BIOSPECTRA

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SODIUM HYDROXIDE 10N TESTING METHODS

TABLE OF CONTENTS

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. **Hydrochloric Acid 0.1N** - Purchased commercially.
7.2. **0.02N HCl** - Dilute 20 ml of Hydrochloric Acid 0.1N to 100 mL with purified water. Can be purchased commercially.
7.3. **LAL Reagent Water** - Purchased commercially.
7.4. **Endosafe PTS Cartridge 1-0.01 EU/mL** - Purchased commercially.
7.5. **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.
7.6. **0.1N Silver Nitrate** - Purchased commercially.
7.7. **1N Sulfuric Acid** - Purchased commercially.
7.8. **6N Sulfuric Acid**- add slowly (use caution) 169 mL of 96% sulfuric acid in small increments allowing to cool and dilute to 1 L, mix thoroughly.
7.9. **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.
7.10. **Ammonium Thiocyanate:** Dissolve 150 g of ammonium thiocyanate in purified water, and dilute with water to 500 mL.
7.11. **Iron Standard (0.01 mg of Fe in 1 mL):** Dissolve 0.702 g of ferrous ammonium sulfate hexahydrate in 10 mL of 10% sulfuric acid reagent solution, and dilute with water to 100 mL. Immediately before use to 10 mL of this solution, add 10 mL of 10% sulfuric acid reagent solution, and dilute with water to 1 L.
7.12. **10% Sulfuric Acid Reagent Solution:** Slowly add 30 mL of 96% sulfuric acid to 375 mL of purified water. Cool and dilute with water to 500 mL.
7.13. **Ammonium Peroxydisulfate:** Purchased commercially.
7.14. **Concentration Hydrochloric Acid:** Purchased commercially.
7.15. **Lead Stock Solution (0.1 mg of Pb in 1 mL):** Dissolve 0.160 g of lead nitrate in 100 mL of dilute nitric acid (1:99), and dilute with purified water to 1 L.
7.16. **Dilute nitric acid (1:99):** Dilute 1 mL of Concentrated Nitric acid to 100 mL with purified water.
7.17. **10% Ammonium Hydroxide:** Dilute 35 mL of 29% Ammonium Hydroxide to 100 mL with purified water.
7.18. **Glycerin Base:** To 200 g of glycerin, add water to a total weight of 235 g. Add 140 mL of 1N NaOH, 50 mL of purified water and mix.
7.19. **Thioacetamide:** Dissolve 4 g of thioacetamide in purified water to make 100 mL.

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 – 7.5 g of sample and add 40 mL of purified water in a clean flask. Stopper the flask and allow to cool to room temperature.
8.1.1.3. Add Phenolphthalein as the indicator and titrate using previously standardized 1N Sulfuric Acid to a colorless endpoint (V1).
8.1.1.4. Add 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 10.0 g of sample into a clean beaker or Nessler tube.
8.1.3.2.3. Dilute to ~20 mL with purified water.
8.1.3.2.4. Slowly, using extreme caution, acidify the sample with ~5 mL of Nitric Acid, testing with litmus paper.
8.1.3.2.5. Dilute to ~40 mL 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 70.5 µL of 0.02N HCl to ~40 mL 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 1 mL of 0.1N Silver Nitrate TS.
8.1.3.4.2. Dilute both to 50 mL 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 can not 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 thesample 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 10.0 g of sample into a clean beaker or Nessler tube.

8.2.3.2.2. Dilute to ~20 mL with purified water.

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

8.2.3.2.4. Dilute to ~40 mL 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 70.5 µL of 0.02N HCl to ~40 mL 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 1 mL of concentrated Nitric Acid and 1 mL of 0.1 N Silver Nitrate TS.

8.2.3.4.2. Dilute both the sample and standard solutions to 50 mL 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 0.200 mL of sample into a sterile vial and add 1.600 mL of LAL reagent water.
8.2.4.2. Add 0.160 mL 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 0.02 mL 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 0.3 mL of buffer.
8.2.4.5. Dilute with LAL reagent water to a final volume of 10 mL.
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, for primary method of analysis.

Alternate Method:
8.2.6.2. Standard and Solution Prep:
8.2.6.2.1. Lead Stock Solution (0.1 mg of Pb in 1 mL): Dissolve 0.160 g of lead nitrate in 100 mL of dilute nitric acid (1:99), and dilute with purified water to 1 L. The solution should be prepared and stored in containers free from lead.
8.2.6.2.2. Lead Standard Solution (0.01 mg of Pb in 1 mL): Dilute 10 mL of lead stock solution to 100 mL with purified water. This must be prepared at the time of use.
8.2.6.2.2.1. Dilute nitric acid (1:99): Dilute 1 mL of 69% nitric acid in 99 mL of purified water.
8.2.6.2.3. 1N Acetic Acid: Dilute 57 mL of glacial acetic acid to 1 L with purified water.
8.2.6.2.4. 10% Ammonium Hydroxide: Dilute 35 mL of 29% ammonium hydroxide to 100 mL with purified water.

8.2.6.2.5. Glycerin Base: To 200 g of glycerin add water to total weight of 235 g. Add 140 mL of 1N NaOH, 50 mL of purified water and mix.

8.2.6.2.6. Thioacetamide: Dissolve 4 g of thioacetamide in purified water to make 100 mL.

8.2.6.2.7. Thioacetamide-glycerin base: Thoroughly mix 1 mL of thioacetamide with 5 mL 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 prep to avoid spattering of sample.

8.2.6.3.2. Sample Preparation: Weigh 30 g of sample into a suitable beaker and carefully add 18 mL of concentrated nitric acid.

8.2.6.3.3. Standard Preparation: Weigh 10 g of sample and add 5 mL of concentrated nitric acid. Add 2 mL of 0.01 mg Lead Standard Solution.

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 20 mL 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 1.2 mL of freshly prepared thioacetamide-glycerin base to each of the solutions and mix. QS each tube to 50 mL and mix.

8.2.6.3.6. Any brown color produced in the sample solution must not exceed that in the standard solution to be reported as ≤ 1 ppm.

8.2.7. **IDENTIFICATION (SODIUM)**

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

8.2.7.2. Add 2 mL 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 4 mL 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, for primary method of analysis.

Alternate Method:

8.2.8.2. Standard and Solution Preparations:

8.2.8.2.1. 30% ammonium thiocyanate: Dissolve 150 g of ammonium thiocyanate in water, and dilute with water to 500 mL.

8.2.8.2.2. Iron Standard (0.01 mg of Fe in 1 mL): Dissolve 0.702 g of ferrous ammonium sulfate hexahydrate in 10 mL of 10% sulfuric acid reagent solution, and dilute with water to 100 mL.

8.2.8.2.2.1. To 10 mL of this solution, add 10 mL of 10% sulfuric acid reagent solution, and dilute with water to 1 L.

8.2.8.2.2.2. 10% sulfuric acid reagent solution: In a well- ventilated fume hood, slowly add 30 mL of 96% sulfuric acid to 375 mL of purified water, cool and dilute with water to 500 mL.

8.2.8.3. Procedure:

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

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

8.2.8.3.3. 0.02 mg Iron Standard Preparation: Pipette 2 mL of 0.01 mg of Iron standard into a graduated cylinder and dilute to 40 mL with purified water. Transfer to a Nessler Color Comparison Tube.

8.2.8.3.4. To the sample and standard solutions add 30-50 mg of ammonium peroxydisulfate crystals, 3 mL of hydrochloric acid, and 3 mL of ammonium thiocyanate reagent solution, and mix.

8.2.8.3.5. Any red color in the sample must not exceed the 0.02 mg 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 25 mL volumetric flask with sample. Quantitatively transfer thealiquot to a 250 mL volumetric flask with purified water. Rinse the 25 mL flask by filling the flask halfway with purified water, shaking it, then transferring the rinse to the 250 mL volumetric flask. Performthe rinse procedure in duplicate. Fill the 250 mL 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 50 mL 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 – 8.2000 g of the previously dried KHP into a 250 mL beaker.

8.2.9.2.2. Add 100 mL 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 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 1 L volumetric flask containing 600 mL of cooled Purified Water, add slowly (using caution) 169 mL 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 48 g of sample in an iodine flask then add 100 mL of 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 phenolphthalein TS usinga 50-mL buret to a precise clear endpoint (V_1); add methyl orange indicator and continue the titration to the first pink endpoint (V_2). Calculate the % Na_2CO_3 using the following equation:

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