

DETERMINATION OF PHOSPHORUS & NITROGEN COMPOUNDS

S.N. D'Souza

National Institute of Oceanography, Dona Paula, Goa.

1. Determination of Soluble Reactive Phosphate

i) Chemistry of phosphorus in seawater

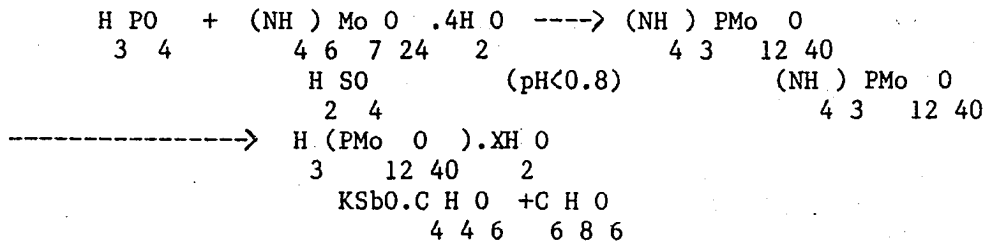
Phosphorus is an abundant and one of the most important element. In sea water it occurs in several chemical forms, both organic and inorganic. Filtration through a membrane filter having a pore size of about 0.5 um (selection of this pore size is purely arbitrary) is generally used to distinguish between particulate and dissolved forms. Phosphorus present in the filtrate is termed as 'dissolved phosphorus' and will consist of not only that fraction which is 'truly' dissolved but also that in colloidal form and very fine particulate material. The dissolved phosphorus is further divided into two fractions: (a) that which reacts rapidly with an acid molybdate reagent under conditions sufficiently mild to prevent hydrolysis of condensed phosphates or 'labile organic phosphorus', is termed reactive phosphorus - this fraction closely corresponds to inorganic orthophosphate (b) the remaining fraction is called dissolved combined phosphorus, or dissolved organic phosphorus.

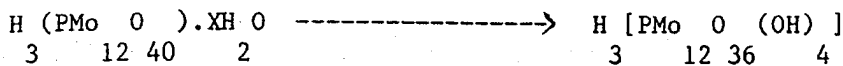
Dissolved inorganic phosphate is present in seawater as ions of orthophosphoric acid  $H_3PO_4$ . About 87% is present as  $HPO_4^{2-}$ , 10% as  $PO_4^{3-}$  and the remaining as  $H_2PO_4$ .

ii) Outline of the method

All the methods for the determination of reactive phosphate in seawater are based on the reaction of the orthophosphate ions with acidified molybdate reagent to yield 12-molybdophosphoric acid and its subsequent reduction to an intensely coloured blue complex "Molybdenum blue", the absorbance of which is measured spectrophotometrically at 880nm against a reagent blank. Ascorbic acid is used as the reducing agent. To obtain a rapid development of colour and to suppress the interference from silicate it is important that the final pH is <1 (-0.8). In order to develop the colour rapidly and to produce a stable complex, antimony ions are introduced in the reagent so that the bluish complex contains antimony and phosphorus in the ratio 1:1 by atoms.

ii) Reaction's





iv) Range: 0.1 - 12  $\mu\text{mol.PO}_4^{3-}$  .1

v) Accuracy:  $\pm 0.02 \mu\text{mol.PO}_4^{3-}$  .1

vi) Interferences:

a) Substances capable of forming heteropoly blue complex with molybdate, like silicate and arsenate may cause interferences if due precautions are not taken. According to Murphy & Riley (1962) silicate concentrations upto  $10 \text{mg.l}^{-1}$  have no effect on phosphate determination. Also the effect of silicate depends on pH so that if the pH is maintained low (<0.8) and if the absorbance is measured after 10 minutes, there is practically no interference from silicate. Arsenate occurring at natural seawater concentrations, i.e.,  $0.01 - 0.03 \mu\text{mol.l}^{-1}$  has no effect on the phosphate determination, however, interference due to higher concentration of arsenate can be eliminated by reducing arsenate to arsenite with metabisulphite before addition of molybdate reagent.

b) Hydrogen sulphide forms colloidal sulphur with greenish colour with molybdate reagent. Sulphide concentrations upto  $2 \text{mg.l}^{-1}$  do not interfere with phosphate determination, however, when the sulphide concentration is high the sulphide interference can be suppressed by (1) diluting the sample, if the phosphate also is high, or (2) by oxidizing sulphide with bromine water.

vii) Sampling

Samples for  $\text{PO}_4^{3-}$  determination should be collected preferably in glass bottles and analysed within 1/2 hour.

## 2. TOTAL PHOSPHORUS DETERMINATION

i) Principle

All the methods used for the total-P determination are based on the principle that all forms of combined phosphorus in seawater are first converted to orthophosphate which is subsequently analysed as above.

ii) Outline of the method

The water samples are autoclaved in closed bottles with alkaline persulphate and the resulting orthophosphate is analysed using acid molybdate as above.

## NITROGENOUS COMPOUNDS

The most important forms of inorganic nitrogen in seawater are ammonia (which consists of free ammonia and  $\text{NH}_4^+$  ions), nitrite and nitrate. In addition to inorganic nitrogen species, seawater contains wide range of organic nitrogen compounds both dissolved and particulate.

### DETERMINATION OF AMMONIA BY INDOPHENOL BLUE METHOD

#### i)) Principle

In a moderately alkaline solution, ammonia is made to react with hypochlorite to give monochloroamine  $\text{NH}_2\text{Cl}$  which, in the presence of phenol, catalytic amount of nitroprusside and excess hypochlorite gives indophenol blue via an intermediate quinone chlorimide as follows:

In order to minimize the production of dichloroamine and the decomposition of urea to ammonia, the formation of monochloroamine has to be carried out between pH 8 and 11. The use of high pH for the development of colour leads to the precipitation of calcium and magnesium compounds causing turbidity which affects the photometric measurements. This interference is overcome by using citrate buffer.

ii) Range:  $0.05 - 40 \mu\text{molNH}_4^{+ -1}$

iii) Interferences: Interference caused by the presence of amino acids, urea and nucleic acids at their normal concentrations in seawater is almost negligible. However, mercuric ions in concentration range  $2 - 40 \text{mg.l}^{-1}$  decrease the indophenol blue by about 20%.

### DETERMINATION OF NITRITE

#### i) Principle

All the methods for photometric determination of nitrite are based on the reaction of nitrite with an aromatic amine to form a diazonium compound which is then further coupled with another aromatic amine to form an azo dye. In most of the laboratories the method followed is that of Bendschneider and Robinson in which the diazotization and coupling are performed with sulphanilamide and N-(1-naphthyl)-ethylenediamine respectively at pH 3 - 4.

#### ii) Reactions

iii) Range: Beer's Law is obeyed in the range  $0.1$  to  $10 \mu\text{molN.l}^{-1}$ , however, above  $3 \mu\text{molN.l}^{-1}$  the samples should be diluted with distilled water.

iv) Interferences: Sulphide ions are reported to interfere in the nitrite determination. Thus when sulphide is suspected to be present in the samples, the gas should be expelled with nitrogen after the addition of the acid sulphanilamide reagent.

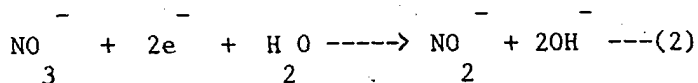
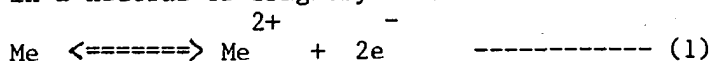
#### DETERMINATION OF NITRATE

Nitrate is the final oxidation product of nitrogen compounds in seawater and is considered to be thermodynamically the most stable oxidation level of nitrogen in the presence of oxygen.

##### i) Principle of determination

The most sensitive methods, for the determination of nitrate which are generally used are based on the reduction of nitrate to nitrite which is subsequently determined via the formation of azo dye. Nitrate occurs in seawater as ion and is not bound nor complexed. The first step in its determination is its reduction to nitrite. This reduction can be carried out either (i) homogeneously in which the samples and the reductor are in the same phase, ex., liquid, or (ii) heterogeneously in which the samples and the reductor are in different phases, ex. liquid and solid respectively. An example of homogeneous reduction is the method developed by Mulin and Riley (1955) in which hydrazine sulphate acts as the reductor in the presence of copper ions as catalyst. However, this method has several short-comings like (a) it is time consuming, (b) not quantitative, and (c) highly dependant on external conditions. The most widely used method for the determination of nitrate in sea water is by its heterogeneous reduction to nitrite in a column containing cadmium (filings or granulated) coated with copper or mercury (amalgamated). The conditions of reduction are adjusted so that nitrite is reduced to nitrate almost quantitatively and not reduced further.

In a neutral or slightly alkaline medium

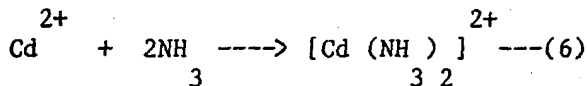
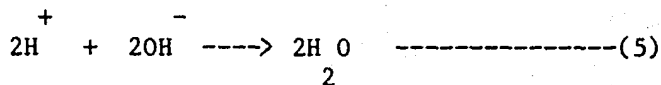
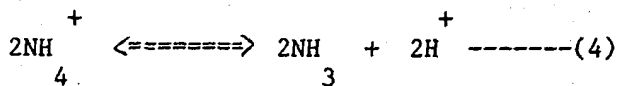


As is evident from eqs. (1) & (2) that the metal ions formed during the reduction of nitrate react with the hydroxyl ions to form a precipitate which is unwanted as it settles in the reductor column thereby reducing the speed of flow. Furthermore the reduction potential needed for the reduction of nitrate is pH dependent as is seen below

$$E = E^{\circ} - \frac{RT}{nF} \ln \left[ \frac{a_{\text{NO}_2} \cdot a_{\text{OH}}^2}{a_{\text{NO}_3}} \right] \quad \text{---(3)}$$

This implies that as the reaction proceeds the pH of the solution changes, especially in the vicinity of the reductor. To overcome both these drawbacks the sample is buffered with ammonium chloride prior to passing it

through the reductor. The buffer ammonium chloride acts as both complexant and buffer

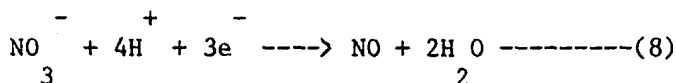
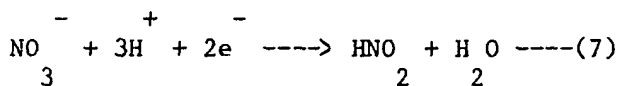


wherein the  $\text{OH}^-$  ions are neutralized by  $\text{H}^+$  ions and the metal  $\text{Cd}^{2+}$  ions are complexed with the ammonia to form a diammine complex.

The flow of the sample through the reductor column has to be controlled within certain limits so that nitrate is quantitatively reduced. Also, the nitrite, if any, originally present in the sample will pass through the reductor unchanged. Hence this amount must be subtracted from the total amount of nitrite measured.

#### ii. Limitations

The yield of the reduction depends on the metal used in the reductor, on pH of the solution, on the activity of the metal surface and on the flow rate. Reaction solutions that are too alkaline, use of inactive metal surfaces, or high flow rate will result in only partial reduction, while solutions which are too acidic, use of highly electronegative metal or too active surfaces will result in nitrate being reduced further than the nitrite step as seen below.



### DETERMINATION OF TOTAL-N

#### i) Principle

Determination of Total-N in seawater is based on the principle that all forms of organic nitrogen are oxidized to nitrate when autoclaved with strong oxidizing agent like potassium persulphate.

#### ii) Outline of the method

Seawater samples are autoclaved in closed glass bottles with potassium persulphate, and the resulting nitrate is analysed by cadmium reduction technique as described above.

References:

1. Riley, J.P. (1975). Analytical Chemistry of sea water. In 'Chemical Oceanography' (Riley, J.P. & Skirrow, G. eds.) vol.3, 2nd ed. Acad. Press Inc.(London) Ltd.
2. Koroleff, F. (1983). Determination of Nutrients. In 'Methods of sea water analysis' (K.Grasshoff, M. Ehrhardt & K. Kremling eds.) 2nd ed. Verlag Chemie, Weinheim.
3. Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31-36.
4. Mulin, J.B. and Riley, J.P. (1955). Anal. Chim. Acta 12, 464.