI-SOP-0019, Result Reporting
6.8. BSI-SOP-0069, Preparation of Samples for Outside Testing
6.9. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
6.10. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.11. BSI-SOP-0098, Balance SOP
6.12. BSI-SOP-0126, Laboratory Notebooks
6.13. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
6.14. BSI-SOP-0134, Pipette SOP
6.15. BSI-SOP-0135, Laboratory Chemicals
6.16. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
6.17. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
6.18. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.19. BSI-SOP-0257, MCP 300 Polarimeter SOP
6.20. BSI-SOP-0303, NexION 350X ICP-MS SOP
6.21. BSI-SOP-0345, Endosafe NexGen PTS Endotoxin Reader SOP
6.22. BSI-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES
6.23. BSI-SOP-0486, Viscometer SOP
6.24. BSI-SOP-0490, MCP 5300 Polarimeter SOP
6.25. BSI-SOP-0574, Anton Paar Lovis 2000M Microviscometer SOP
6.26. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
6.27. ACS, Reagent Chemicals, current edition

7. ANALYTICAL PROCEDURES:

NOTE: These methods are written for Finished Good analyses. If there is In-process from different stages, sample size and method may be adapted for that specific sample set.

7.1. APPEARANCE:

7.1.1. Place 10 g 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 light yellow 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. CLARITY (20% SOLUTION AT 360nm):

7.2.1. Prepare a 20% solution of the specified sample.
  7.2.1.1. Accurately weigh 5.0 g of sample.
  7.2.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water.
  7.2.1.3. Swirl to dissolve completely.
7.2.2. Refer to Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1cm pathlength at 360 nm.

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 0.02N 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 0.50 g 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, neutralize the solution with nitric acid to litmus.
7.3.4. Procedure:
  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 1000 ppm Chloride Standard, report the result as < 1000 ppm.

7.4. ELEMENTAL IMPURITIES:

7.4.1. Refer to 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 (DCN: BSI-ATM-0093), for sample preparation and analysis.
7.4.2. Refer to Analytical Method for the Determination of Manganese by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate (DCN: BSI-ATM-0100), for sample preparation and analysis.

7.5. **ENDOTOXIN:**

7.5.1. Accurately weigh 100 mg of sample into a sterile tube, dilute with LAL water to make 10 mL, dissolve, and mix.

7.5.2. Transfer 2.0 mL of the resulting solution to a separate sterile tube and dilute to 10 mL with LAL reagent water for a final concentration of 0.002 g/mL (2 mg/mL).

7.5.3. Refer to Endosafe NexGen-PTS Endotoxin Reader SOP for further instrument instructions and sample analysis.

7.6. **FREE SULFATE:**

7.6.1. Standard Preparation:

7.6.1.1. Pipette 5 mL 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

7.6.2.1.1. Weigh out 1.0 g of sample, transfer to a 100 mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.

7.6.2.1.2. 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.

7.6.2.2. Pipette 5 mL 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 0.5 mL of hydrochloric acid, dilute (~10%) and 1 mL 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 (< 0.2%).

7.7. **GLUCOSE:**

7.7.1. Sample Preparation:

7.7.1.1. Sample Stock Solution (5 mg/mL Dextran Sulfate): Weigh out 1.0 g of Dextran Sulfate sample into a 200 mL volumetric flask. Fill ~3/4 full with purified water and swirl to dissolve. Fill to volume with purified water and mix by inversion.

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

7.7.2. Standard Preparation:

7.7.2.1. Glucose Standard Stock Solution (440 µg/mL Glucose): Weigh out 110 mg equivalent of Dextrose (D-Glucose) CRS into a 250 mL 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:

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

7.7.2.2. Calibration Standards: Per the "Glucose Calibration Standard Preparations" Table, pipette Glucose Standard Stock Solution into a 100 mL 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	13.2 µg/mL	3.0 mL	100 mL
2	33.0 µg/mL	7.5 mL	100 mL
3	52.8 µg/mL	12.0 mL	100 mL

7.7.3. Procedure:

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

7.7.3.2. Anthrone Solution Preparation:

7.7.3.2.1. Note: Prepare immediately before use.

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

7.7.3.3. Slowly and carefully add 1.0 mL of Anthrone Solution into each of the microcentrifuge tubes, mix, and immediately place the microcentrifuge tubes in a hot bath 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 on ice or 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.

7.7.4.3. Measure the absorbance of the standards and samples at 625 nm 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 Calibration 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 (Colorimetric):**

7.8.1. **Note:** The Identification Test consists of three separate tests, Acrinol, Sulphate, 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. Weigh out 5.0 g of sample, transfer to a 100 mL 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 add 1.0 mL of 1% Acrinol, 5.0 mL 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. Weigh out 1.0 g of sample, transfer to a 100 mL 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 - 100 mg of Anthrone Powder into a 100 mL beaker. Add 50 mL of concentrated sulfuric acid, dissolve, and mix thoroughly.

7.8.3.3. Procedure:

7.8.3.3.1. Into a test tube, pipette 1.0 mL of 1% Dextran Sulfate Solution and 5.0 mL of Anthrone Solution and mix well.

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

7.8.3.3.2.1. **Note:** The test tube should not make direct contact with the bottom of the beaker used as a water bath 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.

7.8.3.3.3. 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 10 mL of purified water into a beaker containing a stir bar.

7.8.4.2. Slowly and with caution add 10 mL 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. Weigh out 1.0 grams 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. **IRON:**

7.9.1. Refer to 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 (DCN: BSI-ATM-0093), for sample preparation and analysis.
7.9.2. Note: Result for ***insoluble iron*** is to be reported from this test method.

7.10. **LOSS ON DRYING:**

7.10.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.
7.10.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
7.10.3. Transfer 1.0 - 1.5 g of the sample to the LOD vial and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5 mm.
7.10.4. Place the LOD vial containing the sample into the oven and dry at 105 ± 2°C for 5 hours.
7.10.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.10.6. Reweigh the LOD vial and sample.
7.10.7. Calculate the %LOD as follows:

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

7.11. **MANGANESE:**

7.11.1. Refer to Analytical Method of Analysis: Determination of Manganese by ICP-MS in Dextran Sulfate, BSI-ATM-0100.

7.12. **MICROBIAL CONTENT:**

7.12.1. Microbial analysis will be performed by an outside testing laboratory.
7.12.1.1. Primary Provider: Mary Paul Laboratories
7.12.1.2. Package and send NLT 35 g of sample to Mary Paul Laboratories with a purchase order and analysis request form.
7.12.2. Analyses:
7.12.2.1. Total Aerobic Microbial Count (TAMC)
7.12.2.1.1. In accordance with USP<61>, The result for ***Total Bioburden*** will be reported from the TAMC result only.
7.12.2.1.2. If there is growth, Identification is required.
7.12.2.2. Total Yeasts and Molds Count (TYMC)
7.12.2.2.1. TYMC will be provided For Information Only and will not be officially reported.
7.12.2.2.2. If there is growth, Identification is required.

7.13. **pH (1% SOLUTION):**

7.13.1. Weigh out 1.0 g of sample, transfer to a 100 mL volumetric flask, dissolve in purified water, dilute to volume with purified water, and mix well.
7.13.2. Follow the appropriate SOP for pH calibration and measurement.

7.14. **PYRIDINE:**

7.14.1. Dry a suitable amount of sample according to the LOD procedure.
7.14.2. Accurately weigh 6 g of Dextran Sulfate sample (dried basis) and transfer to a suitable ~100 mL beaker.
7.14.3. Add 50 mL of purified water and stir to dissolve.
7.14.4. Add 15.0 mL of 0.1N HCl to the test solution.
7.14.5. Follow the appropriate SOP for pH calibration and measurement.

7.14.6. The pH of the resulting solution must be 2.8 or lower to pass test and report as < 2.0%.

7.15. **RESIDUE ON IGNITION:**

7.15.1. Turn on the muffle furnace and allow it to stabilize at 600 ± 50°C. Follow muffle furnace calibration procedure for operation of furnace.

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

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

7.15.4. Ignite quartz crucible at 600 ± 50°C for 30 minutes. Cool in a desiccator for 1.5 hours and weigh using an analytical balance.

7.15.5. Weigh 1.0 g of sample in the previously ignited quartz crucible. Moisten the sample with 1.0 mL of concentrated sulfuric acid.

7.15.6. 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 sample is not lost.

7.15.6.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.15.6.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.

7.15.7. Ignite in the muffle furnace at 600 ± 50°C for 15 minutes or until all carbon has been removed.

7.15.8. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on the analytical balance.

7.15.9. Calculate the %ROI as follows:

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

7.15.10. If the amount of residue exceeds the limit specified, repeat the moistening with sulfuric acid using 1.0 mL, heat to char, then ignite at 600 ± 50°C for 30 minutes until two consecutive weightings of the residue do not differ by more than 0.0005 g or until the specification is met.

7.16. **SPECIFIC ROTATION $[\alpha]_D^{20}$:**

7.16.1. Sample Preparation (5% Dextran Sulfate Solution):

7.16.1.1. Transfer 5.0 g of sample to a 100 mL volumetric flask, dissolve, and dilute to volume with purified water. Mix thoroughly.

7.16.2. Refer to the MCP 5300 Polarimeter SOP for instrument analysis.

7.16.2.1. Analysis: Perform at 20°C utilizing the BSI – Specific Rotation (USP/NF; 20°C) method.

7.16.2.2. The following information will be required to perform the analysis: Volume (dryness) mL, Mass (dryness) (g), and the Loss on Drying (%) result.

7.16.3. Refer to the MCP 300 Polarimeter SOP for instrument analysis.

7.16.3.1. Analysis: Perform at 20°C utilizing the Specific Rotation @ 20°C – BioSpectra method.

7.16.3.2. Calculate the result using the following calculation:

7.16.3.2.1. Specific Rotation = (Raw Result)*(100/(100-Drying Loss))

7.16.3.2.2. Drying Loss result is the Loss on Drying result.

7.17. **SPECIFIC VISCOSITY (IN 1.0M NaCl @ 25°C):**

7.17.1. **Primary Method of Analysis:**

7.17.1.1. 1.0M Sodium Chloride Preparation:

7.17.1.1.1. Transfer 58.44 g of Sodium Chloride to a 1000mL volumetric flask, dissolve, and dilute to volume with Purified Water.

7.17.1.2. Sample Preparation (1% Dextran Sulfate in 1.0M sodium Chloride):

7.17.1.2.1. Using an analytical balance, weigh 1.0 g of sample, transfer to a 100mL volumetric flask, dissolve, and dilute to volume with 1.0M Sodium Chloride.

7.17.1.3. Analysis:

7.17.1.3.1. Perform analysis at 25°C.

7.17.1.3.2. Refer to the Anton Paar Lovis 2000M Microviscometer SOP for viscometer setup and analysis.

7.17.1.3.3. Calculate the Specific Viscosity using the following equation:

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

7.17.1.3.4. Where

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

7.17.1.3.4.2. η_0 = Kinematic Viscosity of 1.0M Sodium Chloride

7.17.2. **Alternative Method of Analysis:**

7.17.2.1. Note: Solutions may be scaled as needed. 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 through the fill tube to fill the lower reservoir until the meniscus is between the minimum and the maximum fill lines marked on the reservoir."

7.17.2.2. 1.0M Sodium Chloride Preparation:

7.17.2.2.1. Transfer 58.44 g of Sodium Chloride to a 1000 mL volumetric flask, dissolve, and dilute to volume with purified water.

7.17.2.3. Sample Preparation (1% Dextran Sulfate in 1.0M Sodium Chloride):

7.17.2.3.1. Using an analytical balance, weigh 1.0 g of sample, transfer to a 100 mL volumetric flask, dissolve, and dilute to volume with 1.0M sodium chloride.

7.17.2.4. Analysis:

7.17.2.4.1. Perform analysis at 25°C.

7.17.2.4.2. Refer to the Viscometer SOP for viscometer setup, use, and efflux time measurements.

7.17.2.4.3. Note: All samples and the 1.0M Sodium Chloride are to be analyzed five (5) times and the average efflux time used in calculating the specific viscosity.

7.17.2.4.4. Calculate the Specific Viscosity using the following equation:

$$Specific\ Viscosity = \frac{\left(\left(\frac{Average\ Efflux\ Time\ of\ Sample\ (seconds)}{Average\ Efflux\ Time\ of\ 1.0M\ NaCl\ (seconds)}\right) - 1\right)}{Sample\ Weight\ (g)\ \times \left(\frac{100 - LOD\ (\%)}{100}\right)}$$

7.18. **SULFUR:**

7.18.1. 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.