Nitrate plus Nitrite Analysis Reagent Recipes

v1.0

Safety

Consult the Material Safety Data Sheet for each reagent before handling or preparing the reagents. Reagents must be made by appropriately trained and qualified personnel equipped with proper safety equipment (e.g. safety glasses, gloves, lab jacket, etc.) within a properly equipped work area (e.g. fume hood, eyes wash, etc.). **Green Eyes LLC (Green Eyes) is not responsible or liable for accidents during reagent preparation or other activities related to testing or deploying Green Eyes equipment.**

General Preparation Considerations

For optimal results, all reagents should be made with high quality (18 M ohm/cm) deionized water (DIW) in labware previously acid washed with a 10% (1.2 N) Hydrochloric Acid (HCl) solution. After acid washing, labware should be rinsed three times with DIW. Reagent storage bottles should also be acid washed and DIW rinsed. Reagent salts or solutions used should be of reagent grade or better unless otherwise specified.

Method Description

A buffered (imidazole) sample is passed in and out of a copper coated, cadmium tube (column) to reduce nitrate to nitrite. The nitrite that was originally present, plus the nitrite reduced from nitrate, (N+N), is determined via a Griess reaction by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride. The intensity of the resulting colored red azo dye is measured colorimetrically. Nitrite can be measured individually by eliminating the cadmium column and buffering steps.

Preparation

Reagent 1 – Imidazole buffer (2000 ml)

Reagents and amounts:

1. Imidazole ($C_3H_4N_2$) – 20.0 g
2. 2% (w/v) Copper Sulfate Solution ($CuSO_4$) – 0.4 ml
3. Concentrated Hydrochloric Acid (37% HCl) - appx. 6 ml
4. DIW – 2000 ml

Preparation: Dissolve Imidazole into 2000 ml of DIW and mix thoroughly. Add copper sulfate solution and continue mixing. While stirring, add HCl dropwise until pH falls to between 7.80 and 7.85. It is important that the pH meter be recently calibrated.

Copper Sulfate Solution: Dissolve 2.0 g of cupric sulfate (anhydrous) into 100 ml of DIW.

Note: It is best to stop stirring when measuring the pH of the buffer solution as the moving liquid will alter the readings slightly.

Storage: Stable in the dark at room temperature for 2 months or more.

Reagent 2 – Sulfanilamide (500 ml)

Reagents and amounts:

1. Sulfanilamide ($C_6H_8N_2O_2S$) – 5.0 g
2. Concentrated Hydrochloric Acid (37% HCl) – 50 ml
3. DIW – 450 ml

Preparation: Slowly add the HCl to the DIW while stirring then add the sulfanilamide. Stir until all the sulfanilamide is dissolved.

Storage: Stable at room temperature.

Reagent 3 – NEDA (250ml)

Reagents and amounts:

1. N-(1-Naphthyl)ethylenediamine dihydrochloride ($C_{12}H_{14}N_2$ – 2HCl) - 0.25 g
2. DIW – 250 ml

Preparation: Add N-1 reagent to DIW and mix until fully dissolved

Storage: When protected from light the reagent is stable for two months or more at room temperature. It will eventually turn "tea colored", but is still effective if not dark brown in color.

Cadmium Column Activation

Acid Pitting:

1. Place appx. 50 mm of chemically resistant tygon tubing to one end of the column and appx. 200 mm to the other end. Add a pinch clamp to the 200mm length of tubing and then connect it to a 60mm syringe. When emptying the syringe in later steps, pinch off the tubing so the solution in the column does not leak out.
2. Insert the 50mm length of tubing into a beaker of DIW and pull enough DIW through the column with the syringe to fill it.
3. Slowly pull 10 ml of 2.5 N nitric acid through the column followed by 25 ml of DIW. An orange haze may form in the syringe.
4. Slowly pull 25 ml of concentrated HCl through the column followed by 25 ml of DIW.

Copper Activation:

5. Pull 25 ml of imidazole buffer through the column and then 15 ml of an activation solution made from equal parts imidazole buffer reagent and 2% Copper Sulfate solution. Repeat twice more with 5 minutes between each treatment with the activation solution. Once the

column has been activated with Copper, it should be protected from air as oxygen will poison the column.

6. Flush the activation solution by rapidly pulling 50 ml of imidazole buffer through the column.

Conditioning:

7. Mix roughly 20 ml of approximately 5 mg / L (350 micro M) nitrate solution with 20 ml of imidazole buffer and slowly pull the conditioning solution through the column. Try not to pull air bubbles through the column, but small amounts of air are not harmful if quickly flush out.
8. Flush the conditioning solution out by pulling another 35 ml of imidazole through the column.
9. Store with both ends of the tygon tubing pinch off.

Waste: All waste should be collected and disposed of in accordance with local regulations.

Air or Inert Gas

Reagent:

1. Air free of dust particles or inert gas such as argon.

References:

J. D. H. Strickland and T. R. Parsons: A Practical Handbook of Seawater Analysis. Ottawa: Fisheries Research Board of Canada, Bulletin 167, 2nd Ed., 1972. 293 pp.

Grasshoff, K: Methods of Seawater Analysis, Verlag Chemie, Weinheim and New York, 1976, pp.149 – 156