NUTRIENT REAGENT PROTOCOL - SIO/STS/ODF

NITRATE/NITRITE ANALYSIS

A modification of the Armstrong et al. (1967) procedure is used for the analysis of nitrate and nitrite. For nitrate analysis, a seawater sample is passed through a cadmium column where the nitrate is reduced to nitrite. This nitrite is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form a red dye. The sample is then passed through a 10mm flowcell and absorbance measured at 540nm. The procedure is the same for the nitrite analysis but without the cadmium column.

REAGENTS

Sulfanilamide
Dissolve 10g sulfamilamide in 1.2N HCl and bring to 1 liter volume. Add 2 drops of 40% surfynol 465/485 surfactant.
Store at room temperature in a dark poly bottle.

Note: 40% Surfynol 465/485 is 20% 465 plus 20% 485 in DIW.

N-(1-Naphthyl)-ethylenediamine dihydrochloride (N-1-N)
Dissolve 1g N-1-N in DIW, bring to 1 liter volume. Add 2 drops 40% surfynol 465/485 surfactant.
Store at room temperature in a dark poly bottle. Discard if the solution turns dark reddish brown.

Imidazole Buffer
Dissolve 13.6g imidazole in ~3.8 liters DIW. Stir for at least 30 minutes to completely dissolve. Add 60 ml of CuSO4 + NH4Cl mix (see below). Add 4 drops 40% Surfynol 465/485 surfactant. Let sit overnight before proceeding
Using a calibrated pH meter, adjust to pH of 7.83-7.85 with 10% (1.2N) HCl (about 20-30 ml of acid, depending on exact strength). Bring final solution to 4L with DIW.
Store at room temperature.

NH4Cl + CuSO4 mix:
Dissolve 2g cupric sulfate in DIW, bring to 100 m1 volume (2%)
Dissolve 250g ammonium chloride in DIW, bring to l liter volume.
Add 5ml of 2% CuSO4 solution to this NH4Cl stock. This should last many months.

Nitrate Standard:
In a 1 liter calibrated volumetric "A" flask, dissolve ~1.5xxgm of high purity dried KNO3 in DIW to make a 1 liter final volume solution. Record temperature of the final solution. Calculate the concentration of this primary nitrate standard using the volumetric flask volume, temperature and exact weight of standard using program nutstd.exe.
Dilute a secondary and working standards as necessary. (See stds.doc).

Nitrite Standard:
In a 1 liter calibrated volumetric "A" flask, dissolve ~0.34xxgm of high purity dried $NaNO_2$ in DIW to make a 1 liter final volume solution. Record temperature of the final solution. Calculate concentration of this primary standard solution using program nutstd.exe.
Dilute secondary standard as necessary (See stds.doc) . Prepare secondary NO_2 std daily.

PHOSPHATE ANALYSIS

Ortho-Phosphate is analysed using a modification of the Bernhardt and Wilhelms (1967) method. Acidified ammonium molybdate is added to a seawater sample to produce phosphomolybdic acid, which is then reduced to phosphomolybdous acid (a blue compound) following the addition of dihydrazine sulfate. The sample is passed through a 10mm flowcell and absorbance measured at 820nm.

REAGENTS

Ammonium Molybdate
H_2SO_4 sol'n:
Pour 420 ml of DIW into a 2 liter Ehrlenmeyer flask or beaker, place this flask or beaker into an ice bath. SLOWLY add 330 ml of conc H_2SO_4.
This solution gets VERY HOT!! Cool in the ice bath. Make up as much as necessary in the above proportions.

Dissolve 27g ammonium molybdate in 250ml of DIW. Bring to 1 liter volume with the cooled sulfuric acid sol'n. Add 3 drops of 15% DDS surfactant. Store in a dark poly bottle.

Dihydrazine Sulfate
Dissolve 6.4g dihydazine sulfate in DIW, bring to 1 liter volume and refrigerate.

Note: Hydrazine sulfate may also be used. Dilute 10.0g to 1 liter with DIW.

Phosphate Standard:
In a 1 liter calibrated volumetric "A" flask, dissolve ~0.81xxgm of dried high purity KH_2PO_4 in DIW. Record the temperature. Dilute to the mark with DIW. Calculate concentration with nutstd.exe. Dilute a secondary and working standards as necessary. (See stds.doc).

SILICATE ANALYSIS

Silicate is analyzed using the basic method of Armstrong et al. (1967). Acidified ammonium molybdate is added to a seawater sample to produce silicomolybdic acid which is then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. The sample is passed through a 10mm flowcell and measured at 660nm.

REAGENTS

Tartaric Acid

Dissolve 200g tartaric acid in DW and bring to 1 liter volume. Store at room temperature in a poly bottle.

Ammonium Molybdate
Dissolve 10.8g Ammonium Molybdate Tetrahydrate in 1000ml dilute H2SO4*.
*(Dilute H2SO4 = 2.8ml conc H2SO4 or 6.4ml of H2SO4 diluted for PO4 moly per liter DW)
(dissolve powder, then add H2SO4)
Add 3-5 drops 15% SDS surfactant per liter of solution.

Stannous Chloride
stock: (as needed)
Dissolve 40g of stannous chloride in 100 ml 5N HCl. Refrigerate in a poly bottle.

NOTE:
Minimize oxygen introduction by swirling rather than shaking the solution. Discard if a white solution (oxychloride) forms.

working: (every 24 hours)
Bring 5 ml of stannous chloride stock to 200 ml final volume with 1.2N HCl. Make up daily - refrigerate when not in use in a dark poly bottle.

Silicate Standard:
In a plastic flask, dissolve 0.5642g dried high purity Na2SiF6 in about 300ml DIW. This solution will take 4-6 hrs to dissolve. Using this 300ml solution, make up a mixed secondary standard (NO3, PO4, SiO3) according to oceanic nutrient ranges (See stds.doc).

At 1 liter, the silicate concentration is 3000uM.

At 2 liter, the silicate concentration is 1500uM.

AMMONIUM ANALYSIS

Phenol method:
Ammonium is analyzed via the Berthelot reaction in which hypochlorous acid and phenol react with ammonium in an alkaline solution to form indophenol blue. The sample is passed through a 10 mm flowcell and measured at 640nm. The method below is a modification of the procedure by Koroleff (1969,1970).

REAGENTS

Sodium Citrate (Complexing Reagent)
Dissolve 280gm Sodium Citrate in approximately 990ml DIW; adjust to pH7; bring to a 1 liter final volume with DIW. Store in a poly bottle.

Alkaline Phenol

Add 60ml 10N NaOH to 700ml DIW. Add 12ml or 12gm Phenol (liquid or solid).
Dilute to 1L with DIW. Store in a dark poly bottle.
Make up new as necessary if sensitivity drops off.

10N NaOH
Dissolve 400gm NaOH in 1L DIW

NOTE:
Optimum pH of analytical effluent should be ~10.5.
Adjust accordingly by adjusting amount of NaOH added.

Sodium Hypochlorite (NaOCl)
Add 2.5ml of 5.25% NaOCl (ie. Bleach) to 100ml DIW.
Prepare daily.

Sodium Nitroprusside
Dissolve 0.5gm in 800ml DIW. Bring to 1L with DIW.
Store in dark poly container. Keep away from light at all times.

Flurometric method:

Ammonia is analyzed using the method described by Kerouel and Aminot [Kero97]. The sample is combined with a working reagent made up of ortho-phthalaldehyde, sodium sulfite and borate buffer and heated to 75degC. Fluorescence proportional to the NH4 concentration is emitted at 460nm following excitation at 370nm.

REAGENTS

Ortho-phthalaldehyde stock (OPH):
Dissolve 8g of ortho-phthalaldehyde in 200mls ethanol and mix thoroughly. Store in a dark glass bottle and keep refrigerated.

Sodium sulfite stock:
Dissolve 0.8g sodium sulfite in DIW and dilute up to 100ml. Store in a glass bottle, replace weekly.

Borate buffer
Dissolve 120g disodium tetraborate in DIW and bring up to 4L volume.

Working reagent:
In the following order and proportions combine:
1L borate buffer
20ml stock orthophthalaldehyde,
2 ml stock sodium sulfite,

4 drops 40% Surfynol 465/485 surfactant and mix. Store in a glass bottle and protect from light. Replace weekly.

Make this up at least one day prior to use. Store in dark bottle and protect from outside air/nh4 contamination.

Ammonium Standard:
In a 1 liter calibrated volumetric "A" flask, dissolve ~0.26xxgm of dried high purity high purity (NH4)2SO4 (ammonium sulfate) in DIW. Record the temperature. Dilute to the mark with DIW. Calculate concentration with nutstd.exe. Dilute a secondary and working standards as necessary. (See stds.doc).