**Determination of Nitrate**  
*(hydrazine reduction method)*


The nitrate in seawater is reduced with hydrazine (catalyzed by copper) in a solution buffered at pH 9.6. The nitrite thus produced is determined by diazotizing with sulphanilamide and coupling with N-(1-naphthyl)-ethylene-diamine to form a highly coloured azo dye, the absorbance of which is measured at 543 nm. If much nitrite is present in a sample of low nitrate concentration, the nitrite content of the sample must be determined and a correction made to the nitrate result.

**Reagents:**

**Phenol Solution:**
Dissolve 9.2 g of dry phenol in 200 mL distilled water. Store at 4°C. Stable for many weeks.

**Sodium hydroxide solution:**
Dissolve 2.9 g of sodium hydroxide pellets in 200 mL distilled water. Store at 4°C. Renew every few months to avoid excess carbonation.

**Copper sulphate solution:**
Dissolve 0.02 g copper sulphate in 200 mL distilled water. Store at 4°C. Stable a few weeks.

**Sulphanilamide solution:**
Dissolve 2.5 g sulphanilamide in a mixture of 25 mL conc. HCl and 150 mL distilled water. Dilute to 250 mL. Store at 4°C. Stable many months.

**N-(1-naphthyl)-ethylene-diamine:**
Dissolve 0.25 g of N-(1-naphthyl)-ethylene-diamine dihydrochloride in 250 mL distilled water. Store in a dark bottle at 4 °C. Stable a few weeks until a brown coloration develops.

**Hydrazine sulfate solution:**
Dissolve 1.45 g in 200 mL distilled water. Store at 4°C. Stable a few weeks.
30 psu water (blank solution):
Dissolve 30 g NaCl in 1 L of high purity water.

Standard nitrate solutions:
Dissolve 0.1011 g of analytical reagent quality potassium nitrate, KNO₃ in 100 mL distilled water. The solution is stable indefinitely in the absence of evaporation. Dilute 1.5 mL of this solution to 50 mL of 30 psu water for a 300 µM NO₃ standard. Prepare several standards down to 3 µM NO₃, the detection limit using this method.

Standard nitrite solution:
Dissolve 0.069 g NaNO₂ in 100 mL of Q-water. Prepare several dilutions as per nitrate.

Buffer reagent:
Mix phenol solution and sodium hydroxide solution 1:1. Use within 60 minutes.

Reducing reagent:
Mix copper sulphate solution and hydrazine sulphate solution 1:1. Use within 60 minutes.

Sample Collection:

1. All collecting jars, flasks, syringes must be carefully acid washed (20% HCl). Rinse at least 3 times to make sure any residual acid is removed. Rinsing is VERY important.
2. Use super distilled or high quality RO or Q-water only.
3. All jars and covers should be quickly air-dried and capped.
4. All flasks, syringes, etc. must be quickly covered in aluminum foil to avoid contamination.
5. All jars must be wide mouth and pre-labeled as to the site, date, depth, and type of sample (NH₄ or NO₃). These must have tight fitting lids.
6. Collect 3 replicate samples for each depth and type of nutrient.
7. Keep the bottles from each depth together with a rubber band or tape.
8. Water samples must be kept cool and in the dark during sampling, then frozen upright ASAP until they can be processed.
9. Filter samples through a GFF or 0.2 µM filter prior to analysis.
Nitrate Determination:

1. Place 30 mL samples (+ blanks and standards) in a 23°C water bath along with the prepared buffer reagent and reducing reagent. You will need to count the number of samples and prepare the appropriate amount of each of these solutions.

2. Once samples are at bath temperature, add 1.2 mL of the buffer reagent, cap and mix. Then add 0.6 mL of the reducing reagent, cap, mix and return to the water bath.

3. Shield the samples from direct light and let sit in water bath for as long as possible. For best results the samples should stay in the 23°C water bath for at least 8 hours, then a further 12 hours at any temp between 18°C and 25°C for a total of 20 hours. For the purposes of this exercise you can stop the reaction earlier, just keep in mind you will not have 100% reduction of nitrate to nitrite. To measure nitrite, simply omit the reducing agent from the reaction.

4. Add 1.2 mL acetone to the sample, cap, mix and let sit for 2 minutes.

5. Add 0.6 mL sulphanilamide solution, cap, mix and let sit 5 minutes.

6. Add 0.6 mL N-(1-naphthyl)-ethylene-diamine dihydrochloride solution, cap, mix and let sit 10 minutes.

7. Read the absorbance on spectrophotometer at 543 nm using a 10 cm cell (requires ~25 mL of sample).

Data Analysis:

1. Take the mean of the blank absorbances.

2. Subtract this from each standard absorbance.

3. Plot the standard curve (abs. versus concentration in µM)

4. Calculate the concentration of the unknown samples. According to the equation of the standard curve.

5. Ensure that sample measurements fall within the range of the standards.