Green Eyes
Environmental Observing Systems

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Reagent Recipes v1.0

(Nutrient Monitors)

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Contents

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.

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

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

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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.

Air or Inert Gas

Reagent 1:

1. Air free of dust particles or inert gas such as argon in a reagent bag.

Note: If deploying a NuLAB or an EcoLAB out of the water, an inlet filter can be placed on the air port of the valve for an unlimited supply of filtered air.

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 in a reagent bag.

Note: If deploying a NuLAB or an EcoLAB out of the water, an inlet filter can be placed on the air port of the valve for an unlimited supply of filtered air.

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

Ortho-Phosphate Analysis Reagent Recipes

v1.0

Safety

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General Preparation Considerations

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Method Description

This method is based on an optimization of the popular chemistry published by Murphy and Riley (1962) by Drummond and Maher (1995). Phosphate is reacted with molybdenum to form 12-molybdophosphoric acid that when reduced by ascorbic acid forms a colored molybdenum blue compound that is measured colorimetrically.

Preparation

Reagent 1 – Molybdic Acid (500 ml)

Reagents and amounts:

1. Amonium Molybdate 4-hydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) – 3.2 g
2. Potassium Antimony Tartrate solution – 8 ml
3. Concentrated Sulfuric Acid (36 N) – 35 ml
4. SDS solution – 5 ml
5. DIW – 250 ml, 215 ml

Preparation:

While stirring, slowly add the sulfuric acid to 250 ml of DIW in a one liter flask. Then add the ammonium molybdate and continue stirring until all the molybdate is dissolved. While stirring, slowly add 8.0 ml of the potassium antimony tartrate solution and insure that it mixes completely. Add 215 ml of DIW followed by the SDS solution, mix well and store in a tightly sealed container.

Storage: Stable for 6 months at under refrigeration and 3 months at room temperature.

Note: If also running ammonium analysis on an EcoLAB, replace the ammonium molybdate with 4.5g of sodium molybdate (Na_2MoO_4 - $2H_2O$). Sodium molybdate is very sensitive to ambient electrical fields and thus particles tend to "dance" around the weighing containers. It is recommended to weigh the salt inside a clean, dry bottle and seal until it is added to the acid. Wash the remaining crystals out of the bottle with the reagent.

Potassium Antimony Tartrate ($C_8H_4K_2O_{12}Sb_2$ – $3H_2O$) solution: Add 3.5 g potassium antimony tartrate to 200ml DIW and mix thoroughly. Stable for 6 months under refrigeration.

Sodium Dodecyl Sulfate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) solution: Add 3 g of SDS to 100 ml of DIW and stir until fully dissolved. Stable for 6 months under refrigeration.

Reagent 2 – Ascorbic Acid (500 ml)

Reagents and amounts:

1. Ascorbic Acid ($C_6H_8O_6$) – 4.5 g
2. DIW – 500 ml

Preparation: Add the ascorbic acid to the DIW and stir until completely dissolved.

Storage: Store solutions in dark bottles and refrigerate. Ascorbic acid is reported to be unstable but if protected from light and air (oxygen), the reagent can be stable for eight weeks. Discard if a yellow color forms.

Air or Inert Gas

Reagent:

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

References:

L. Drummond and W. Maher: Determination of phosphorus in aqueous solution via formation of the phosphoantimonylmolybdenum blue complex - Rexamination of optimium conditions for the analysis of phosphate. Analytica Chimica Acta 302 (1995) pp. 69 – 74.

J. Murphy and J. P. Riley: A modified single solution method for the determination of phosphate in natural waters, Analytical Chimica Acta, 27 (1962) p. 31

Ammonium Analysis Reagent Recipes

v1.10

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.**

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Method Description

Ammonium (NH_4^+) and Ammonia (NH_3) are both determined by the Berthelot Reaction in which an indophenol blue chromophore forms when ammonium and ammonia react with sodium phenoxide (phenol and sodium hydroxide) followed by the addition of sodium hypochlorite. Sodium citrate is used to prevent precipitation of calcium and magnesium hydroxides at the elevated reaction pH.

Preparation

Reagent 1 – Complexing Reagent (1000 ml)

Reagents and amounts:

1. Sodium Citrate [$HOC(COONa)(Ch_2OONa)_2 \cdot 2H_20$] – 140 g
2. Sodium Hydroxide (NaOH) – 5 g
3. DIW – 1000 ml

Preparation: Add 800 ml of DIW to a clean 1000ml flask. Add the sodium citrate and stir until dissolved. Then add the sodium hydroxide and make to a final volume of 1000 ml. Mix well before transferring to a bottle or reagent bag.

Storage: Store in a refrigerator until use. Stable for 2 months or more.

Reagent 2 – Color forming reagent (250ml)

Reagents and amounts:

1. Phenol (C_6H_5OH) solution (88%) - 8.75 ml
2. Sodium Nitroprusside Dihydrate [$Na_2Fe(CN)_5NO \cdot 2H_20$] – 0.1 g
3. DIW – 250 ml

Preparation: Phenol is a toxic chemical. All staff working with it should read the MSDS and observe proper safety procedures to eliminate skin contact, inhalation or ingestion. In a fume hood, add 200 ml of DIW to a 250 ml flask. Add the phenol solution and mix well (magnetic stirrer recommended). Add the nitroprusside and 50 ml of DIW, and mix well.

Storage: Store protected from light in refrigerator. Reagent is stable for two months or more.

Reagent 3 – Oxidizer (250ml)

Reagents and amounts:

1. Sodium Hypochlorite (commercial bleach with 5.25% free chlorine) – 12.5 ml
2. DIW – 240 ml

Preparation: Add 240 ml of DIW to a clean flask and then add the hypochlorite. Mix well.

Storage: When protected from light the reagent is stable for two months or more at room temperature.

Wash

Reagent:

1. Nutrient free deionized water.

Air or Inert Gas

Reagent:

1. Air free of ammonia (gas) and particles or inert gas such as argon.

Note: If deploying a NuLAB or an EcoLAB out of the water, an inlet filter can be placed on the air port of the valve for an unlimited supply of filtered air.

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.

L Solorzano: Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceangr. Vol.14(5). 1969. pp. 799-801.

Silicate Analysis Reagent Recipes

v1.0

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Method Description

EPA Method 366.0

Preparation

Reagent 1 – Ascorbic Acid Solution (250 ml)

Reagents and amounts:

1. Ascorbic Acid - 4.4g
2. DIW – 250 ml

Preparation: Fill beaker with 250ml of DIW and add 4.4g of Ascorbic Acid and stir until completely dissolved.

Storage: Store solutions in dark bottles and refrigerate. Ascorbic acid is reported to be unstable but if protected from light and air (oxygen), the reagent can be stable for eight weeks. Discard if a yellow color forms.

Reagent 2 – Oxalic Acid Solution (250ml)

Reagents and amounts:

1. Oxalic Acid – 12.5g
2. DIW – 250 ml

Preparation: Fill beaker with 250ml of DIW and 12.5g of Oxalic Acid and stir until completely dissolved.

Storage: Store in dark bottles in refrigerator. Reagent is stable for two months or more.

Reagent 3 – Sodium Molybdate Preparation Solutions

3.1 10% Sulfuric Acid (100mL)

Reagents and amounts:

1. Concentrated Sulfuric Acid – 10 mL
2. DIW – 90 mL

Preparation: Slowly add 10 ml of concentrated sulfuric acid to 90ml of deionized water and mix gently.

Storage: Acid reagent is stable indefinitely, in a sealed poly bottle.

3.2 Sodium Dodecyl Sulfate (100ml)

Reagents and amounts:

1. SDS –3g
2. DIW – 100ml

Preparation: Fill beaker with 100ml of deionized water, and add 3g of SDS and mix until completely dissolved.

Storage: Store solution in dark bottles and refrigerate.

Reagent 4 – Sodium Molybdate Solution (250ml)

Preparation:

1. Add 10ml of 10% sulfuric acid to 240ml of DIW.
2. Add 3.3g of Sodium Molybdate and mix well
3. Add 2.5ml of Sodium Dodecyl Sulfate, mix.

Wash

Reagent 1:

1. Nutrient free deionized water.

References:

Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

Jia-Zhong Zhang, Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science.

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