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MES HYDRATE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with procedures for analyzing MES Hydrate (MES Monohydrate) Raw Materials, Finished Goods, and Stability.

2. SCOPE:

2.1. These procedures apply to the testing of MES Hydrate (MES Monohydrate) in the Laboratory.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager or designee is responsible for training, maintenance, and implementation of this procedure.
3.2. The Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the appropriate personnel if any analyses fail to meet their respective specifications.
3.3. Standard laboratory safety regulations apply. Read and understand the safety data sheet (SDS) before handling or working with any chemical.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Oven
4.3. Metrohm Auto-Titrator
4.4. Muffle Furnace
4.5. OPI-180 OD Handheld Colorimeter SOP
4.6. Perkin-Elmer NexION 350X
4.7. Perkin-Elmer Spectrum Two UATR
4.8. XL200 pH/Conductivity Meter, or equivalent
4.9. UV/Vis Spectrophotometer
4.10. Endosafe nexgen-PTS Endotoxin Reader
4.11. Turbidimeter

5. REAGENTS:

5.1. **0.1N Sodium Hydroxide (NaOH):** Purchased Commercially.
5.2. **1N Sodium Hydroxide (NaOH):** Purchased Commercially.
5.3. **Composite 5:** Purchased Commercially.
5.4. **Formamide:** Purchased Commercially.
5.5. **LAL Reagent Water:** Purchased Commercially.
5.6. **Methanol:** Purchased Commercially.
5.7. **Purified Water:** Generated in-house or purchased commercially.

6. REFERENCES:

6.1. BSI-ATM-0115, Analytical Method: Determination of Elemental Impurities in MES Hydrate
6.2. BSI-SOP-0019, Result Reporting
6.3. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
6.4. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
6.5. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.6. BSI-SOP-0095, DNase (Endonuclease) Assay
6.7. BSI-SOP-0096, RNase (Ribonuclease) Assay
6.8. BSI-SOP-0098, Balance SOP
6.9. BSI-SOP-0126, Laboratory Notebooks
6.10. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
6.11. BSI-SOP-0138, DNase (Exonuclease) Assay

6.12. BSI-SOP-0139, Protease Assay
6.13. BSI-SOP-0140, Standardization of Titrants
6.14. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
6.15. BSI-SOP-0254, Spectrum Two UATR SOP
6.16. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.17. BSI-SOP-0303, NexION 350X ICP-MS SOP
6.18. BSI-SOP-0345, Endosafe nexgen PTS Endotoxin Reader SOP
6.19. BSI-SOP-0595, DNase (NICKase) Assay
6.20. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP

7. PROCEDURES:

7.1. ABSORBANCE (0.1M):

7.1.1. Weigh 0.53 g of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
7.1.2. Swirl to dissolve completely.
7.1.3. Refer to the Lambda 25 UV/Vis Spectrophotometer to determine the Absorbance of the sample.
7.1.3.1. Measure the sample at the following wavelengths: 260 nm and 280 nm.

7.2. ABSORBANCE (20% W/W):

7.2.1. Weigh 5.0g of sample into a suitable beaker and add 20.0g of purified water.
7.2.2. Swirl to dissolve completely.
7.2.3. Refer to the Lambda 25 UV/Vis Spectrophotometer to determine the Absorbance of the sample.
7.2.3.1. Measure the sample at the following wavelength: 290nm.

7.3. APPEARANCE:

7.3.1. Weigh a suitable amount of the sample into a clean, dry glass beaker.
7.3.2. In an area with sufficient lighting, view the sample from all sides.
7.3.3. The sample should be white in color and characteristic of crystals.
7.3.4. 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.

7.4. ASSAY (anhydrous basis):

7.4.1. Standardize 0.1 N sodium hydroxide in accordance with Standardization of Titrants utilizing the Metrohm Auto Titrator.
7.4.2. Accurately weigh 0.8 g of sample (measured as-is) and transfer to a suitable beaker.
7.4.3. Add 50 mL of purified water and stir to dissolve.
7.4.4. Titrate to the potentiometric endpoint with 0.1N sodium hydroxide.
7.4.5. Submerge the probe in storage solution after analysis is completed to condition the glass electrode. To calculate assay on the anhydrous basis, use below equation:

$$\%\ MES, Hydrate\ (as-is, anhydrous\ basis) = \frac{(mL \times N\ of\ NaOH)(19.524)}{Sample\ Weight\ (g)}$$

$$\%\ Mes, Hydrate\ (anhydrous) = \frac{As-Is\ Assay\ \%}{(100-KF\ Value)} * 100$$

7.5. **CYTOTOXICITY (50% CONCENTRATION):**

7.5.1. Cytotoxicity at the 50% Dilution Concentration analysis will be performed by an outside testing laboratory.

7.5.1.1. Request the following: Modified MTT Cytotoxicity Test Protocol as a GMP Compliance Study from the Approved Contract Laboratory.

7.5.1.2. Package and send NLT 10 g of sample to Approved Contract Laboratory.

7.5.2. Analyses to be reported:

7.5.2.1. MTT Cytotoxicity Test at the 50% test article dilution.

7.5.2.2. Specification Required on Report to Pass: No Cytotoxic Potential

7.5.2.3. Specification on Report that Does Not Pass: Cytotoxic Potential

7.6. **ENDOTOXIN:**

7.6.1. Accurately weigh 20 mg of sample into a sterile tube.

7.6.2. Add 70 µL of 1N NaOH.

7.6.3. Dilute to 10 mL with LAL reagent water.

7.6.4. To 1 mL of this solution, add 4 mL of LAL reagent water. Mix thoroughly for a final concentration of 0.0004 g/mL.

7.6.5. Follow the Endosafe nexgen-PTS Endotoxin Reader SOP to analyze sample.

7.7. **ENZYME ACTIVITY:**

7.7.1. RNase, DNase, and Protease per procedures referenced in section 4.

7.7.2. NICKase per procedure referenced in section 4.

7.8. **IDENTIFICATION (IR) (As-is):**

7.8.1. Follow Spectrum Two UATR SOP.

7.8.2. Analyze sample as-is.

7.9. **MICROBIAL:**

7.9.1. Microbial analysis will be performed by an outside testing laboratory

7.9.1.1. Package and send NLT 35 g of sample to Approved Contract Laboratory

7.9.2. Analyses:

7.9.2.1. Total Aerobic Microbial Count (TAMC)

7.9.2.2. Total Yeast Microbial Count (TYMC)

7.10. **pH of a 1% SOLUTION:**

7.10.1. Weigh 1.0 g of sample. Transfer to a suitable beaker.

7.10.2. Add 100 mL of purified water and stir to mix.

7.10.3. Follow the appropriate SOP for calibration and pH measurement.

7.11. **SOLUBILITY (0.1M):**

7.11.1. Weigh 0.53 g of sample and quantitatively transfer the aliquot to a 25-mL volumetric flask and dissolve in ~15-20 mL of purified water.

7.11.2. Q.S. to 25 mL with purified water. Scale as required.

7.11.3. View sample from all sides under sufficient light noting any apparent color or undissolved particulate. Solution should be clear (complete) and colorless to pass test.

7.12. **SOLUBILITY 20% w/v:**

7.12.1. Sample Preparation:

7.12.1.1. Weigh 20.0g of sample and transfer to a suitable beaker.

7.12.1.2. Add 80mL of purified water and dissolve.

7.12.2. Color:

7.12.2.1. In an area with sufficient lighting, compare the color of the *Sample Preparation* to Purified Water.

7.12.2.2. The color of the *Sample Preparation* may not be more intense than the color of purified water to report as colorless.

7.12.3. Turbidity:
- 7.12.3.1. Analyze the *Sample Preparation* for turbidity using a calibrated turbidimeter.
- 7.12.3.2. The turbidity result may not exceed 3NTU to report as Clear.

7.13. **TRACE ELEMENTS:**

7.13.1. Refer to Analytical Method: Determination of Elemental Impurities in MES Hydrate, DCN: BSI-ATM-0115 and NexION 350X ICP-MS SOP, DCN: BSI-SOP-0303.

7.14. **WATER BY KARL FISCHER:**

7.14.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.14.2. Immediately weigh 0.1 g of as-is sample into the glass weighing spoon and tare it.

7.14.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
- 7.14.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

7.14.4. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, transfer the sample weight to the auto-titrator software.

7.14.5. Check to make sure there is no residual sample stuck to the sides of the titration vessel.

7.14.6. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

7.14.7. The moisture content will be determined by the Metrohm Auto Titrando 907, using the following equation:

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)(\frac{mg}{mL} of\ Composite\ 5)(0.1)}{Sample\ Weight\ (g)}$$