**Chemical Analysis of Water: Nutrients.**

These assays are based on spectrophotometric measurement of coloured products. The reactions are carried out using an excess of reagents so that the limiting factor is the amount of the analyte, thus the intensity of the colour is in direct proportion to the concentration of the analyte. The volume of sample analysed is 5 or 10 ml, whereas the volume of reagents added is much smaller. Since an excess of reagent is used, the absolute accuracy of the volume added is not important as long as it is the same for all samples, standards and blanks. The accuracy of measurement of the samples and standards, and more particularly of dilutions carried out is of much greater significance. Pipettors should be checked and set for the volume required, calibrated by weighing water (1ml=1g). Pipettors are generally only accurate for the volume at which they are calibrated.

**REAGENTS**

**Ammonium**: [ref. Chaney and Marbach, 1962: see Mackereth, Heron and Talling, 1978]

a: Phenol - nitroprusside: *caution: caustic & toxic: must be used in a fume hood*
Phenol 15g
Sodium nitroprusside 0.015g
dissolve in 500ml distilled $H_2O$: Store in fridge for 3 months

b: Alkaline hypochlorite: *caution: caustic*
(Solution should contain 10gNaOH and 0.265g available Cl in 500ml: in case of difficulty the volume of bleach* can be increased )
NaOH 10g: dissolve in 400ml distilled $H_2O$ and allow to cool: place in volumetric flask.
Add bleach 2ml*
Make up to 500ml with $H_2O$. Store in fridge for 3 months.

*more may be required if the reagent does not give the correct absorbance with standard $NH_4Cl$: 500µg/l standard should give an $A_{635}$ of approx. 0.25 in a 1 cm cell. Commercial bleach (e.g. Safeways own brand. plain bleach, not scented or thickened) may prove unsatisfactory if the available Cl is less than ~8%: alternatively use BDH sodium hypochlorite solution GPR, 12% available Cl.
The amount of available Cl can be checked as follows:
25 ml $H_2O$ + 2g potassium iodide + 10ml glacial acetic acid + 5ml of a 1:10 dilution of the bleach;
titrate with 0.100M sodium thiosulphate, using starch as an indicator.
(0.1M thiosulphate: 24.82g $Na_2S_2O_3.5H_2O$, dissolved in water to 1000 ml @20°C.
indicator: 1g soluble starch boiled in 100ml water, then filtered. Use a few drops).
Calculate available Cl as: mg Cl (as $Cl_2$)/ml = $\frac{\text{Volume of thiosulphate x Normality of thiosulphate x 35.45}}{\text{volume of bleach (= 0.5ml)}}$

(APHA 1995, p 4 -38)

Standard Ammonium Chloride:
$NH_4Cl$ 3.821g Dissolve in $H_2O$ to 1000ml (volumetric flask) @ 20°C:
add 2 ml Chloroform as preservative: keep for no longer than 1 week.
This stock is 1000mg/l **$NH_4$ - N**. Dilute to 1000µg/l for working strength: this solution should not be stored.

**Nitrite:** [ref. Bendshneider and Robinson, 1952; see see Mackereth, Heron and Talling, 1978]

Sulphanilamide: (1% in 10% HCl) *caution: caustic*
$H_2O$ 90ml
Add 10ml conc. HCl
Dissolve 1g Sulphanilamide: store in dark bottle in fridge - will keep satisfactorily over a long period.

N-1-naphthylethylenediamine dihydrochloride (NEDD)
1g dissolved in 100ml $H_2O$: store in dark bottle in fridge - will keep satisfactorily over a long period.

Standard Nitrite stock solution:
$NaNO_2$ (AR grade) 2.46g dissolve in distilled $H_2O$, make up to 1 litre @ 20°C.
Add 2 ml Chloroform as preservative: keep for no longer than 1 week.
This stock is 500mg/l **$NO_2$ -N**. Dilute to 1000µg/l for working strength: this solution should not be stored.

**Nitrate** (actually Total Oxidised Nitrogen -**TON**): [modified from Hydrazine Reduction method; APHA, 1995]

NaOH reagent: 250ml *caution: caustic*

NaOH 25g, add to 237.5 ml $H_2O$, store in air tight plastic bottle at room temp. for 1 month.

Reducing reagent: *caution: poisonous, highly reactive*

| | | | |
|---|---|---|---|
| A: | Hydrazine sulphate | 2.7g in 100ml distilled $H_2O$ | *caution: poisonous* |
| B: | Copper sulphate ($CuSO_4 \cdot 5H_2O$) | 0.25g in 100ml distilled $H_2O$ | *caution: harmful* |
| C: | Zinc sulphate ($ZnSO_4 \cdot 7H_2O$) | 5.3 g in 100ml distilled $H_2O$ | *caution: harmful* |

Store all solutions at room temp: stable indefinitely until mixed.

On day of use mix: 27.4 ml $H_2O$; 10ml A; 1.5ml B; 1.1ml C.
(do not use if more than 1 day old)

Colour Reagent: add in the following order:

| | |
|---|---|
| distilled $H_2O$ | 90ml |
| Orthophosphoric acid | 50ml |
| Sulphanilamide | 5g |
| N-1-naphthylethylenediamine dihydrochloride (NEDD) | 0.25g |
| dissolve and add (use for rinsing reagents in, etc) distilled $H_2O$ | 57.5 ml |

store refrigerated in a dark bottle: renew monthly

Standard Nitrate solution:

$KNO_3$ (anhydrous) 7.221g
Dissolve in $H_2O$ to 1000ml (volumetric flask) @ 20°C: add 2 ml Chloroform as preservative: keep for no longer than 1 week.
This stock is 1000mg/l **$NO_3$ - N**. Dilute to 1000µg/l for working strength: this solution should not be stored.

(Artificial Sea Water for analysis of marine samples: g per litre: NaCl 24.7; KCl 0.7; $CaCl_2.2H_2O$ 1.32; $MgSO_4.7H_2O$ 4.2;$NaHCO_3$ 0.18)

**Phosphorus: Ascorbic acid method**

This is a single-solution method which is not time dependent. The reagent has to be made up fresh from stocks just before use.
Summary of Method: Some forms of phosphate in acidic molybdate solution form a yellow phospho-molybdate complex, which is reduced with ascorbic acid to a more stable blue complex that has a higher extinction coefficient than the yellow one. The reaction is catalysed by antimony and the blue colour is formed in amounts proportional to the soluble reactive phosphate (SRP) present.

Reagents:

| | |
|---|---|
| 2.5M $H_2SO_4$ 500 ml | 70 ml conc. $H_2SO_4$ diluted slowly in about 400 ml deionised $H_2O$ and made up to when cool. *Caution highly corrosive, great heat emitted during dilution: dilute into beaker in a basin of cold water, in fume hood.*. Store in glass bottle |

Ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ : 4g dissolved in 100ml deionised $H_2O$. Store in glass bottle. (precipitates on long storage & will not redissolve)

Potassium Antimonyl Tartrate (PAT) 0.274g PAT dissolved in 100 ml deionised $H_2O$. Store in a dark glass bottle. *PAT is hazardous: wear dust mask and gloves when handling*.

Ascorbic acid Make up fresh amount of solution as required for each batch of analyses, according to Recipes Table for Mixed Reagent

**Mixed Reagent:** Mix according to recipe sheet for the number of sample, standard and blank tubes required **once the tubes are ready**

**Recipes for SRP Mixed Reagent** (add 1 ml per 5 ml sample volume: reagent volume (ml) = no of tubes)

| Reagent Volume, ml | $H_2O$ ml | Ascorbic acid g | 2.5M $H_2SO_4$ ml | Ammonium Molybdate, ml | PAT ml |
|---|---|---|---|---|---|
| ***add in order:*** | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** |
| 30 | 9 | 0.18 | 15 | 4.5 | 1.5 |
| 40 | 12 | 0.24 | 20 | 6.0 | 2.0 |
| 50 | 15 | 0.30 | 25 | 7.5 | 2.5 |
| 60 | 18 | 0.36 | 30 | 9.0 | 3.0 |
| 70 | 21 | 0.42 | 35 | 10.5 | 3.5 |
| 80 | 24 | 0.48 | 40 | 12.0 | 4.0 |
| 90 | 27 | 0.54 | 45 | 13.5 | 4.5 |
| 100 | 30 | 0.60 | 50 | 15.0 | 5.0 |
| 110 | 33 | 0.66 | 55 | 16.5 | 5..5 |
| 120 | 36 | 0.72 | 60 | 18.0 | 6.0 |
| 130 | 39 | 0.78 | 65 | 19.5 | 6.5 |
| 140 | 42 | 0.84 | 70 | 21.0 | 7.0 |
| 150 | 45 | 0.90 | 75 | 22.5 | 7.5 |
| 160 | 48 | 0.96 | 80 | 24.0 | 8.0 |
| 170 | 51 | 1.02 | 86 | 25.5 | 8.5 |
| 180 | 54 | 1.08 | 90 | 27.0 | 9.0 |
| 190 | 57 | 1.14 | 95 | 28.5 | 9.5 |
| 200 | 60 | 1.20 | 100 | 30.0 | 10.0 |
| 210 | 63 | 1.26 | 105 | 31.5 | 10.5 |
| 220 | 66 | 1.32 | 110 | 33.0 | 11.0 |
| 230 | 69 | 1.38 | 115 | 34.5 | 11.5 |
| 240 | 72 | 1.44 | 120 | 36.0 | 12.0 |
| 250 | 75 | 1.50 | 125 | 37.5 | 12.5 |
| 260 | 78 | 1.56 | 130 | 39.0 | 13.0 |
| 270 | 81 | 1.62 | 135 | 40.5 | 13.5 |
| 280 | 84 | 1.68 | 140 | 42.0 | 14.0 |
| 290 | 87 | 1.74 | 145 | 43.5 | 14.5 |
| 300 | 90 | 1.80 | 150 | 45.0 | 15.0 |

Standard Phosphate solution:

$KH_2PO_4$(oven dried) 2.197g. Dissolve in $H_2O$ to 1000ml (volumetric flask) @ 20°C: transfer to a dark bottle: keep for no longer than 1 week.
This stock is 500mg/l **$PO_4$-P**. Dilute to 1000µg/l for working strength: this solution should not be stored.

Dilute this working stock to provide standards from 0 to 1000 µg/l (if using a 1cm spectrophotometer cell: the method is more sensitive with a 4 cm cell, so the maximum standard may need to be only 200 µg/l)

References

Mackereth, FJH, Heron, J and Talling, JF. 1978. Water Analysis: some revised methods for limnologists. Freshwater Biological Association; Scientific Publication no. 36.
APHA. 1995. Standard Methods for the Examination of Water and Wastewater, 19th edition. American Public Health Association.
Murphy, J and Riley, JP. 1962. A modified single-solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. **27.** 31-36.

Modifications:

These methods are intended for the analysis of fresh, relatively colourless waters, and may not work in saline water. In particular, in the TON assay, the NaOH reagent causes a precipitate to form by reacting with Ca and Mg salts in sea water. It is necessary to centrifuge this out, then carry out the analysis with the supernatant (see procedure for marine samples). When assaying marine samples the standards should be made up in Artificial Seawater (see recipe attached to TON assay).

Samples coloured by humic materials may also need special treatment. The absorbance of the sample at the relevant wavelength cannot simply be subtracted as addition of some of the reagents changes the colour (by altering the pH). For Ammonium, Nitrite and Phophate, add only the first reagent and read the absorbance, to be subtracted from those tubes which have received the full treatment. For TON, it is necessary to prepare a 'false colour reagent', containing all components apart from the NEDD, and add this to the tubes for sample colour correction in the same manner as in the full analysis. (Alternatively, see the recommendations in Standard Methods).

Notes:

Glass and plasticware preparation - all tubes, flasks, tips and beakers must be acid washed in 1- 5M HCl prior to use. Fill the vessels with acid in a fume hood and allow to stand for a period: the time required is less with a more concentrated acid but beware, 5M HCl fumes. Tip out the acid (retain for further use until it becomes discoloured), and rinse the vessels **4 times** in deionised water; dry. Failure to clean tubes results in high and erratic absorbances in replicates. After use, rinse the vessels in deionised water and repeat the acid washing. Sampling bottles should be similarly washed prior to use, in a different batch of acid from that used for the test tubes and reagent vessels.

**Disposal of wastes:**
Excess reagents, contents of reaction tubes, and samples collected in the waste jar of the spectrophotometer must be emptied into to a waste drum for disposal.

**Schedule of dilutions of standards for chemical analysis:**
Dilutions from 1000µg $l^{-1}$ working standards using 5 ml and 1 ml pipettors

5 ml reaction volumes:

| Concentration µg $l^{-1}$ | Working std. ml | $H_2O$ ml |
|---|---|---|
| 1000 | 5 | 0 |
| 800 | 4 | 1 |
| 600 | 3 | 2 |
| 400 | 2 | 3 |
| 200 | 1 | 4 |
| 100 | 0.5 | 4.5 |
| 50 | 0.25 | 4.75 |
| 25 | 0.125 | 5 - 0.125 |
| 10 | 0.05 | 5 – 0.05 |
| **0** | **0** | **5** |

**Analysis Procedure:**

***!*** *Pour small amounts of reagents into labelled beakers, top up as required. Unused reagent must* ***not*** *be returned to the bottles.*

**Ammonium:** *Phenol is toxic: must be carried out in a fume hood*

1 Pipette 5 ml aliquots of standards, blanks and samples to acid-washed glass tubes in triplicate.
2 Add 0.5 ml Phenol-Nitroprusside reagent, mix well (vortex).
3 Add 0.5 ml Alkaline Hypochlorite reagent, mix well.
4 Leave in the dark at room temperature (eg. in a cupboard) for 60 minutes.
5 Mix well and read at 635 nm. Absorbance in a 1 cm cell should be 0.2-0.3 for a 500µg/l standard.

**Nitrite:**

1 Pipette 5 ml aliquots of standards, blanks and samples to acid-washed glass tubes in triplicate.
2 Add 0.1 ml Sulphanilamide reagent, mix well (vortex).
3 Allow to stand for 5 minutes.
4 Add 0.1 ml NEDD reagent, mix well.
5 Allow 10 minutes for full colour development, but read all tubes before 60 minutes.
6. Mix tubes and read absorbance at 543nm.
For a 500µg/l standard in a 1 cm cell this should be approx. 2.0

**Nitrate (Total Oxidised Nitrogen=TON):**

1 Pipette 5 ml aliquots of standards, blanks and samples to acid-washed glass tubes in triplicate
2 Add 0.73 ml NaOH reagent, mix well by vortexing
3 Add 420µl of freshly made reducing agent and mix as above.
4 Wait 4-5 minutes.
5 Add 0.73 ml Colour reagent, Mix.
6 Leave for 30 min for full colour development, mix and read at 535nm.
Absorbance of a 500µg/l standard in a 1 cm cell should be approx. 0.5-0.7.

**Nitrate (TON) in Marine samples:**

1 Pipette 9 ml aliquots of standards, blanks and samples to acid-washed Hach tubes in duplicate (make up standards and blanks in Artificial Sea Water)
2 Add 1.30 ml NaOH reagent, cap and mix well
3 Centrifuge to pellet the precipitate (15 min at 2500rpm), pipette out 5ml of supernatant into 3 fresh test tubes for each sample/standard (measure 5.0g by weight on a balance)
4 Add 370µl of freshly made reducing agent and mix (vortex).
5 Wait 4-5 minutes.
6 Add 0.64 ml Colour Reagent, mix.
7 Leave for 30 min for full colour development, mix and read at 535nm.

**Phosphorus: ascorbic acid method (Soluble Reactive Phosphorus=SRP)**

1 Pipette 5 ml aliquots of standards, blanks and samples to acid-washed glass tubes in triplicate
2 Prepare the required amount of Mixed Reagent
3 Add 1 ml mixed reagent to each tube and vortex thoroughly.
4 Allow to stand for 20-30 minutes.
5 Read absorbance at 882nm against a deionised $H_2O$ blank (use a flowcell if possible)

**Schedule of dilutions of standards for chemical analysis:** 5 ml reaction volumes:
Dilutions from 1000µg $l^{-1}$ working standards using 5 ml and 1 ml pipettors

| Concentration µg $l^{-1}$ | Working std. ml | $H_2O$ ml |
|---|---|---|
| 1000 | 5 | 0 |
| 800 | 4 | 1 |
| 600 | 3 | 2 |
| 400 | 2 | 3 |
| 200 | 1 | 4 |
| 100 | 0.5 | 4.5 |
| 50 | 0.25 | 4.75 |
| 25 | 0.125 | 5 - 0.125 |
| 10 | 0.05 | 5 – 0.05 |
| 0 | 0 | 5 |

**Disposal of wastes:** Excess reagents, contents of reaction tubes, and solutions collected in the waste jar of the spectrophotometer must be discarded into to a waste drum for disposal.