BIOSPECTRA

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SODIUM HYDROXIDE 10N TESTING METHODS

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1. PURPOSE:

1.1. To provide the laboratory personnel with a procedure for analyzing Sodium Hydroxide 10N In-Process, Stability, and Finished Good samples.

2. SCOPE:

2.1. Applies to the analysis of Sodium Hydroxide 10 N In-Process, Stability, and Finished Goods in the Laboratory. Methods include testing for all grades of Sodium Hydroxide 10 N sold by BioSpectra; only the specific tests required for the requested grade must be tested.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager is responsible for training, maintenance and implementation of this procedure.

3.2. The laboratory personnel 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. SAFETY:

4.1. Causes SEVERE skin burns and eye damage. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

5. REFERENCES:

5.1. BSI-ATM-0074, Analytical Method of Analysis: Sodium Hydroxide via ICP-MS
5.2. BSI-ATM-0132, Analytical Method for Determination of Trace Metals in Sodium Hydroxide
5.3. BSI-FRM-0717, Sodium Hydroxide 10N Analytical Procedure
5.4. BSI-RPT-2117, Analytical Method Validation Report: Protease Assay for 10N Sodium Hydroxide
5.5. BSI-RPT-2118, Analytical Method Validation Report: DNase (Exonuclease) Assay for 10N Sodium Hydroxide
5.6. BSI-RPT-2119, Analytical Method Validation Report: RNase (Ribonuclease) Assay for 10N Sodium Hydroxide
5.7. BSI-RPT-2120, Analytical Method Validation Report: DNase (Endonuclease) Assay for 10N Sodium Hydroxide
5.8. BSI-SOP-0019, Result Reporting
5.9. BSI-SOP-0095, DNase (Endonuclease) Assay
5.10. BSI-SOP-0096, RNase (Ribonuclease) Assay
5.11. BSI-SOP-0098, Balance SOP
5.12. BSI-SOP-0126, Laboratory Notebooks
5.13. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
5.14. BSI-SOP-0135, Laboratory Chemicals
5.15. BSI-SOP-0138, DNase (Exonuclease) Assay
5.16. BSI-SOP-0139, Protease Assay
5.17. BSI-SOP-0140, Standardization of Titrants
5.18. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration SOP
5.19. BSI-SOP-0255, XL200 pH mV Conductivity Meter SOP
5.20. BSI-SOP-0303, NexION 350X ICP-MS SOP
5.21. BSI-SOP-0345, Laboratory Nexgen-PTS Endotoxin Reader SOP
5.22. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
5.23. *ACS Reagent Chemicals*, current edition
5.24. *USP-NF* current edition

6. EQUIPMENT:

6.1. Analytical Balance
6.2. Hach Portable Turbidimeter Model 2100 Q, or equivalent
6.3. Endosafe PTS Endotoxin Reader, or equivalent
6.4. NexION 350X ICP-MS
6.5. XL200 pH mV Conductivity Meter

7. REAGENTS:

7.1. **0.02N HCl -** Dilute 20mL of Hydrochloric Acid 0.1N to 100mL with purified water. Can be purchased commercially.
7.2. **0.1N Silver Nitrate** - Purchased commercially.
7.3. **10% Ammonium Hydroxide:** Dilute 35mL of 29% Ammonium Hydroxide to 100mL with purified water.
7.4. **10% Sulfuric Acid Reagent Solution:** Slowly add 30mL of 96% sulfuric acid to 375mL of purified water. Cool and dilute with water to 500mL.
7.5. **1N Sulfuric Acid** - Purchased commercially.
7.6. **6N Sulfuric Acid**- add slowly (use caution) 169mL of 96% sulfuric acid in small increments allowing to cool and dilute to 1L, mix thoroughly.
7.7. **Ammonium Peroxydisulfate:** Purchased commercially.
7.8. **Ammonium Thiocyanate, 30%:** Dissolve 150g of ammonium thiocyanate in purified water, and dilute with water to 500mL.
7.9. **Concentration Hydrochloric Acid:** Purchased commercially.
7.10. **Dilute nitric acid (1:99):** Dilute 1mL of Concentrated Nitric acid to 100mL with purified water.
7.11. **Endosafe PTS Cartridge 1-0.01 EU/mL** - Purchased commercially.
7.12. **Glycerin Base:** To 200g of glycerin, add water to a total weight of 235g. Add 140mL of 1N NaOH, 50mL of purified water and mix.
7.13. **Hydrochloric Acid 0.1N** - Purchased commercially.
7.14. **Iron Standard (0.01 mg of Fe in 1 mL):** Dissolve 0.702g of ferrous ammonium sulfate hexahydrate in 10 mL of 10% sulfuric acid reagent solution, and dilute with water to 100mL. Immediately before use to 10mL of this solution, add 10mL of 10% sulfuric acid reagent solution, and dilute with water to 1L.
7.15. **LAL Reagent Water** - Purchased commercially.
7.16. **Lead Stock Solution (0.1 mg of Pb in 1 mL):** Dissolve 0.160g of lead nitrate in 100mL of dilute nitric acid (1:99), and dilute with purified water to L.
7.17. **Potassium Hydrogen Phthalate (KHP) -** Prepare an appropriate sample container at 120°C for 30 minutes. Allow to cool in desiccator. Crush and dry a suitable amount of potassium hydrogen phthalate. Dry at 120°C for 2 hours. Cool and store in desiccator in a closed container. Stable for 3 months.
7.18. **Thioacetamide:** Dissolve 4g of thioacetamide in purified water to make 100mL.
7.19. **Tris-** Prepare an appropriate sample container at 105°C for 30 minutes. Allow to cool in desiccator and weigh the appropriate amount Tris. Dry at 105°C for 3 hours. Cool and store in desiccator in a closed container. Stable for 3 months.

8. ANALYTICAL PROCEDURES:

8.1. IN-PROCESS TESTING:

8.1.1. ASSAY

8.1.1.1. Perform a manual standardization or titrant check of 1N Sulfuric Acid per Standardization of Titrants.
8.1.1.2. Accurately weigh 3.5 – 7.5g of sample and add 40mL of purified water in a clean flask. Stopper the flask and allow to cool to room temperature.
8.1.1.3. Add 150 µL of Phenolphthalein as the indicator and titrate using previously standardized 1N Sulfuric Acid to a colorless endpoint (V1).
8.1.1.4. Add 150 µL of Methyl Orange as the indicator.
8.1.1.5. Titrate using previously standardized 1N Sulfuric Acid to a pink endpoint (V2).
8.1.1.6. Calculate the percentage of Sodium Hydroxide using the following equation:

$$\%NaOH = \frac{(V_2) \times N\ H_2SO_4\ \times 4.00}{Sample\ Weight\ (g)}$$

8.1.2. DENSITY @ 20-25°C

8.1.2.1. QC or Manufacturing to perform a density check of the material.
8.1.2.2. Perform a water check on the DMA 35 Density Meter before the sample analysis. Refer to BSI-SOP-0350 for instrument operation and water check analysis.
8.1.2.3. Record the Density of the sample from the DMA 35 Density Meter. Refer to BSI-SOP-0350 for instrument operation and sample analysis.
8.1.2.4. Ensure that the sample is at 20-25°C for analysis.
8.1.2.5. Clean immediately after use following DMA 35 Density Meter SOP

8.1.3. CHLORIDE

8.1.3.1. ***Note:*** *Record < 5ppm or >5 ppm in the batch record.*
For QC Release to Dilute to Normality, the confirmation 1 and 2 samples must be run against a freshly prepared 5 ppm standard only.

8.1.3.2. **Sample preparation:**
8.1.3.2.1. Thoroughly rinse Nessler tubes and glassware using purified water prior to use.
8.1.3.2.2. Weigh 10.0g of sample into a clean beaker or Nessler tube.
8.1.3.2.3. Dilute to ~20mL with purified water.
8.1.3.2.4. Slowly, using extreme caution, acidify the sample with ~5mL of Nitric Acid, testing with litmus paper.
8.1.3.2.5. Dilute to ~40mL with purified water.
8.1.3.2.6. Mix thoroughly and transfer to a Nessler tube.
8.1.3.3. **5 ppm Standard Preparation**: Standard preparation for internal reporting only.
8.1.3.3.1. 5 ppm Limit: Dilute 70.5µL of 0.02N HCl to ~40mL with purified water in a Nessler tube.
8.1.3.4. **Analysis Procedure:**
8.1.3.4.1. To both the standard and sample solutions, add 1 mL of concentrated Nitric Acid and 1mL of 0.1N Silver Nitrate TS.
8.1.3.4.2. Dilute both to 50mL with purified water.
8.1.3.4.3. Mix and allow to sit for 5 minutes, using a calibrated timer.

8.1.3.4.4. Acceptance Criteria: After 5 minutes, the turbidity in the sample solution does not exceedthe turbidity produced by the standard when viewed against a dark background. If the sample cannot be determined visually, analyze turbidity utilizing the turbidimeter and record the sample NTU results. The sample NTU must be > 2 NTU from the standard NTU in order to be considered acceptable. If the sample NTU value falls within 2 NTU of the standard, run the sample in triplicate. **Notify Laboratory Management prior to proceeding.**

8.1.3.4.5. For Cold Water Regen samples, the turbidity in the sample solution cannot exceed that of the standard in order to report as < 5 ppm. If the turbidity of the sample exceeds that of the standard, report as > 5 ppm and **notify QA/Laboratory Management and Process Technology.**

8.1.4. **NORMALITY**

8.1.4.1. Refer to Section 8.2.9 for sample preparation and testing.

8.2. **FINISHED GOOD TESTING:**

8.2.1. **ABSORBANCE (NEAT) :**

8.2.1.1. Analyze the sample neat, utilizing a quartz, or otherwise UV compatible cuvette.

8.2.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample at the required wavelength.

8.2.2. **APPEARANCE AND COLOR**

8.2.2.1. Transfer 50mL of sample into a Nessler tube.

8.2.2.2. In order to pass, test solution is complete, clear, and colorless. Verify the solution appearance against a clear and colorless reference solution, such as purified water, and view against a color comparison plate with suitable lighting.

8.2.3. **CHLORIDE**

8.2.3.1. Thoroughly rinse Nessler tubes and glassware using purified water, prior to use.

8.2.3.2. **Sample Preparation:**

8.2.3.2.1. Weigh 10.0g of sample into a clean beaker or Nessler tube.

8.2.3.2.2. Dilute to ~20mL with purified water.

8.2.3.2.3. Slowly, using extreme caution, acidify the sample with ~5mL of Nitric Acid, testing with litmus paper.

8.2.3.2.4. Dilute to ~40mL with purified water.

8.2.3.2.5. Mix thoroughly and transfer to a Nessler tube.

8.2.3.3. **5 ppm Standard Preparation:**

8.2.3.3.1. Dilute 70.5µL of 0.02N HCl to ~40mL with purified water in a Nessler tube.

8.2.3.4. **Analysis Procedure:**

8.2.3.4.1. To both the sample and standard solutions, add 1mL of concentrated Nitric Acid and 1mL of 0.1 N Silver Nitrate TS.

8.2.3.4.2. Dilute both the sample and standard solutions to 50mL with purified water.

8.2.3.4.3. Mix and allow solutions to sit for 5 minutes, using a calibrated timer.

8.2.3.4.4. Acceptance Criteria: After 5 minutes, the turbidity in the sample solution does not exceed the turbidity produced by the standard when viewed against a dark background. If the sample cannot be determined visually, analyze turbidity using a turbidimeter, and record the NTU results. The sample must be less than the standard NTU in order to be considered acceptable.

8.2.4. **ENDOTOXINS**

8.2.4.1. Pipet 0.200mL of sample into a sterile vial and add 1.600mL of LAL reagent water.
8.2.4.2. Add 0.160mL of concentrated Hydrochloric acid to acidify.
8.2.4.3. Check the pH of the solution with pH paper: solution must be acidic.
8.2.4.3.1. If basic add HCl in increments until acidic.
8.2.4.3.1.1. Add approximately 0.02mL of HCl.
8.2.4.4. Once acidic add sufficient buffer of a pH range ~9-10 until the solution is between pH 6-8.
8.2.4.4.1. Add approximately 0.3mL of buffer.
8.2.4.5. Dilute with LAL reagent water to a final volume of 10mL.
8.2.4.6. Follow the Endosafe Nexgen PTS Endotoxin Reader SOP for sample analysis.
8.2.4.6.1. The dilution factor is 50.

8.2.5. **ENZYMES**

8.2.5.1. Sample Solution: 15µL of 10N NaOH Sample (0.02g) and 0.07g of enzyme free HEPES free acid, dissolved and diluted with 985µL of test specific enzyme buffer to a total volume of 1mL.
8.2.5.2. Analysis:
8.2.5.2.1. BSI-SOP-0095, DNase (Endonuclease) Assay
8.2.5.2.2. BSI-SOP-0096, RNase (Ribonuclease) Assay
8.2.5.2.3. BSI-SOP-0138, DNase (Exonuclease) Assay
8.2.5.2.4. BSI-SOP-0139, Protease Assay

8.2.6. **HEAVY METALS (Pb)**

8.2.6.1. Refer to Analytical Method of Analysis: Sodium Hydroxide via ICP-MS, BSI-ATM-0074.

Alternate Method:

8.2.6.2. Standard and Solution Preparation:
8.2.6.2.1. Lead Standard Solution (0.01mg of Pb in 1mL): Dilute 10mL of lead stock solution to 100mL with purified water. This must be prepared at the time of use.
8.2.6.2.2. Thioacetamide-glycerin base: Thoroughly mix 1mL of thioacetamide with 5mL of Glycerin base. Heat in a boiling bath for 20 seconds. Prepare immediately before use.
8.2.6.3. Procedure:
8.2.6.3.1. Note: Prepare in hood, and use caution for standard and sample preparation to avoid spattering of sample.
8.2.6.3.2. Sample Preparation: Weigh 30g of sample into a suitable beaker and carefully add 18 mL of concentrated nitric acid.
8.2.6.3.3. Standard Preparation: Weigh 10g of sample and add 5mL of concentrated nitric acid. Add 2mL of 0.01mg Lead Standard Solution (0.01mg/ml Pb).

8.2.6.3.4. Place both the standard and sample on a hot plate and evaporate to dryness. Cool and dissolve each residue with 20mL of purified water. Adjust the pH to between 3 and 4 utilizing a pH meter, with 1N acetic acid, or 10% ammonium hydroxide.

8.2.6.3.5. Transfer the solutions to separate Nessler Color Comparison Tubes. Add 1.2mL of freshly prepared thioacetamide-glycerin base to each of the solutions and mix. QS each tube to 50mL and mix.

8.2.6.3.6. Any brown color produced in the sample solution must not exceed that in the standard solution.

8.2.7. **IDENTIFICATION (SODIUM)**

8.2.7.1. Pipette 1mL of sample into a test tube containing 25mL of purified water.

8.2.7.2. Add 2mL of 15% Potassium Carbonate and heat to boiling

8.2.7.3. Allow to cool in ice bath and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation

8.2.7.4. No precipitate should be formed at this stage of analysis.

8.2.7.5. Add 4mL Potassium Pyroantimonate TS and heat to boiling.

8.2.7.6. Allow to cool in ice bath and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation.

8.2.7.7. A dense precipitate must form in order to pass test.

8.2.8. **IRON**

8.2.8.1. Refer to Analytical Method of Analysis: Sodium Hydroxide via ICP-MS, BSI-ATM-0074.

Alternate Method:

8.2.8.2. Procedure:

8.2.8.2.1. Thoroughly rinse glassware with purified water prior to use.

8.2.8.2.2. Sample Preparation: To 10g of sample, add 0.1mL of phenolphthalein indicator solution, neutralize with hydrochloric acid (solution will turn from pink to clear) and dilute with water to 40mL in a graduated cylinder. Transfer to a Nessler Color Comparison Tube.

8.2.8.2.3. 2ppm Iron Standard Preparation: Pipette 2.0mL of Iron standard (0.01 mg/mL Fe) into a graduated cylinder and dilute to 40mL with purified water. Transfer to a Nessler Color Comparison Tube.

8.2.8.2.4. 0.500ppm Iron Standard Preparation: Pipette 0.5mL of Iron standard (0.01 mg/mL Fe) into a graduated cylinder and dilute to 40mL with purified water. Transfer to a Nessler Color Comparison Tube.

8.2.8.2.5. To the sample and standard solution, add 30-50mg of ammonium peroxydisulfate crystals, 3mL of Hydrochloric acid, and 3mL of 30% Ammonium Thiocyanate reagent solution, and mix.

8.2.8.2.6. Any red color in the sample must not exceed the standard solution.

8.2.9. **NORMALITY**

8.2.9.1. KHP (Potassium Hydrogen Phthalate) preparation:

8.2.9.1.1. Crush and dry a suitable amount of KHP at 120°C for 2 hours. Allow to cool to ambient temperature in a desiccator.

8.2.9.1.2. Fill a 25mL volumetric flask with sample. Quantitatively transfer the aliquot to a 250mL volumetric flask with purified water. Rinse the 25mL flask by filling the flask halfway with purified water, shaking it, then transferring the rinse to the 250mL volumetric flask. Perform the rinse procedure in duplicate. Fill the 250mL volumetric flask to volume with purified water. Mix well and cool to 25° ± 2°C. QS the sample solution to 250 mL after cooling is complete.

8.2.9.1.3. Prime the 50mL burette by filling it with the diluted sample solution. Empty the burette and repeat.

8.2.9.1.4. Fill the burette to the required volume with the prepared sample solution.

8.2.9.2. Sample preparation:

8.2.9.2.1. Weigh 8.0000 – 8.2000g of the previously dried KHP into a 250mL beaker.

8.2.9.2.2. Add 100mL of purified water down the sides of the beaker to avoid the loss of KHP.

8.2.9.3. Analysis Procedure:

8.2.9.3.1. To the KHP solution, add 150 µL of phenolphthalein indicator.

8.2.9.3.2. Titrate the KHP using the sample solution in the burette, to a pink endpoint.

8.2.9.3.3. Calculate the normality using the following equation:

$$N = \frac{(KH\ \ Pweight\ g)(KHP\ Purity)(10)}{(0.20423)(mL\ of\ NaOH\ sample\ solution)}$$

8.2.10. **TRACE METALS**

8.2.10.1.1. Refer to Analytical Method of Analysis: Sodium Hydroxide via ICP-MS, BSI-ATM-0074, for elemental impurity sample preparation and analysis.

8.2.10.1.2. For BioTech product analysis, refer to Analytical Method for Determination of Trace Metals in Sodium Hydroxide, BSI-ATM-0132, for trace metal sample preparation and analysis.

8.2.11. **SODIUM CARBONATE**

8.2.11.1. **Preparation of 6N sulfuric acid Solution:**

8.2.11.1.1. To a 1L volumetric flask containing 600mL of cooled Purified Water, add slowly (using caution) 169mL of 96% sulfuric acid in small increments allowing to cool in between each addition. Dilute to the mark, mix thoroughly. Reagent may already be prepared.

8.2.11.1.2. Following the Standardization of Titrants SOP, perform a single check of the 6N sulfuric Acid normality concentration when the reagent is first prepared:

8.2.11.2. **Sample Analysis:**

8.2.11.2.1. Accurately weigh 48g of sample in an iodine flask then add 100mLof purified water. Stopper, swirl to mix, water seal the flask, and chill to room temperature in an ice bath.

8.2.11.2.2. While in an ice bath, slowly add the calculated volume of 6N sulfuric acid reagent required from the calculation below. Wash down the flask sides with purified water, swirl to mix, water-seal the flask, and then chill to room temperature.

$$mL\ of\ 6N\ sulfuric\ acid\ to\ add = \frac{(29.9)^1(sample\ weight)}{(4.00)(N\ of\ 6N\ Sulfuric\ Acid)} - 5\ mL$$

[1] Theoretical assay value of a 9.95N Sodium Hydroxide. (Low end of target range to avoid the over addition of 6N Sulfuric Acid)

8.2.11.2.3. Titrate with a standardized 1N H_2SO_4 and 150µL of phenolphthalein TS usinga 50-mL buret to a precise clear endpoint (V_1); add 150µL of methyl orange indicator and continue the titration to the first pink endpoint (V_2). Calculate the % Na_2CO_3 using the following equation:

$$\%\ Na_2CO_3 = \frac{(V_2 - V_1)\ x\ N\ of\ Titrant\ x\ 10.6}{sample\ weight\ (g)}$$