



# REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

DECEMBER 1991

## *Standard chemical methods for marine environmental monitoring*

*Reference Methods For Marine Pollution Studies No. 50*

Prepared in co-operation with



IOC



IAEA

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## PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it (1),(2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The Methods recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

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which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

This Reference Method was designed to provide the user with reliable techniques for the determination of ten chemical parameters of general application to basic oceanographic studies, whether at sea or within coastal lagoons and estuaries. It is interesting to note that, although techniques have existed for these parameters for more than half a century, the general analytical precision and accuracy of them has been poor. This is partly due to the ease with which samples may be contaminated during handling and the tendency (particularly with nutrients) for the analytes to break down or react during storage. Both issues are addressed in this manual but cannot be overstressed.

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- (1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.
  - (2) P. HULM: A Strategy for the Seas. The Regional Seas Programme: Past and Future, UNEP 1983.
  - (3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessments. UNEP 1990.

This first edition of the Reference Method for Marine Pollution Studies No. 50 was prepared in co-operation with the International Atomic Energy Agency (IAEA) and the Intergovernmental Oceanographic Commission (IOC). It includes comments received from the joint IOC/UNEP Group of Experts on Methods, Standards and Intercalibration (GEMSI) of GIPME who reviewed the method and tested it. The assistance of all those who contributed to the preparation of this reference method is gratefully acknowledged.

## CONTENTS

1.	Introduction	1
2.	Determination of pH	1
3.	Determination of dissolved oxygen	7
4.	Determination of hydrogen sulphide	15
5.	Determination of dissolved inorganic phosphate	20
6.	Determination of reactive silicate	25
7.	Determination of ammonia	29
8.	Determination of nitrite	33
9.	Determination of nitrate	36
10.	Simultaneous persulphate oxidation for the determination of total nitrogen and phosphorus compounds	42



## 1. INTRODUCTION

The marine analytical chemist is faced with two major problems. Firstly, to produce correct analytical results in the rather complicated seawater matrix; and secondly, to obtain representative samples from a highly variable environment over which he has no control. The latter problem is complicated by the fact that the constituents (dissolved or dispersed) in the sea have a three-dimensional pattern of distribution, i.e., they vary from place to place, with depth and with time because of physical and biogeochemical processes. In addition, the sample itself may drastically change its composition after having been enclosed in the sampler and removed from its natural environment. Therefore, the most refined techniques and skilled work on the part of the analyst will not produce automatically a representative value if the sampling procedure is influenced by significant errors.

To select an adequate sampling strategy (e.g., station plan, frequency of sampling, depth of sampling, etc.) it is necessary to understand what happens in the environment. This implies that the marine analytical chemist is forced to have a reasonable knowledge of the physical, chemical and biological processes prevailing in the area of interest. Furthermore, the analytical chemist should be familiar with a number of additional questions before he decides on a particular sampling programme; i.e., questions such as: (1) What is the optimum type of sampler for the different constituents? (2) How much sample volume is required for the analysis and for rinsing the bottles? (3) How should the subsampling and conservation of sample be arranged? (4) Are there systematic errors to be expected from the vessel, from the hydrowire or from the sampler itself?

It is beyond the scope and intention of this Manual to be comprehensive in the different aspects of sampling methods. It is, however, important to emphasize here that the sampling procedure is the first and often most essential step in the analysis. Therefore, a marine analytical chemist should be aware of the various potential errors and be able to estimate their effects before any field observations are started.

## 2. DETERMINATION OF pH

### 2.1 SCOPE AND FIELD OF APPLICATION

The determination of pH described here is performed electrometrically by means of ion specific electrodes (glass electrodes) with a silver-silver-chloride or calomel electrode as reference. The potentiometric procedure represents normal practice in oceanography given the ready availability of glass electrodes and reliable voltmeters with an impedance of more than  $10^{12}$  "ohms". Other techniques (e.g., spectrophotometric measurement with suitable indicators) are, therefore, not considered here.

## 2.2 REFERENCES

- BATES, R.G. (1973): Determination of pH, Theory and Practice, 2nd ed., John Wiley and Sons, Inc., New York.
- BATES, R.G. (1982): pH measurements in the marine environment. Pure and Applied Chemistry, 54, 230-232.
- DICKSON, A.G. (1984): pH scales and proton-transfer reactions in saline media such as sea water. Geochim et Cosmochim. Acta, 48, 2299-2308.
- GRASSHOFF, K. (1983): Determination of pH. In: GRASSHOFF, K., EHRHARDT, M. and KREMLING, K. (editors), Methods of Seawater Analysis. Verlag Chemie, Weinheim, 85-97.

## 2.3 PRINCIPLE

The pH is defined by the equation

$$\text{pH} = -\log a_{\text{H}^+},$$

where  $a_{\text{H}^+}$  is the activity of the hydrogen ions. The relationship of  $a_{\text{H}^+}$  with the hydrogen ion concentration ( $C_{\text{H}^+}$ ) is given by  $a_{\text{H}^+} = C_{\text{H}^+} \cdot \tau_{\text{H}^+}$  where  $\tau_{\text{H}^+}$  is the thermodynamic activity coefficient of hydrogen ion in the specific solution. This activity coefficient is a function of the solvent (e.g., water), the ionic strength, and the specific ions dissolved in the solvent.

The fundamental principle of the glass electrode is based on the fact that some special conductive glasses are able to build up a hydrated boundary layer at the glass surface when in contact with aqueous solutions. Because of ion exchange processes between the boundary layer and the solution, a membrane potential exists which is sensitive to  $a_{\text{H}^+}$  in the solution. The weak current in the glass is conducted by the alkali ions.

The potential of the glass electrode (indicator electrode) is measured against a pH-independent reference electrode (usual types are the silver-silver-chloride and calomel electrodes). However, since the electrode chain potential of this glass-reference electrode couple is not defined in absolute terms, the pH electrode couple must be calibrated with solutions of known hydrogen ion activities. The unknown  $\text{pH}_x$  value is then found from the equation:

$$\text{pH}_x = \text{pH}_s + (\epsilon_x - \epsilon_s)/Nf$$

where

$\text{pH}_s$  = pH of the standard solution,  
 $\epsilon_x$  and  $\epsilon_s$  = electrode chain potential in the test and standard, respectively,  
 $Nf$  = Nernst factor (slope of the pH couple at the working temperature in  $\text{mV} \cdot \text{pH}^{-1}$ ).

Usually, the pH meter automatically converts the measured potential into units of pH.



The presently described technique should generate useful data easily comparable with that of other authors for the purpose of providing supporting environmental information to contamination monitoring programmes. It should be noted, however, that there is a considerable debate in the scientific literature concerning the poor reproducibility of the liquid junction when calibrating the electrodes with low ionic strength NBS buffers and subsequently employing them for seawater which has a completely different major ion composition. An alternative seawater based buffer scale is available but is not as yet widely employed. pH values obtained with the two techniques are somewhat different and extreme caution should be employed when making thermodynamic interpretations of pH (NBS). For further information, the excellent review by Dickson (1984) should be consulted.

## 2.4 REAGENTS

The National Bureau of Standards (N.B.S.) has compiled a list of standard buffer solutions for pH calibration. Some suitable ones are listed in Table 1 together with their temperature dependencies.

### 2.4.1 Phosphate Standard (1:1)

3.402 g potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and 3.550 g disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) are dried at  $80^\circ\text{C}$ , dissolved in  $\text{CO}_2$ -free distilled water and diluted to a total weight of 1 kg. The solution contains  $0.0024 \text{ mol}\cdot\text{kg}^{-1}$  of each salt.

### 2.4.2 Phosphate Standard (1:3.5)

1.183 g potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and 4.321 g disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) are dried at  $80^\circ\text{C}$ , dissolved in  $\text{CO}_2$ -free distilled water and diluted to 1 kg. The solution contains  $0.008695$  and  $0.03043 \text{ mol}\cdot\text{kg}^{-1}$  of each salt, respectively.

### 2.4.3 Potassium hydrogen phthalate

10.211 g potassium hydrogen phthalate is dissolved in distilled water and diluted to 1 kg. The solution contains  $0.05 \text{ mol}\cdot\text{kg}^{-1}$ .

NOTE: All standard solutions should be prepared in  $\text{CO}_2$ -free distilled water (preferably prepared by boiling for a while and passing nitrogen gas through during the cooling) and should be stored in tightly stoppered glass bottles. They are stable for several weeks but should be discarded if they eventually become turbid.

Table 1: pH of NBS standard solutions

	0.05 mol·kg <sup>-1</sup> KH-phthalate	0.025 mol·kg <sup>-1</sup> KH <sub>2</sub> PO <sub>4</sub> 0.025 mol·kg <sup>-1</sup> Na <sub>2</sub> HPO <sub>4</sub>	0.008695 mol·kg <sup>-1</sup> KH <sub>2</sub> PO <sub>4</sub> 0.03043 mol·kg <sup>-1</sup> Na <sub>2</sub> HPO <sub>4</sub>
5°C	3.999	6.951	7.500
10°C	3.998	6.923	7.472
15°C	3.999	6.900	7.448
20°C	4.002	6.881	7.429
25°C	4.008	6.865	7.413
30°C	4.015	6.853	7.400

#### 2.4.4 Commercially available buffer solutions

These are readily available from a number of suppliers. They should be of very high quality (accurate to  $\pm 0.001$  pH units) and careful attention must be paid to the maker's instructions on storage and the calibration temperature.

## 2.5 APPARATUS

### 2.5.1 Electrodes

Combined electrodes, where the glass electrode and the reference electrode are combined in one shaft, should be used.

The reference electrode should be of the same type as the "pick-up electrode" in the glass electrode (symmetrical chain). The glass bulb should never dry out, i.e., the electrode should be immersed in seawater during pauses (or in distilled water during longer storage). Normally, the manufactures (e.g., W. Ingold or Metrohm) provide guidance on how their electrodes should be treated in order to produce reliable readings.

### 2.5.2 pH Meter

Numerous pH meters of good quality are commercially available. An instrument with digital display to at least two decimal places is preferred for work at sea. It should have controls for temperature, slope, and zero adjustment, and should be insensitive to slight changes of the line voltage.

### 2.5.3 Glass Bottles with glass stoppers of 50 - 100 cm<sup>3</sup> volume.

### 2.5.4 Water Bath and Measuring Cell

Since pH is dependent on temperature, measurements must be performed with thermostated samples. For high precision experiments, a measuring cell with connected heat exchanger is recommended (Fig. 1). They are made of glass and require a water bath with a pump for external circulation of the thermostated water. Connecting tubes should be as short as possible in order to reach thermal equilibrium ( $\pm 0.2$  °C) quickly and to keep the sample volume small.

It is also general practice today to equilibrate the samples in a simple water bath. It offers the advantage of faster measurement (due to simultaneous thermal equilibrium of a greater amount of samples) but may cause problems during severe movements of the ship implying lower precisions in pH measurement.

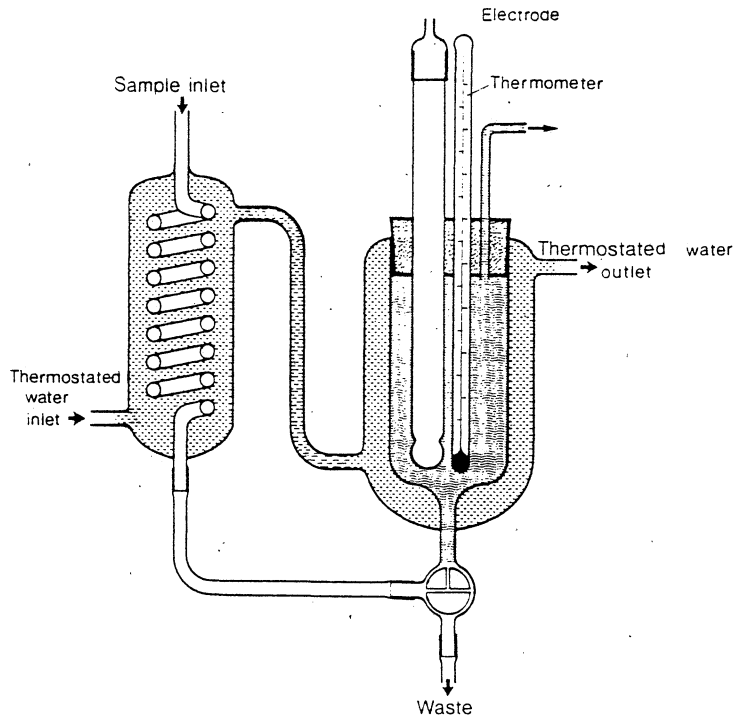


Fig. 1: Set-up for thermostated pH cell.

## 2.6 SAMPLING PROCEDURE

The same care and precautions as for oxygen sampling should be followed (see Section 3.) to minimize interaction with the carbon dioxide in the air. The sample is drawn via a hose from the water sampler (immediately after the oxygen sample) which should be inserted to the bottom of the pH glass bottles and then slowly withdrawn during the final stages of filling. At least one full volume of the pH bottle should be allowed to run over before stoppering. It is important that no air bubble is trapped under the stopper. Measurement of pH should be done within one hour after sampling.

## 2.7 METHOD

### 2.7.1 Calibration of pH Meter

Set the temperature control of the thermostat to 20 or 25 °C (selection depends on the water temperature and cooling facilities on board). Rinse the measuring cell carefully with distilled water and the standard solution (phosphate standard 1:1) before filling with the latter. Set the slope control of the pH meter (if any) to 98% of the theoretical slope and adjust the pH meter to the assigned value of the standard (Table 1) by the pH control. Drain the cell and fill it again with the Phosphate standard (1:1) solution. The reading should be within  $\pm 0.002$  pH units (or  $\pm 0.01$  if a reading of three (or two) decimal places is available at the pH meter).

Drain the cell again and rinse at least twice with distilled water and then twice with phthalate standard solution before finally filling it with this buffer. Set the pH meter to the theoretical value (Table 1) by means of slope control (if any) or otherwise with the temperature control (the latter is only allowed if calibration and measurement are performed at the same temperature). Drain and rinse the cell and fill it again with the 1:1 phosphate standard. If the pH reading deviates more than  $\pm 0.003$  units from the theoretical value then adjust by means of the pH control and repeat the calibration procedure also with phthalate standard solution.

After completion of the calibration procedure rinse the cell thoroughly with distilled water and keep it filled with seawater of about the salinity to be expected in the samples.

NOTE 1: Instead of the phthalate standard solution, the phosphate standard 1:3.5 can be used for calibration. This offers the advantage that the second calibration point is closer to the pH range of seawater, but has the disadvantage of being quite close to the first calibration point, set by the 1:1 phosphate standard. The use of the alkaline N.B.S. borax standard solution (about pH 9.2 at 25°C) is not recommended because of its instability as a result of carbon dioxide uptake.

NOTE 2: Samples from very cold waters can de-gas when warmed. If this appears to be a problem (for example in arctic, antarctic or deep-sea samples) samples should be kept cool and measurements made at a low, constant (e.g., 5°C) temperature.

### 2.7.2 Measurement of pH in seawater samples

Drain the cell and rinse at least twice with the sample. Fill the measuring cell completely (slowly through the heat exchanger; see Fig. 1) and let the sample reach constant temperature. The pH reading should be constant after about one minute if the electrode is working correctly. Proceed with the adjacent sample in uninterrupted order from surface to bottom or vice-versa.

When simply a water bath is used for thermal equilibrium, the electrode is directly immersed into the sampling bottles (remove the stopper just before pH measurement.) After pH reading is constant proceed with the adjacent sample without further rinsing of the electrode. (The remaining seawater film on the electrode from the previous measurement has been proved to be of negligible influence when the difference from one sample to another is only a few tenths of a pH unit. This method can be considered almost as good as the procedure described in Fig. 1.)

## 2.8 CALCULATION OF RESULTS

The measured pH value must be corrected for in situ conditions. The correction for the temperature difference between measurement conditions and those at the sample depth in the water can be calculated from:

$$\text{pH}_{\text{in situ}} = \text{pH}_{\text{measured}} + 0.0114 (t_2 - t_1) ,$$

where

$$\begin{aligned} t_1 &= \text{in situ temperature} \\ t_2 &= \text{measurement temperature} \end{aligned}$$

NOTE: The temperature coefficient is uncertain by about  $\pm 0.0010$ , i.e., the accuracy of the correction will be  $\pm 0.01$  pH units, if  $t_1$  and  $t_2$  differ by  $10^\circ\text{C}$ .

For samples taken from 500m or deeper a further correction for the difference in hydrostatic pressure should be applied. However, the effects of pressure on pH changes (i.e., pressure effects on the dissociation, ion pairing formation or activity coefficients of the different components) are far from understood. Because of these uncertainties and because of the fact that monitoring studies are performed, in general, in shallower water bodies, a correction factor will not be given here.

## 2.9 ESTIMATION OF PRECISION AND ACCURACY

It is clear that precision and accuracy of the pH measurement are dependent not only on the handling of samples and the quality of the standard solutions. The pH meter, the electrodes as well as its cable connections are also important factors. It is, therefore, essential that these are of good quality and maintained according to instructions given by the manufacturers. The precision (standard deviation) of the measurement can be, under optimal conditions, as good as  $\pm 0.002$  pH units may be obtained with proper equipment.

# 3. DETERMINATION OF DISSOLVED OXYGEN

## 3.1 SCOPE AND FIELD OF APPLICATION

The chemical determination of oxygen concentrations is based on the well-known method first proposed by Winkler in 1888, and has been modified for the determination of oxygen in seawater by several authors. The chemical procedure outlined here mainly follows the description by Grasshoff (1983). For the application of electrochemical methods, which are of special interest for a continuous record of oxygen consumption or oxygen profiles, the reader is referred to the literature (e.g. Whitfield and Jagner (ed.) "Marine Electrochemistry", John Wiley and Sons, 1981).

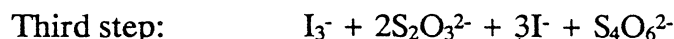
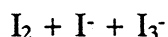
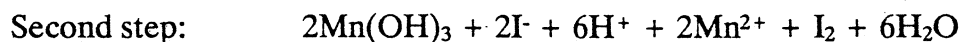
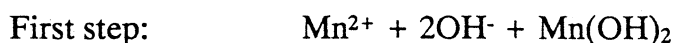
## 3.2 REFERENCES

GRASSHOFF (1983): Determination of oxygen. In: GRASSHOFF, K., EHRHARDT, M., KREMLING, K. (EDITORS), Methods of Seawater Analysis, Verlag Chemie, Weinheim, p. 61-72.

## 3.3 PRINCIPLE

The Winkler method is based on the following principle: Manganese (II) ions are precipitated in an alkaline solution as manganese (II) hydroxide, which is oxidized by the dissolved oxygen (in a known amount of seawater) to manganese (III) hydroxide (first step of the procedure). After complete fixation of oxygen, the hydroxide is dissolved with acid. The liberated manganese (III) ions then react with previously added iodide ions. These are oxidized to iodine, which in turn forms a complex with the surplus iodide, thus protected from partial evaporation (second step of the procedure). In the third step of the method, the iodine (or  $I_3$  produced is then determined by titration with thiosulphate.

The stoichiometric equations for the reactions described above are:



The equations show that one mole (two atoms) of oxygen is equivalent to four moles of thiosulphate.

## 3.4 REAGENTS

### 3.4.1. Manganese (II) chloride solution

40 g  $MnCl_2 \cdot 5H_2O$  is dissolved in distilled water and diluted to 100 cm<sup>3</sup>.

### 3.4.2 Alkaline Iodide solution

60 g KI and 30 g KOH are dissolved separately in a minimum amount of distilled water and combined. The solution is diluted to 100 cm<sup>3</sup> with distilled water. (If the saturated solution contains some residual salts add some more distilled water for dissolution.)

### 3.4.3 Sulphuric acid (1:1)

50 cm<sup>3</sup> concentrated sulphuric ( $d = 1.84 \text{ g} \cdot \text{cm}^{-3}$ ) acid is added slowly to 50 cm<sup>3</sup> distilled water with constant mixing and cooling.

#### 3.4.4 Sodium thiosulphate stock solution (ca. 0.2 mol. dm<sup>-3</sup>)

49.5 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O is dissolved and made up to 1000 cm<sup>3</sup> with distilled water. This stock solution is diluted with distilled water 1:10 for use; i.e. 100 cm<sup>3</sup> of the stock solution are diluted with distilled water to a total volume of 1000 cm<sup>3</sup> to give around 0.02 mol·dm<sup>-3</sup>.

#### 3.4.5 Iodate standard

Exactly 325 mg (0.0833·10<sup>-2</sup> mol) KH(IO<sub>3</sub>)<sub>2</sub> or 356.7 mg (0.1667·10<sup>-2</sup> mol) KIO<sub>3</sub>, after drying at 115°C to constant weight, is dissolved in distilled water and diluted to 1000 cm<sup>3</sup>. The solution has an "oxidation concentration" of 0.0100 mol·dm<sup>-3</sup> of electrons.

#### 3.4.6 Starch solution

1 g soluble starch is dissolved in 100 cm<sup>3</sup> distilled water and heated quickly until the solution becomes clear (the solution may be stabilized with 1 cm<sup>3</sup> phenol). Instead of this solution, a commercially available zinc starch solution (readily dissolvable in water) may be used.

### 3.5 APPARATUS

#### 3.5.1 Glass bottles (ca. 50 cm<sup>3</sup>)

A special type of bottle should be used for the subsampling of oxygen samples (see Fig. 2). Only glass stoppers should be used, and each bottle must have its own stopper. The volume of each stoppered bottle must be determined by weighing the bottle empty and filled with distilled water to  $\pm 0.05$  g. Measure the water temperature immediately after weighing in order to apply the appropriate water density for calculating the bottle volume.

#### 3.5.2 Reagent dispenser

Automatic or semi-automatic syringe pipettes (with long plastic or stainless steel tips) should be used for reagent additions. They do not require filling via the dispenser nozzle and allow swift additions. A fixed set-up of a double reagent dispenser is shown in the diagramme of Fig. 3.

#### 3.5.3 Precision burette (10 cm<sup>3</sup>)

A calibrated piston burette (hand-operated or motor-driven) with a reading to 0.01 cm<sup>3</sup> should be used for titration. The piston burette offers the advantage of better precision, higher speed of work and is less fragile in comparison to ordinary burettes.

The titration procedure can be optimized by positioning the whole equipment in a box, as illustrated in Fig. 4. The equipment can also easily be transported without risk of damage.

### 3.6 SAMPLING AND OXYGEN FIXATION PROCEDURES

The oxygen sample should be the first sample drawn from the water sampler, and the subsampling should be carried out as soon as possible after the sampler has been recovered (in oxygen deficient areas, oxygen subsampling should be carried out starting with the bottom sampler). For this purpose the 50 cm<sup>3</sup> glass bottles are unstoppered, and the nozzle tube of the sampler is inserted to the bottom of the sample bottle. (The nozzle should be preferably of transparent tubing, sufficiently narrow to ensure that the sample stream carries away any air bubbles in the tube, but wide enough to fill the bottle at a moderate speed). The bottles flushed with at least twice the volume of the bottle (ca. 100 cm<sup>3</sup>). At the final stages of filling the tube is carefully removed from the bottle. The bottle must be brim full. If air bubbles have nevertheless been trapped on the walls of the bottle, they should be driven out by gently tapping at the bottle.

The reagents are added simultaneously (0.5 cm<sup>3</sup> each of manganese (II)-chloride and alkaline iodide solution) with the special dispenser (see Fig 3). the syringe pipette tips should extend deep into the bottle. The tips are slowly withdrawn while the reagents are added. Then the stopper is inserted avoiding the trapping of air bubbles. The bottles are shaken vigorously for about 1 min to achieve the chemical fixation of oxygen. The precipitate is then allowed to settle (ca. 10-20 min) before analysis is continued.

Oxygen samples may be stored up to 10-12 h after addition of the reagents. The bottles, however, should be kept in the dark and at constant temperature. If titration cannot be achieved within 12 hours, or, if a large temperature difference exists between subsamples and the (e.g. air-conditioned) laboratory, the bottles should be kept under water (in order to prevent contamination from atmospheric oxygen by expansion and contraction of the samples).

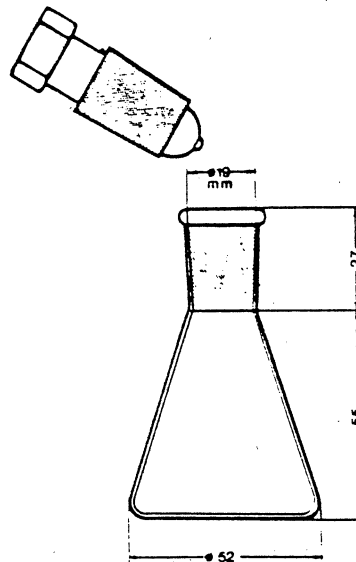


Fig. 2: Recommended subsampling bottle for the determination of oxygen (ca. 50 cm<sup>3</sup>; Duran glass, available from Schott, F.R.G.)



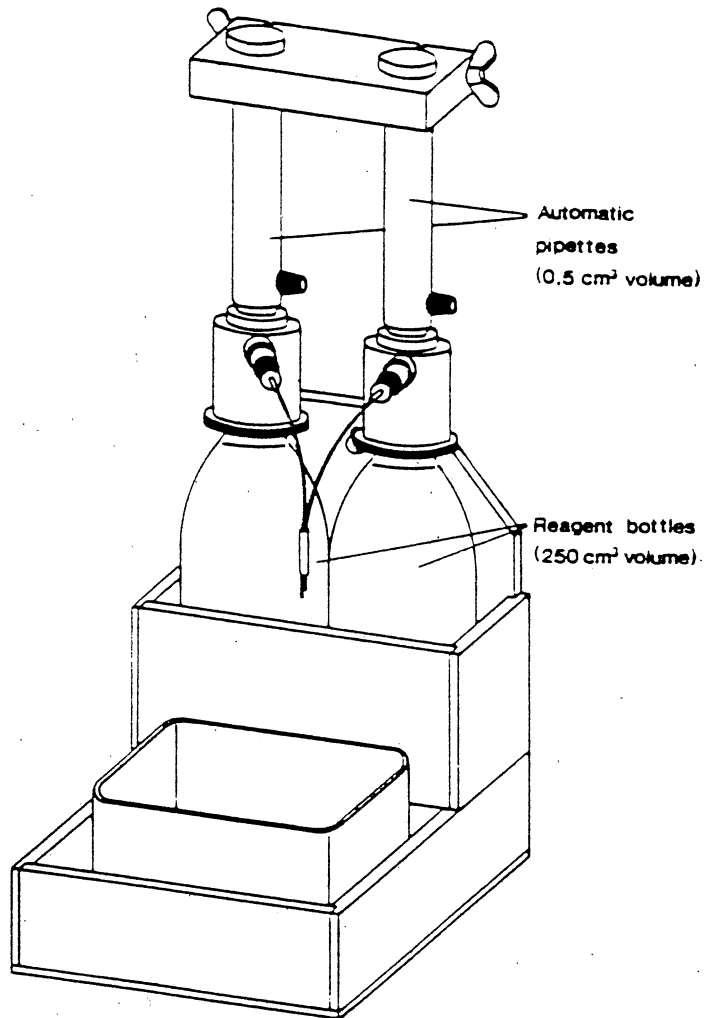


Fig. 3: Double reagent dispenser for oxygen reagents (available from Brand, F.R.G.)

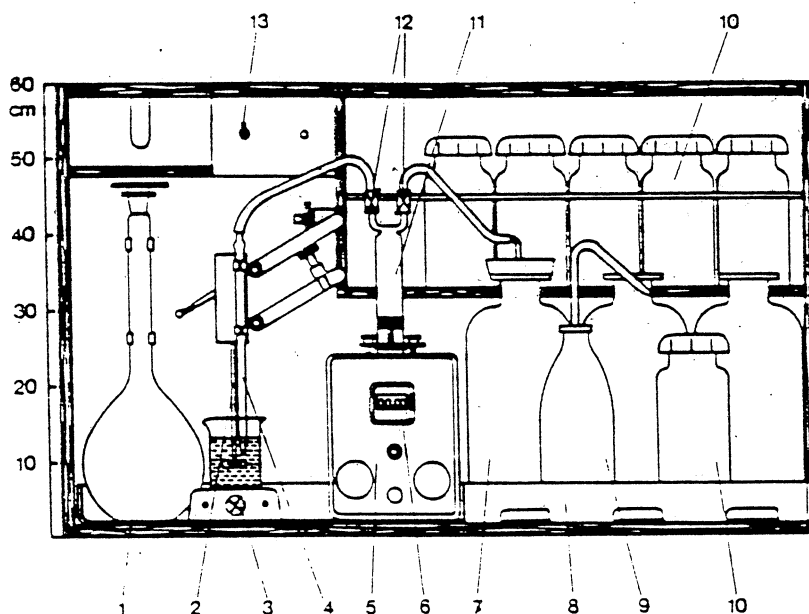


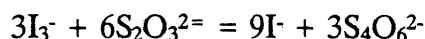
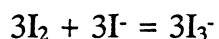
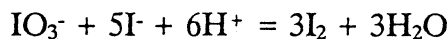
Fig. 4: Complete set-up for oxygen determination:

1 1 dm<sup>3</sup> volumetric flask; 2 stirrer; 3 illumination from below; 4 burette tip; 5 burette; 6 digital reading; 7 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> bottle; 8 plastic lining; 9 splashing bottle; 10 reagent bottles for H<sub>2</sub>SO<sub>4</sub>, KOH+KI, MnCl<sub>2</sub>; 11 burette cylinder; 12 valves; 13 main switch.

### 3.7 METHOD

#### 3.7.1 Standardization of the thiosulphate solution

The exact titre of the sodium thiosulphate is determined by titration against the iodate standard (reagent 3.4.5). This standardization is based on a quantitative co-proportionation reaction (only in acidic solutions) of iodide with iodate resulting in the formation of iodine. The next steps - the fixation of the liberated iodine and subsequent titration with thiosulphate - are identical with the second and third step described in paragraph 3.3. The stoichiometric equations involved in the standardization are:



For the standardization procedure (with the equipment shown in Fig. 4) place about 80 cm<sup>3</sup> distilled water in 150 cm<sup>3</sup> titration beaker and add separately 1 cm<sup>3</sup> sulphuric acid (1:1), 0.5 cm<sup>3</sup> alkaline iodide solution and 0.5 cm<sup>3</sup> manganese (II) chloride solution (see reagents 3.4.1-3.4.3). Mix thoroughly after each addition. (Do not add manganese(II) chloride solution before alkaline iodide solution!) Then add 10 cm<sup>3</sup> (with the calibrated pipette) of the iodate standard solution, and titrate the liberated iodine with the thiosulphate solution as described below for oxygen analysis. Note the volume of thiosulphate solution consumed.

### 3.7.2 Analysis of the sample

Dissolve the precipitated hydroxides (see 3.6) with sulphuric acid. For this, pipette 1 cm<sup>3</sup> sulphuric acid (1:1) into the sample bottle immediately after unstoppering. Insert the tip of the pipette almost to the level of the precipitate and then slowly withdraw without disturbing the precipitate. Stopper the bottle again and dissolve the precipitate by vigorous shaking. Then remove the stopper and rinse the film of the sample on it (containing liberated iodine) carefully into the titration beaker. Transfer the content of the bottle quantitatively into the 150 cm<sup>3</sup> beaker by rinsing twice with a few cm<sup>3</sup> of distilled water.

Titrate the liberated iodine with the thiosulphate solution to a light yellow colour (see Fig. 4). Then add 1 cm<sup>3</sup> starch indicator solution and continue (at an even speed) until the blue colour disappears (diffuse illumination from below and moderate room illumination facilitates detection of the endpoint). Note the volume of thiosulphate solution consumed.

*NOTE: The liberated iodine should be titrated quickly and without delay in order to prevent losses by evaporation. It is also important, for the same reasons, that the entire volume of the sample is titrated and representative aliquots are not taken.*

### 3.7.3 Determination of the reagent blank

Determine the overall reagent blank by the following procedure: Fill three oxygen bottles with distilled water or any seawater sample. Use the same water for all bottles, and take extreme care that the oxygen content in the three bottles is identical (see 3.6). Then add to the first bottle one set of fixation reagents; to the second two sets; to the third three sets. Proceed with titration as described above (3.7.2). Note the volumes of thiosulphate solution consumed.

### 3.8 CALCULATION OF RESULTS

#### 3.8.1 Titre of thiosulphate solution

The exact concentration of the thiosulphate solution, C, is determined from the titration with the iodate standard as

$$c = \frac{10 \text{ cm}^3 \cdot 0.01 \text{ mol dm}^{-3}}{V \text{ cm}^3} = \frac{0.1000}{V} \text{ mol dm}^{-3}$$

where V is the volume of thiosulphate solution consumed (see 3.7.1). Since C should be around  $0.02 \text{ mol} \cdot \text{dm}^{-3}$  (see 3.4.4), the factor f, with which the consumed volume (V) must be multiplied to get the corresponding amount of  $0.02 \text{ mol} \cdot \text{dm}^{-3}$  solution is then

$$0.02 = \frac{0.1000}{f \cdot V}$$

or

$$f = \frac{5}{V}$$

#### 3.8.2 Concentration of dissolved oxygen

One mole of thiosulphate corresponds to 1/4 mole of oxygen (or 8 g; see 3.3). Therefore,  $1 \text{ cm}^3$  of  $0.02 \text{ mol} \cdot \text{dm}^{-3}$  thiosulphate solution corresponds to 0.160 mg oxygen or  $0.112 \text{ cm}^3$  oxygen (at  $0^\circ\text{C}$  and normal pressure). If a  $\text{cm}^3$  is the amount of thiosulphate solution used to titrate a sample and b  $\text{cm}^3$  is the volume of the oxygen bottle (actually (b-1) will be employed in the formula shown below in order to allow for the added volume of added reagents), then the total amount of oxygen in the water sample (enclosed in the oxygen bottle) is:

$$a \cdot f \cdot 0.160 \text{ mg O}_2$$

or

$$a \cdot f \cdot 0.112 \text{ cm}^3 \text{ O}_2$$

Referred to a sample volume of  $1 \text{ dm}^3$ , the concentration is

$$\frac{a \cdot f \cdot 0.112 \cdot 10^3}{b-1} \text{ cm}^3 \cdot \text{dm}^{-3} \text{ O}_2$$

(For convenience a "bottle factor"  $F = 112/(b-1)$  may be calculated and listed together with the bottle number. The final calculation is then  $a \cdot f \cdot F = \text{cm}^3 \cdot \text{dm}^{-3} \text{ O}_2$ ).

NOTE: In compliance with SI units it is recommended to report the oxygen concentrations in  $\text{mmol} \cdot \text{m}^{-3}$  in which case these values should be multiplied by 44.66.

#### 3.8.3 Reagent blank value

The apparent amount of oxygen in each bottle (from the three blank titrations; see 3.7.3) is calculated as described above; but a correction is applied for the

additional volume of the double and triple sets of reagents. The results are plotted on a graph (apparent concentration against number of sets). The slope (if any) of the resulting straight line gives the reagent blank which must be subtracted from the estimated oxygen concentration of the sample (it is convenient to calculate the blank correction for a sample volume of 1 dm<sup>3</sup>, assuming an average value of the oxygen bottles). If the reagent blank has a higher concentration of 0.1 cm<sup>3</sup>.dm<sup>-3</sup> O<sub>2</sub>, the reagents should be discarded and new ones prepared.

### 3.9 ESTIMATION OF PRECISION AND ACCURACY

The accuracy in oxygen analysis is very difficult to ascertain since the major sources for systematic errors originate from the sampling and subsampling procedures. The effect of possible outgassing of oxygen during retrieval of the sampler, for example, can hardly be estimated and can only be minimized by proper equipment and by a careful sampling routine.

If considerable care is taken to avoid handling errors, the precision (standard deviation) for the remaining procedure can be as good as  $\pm 0.02$  cm<sup>3</sup>.dm<sup>-3</sup> O<sub>2</sub> for concentrations of less than 2 cm<sup>3</sup>.dm<sup>-3</sup> and  $\pm 0.04$  cm<sup>3</sup>.dm<sup>-3</sup> for higher oxygen concentrations.

## 4. DETERMINATION OF HYDROGEN SULPHIDE

### 4.1 SCOPE AND FIELD OF APPLICATION

Hydrogen sulphide is mainly found in the deep waters of stagnant basins, where it is formed by microbiological reduction of sulphate ions. The method described here is a well established colorimetric procedure which is based on the formulation of methylene blue. The method has been critically reviewed for seawater analysis by several authors (e.g., Cline, 1969; Fonselius, 1962, 1983) and is applicable for direct determination to concentrations up to about 50  $\mu$ mol.dm<sup>-3</sup> H<sub>2</sub>S. At higher concentrations the highly coloured solutions have to be diluted prior to the absorbance measurement.

It is also possible to determine the hydrogen sulphide content by a rather simple titration method. It has the advantage that it only requires the reagents for oxygen determination, however, suffers from the fact that it is not specific for H<sub>2</sub>S. Details of the procedure are given by Fonselius (1983).

### 4.2 REFERENCES

- CLINE, J.D. (1969): Spectrophotometric determination of hydrogen sulfide in natural water. *Limnology and Oceanography*, 14, 454-458.
- FONSELIUS, S.H. (1962): Hydrography of the Baltic deep basins. Fishery Board of Sweden, Ser. Hydrog. No. 13.

FONSELIUS, S.H. (1983): Determination of hydrogen sulphide. In: GRASSHOFF, K., EHRHARDT, M. and KREMLING, K. (editors), *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, 73-80.

### 4.3 PRINCIPLE

The method is based on the following principle: Hydrogen sulfide and dimethyl-p-phenylene diamine react in an acidified sample with ferric chloride as catalyst to form a heterocyclic (thiazine) dye called methylene blue. The light absorption by the coloured sample is then measured in 1, 5 or 10 cm cells at or close to 670 nm in a spectro- or filter photometer.

### 4.4 REAGENTS

#### 4.4.1 N,N-dimethyl-p-phenylene diamine dihydrochloride

2 g  $(\text{CH}_3)_2\text{N} \cdot \text{C}_6\text{H}_4 \cdot \text{NH}_2 \cdot 2 \text{HCl}(1,4)$ , a.g. is dissolved in 500 cm<sup>3</sup> of about 6 mol.dm<sup>-3</sup> HCl (the acid is prepared by diluting concentrated HCl with an equal amount of distilled water). The reagent is stable for several months.

#### 4.4.2 Ferric chloride solution

8 g FeCl<sub>3</sub> (a.g.) is dissolved in 500 cm<sup>3</sup> of about 6 mol.dm<sup>-3</sup> HCl (see above).

#### 4.4.3 Oxygen-free distilled water

A suitable volume of distilled water is boiled for at least 30 minutes. When the water cools down to room temperature, nitrogen gas must be continuously bubbled through the water. Since proper storage is difficult, this water should be prepared just before use.

#### 4.4.4 Sulphide stock solution (3.12 μmol.cm<sup>-3</sup>)

A few crystals of Na<sub>2</sub>S.9H<sub>2</sub>O (a.g.) are washed quickly with distilled water by squirting from a washing bottle. The crystals are immediately dried with filter papers and placed in a pre-weighed glass-stoppered weighing glass. About 0.750 g is weighed and dissolved in oxygen-free distilled water (added by means of a siphon) to 1000 cm<sup>3</sup> in a volumetric flask. The solution should be stored in an amber glass bottle which has been flushed with nitrogen gas before filling. To prevent any penetration from atmospheric oxygen, the stock solution should be covered by a thin layer of paraffin oil. The solution is then removed by means of a glass tube which is inserted (through the stopcock) nearly to the bottom of the bottle.

#### 4.4.5 Sulphide working solution ( 0.16 μmol.cm<sup>-3</sup>)

25 cm<sup>3</sup> sulphide stock solution are pipetted into a 500 cm<sup>3</sup> volumetric flask containing oxygen-free distilled water. The flask is filled up to the mark with the same

water. The solution should be prepared just before use and is stable for only about 30 minutes. Its accurate concentration is determined by the titration procedure described below.

#### 4.4.6 Potassium iodide, KI (a.g.), crystals

4.4.7 The sodium thiosulphate solution ( $0.02 \text{ mol}\cdot\text{dm}^{-3}$ ), the potassium iodate solution, the sulphuric acid solution (1:1), and the starch solution which are used for standardization of the sulphide working solution are the same solutions as described for the determination of oxygen (see 3.4).

### 4.5 APPARATUS

#### 4.5.1 Glass bottles (ordinary 50 cm<sup>3</sup> "oxygen bottles")

The same type of bottles (volume calibrated) as described for oxygen determination should be used (see Fig. 2).

4.5.2 Two automatic syringe pipettes, adjusted to a volume of  $0.5 \text{ cm}^3$  (commercially available from companies such as Brand or Eppendorf.)

4.5.3 Spectro- or filter photometer, with a filter at or close to 670 nm; with 1 cm, 5 cm or 10 cm cells.

### 4.6 SAMPLING

The samples must be collected by water samplers completely made of plastic or at least plastic coated on the inside, since the sulphide reacts with metal. Subsampling should be performed in the same way as described for oxygen sampling under paragraph 3.6. (i.e., with "oxygen bottles", and flushing by at least once the volume before final filling). It is also recommended to start with oxygen sub-sampling first. Only in case of pure white precipitate after oxygen reagent addition, existence of  $\text{H}_2\text{S}$  in the sample is possible).

If the samples cannot be analyzed on board the ship, they may be preserved by adding  $1 \text{ cm}^3$  of zinc acetate (or chloride) solution which precipitates the sulphide as zinc sulphide. The samples should be kept in a dark place until the other reagents are added later on.

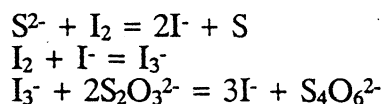
### 4.7 METHOD

#### 4.7.1 Calibration

4.7.1.1 Standardization of the thiosulphate solution. Proceed as described for the analysis of oxygen (paragraphs 4.7 and 4.8).

4.7.1.2 Standardization of the sulphide working solution. This standardization is based on the quantitative reduction of iodine (available in excess) by the hydrogen

sulfide (in acid solutions only), and on the subsequent titration of the remaining iodine with thiosulphate (see also 3.7):



The standardization must be carried out immediately after preparation of the sulphide working solution (4.4.5) and together with the preparation of the photometric standard samples (4.7.1.3, see below).

To each of six Erlenmeyer flasks (150 or 200 cm<sup>3</sup> with glass stoppers) add 10 cm<sup>3</sup> distilled water and around 2 g potassium iodide (4.4.6). Then pipette into each flask 10.00 cm<sup>3</sup> iodate solution and 1.0 cm<sup>3</sup> sulphuric acid (see reagents 4.4.7). Into three of the flasks pipette 50 cm<sup>3</sup> of the sulphide working solution, and to the other three flasks add 50 cm<sup>3</sup> distilled water. Set all flasks aside (in a dark place) for about 10 min, then begin titration with thiosulphate using starch as indicator in the same way as described under section 3.7.

From the stoichiometric equations involved (see above) it is obvious that 2 moles Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> correspond to 1 mole H<sub>2</sub>S; i.e., 1 cm<sup>3</sup> of the 0.02 mol.dm<sup>-3</sup> thiosulphate solution corresponds to 1·10<sup>-5</sup> mole hydrogen sulphide (or 0.22414 cm<sup>3</sup> H<sub>2</sub>S at 0 °C and normal pressure). Therefore, the H<sub>2</sub>S concentration of the working solution is calculated from the formula:

$$\underline{C} \text{ (cm}^3\text{.dm}^{-3}\text{)} = \frac{(a-b) \cdot f \cdot 0.22414 \cdot 10^3}{50} = \frac{(a-b) \cdot f \cdot 224.14}{50}$$

$$\underline{C} \text{ (}\mu\text{mol.dm}^{-3}\text{)} = \frac{(a-b) \cdot f \cdot 10^{-5} \cdot 10^3 \cdot 10^6}{50} = \frac{(a-b) \cdot f \cdot 10^4}{50}$$

where

a = cm<sup>3</sup> thiosulphate for solutions with no added sulphide (mean of the titrations),  
 b = cm<sup>3</sup> thiosulphate for solutions containing sulphide (mean of the titrations),  
 f = factor for the thiosulphate solution (see also 3.7).

**4.7.1.3 Photometric standard samples.** From the sulphide working solution (4.4.5) prepare a standard series in the following way. To 100 cm<sup>3</sup> volumetric flasks (purged with nitrogen gas) add by means of pipette or burette the following volumes and fill up with oxygen-free distilled water to the 100 cm<sup>3</sup> mark (before mixing purge some nitrogen into the residual volume of the flask again). Then the standard concentrations are:

$$\begin{aligned} 4.0 \text{ cm}^3 &= 6.3 \mu\text{mol}\cdot\text{dm}^{-3} \text{ S}^{2-} \\ 8.0 \text{ " } &= 12.5 \text{ " " " } \\ 12.0 \text{ " } &= 18.7 \text{ " " " } \\ 16.0 \text{ " } &= 25.0 \text{ " " " } \\ 20.0 \text{ " } &= 31.2 \text{ " " " } \end{aligned}$$

Correct the approximate sulphide concentrations according to the value found in the standardization procedure described above (4.7.1).



Add immediately (with the syringe pipettes) 1 cm<sup>3</sup> of each of the sulphide reagents (reagents 4.4.1 and 4.4.2) and mix thoroughly. After 60 min, measure the standard samples against the blank at 670 nm in 1 cm or 5 cm cells depending on the colour intensity. From the results prepare a calibration curve by plotting the concentrations (minus blank) as abscissa against the absorbances which should be a straight line passing through the origin.

#### 4.7.2 Analysis of the sample

Add the reagents, 0.5 cm<sup>3</sup> dimethyl-p-phenylene-diamine and 0.5 cm<sup>3</sup> ferric chloride (4.4.1 and 4.4.2) to the sample, preferably with syringe pipettes, immediately after the subsampling procedure. Let the pipette tips extend to the bottom of the bottle. Insert the stopper avoiding air bubbles and mix thoroughly. After 60 min, measure the absorbance against H<sub>2</sub>S-free distilled water at 670 nm using 1 cm cells for high concentrations, and 5 or 10 cm cells for low concentrations. The blue colour intensity which develops within a few minutes is constant for at least 24 h.

Blank values are usually negligible. Determine the value by adding the reagents to distilled water or filtered seawater and measure this against the same water without reagents.

If the sulphide concentration of the sample exceeds 50 μmol·dm<sup>-3</sup>, dilute the blue colour solution with distilled water, and take account of the dilution factor when calculating the results.

### 4.8 CALCULATION OF RESULTS

The concentration of hydrogen sulphide is determined from the standard curve as described in section 4.7.1.3. For routine analysis calculate the slope  $b$  of the calibration curve (for the individual cell length used) which follows the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

Then the sulphide concentration of the sample is obtained from:

$$C (\mu\text{mol. dm}^{-3}) = (A_s - A_{bl})/b$$

where  $A_s$  and  $A_{bl}$  are the absorbances of the sample and the blank, respectively.

### 4.9 ESTIMATION OF PRECISION AND ACCURACY

Systematic errors in the analysis described above mainly originate from difficulties in preparing accurate concentrations of the standard sulphide concentrations. This error, however, is estimated being below 2%. The precision of the method (from replicate measurements at a concentration level of about 30 μmol·dm<sup>-3</sup>) can be given as about  $\pm 1\%$ .

## 5. DETERMINATION OF DISSOLVED INORGANIC PHOSPHATE

### 5.1 SCOPE AND FIELD OF APPLICATION

The present methods in the analysis of inorganic phosphate in seawater follow essentially the colorimetric method by Murphy and Riley (1962) which is based on the formation of a highly coloured blue phosphomolybdate complex. The modified procedure described here mainly follows the method outlined by Koroleff (1983). The method shows no measurable effects from salinity but interference may be observed with relatively high concentrations of silicate, arsenate or hydrogen sulphide. The absorbances are proportional to the phosphate concentrations up to about  $28 \mu\text{mol}\cdot\text{dm}^{-3}$  when measured in a 1 cm cell.

### 5.2 REFERENCES

KOROLEFF, F. (1983): Determination of phosphorus. In: GRASSHOFF, K., EHRHARDT, M. and KREMLING, K. (editors), *Methods of Seawater Analysis*, Verlag Chemie, Weinheim, 125-139.

MURPHY, J. and RILEY, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31-36.

### 5.3 PRINCIPLE

The phosphate ions in the sample react in acidic solution with ammonium molybdate to yield a phosphomolybdate complex. This heteropoly acid is reduced by ascorbic acid with trivalent antimony ions as catalyst to a blue-coloured complex (with molar absorptivity of about 22,700), the absorbance of which is then measured in a spectro- or filter photometer at 882 nm. In order to obtain a rapid colour development and to depress the interference of silicate, it is important that the final reaction pH is less than 1, and that the ratio between sulphuric acid (in  $\text{mol}\cdot\text{dm}^{-3}$ ) and molybdate is kept between 2 and 3 per cent.

### 5.4 REAGENTS

#### 5.4.1 Sulphuric acid ( $4.5 \text{ mol}\cdot\text{dm}^{-3}$ )

$250 \text{ cm}^3$  concentrated acid ( $d = 1.84 \text{ g}\cdot\text{cm}^{-3}$ ) are slowly added, under cooling and mixing, to about  $700 \text{ cm}^3$  distilled water. Finally, the volume is adjusted to  $1000 \text{ cm}^3$  with distilled water.

#### 5.4.2 Ammonium heptamolybdate solution

$9.0 \text{ g}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  is dissolved in about  $90 \text{ cm}^3$  distilled water and diluted to  $100 \text{ cm}^3$ . The solution should be stored in a polyethylene bottle and should be renewed if any precipitation occurs.

#### 5.4.3 Potassium antimonytartrate solution

3.25 g  $K(SbO)C_4H_4O_6$  is dissolved in distilled water and diluted to 100 cm<sup>3</sup>. The solution should be renewed if any precipitation occurs.

#### 5.4.4 Mixed reagent

200 cm<sup>3</sup> sulphuric acid (5.4.1) is mixed under continuous stirring with 45 cm<sup>3</sup> molybdate solution (5.4.2). Then 5 cm<sup>3</sup> tartrate solution (5.4.3) is added. If the reagent is stored cool, it is stable for several months.

#### 5.4.5 Ascorbic acid solution

7.0 g  $C_6H_8O_6$  is dissolved in distilled water and diluted to 100 cm<sup>3</sup>. The solution is stable for at least a week (as long as it remains colourless) if stored in a dark bottle and in a refrigerator.

#### 5.4.6 Phosphate stock solution (10.0 $\mu\text{mol.cm}^{-3}$ )

Potassium dihydrogen-phosphate ( $KH_2PO_4$ , a.g.) is dried in an oven at 110°C. Then exactly 1.361 g is dissolved in distilled water and diluted to 1000 cm<sup>3</sup> in a volumetric flask. The solution is stable for at least several months. A long-term storage test performed by the authors at ca. 20°C proved that stock solutions of 10  $\mu\text{mol.cm}^{-3}$  phosphate, silicate, ammonia, nitrate and nitrate are even stable, within the analytical precision, for more than 5 years when stored in sealed glass-ampoules ("Standard Seawater" quality) or in tight plastic bottles (for silicate only!).

#### 5.4.7 Phosphate working solution (0.100 $\mu\text{mol.cm}^{-3}$ )

10.0 cm<sup>3</sup> of the stock solution are diluted with distilled water to 1000 cm<sup>3</sup> in a volumetric flask. This solution should be prepared daily.

### 5.5 APPARATUS

5.5.1 Glass stoppered bottles (ca. 50 cm<sup>3</sup>) or other suitable containers (e.g. polypropylene bottles, Nalgene type No. 2105-002) for subsampling.

5.5.2 Graduated cylinder (glass or plastic made) for the quick subsampling of 50 cm<sup>3</sup> nutrient sample portions (Fig. 5).

*NOTE: All glass and plastic ware to be used must be cleaned and should be reserved solely for phosphate analysis. The procedure used for cleaning must be tested for phosphate contamination (common detergents also usually contain phosphates, so that care must be taken when choosing cleaning chemicals). This is also necessary for the distilled or de-ionized water used in the cleaning and analytical procedure described here. It is also recommended to store the sample bottles, when not in use, in 0.1% v/v HCl solution (after the previous major cleaning) and to rinse them, before subsampling, three times with the sample solution.*

5.5.3 Automatic syringe pipettes of 1 cm<sup>3</sup> or 2 cm<sup>3</sup> volume for reagent additions (see 4.5.2).

5.5.4 Spectro-or filter photometer with filter at or close to 882 nm and cells of 1 cm, 5 cm and 10 cm length.

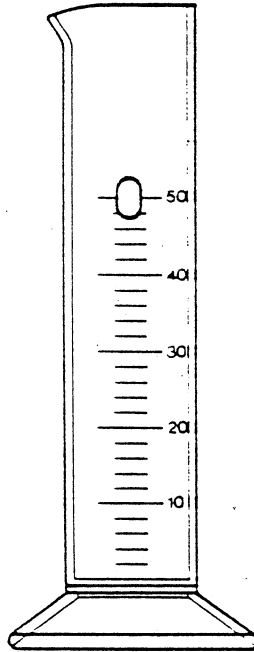


Fig. 5: Special type of graduated cylinder (glass or plastic made) for the quick subsampling of 50 cm<sup>3</sup> portions in nutrient analysis (with the hole as sample overflow).

## 5.6 SAMPLING

The subsampling of nutrients should be done in stoppered glass or special plastic bottles (e.g. polypropylene) in a convenient way by means of the graduated cylinder shown in Fig. 5. The samples should be stored in a cool dark place until analysis which should be started as soon as possible, certainly within 2 hours. If the analysis must be delayed for longer the samples have to be preserved. It must, however, be emphasized that no entirely satisfactory preservation method exists for samples collected for nutrient analysis. Prolonged storage in plastic (e.g. polythene) containers may result in rapid and considerable losses in phosphate from the samples unless these are shock frozen. The use of such materials is not recommended unless their properties for nutrient storage have been carefully checked. From the authors' experience and knowledge of literature, quick freezing (e.g. in a dry ice/acetone bath) with subsequent storage in a freezer may preserve the inorganic phosphate concentration at least for 30 days.

If a sample has a visible turbidity, it must be centrifuged or filtered immediately after collection. Centrifugation is difficult to perform on board. Filters, however, can affect samples by absorption or leaching of components, or the filtration characteristics of the filter will change after the deposition of the first layer of particulate matter. Unfortunately, only very few systematic studies have been performed on this subject so far. We have obtained good results with 0.4  $\mu\text{m}$  Nuclepore filters (cleaned in cold 6 mol.dm<sup>-3</sup> a.g. HCl for 3 days and rinsed with distilled water) or with glass fibre filters from Sartorius (Type S 13400, cleaned for a maximum of 4 hours in 0.1 mol.dm<sup>-3</sup> HCl). Filtration can be carried out by suction or with a pressure filtration system (an all-plastic filtration apparatus, for example, is available from sartorius (Type SM 16510)). In any case, however, the applied filtration procedures should be examined carefully and independently for each nutrient component of interest. Furthermore, analytical results from filtered samples should only be reported when "filtration blanks" are quoted.

## 5.7 METHOD

### 5.7.1 Calibration

Prepare a series of working standards from the phosphate working solution (5.4.7). To 100 cm<sup>3</sup> volumetric flasks add (by means of pipette or burette) the following volumes and fill up with distilled water to the 100 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

0.5 cm <sup>3</sup> of working solution	=	0.50 $\mu\text{mol dm}^{-3}$ PO <sub>4</sub> <sup>3-</sup>
1.0 " " " "	=	1.00 " "
2.0 " " " "	=	2.00 " "
3.0 " " " "	=	3.00 " "
4.0 " " " "	=	4.00 " "
5.0 " " " "	=	5.00 " "

To 50 cm<sup>3</sup> portions of these working standards add the reagents and follow the same procedure as described below for analysis of the sample (see 5.7.2). Prepare a reagent blank from the same volumes of distilled water and reagents. Plot the standard concentrations as abscissa versus the absorbances (corrected for the reagent blank). The calibration curve should be linear over the range of concentration.

### 5.7.2 Analysis of the sample

Transfer two 50 cm<sup>3</sup> portions of the sample to two reaction flasks (by means of the graduated cylinder, see Fig. 5). One of the portions is regarded as the sample, the other one as the turbidity blank. To each of the portions add 1.5 cm<sup>3</sup> (5.4.5) and to the sample add also 1.5 cm<sup>3</sup> of the mixed reagent ascorbic acid solution (5.4.4). Mix well between the additions. After 10 min (but within half an hour) measure the absorbance of the sample and the turbidity blank at 882 nm against acidified distilled water as reference (the turbidity blank may be negligible especially if filtered samples are employed, and can therefore be omitted at high phosphate concentrations and insignificant turbidity).

### 5.7.3 Interferences

There are some other ions occurring in seawater which potentially may interfere with the formation of the blue phosphomolybdic complex. The major interferences are briefly considered in the following paragraph.

It is well known that silicate and arsenate ions gradually form similar blue heteropoly acid complexes with molybdate ions. As a general rule, however, it may be stated that if the colour is measured after 10 min, there are practically no interferences caused by silicate (up to  $200 \mu\text{mol}\cdot\text{dm}^{-3}$ ) or arsenate (at "normal" total arsenic seawater concentrations of around  $25 \text{ nmol}\cdot\text{dm}^{-3}$ ). If measurements are performed after 30 min, for example,  $200 \mu\text{mol}\cdot\text{dm}^{-3}$  silicate gives - according to Koroleff (1983) - a net but almost negligible increase in absorbance of 0.003 in a 10 cm cell.

The analysis of phosphate may also be influenced by high concentrations of hydrogen sulphide. It has been found that sulphide concentrations up to about  $60 \mu\text{mol}\cdot\text{dm}^{-3}$  do not interfere with the phosphate determination. At higher concentrations antimony sulphide is formed (with greenish colour) when the acid-molybdate reagent is added to these waters. Since high sulphide concentrations are mostly associated with elevated phosphate concentrations, the effect of sulphide can be simply eliminated by diluting the sample with distilled water. If this step is not possible, the sulphide ions should be oxidized by adding bromine water drop by drop to an acidified sample (add  $0.2 \text{ cm}^3$  of  $4.5 \text{ mol}\cdot\text{dm}^{-3}$  acid to  $100 \text{ cm}^3$  sample). The excess bromine is then removed by passing a stream of air or nitrogen through the sample (for about 15 min) before commencing the phosphate determination.

## 5.8. CALCULATION OF RESULTS

The concentration of phosphate is determined from the standard curve as described in section 5.7.1. Calculate the slope  $b$  of the calibration curve (for the individual cell length used) which follows the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

Then the phosphate concentration of the sample is obtained from:

$$C (\mu\text{mol}\cdot\text{dm}^{-3}) = (A_s - A_{bl} - A_t)/b$$

where  $A_s$ ,  $A_{bl}$ , and  $A_t$  are the absorbances of the sample, the reagent blank, and the turbidity blank, respectively.

## 5.9 ESTIMATION OF PRECISION AND ACCURACY

Systematic errors in the phosphate analysis mainly originate from an improper cleaning of the glassware, from difficulties during the subsampling and from prolonged storage of the untreated samples. The precision of the method can be considered as being between  $\pm 2\%$  at a relatively high concentration level (about  $3 \mu\text{mol}\cdot\text{dm}^{-3}$ ) and  $\pm 15\%$  at the low level of around  $0.2 \mu\text{mol}\cdot\text{dm}^{-3}$ .

## 6. DETERMINATION OF REACTIVE SILICATE

### 6.1 SCOPE AND FIELD OF APPLICATION

The determination of dissolved silicon compounds is based on the formulation of a heteropoly acid when the sample is treated with a molybdate solution. This silicomolybdic acid (occurring in two isomeric forms) is then reduced to an intensely blue-coloured complex by adding ascorbic acid as a reductant. The colour is formed within 30 min, determined at 810 nm, and is stable for several hours.

The method outlined here mainly follows a procedure described by Koroleff (1983). It has a concentration range up to  $80 \mu\text{mol}\cdot\text{dm}^{-3}$  (samples with higher concentrations are diluted with distilled water), and a detection limit of about  $0.1 \mu\text{mol}\cdot\text{dm}^{-3}$  (in a 5 cm cell). However, only silicic acid and its dimer react with molybdate; therefore, the method gives only the amount of "reactive" silicate.

Interferences are observed from salinity which reduces the final colour intensity to some extent. Hydrogen sulphide can be tolerated up to about  $150 \mu\text{mol}\cdot\text{dm}^{-3}$  without problems.

### 6.2. REFERENCE

KOROLEFF, F. (1983): Determination of dissolved inorganic silicate. In: GRASSHOFF, K., EHRHARDT, M. and KREMLING, K. (editors), *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, 175-180.

### 6.3 PRINCIPLE

Similar to phosphate ions, "reactive" silicate forms, in acid solution, a heteropoly acid when treated with molybdate ions. This silicomolybdic complex exists in two isomeric forms ( $\alpha$ - and  $\beta$ -isomer) depending on the pH at formation. The isomers have different stabilities, are both yellow coloured, showing however, only low molar absorptivities in the range of about 1200 and 3300 absorbance units/mole for the  $\alpha$ - and  $\beta$ -isomer, respectively. Major analytical efforts were therefore dedicated to the development of methods in which the heteropoly acids are reduced to intensely blue-coloured complexes. The method outlined here involves the addition of oxalic acid (to avoid the reduction of any excess molybdate reagent and to eliminate the influence of any phosphate present), and the use of ascorbic acid as the reductant. The blue complex shows a molar absorptivity in ocean waters of about 19 000, with stability of the colour for at least several hours.

### 6.4 REAGENTS

All solutions should be prepared from reagents of known analytical grade only, using de-ionized or distilled water from an all-quartz apparatus. The silicate content

of this water should be checked (as a precaution) at frequent intervals according to the procedure described below, and the solutions should preferably be stored in plastic bottles.

#### 6.4.1 Sulphuric acid ( $4.5 \text{ mol.dm}^{-3}$ )

250 cm<sup>3</sup> concentrated acid ( $d = 1.84 \text{ g.cm}^{-3}$ ) are slowly added, under cooling and mixing, to about 700 cm<sup>3</sup> distilled water. Finally the volume is adjusted to 1000 cm<sup>3</sup> with distilled water. The solution should be stored in a polyethylene bottle.

#### 6.4.2 Ammonium heptamolybdate solution

20 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  is dissolved in about 80 cm<sup>3</sup> distilled water (in a plastic beaker) by moderate heating and diluted to 100 cm<sup>3</sup>.

#### 6.4.3 Mixed reagent

A measured volume of the molybdate solution (6.4.2) is added to an equal amount of the sulphuric acid (6.4.1) under mixing. **DO NOT** add acid to the molybdate solution. The solution should be stored in a polyethylene bottle protected from sunlight and should be stable for several months.

#### 6.4.4 Oxalic acid solution

10 g  $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$  is dissolved in 100 cm<sup>3</sup> distilled water. The saturated solution is stored in a plastic bottle and is stable indefinitely.

#### 6.4.5 Ascorbic acid solution

1.75 g  $\text{C}_6\text{H}_8\text{O}_6$  is dissolved in 100 cm<sup>3</sup> distilled water. The solution is stored in an amber glass bottle in a refrigerator. The reagent is effective as long as it remains colourless.

#### 6.4.6 Silicate stock solution ( $10 \mu\text{mol.cm}^{-3}$ )

Disodium hexafluoro silicate,  $\text{Na}_2\text{SiF}_6$ , is dried at 105°C to constant weight. Then 1.8807 g of the salt is dissolved in distilled water (in a plastic beaker) and diluted to 1000 cm<sup>3</sup> in a volumetric flask. The solution is immediately transferred to a polyethylene bottle (see also 5.4.6).

#### 6.4.7 Silicate working solution ( $0.5 \mu\text{mol.cm}^{-3}$ )

5 cm<sup>3</sup> of the stock solution (7.4.6) is diluted with distilled water to a final volume of 100 cm<sup>3</sup> in a volumetric flask, preferably made of plastic material. This solution must be renewed daily.

### 6.5 APPARATUS

6.5.1 Stoppered bottles made of plastic (e.g. polyethylene bottles of ca. 100 cm<sup>3</sup>).



6.5.2 Automatic syringe pipettes of 2 cm<sup>3</sup> volume for reagent additions.

6.5.3 Spectro or filter photometer with filter at or close to 810nm and cells of 1 cm, 5 cm and 10 cm length as required.

## 6.6 SAMPLING

The subsampling for silicate analysis should be performed with plastic bottles only (made of polyethylene, polypropylene or PVC bottles). The samples should be stored in a cool dark place and not longer for a day until analysis. If longer storage is necessary quick freezing of the samples is recommended (see the general remarks for phosphate sampling in paragraph 5.6). Special problems for silicate samples may arise from its tendency to polymerise when stored frozen, but this phenomenon is favoured more in less saline waters. Frozen samples should be thawed well before analysis, shaken to thoroughly mix them and visually inspected for any extraneous precipitate.

## 6.7 METHOD

### 6.7.1 Calibration

Prepare a series of working standards from the silicate working solution (6.4.7). To 100 cm<sup>3</sup> volumetric flasks add (by means of pipette) the following volumes and fill up with distilled water to the 100 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

0.5 cm <sup>3</sup> of working solution	=	2.50 μmol dm <sup>-3</sup> Si
1.0 " " " "	=	5.00 " "
2.0 " " " "	=	10.00 " "
5.0 " " " "	=	25.00 " "
10.0 " " " "	=	50.00 " "
15.0 " " " "	=	75.00 " "

To 50 cm<sup>3</sup> portions of this working standards add the reagents and follow the procedure outlined below for analysis of the sample (6.7.2). In addition, prepare a blank from the same volumes of distilled water and the reagents. The blank sample compensates for the silicate content in the reagents as well as in the distilled water used for preparation of the standard solutions. Plot the measured absorbances versus the standard concentrations (corrected for the reagent blank). The calibration curve should be linear over the entire range of concentration.

### 6.7.2 Analysis of the sample

Measure 50 cm<sup>3</sup> of the sample with a graduated cylinder (see Fig. 5) and transfer it into the plastic reaction bottle. Add 1.5 cm<sup>3</sup> of the mixed reagent (6.4.3) and mix well. After 10 - 20 min add 1 cm<sup>3</sup> oxalic acid (6.4.4) immediately followed by 1 cm<sup>3</sup> ascorbic acid (7.4.4). Mix well between the additions. Measure the absorbance after 30 - 40 min in a cell of suitable length at 810 nm against distilled water as reference.

As distilled water usually contains detectable amounts of silicate (of the order of  $0.2 \mu\text{mol.dm}^{-3}$  Si), the reagent blank for analysis of the sample must be determined in a different way than described for the calibration procedure. For this reason the blank is best prepared by carrying out the above procedure using  $50 \text{ cm}^3$  of distilled water. The absorbance is denoted  $A_{1.5}$ . Repeat the determination, but add only  $1.0 \text{ cm}^3$  of the mixed reagent (6.4.3). After reduction, add  $0.5 \text{ cm}^3$  distilled water to the sample with  $1.0 \text{ cm}^3$  reagent. Measure the absorbance ( $A_{1.0}$ ). The absorbance caused by the reagents only is calculated from:

$$A_{rb} = 3(A_{1.5} - A_{1.0})$$

Determine the reagent blank for each new batch of mixed reagent.

### 6.7.3 Interferences

Interferences are observed from salinity which reduces the final colour intensity to some extent. Hydrogen sulphide can be tolerated up to about  $150 \mu\text{mol dm}^{-3}$  without problems.

For precise estimates of low silicate concentrations (using a  $10 \text{ cm}$  cell), measure a reference absorbance for every sample to compensate for its natural turbidity. For this reason add  $3 \text{ cm}^3$  sulphuric acid ( $0.25 \text{ mol.dm}^{-3}$ ) to  $50 \text{ cm}^3$  of the sample and measure the absorbance against distilled water as reference (if the sample has a visible turbidity centrifuge or filter it, before analysis, through a well-rinsed  $0.4 \mu\text{m}$  Nuclepore filter).

Samples containing more than  $150 \mu\text{mol.dm}^{-3}$  sulphide must be treated with bromine water in the same way as already described in paragraph 6.7.3.

## 6.8 CALCULATION OF RESULTS

Calculate the slope  $b$  of the calibration curve described above (for the individual cell length used) which follows the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

Then the "reactive" silicate concentration of the sample is obtained from

$$C (\mu\text{mol.dm}^{-3}) = \text{SF} \cdot (A_s - A_{rb} - A_t)/b$$

where  $A_s$ ,  $A_{rb}$ , and  $A_t$  are the absorbances of the sample, the reagent blank, and the turbidity blank, respectively.

## 6.9 ESTIMATION OF PRECISION AND ACCURACY

Several national and international intercalibration studies in the past have proven the blue silicomolybdic acid method as a very accurate procedure with a rather low tendency to systematic errors. The precision of the method can be considered as being between  $\pm 2.5$  and  $\pm 4\%$  at concentration levels of about  $50 \mu\text{mol.dm}^{-3}$  and  $5 \mu\text{mol.dm}^{-3}$ , respectively.

## 7. DETERMINATION OF AMMONIA

### 7.1 SCOPE AND FIELD OF APPLICATION

The method is specific for ammonia and is based on the formation of the blue coloured indophenol by phenol and hypochlorite in the presence of the  $\text{NH}_4^+$  and  $\text{NH}_3$  species. The reaction requires an elevated temperature or a catalyst. The colour is measured at 630 nm and is stable for at least 30 h.

The procedure outlined here, mainly follows the methods described by Grasshoff and Johanssen (1973) and by Koroleff (1983). The detection limit of the method is about  $0.05 \mu\text{mol.dm}^{-3}$  (in a 10 cm cell), and the Lambert-Beers's Law is followed up to an ammonia concentration of about  $40 \mu\text{mol.dm}^{-3}$ .

Interferences from amino acids and urea (at seawater levels) can be neglected but may be significant in estuarine or brackish waters, especially where these are contaminated with urban waste. Hydrogen sulphide can be tolerated up to about  $60 \mu\text{mol.dm}^{-3}$ . Samples with higher  $\text{H}_2\text{S}$  concentrations should be diluted. The blue colour of the indophenol, however, is influenced by salinity which has to be compensated by the application of a salt factor.

### 7.2 REFERENCES

GRASSHOFF, K. and JOHANNSEN, H. (1973): A new sensitive and direct method for the automatic determination of ammonia in sea water. *Journal du Conseil International pour l'Exploration de la Mer*, 34, 516-521.

KOROLEFF, F. (1983): Determination of ammonia. In: GRASSHOFF, K., EHRHARDT, M. and KREMLING, K. (editors), *Methods of Seawater Analysis*, Verlag Chemie, Weinheim, 150-157.

### 7.3 PRINCIPLE

In moderately alkaline solution ammonia reacts with hypochlorite to form monochloramine which, in the presence of phenol, catalytic amounts of nitroprusside ions and excess hypochlorite, gives indophenol blue (with a molar absorptivity of about 20,000). The reaction is quantitative in the pH range between 10.8 and 11.4. The reaction mechanism, however, is complicated and not yet fully understood.

The mentioned salt effect depends on the fact that the final reaction pH is a function of the sample salinity (i.e. increasing salinity with increasing buffer capacity decrease the final reaction pH). The precipitation of magnesium and calcium hydroxides in the sample solution (occurring at a pH higher than 9.6) is avoided by the addition of a complexing reagent (citrate) which keeps the Mg and Ca ions in solution.

## 7.4 REAGENTS

### 7.4.1 "Ammonia-free" water

There is no standard procedure for the preparation of water with a very low ammonia content. De-ionised water may sometimes be used without subsequent distillation, but it must be noticed that ion exchange resins potentially bleed out organic substances and ammonia. In case the ammonia blank concentrations are higher than  $0.3 \mu\text{mol}\cdot\text{dm}^{-3}$ , the water should be subjected to subsequent distillation. In this second step, 0.3 g NaOH and 1 g  $\text{K}_2\text{S}_2\text{O}_8$  are added to  $1000 \text{ cm}^3$  of water (in a  $2 \text{ dm}^3$  flask). The solution should be boiled for 10 min to remove ammonia (without the condenser) and then distilled until a residue of about  $150 \text{ cm}^3$ . The distilled water should be stored in a tightly sealed container, preferably made of glass.

### 7.4.2 Buffer solution

240 g tri-sodium citrate dihydrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2 \text{ H}_2\text{O}$ ) and 0.4 g NaOH are dissolved in about  $600 \text{ cm}^3$  distilled water. The solution is boiled (to remove ammonia) until the volume is below  $500 \text{ cm}^3$ . It is then cooled and diluted to  $500 \text{ cm}^3$  with "ammonia-free" water. The solution is stable and should be stored in a well-stoppered polyethylene bottle.

### 7.4.3 Phenol reagent

38 g colourless phenol ( $\text{C}_6\text{H}_5\text{OH}$ ) and 400 mg sodium nitroprusside dihydrate ( $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2 \text{ H}_2\text{O}$ ) are dissolved in "ammonia-free" water and diluted to  $1000 \text{ cm}^3$ . When stored in a tightly closed dark bottle and in a refrigerator, the solution should be stable for several months.

### 7.4.4 Hypochlorite reagent

1 g "Trione", the commercial name for dichloroisocyanuric acid (dichloro-s-triazine-2,3,6(1H, 3H, 5H)-trione) and 8 g NaOH are dissolved in "ammonia-free" water and diluted to  $500 \text{ cm}^3$ . The reagent "Trione" is employed as a hypochlorite donor (in comparison to generally used commercial hypochlorite solutions) has the advantage of being a stable solid, and that it is easy to prepare. The solution should be stored in a dark bottle in a refrigerator and is stable for at least a week.

### 7.4.5 Ammonia stock solution ( $10 \mu\text{mol}\cdot\text{cm}^{-3}$ )

Ammonium sulphate ( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> or ammonium chloride (NH<sub>4</sub>Cl) is dried at  $100 \text{ }^\circ\text{C}$  to constant weight. Then 0.661 g ( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> (or 0.0535 g NH<sub>4</sub>Cl) is dissolved in "ammonia-free" water and diluted to  $1000 \text{ cm}^3$  in a volumetric flask. When kept in a glass bottle (protected from sunlight) and in a refrigerator, the solution should be stable for at least several weeks (see also 5.4.6).

### 7.4.6 Ammonia working solution ( $0.10 \mu\text{mol}\cdot\text{cm}^{-3}$ )

Exactly  $10.0 \text{ cm}^3$  of the stock solution (7.4.5) is diluted with "ammonia-free" water to a final volume of  $1000 \text{ cm}^3$  in a volumetric flask made of glass. This solution must be prepared daily.

## 7.5 APPARATUS

7.5.1 Stoppered 50 cm<sup>3</sup> flasks (of glass, reserved solely for this determination and stored closed and filled with "ammonia free" water between analysis).

7.5.2 Automatic syringe pipettes of 2 cm<sup>3</sup> volume for reagent additions (see 4.5.2).

7.5.3 Spectro- or filter photometer with a filter having maximum transmission at 630 nm, and cells of 1 cm, 5 cm and 10 cm length as required.

*NOTE: All flasks and tubes to be used should be cleaned with acid, rinsed well with "ammonia-free" water and kept closed between analyses. The analysis should be performed in a well-ventilated room with no ammoniacal solutions stored. Smoking should be forbidden.*

## 7.6 SAMPLING

The analysis of ammonia should be commenced without delay after subsampling. If a short-term storage is necessary, the samples must be cooled in a refrigerator but should be analysed within about 3 h. A preservation for longer periods, although discussed in the literature, is not recommended here and should be avoided if possible.

Filtration of samples should also be avoided, if possible, because it is nearly impossible to obtain filters free of ammonia. Waters with high turbidity frequently contain high concentrations of ammonia and may therefore be diluted before the analysis (the residual turbidity may then be compensated with a similarly diluted sample in the reference cell).

## 7.7 METHOD

### 7.7.1 Calibration

Prepare a series of working standards from the ammonia working solution (7.4.6). To 100 cm<sup>3</sup> volumetric flasks add (by means of pipette or burette) the following volumes and fill up with "ammonia-free" water to the 100 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

0.5 cm <sup>3</sup> of working solution	=	0.50 μmol.dm <sup>-3</sup> NH <sub>3</sub>
1.0 " " " "	=	1.0 " " "
2.0 " " " "	=	2.0 " " "
3.0 " " " "	=	2.0 " " "
5.0 " " " "	=	5.0 " " "
7.0 " " " "	=	7.0 " " "
10.0 " " " "	=	10.0 " " "

To 50 cm<sup>3</sup> portions of these working standards add the reagents and follow the procedure outlined below for analysis of the sample (7.7.2). In addition, prepare a

"blank sample" from the same volumes of the distilled water used and the reagents. Use a cuvette of similar length (preferably 5cm) filled with distilled water as reference. Plot the measured absorbances (corrected for the blank) versus the standard concentrations.

### 7.7.2 Analysis of the sample

Measure 50 cm<sup>3</sup> of the sample with a graduated cylinder (see Fig. 6) and transfer it into the reaction flasks. Add 2 cm<sup>3</sup> phenol reagent (7.4.3), 1 cm<sup>3</sup> buffer solution (7.4.2) and 2 cm<sup>3</sup> hypochlorite reagent (7.4.4). Mix well by swirling between the additions. Close the reaction bottles properly and keep them in a dark place during the reaction time.

Measure the absorbance after 6 h in a cell of suitable length at 630 nm, and use a cuvette of similar length filled with distilled water as reference (in ocean waters the reaction requires about 5 h at room temperature; since the colour of the indophenol blue is stable for at least 30 h, it is convenient, in routine analysis, to let the samples react overnight).

With detectable traces of ammonia in the redistilled water, the blank from the reagents added is determined in the following way: First, measure the absorbance ( $A_b$ ) by carrying out the above procedure using 50 cm<sup>3</sup> of distilled water. Repeat the determination by adding, however, 3 cm<sup>3</sup> phenol reagent, 1.5 cm<sup>3</sup> buffer solution and 3 cm<sup>3</sup> hypochlorite reagent to only 47.5 cm<sup>3</sup> of the same distilled water. Measure the absorbance ( $A_{1.5b}$ ). Then the blank caused by the reagents only is calculated from:

$$A_{rb} = 2(A_{1.5b} - A_b).$$

Determine the reagent blank at regular intervals (and for each new batch of solution), because the reagents may absorb ammonia from the air.

### 7.7.3 Interferences

Interferences from amino acids and urea (at seawater levels) can be neglected but may be significant in estuarine or brackish waters, especially where these are contaminated with urban waste. Hydrogen sulphide can be tolerated up to about 60  $\mu\text{mol.dm}^{-3}$ . Samples with higher H<sub>2</sub>S concentrations should be diluted. The blue colour of the indophenol, however, is influenced by salinity which has to be compensated by the application of a salt factor.

## 7.8 CALCULATION OF RESULTS

Calculate the slope  $b$  of the calibration curve described above (for the cell length used) which follows the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

As already mentioned, for any given concentration of ammonia the blue colour produced in seawater is less intensive than in distilled water. Thus, for each sample a correction has to be made with respect to its salinity and the resulting pH. The salt effect factor (SF) can be taken from the following table:

Salinity	0	5	10	15	20	25	30	35
ca. pH	11.0	10.7	10.5	10.4	10.2	10.1	9.9	9.8
SF	1.00	1.03	1.06	1.09	1.14	1.18	1.22	1.25

Thus, the ammonia concentration of the sample is obtained from:

$$C(\mu\text{mol}\cdot\text{dm}^{-3}) = \text{SF}\cdot(A_s - A_{rb} - A_t)/b$$

where  $A_s$ ,  $A_{rb}$  and  $A_t$  are the absorbances of the sample, the reagent blank and the turbidity blank (if any), respectively.

## 7.9 ESTIMATION OF PRECISION AND ACCURACY

Good accuracy and precision in the analysis of ammonia is difficult to obtain, and highly dependent on how successfully the contamination control is achieved during cleaning procedures, sample handling and analytical steps. The main pitfalls are caused by contamination from airborne ammonia (especially tobacco smoke) as well as from the reagents and glassware.

The precision of the method (under ideal circumstances) can be considered as being between 2 and 5% at a concentration level of about  $5 \mu\text{mol}\cdot\text{dm}^{-3}$ .

## 8. DETERMINATION OF NITRITE

### 8.1 SCOPE AND FIELD OF APPLICATION

The method is specific for nitrite ions ( $\text{NO}_2$ ) and is based on the formation of a highly coloured azo dye which is measured colorimetrically at 540 nm. The procedure outlined here is applicable to all types of marine waters and follows the methods by Bendschneider and Robinson (1952) and Grasshoff (1983). It shows a detection limit of  $0.01 \mu\text{mol}\cdot\text{dm}^{-3}$  and a linearity between the amount of the azo dye formed and the initial concentration of nitrite over a wide range of concentration ( $0-10 \mu\text{mol}\cdot\text{dm}^{-3}$ ).

The reaction is widely free from interferences of compounds normally present in ocean or inshore waters. Salinity does not affect the absorbance significantly. If hydrogen sulphide is suspected to be present in a sample (nitrite and sulphide cannot co-exist for long periods in natural seawater), the gas must be expelled with nitrogen after addition of the acid sulphanilamide reagent.

## 8.2 REFERENCES

BENDSCHNEIDER, K. and ROBINSON, R.J. (1952): A new spectrophotometric method for the determination of nitrite in sea water. *Journal of Marine Research*, 11, 87-96.

GRASSHOFF, K. (1983): Determination of nitrite. In: GRASSHOFF, K., M. Ehrhardt, K. KREMLING (editors), *Methods of Seawater Analysis*, Verlag Chemie, Weinheim, 139-142.

## 8.3 PRINCIPLE

The photometric determination of nitrite is based on the reaction of nitrite with an aromatic amine (sulphanilamide) which leads (at pH 1.5 - 2.0) to the formation of a diazonium compound. This diazo compound then couples with a second aromatic amine (N-(1-naphthyl)-ethylenediamine) to form the azo dye with a molar absorptivity of about 46,000.

## 8.4 REAGENTS

### 8.4.1 Sulphanilamide reagent

10 g crystalline sulphanilamide ( $\text{NH}_2\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$ ) is dissolved in a mixture of 100 cm<sup>3</sup> concentrated hydrochloric acid (HCl, a.g.) and about 500 cm<sup>3</sup> distilled water. (Moderate heating accelerates the dissolution.) After cooling, the solution is diluted to 1000 cm<sup>3</sup> with distilled water. The reagent is stable for several months.

### 8.4.2 N-(1-naphthyl)-ethylenediamine solution

0.5g of N-(1-naphthyl)-ethylenediamine dihydrochloride ( $\text{C}_{10}\text{H}_7\text{NH}(\text{CH}_2)_2\text{NH}_2 \cdot 2 \text{HCl}$ ) is dissolved in distilled water and diluted to 500 cm<sup>3</sup>. The solution should be stored in a dark glass bottle in a refrigerator, and must be renewed as soon as it develops a brown colour (usually stable for 1 month).

### 8.4.3 Nitrite stock solution (10 $\mu\text{mol}\cdot\text{cm}^{-3}$ )

Anhydrous sodium nitrite ( $\text{NaNO}_2$ , a.g.) is dried at 100°C (for about 1 h) to constant weight. Then 0.690 g of the dry salt is dissolved in distilled water and diluted to 1000 cm<sup>3</sup> in a volumetric flask. The solution should be stored in a glass bottle and is stable for at least several months (see also 5.4.6).

NOTE: Aged solid reagents, even of analytical grade, may contain less than 100%  $\text{NaNO}_2$  and should, therefore, not be used for the preparation of the nitrite standard solution. For calibration purposes (in distilled water) silver nitrite may be preferred instead because of its higher stability.

### 8.4.4 Nitrite working solution (0.10 $\mu\text{mol}\cdot\text{dm}^{-3}$ )

Exactly 10.0 cm<sup>3</sup> of the nitrite stock solution (8.4.3) is transferred to a volumetric flask and diluted to 1000 cm<sup>3</sup> with distilled water. This solution must be renewed daily.



## 8.5 APPARATUS

8.5.1 Stoppered glass bottles with a capacity of about 100 cm<sup>3</sup>.

8.5.2 Reagent dispensers (automatic syringe pipettes or piston pipettes).

8.5.3 Spectro- or filter photometer, with filter at or close to 540 nm, and cuvettes of 1 cm and 5 cm.

## 8.6 SAMPLING

The sampling procedure for nitrite analysis can be performed without special precaution. Because of its intermediate status in the redox chain ammonia-nitrite-nitrate, however, the samples cannot be properly preserved. The determination of nitrite should, therefore, be carried out without delay, i.e., the reagents should be added within about 30 min after subsampling.

## 8.7 METHOD

### 8.7.1 Calibration

Prepare a series of working standards from the nitrate working solution (8.4.4). To 100 cm<sup>3</sup> volumetric flasks add (by means of micropipettes) the following volumes and fill up with distilled water to the 100 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

0.10 cm <sup>3</sup> of working solution	=	0.10 μmol.dm <sup>-3</sup> NO <sub>2</sub>
0.50 " " " "	=	0.50 " " "
1.00 " " " "	=	1.00 " " "
1.50 " " " "	=	1.50 " " "
2.00 " " " "	=	2.00 " " "
2.50 " " " "	=	2.50 " " "

To 50 cm<sup>3</sup> of these working standards add the reagents and follow the procedure outlined below for analysis of the sample (8.7.2). In addition, prepare a "blank sample" from the same volumes of distilled water and the reagents. Measure the absorbances in a 5 cm cuvette. Plot the measured absorbances (corrected for the blank) versus the standard concentrations.

### 8.7.2 Analysis of the sample

Transfer 50 cm<sup>3</sup> of the sample (with a graduated cylinder see Fig. 6) into the reaction bottle and add 1 cm<sup>3</sup> of the sulphanilamide reagent (8.4.1). Then mix well. After reaction time of about 1 min, add 1 cm<sup>3</sup> of the diamine solution (8.4.2). Shake the flask and allow the azo dye to develop for at least 20-30 min. Measure the absorbance in a cell of suitable length at 540 nm against distilled water as reference. The colour intensity is constant for about two hours. (The dye should not be exposed to bright daylight.).

For precise measurements of low nitrite concentrations, any turbidity in the sample must be compensated by a "turbidity blank". For this reason add 1 cm<sup>3</sup> of the sulphanilamide reagent to 50 cm<sup>3</sup> of the sample and measure the absorbance against distilled water as reference. (The addition of acid to a sample usually changes its turbidity; therefore, it is important that the acidic sulphanilamide reagent is added not only to the sample, but also to the "turbidity blank" sample).

## 8.8 CALCULATION OF RESULTS

Calculate the slope  $b$  of the calibration curve described above (for the individual cell length used) from the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

The concentration of nitrite is then calculated according to

$$C(\mu\text{mol.dm}^{-3}) = (A_s - A_b - A_t)/b$$

where  $A_s$ ,  $A_b$  and  $A_t$  are the absorbance values from the sample, the "blank sample" and the "turbidity blank" sample, respectively.

## 8.9 ESTIMATION OF PRECISION AND ACCURACY

In routine work, the reproducibility of the procedure can be considered as  $\pm 0.02 \mu\text{mol.dm}^{-3}$ . Considerable systematic errors, however, may be introduced by turbidity of the samples (especially at low concentration levels), and by impurities or partial decomposition of the nitrite standard, as being demonstrated by different intercalibration studies.

Therefore, it is important to follow the precautions described under 8.4.3 and 8.7.2 in order to achieve high quality analysis.

# 9. DETERMINATION OF NITRATE

## 9.1 SCOPE AND FIELD OF APPLICATION

The method generally applied for the determination of nitrate ( $\text{NO}_3$ ) is based on its reduction to nitrite, which is then determined colorimetrically via the formation of an azo dye as described in chapter 8. The method outlined here is based on a heterogeneous reaction with copper-coated cadmium granules and follows mainly the procedure by Grasshoff (1983). It has proved to be reliable and useful for work at sea and is widely free from interferences in nearshore and oceanic waters.

The method determines the sum of nitrite and nitrate, therefore, a separate determination of nitrite must be conducted, and the concentration subtracted from that obtained with this method. At concentration levels higher than about  $20 \mu\text{mol}\cdot\text{dm}^{-3}$ , calibration curves for a low and high range must be established.

## 9.2 REFERENCE

GRASSHOFF; K. (1983): Determination of nitrate. In: GRASSHOFF; K., EHRHARDT, M. and KREMLING, K. (editors): *Methods of Seawater Analysis*, Verlag Chemie, Weinheim, 143-150.

## 9.3 PRINCIPLE

Nitrate is reduced to nitrite in a reduction column filled with copper-coated cadmium granules. The yield of the reduction depends on the pH of the solution and on the activity of the metal surface. The conditions of the reduction described in the method are adjusted to a pH of about 8.5, so that nitrate is converted to nitrite almost quantitatively (90 - 95%) and not reduced further. Ammonium chloride buffer is used to control the pH and to complex the liberated cadmium ions.

The nitrite formed is then determined colorimetrically (at 540 nm) according to the reaction already described in section 8.

## 9.4 REAGENTS

### 9.4.1 Ammonium chloride buffer

10 g ammonium chloride ( $\text{NH}_4\text{Cl}$ , a.g.) is dissolved in distilled water and diluted to  $1000 \text{ cm}^3$ . The pH is adjusted to 8.5 by adding about  $1.6 \text{ cm}^3$  of concentrated ammonium hydroxide ( $\text{NH}_4\text{OH}$ ).

### 9.4.2 Sulphanilamide reagent (same reagent as for nitrite determination, see 8.4.1)

10 g crystalline sulphanilamide ( $\text{NH}_2\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$ ) is dissolved in a mixture of  $100 \text{ cm}^3$  concentrated hydrochloric acid ( $\text{HCl}$ , a.g.) and about  $500 \text{ cm}^3$  distilled water and made up to  $1000 \text{ cm}^3$  with distilled water. The reagent is stable for several months.

### 9.4.3 N-(1-naphthyl)-ethylenediamine (same reagent as for nitrite determination, see 8.4.2)

0.5g of N-(1-naphthyl)-ethylenediamine dihydrochloride ( $\text{C}_{10}\text{H}_7\text{NH}(\text{CH}_2)_2\text{NH}_2 \cdot 2 \text{ HCl}$ ) is dissolved in distilled water and diluted to  $500 \text{ cm}^3$ . The solution should be stored cool in a dark glass bottle and should be renewed as soon as it develops a brown colour (usually stable for 1 month).

#### 9.4.4 Filling of reduction column

Commercially available granulated cadmium (e.g. from Merck) is sieved and the fraction between 40 and 60 mesh (i.e. around 0.25 to 0.42 mm) is retained and used. The further treatment is described under 9.7.1.

*WARNING: Cadmium can be poisonous. It should, therefore, be handled with great care. Never incorporate the dust. Perform all operations on the dry metal in a fume hood.*

#### 9.4.5 Copper sulphate solution

10 g copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) is dissolved in about 1000  $\text{cm}^3$  distilled water.

#### 9.4.6 Nitrate stock solution (10 $\mu\text{mol} \cdot \text{cm}^{-3}$ )

1.011 g dry potassium nitrate ( $\text{KNO}_3$ , a.g.) is dissolved in distilled water and diluted to 1000  $\text{cm}^3$  in a volumetric flask. The solution is stable for at least several months (see also 5.4.6).

#### 9.4.7 Nitrate working solution (0.10 $\mu\text{mol} \cdot \text{cm}^{-3}$ )

10  $\text{cm}^3$  of the nitrate stock solution (9.4.6) is transferred to a volumetric flask and diluted to 1000  $\text{cm}^3$  with distilled water. This solution must be renewed daily.

### 9.5 APPARATUS

#### 9.5.1 Reduction column (see Fig. 6)

The major part of the reduction column consists of a U-shaped glass tube with a total length of about 60 cm and an inner diameter of 3 mm. Connections to the 100  $\text{cm}^3$  sample bottle and the 25  $\text{cm}^3$  (marked) Erlenmeyer flask are made from flexible capillary tubing (tygon). The sample is drawn through the column by an aspirator or by a small peristaltic pump (with control of the flow rate by means of a three-way stopcock with a bypass). For practical purpose, the whole set-up can be mounted in a box.

9.5.2 Stoppered glass bottles, reagent dispensers, and a spectro- or filter photometer (with 1 cm and 5 cm cuvettes) as described for the analysis of nitrite are required (see 8.5).

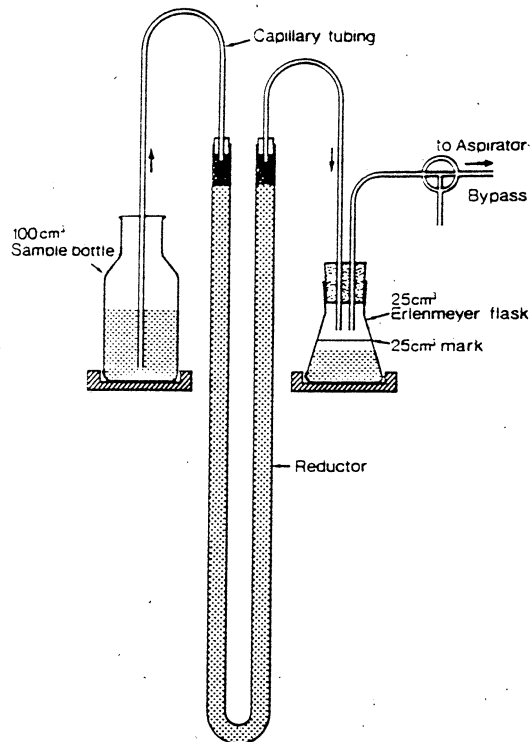


Fig. 6: Reduction column for the analysis of nitrate (details see paragraphs 9.5.1 and 9.7.1).

## 9.6 SAMPLING

The analysis should preferably be started within 1 hour after subsampling. The samples should be stored in a refrigerator, but for not more than 5 h. If longer storage is necessary, quick freezing of samples (to  $-20^{\circ}\text{C}$ ) is recommended (see general remarks in paragraph 5.6).

## 9.7 METHOD

### 9.7.1 Preparation of the reduction column

Free the sieved cadmium granules from oxides by washing them in  $2 \text{ mol}\cdot\text{dm}^{-3}$  hydrochloric acid. Then shake the granules in a  $200 \text{ cm}^3$  beaker vigorously (for about 3 min) with  $100 \text{ cm}^3$  of the copper sulphate solution (reagent 9.4.5). Afterwards rinse

the copperized cadmium granules under gentle shaking, decant the water and continue washing until the water is free from finely dispersed copper.

Then pour the copperized granules into the reduction column (with the aid of distilled water and a funnel). Encourage effective packing by gently tapping the column with a pencil. When one arm is filled, connect the funnel to the other arm and repeat the procedure. Leave some space in both side arms in order to pack in some glass wool.

Activate the metal by passing through about 250 cm<sup>3</sup> buffer solution (reagent 9.4.1.) containing about 100 μmol.dm<sup>-3</sup> nitrate. Then rinse thoroughly with buffer solution before the reducer is used for analysis.

Check the reduction efficiency of the reduction column by analyzing a nitrate standard solution of suitable concentration (see 9.7.3). Compare the determined absorbance with that of a nitrite solution of the same concentration (e.g. if  $A_{\text{NO}_3} = 0.200$  and  $A_{\text{NO}_2} = 0.210$ , the reduction efficiency would be  $(0.200 \times 100)/0.210 = 95.2\%$ ).

NOTE: Repeat the activation procedure if the reduction column has not been used for several days, or, if the column has accidentally been filled up with air bubbles. When not in use, keep the column brimful with the ammonium chloride buffer solution. If the efficiency cannot be brought back to above 90% of the theoretical value, it is preferable to refill the column. If frequently used, however, it should last for several months.

### 9.7.2 Calibration

Prepare, if necessary, two series of working standards for a low and a high range of concentration (up to 40 μmol.dm<sup>-3</sup> nitrate). To 1000 cm<sup>3</sup> volumetric flasks add the following volumes of the nitrate standard solutions (reagents 9.4.6 or 9.4.7, respectively) and fill up with distilled water to the 1000 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

#### Low range

10.00 cm <sup>3</sup> of "working solution" (9.4.7)	=	1.00 μmol.dm <sup>-3</sup> NO <sub>3</sub>
20.00 " " " " "	=	2.00 " " "
40.00 " " " " "	=	4.00 " " "
60.00 " " " " "	=	6.00 " " "
80.00 " " " " "	=	8.00 " " "
100.00 " " " " "	=	10.00 " " "

#### High range

1.00 cm <sup>3</sup> of "stock solution" (9.4.6)	=	10.0 μmol.dm <sup>-3</sup> NO <sub>3</sub>
2.00 " " " " "	=	20.0 " " "
3.00 " " " " "	=	30.0 " " "
4.00 " " " " "	=	40.0 " " "

To 25 cm<sup>3</sup> portions of the low range working standards (1 - 10 μmol.dm<sup>-3</sup>) add 25 cm<sup>3</sup> of the buffer solution (reagent 9.4.1). Prepare a similar solution of the high range working standard, but here take 25 cm<sup>3</sup> of the standard and 75 cm<sup>3</sup> of the buffer solution. In addition, prepare a "blank sample" from 25 cm<sup>3</sup> distilled water and the

same volume of buffer solution. Analyse the standard and blank solutions in the same way as described below for the analysis of the sample (9.7.3). Plot the measured absorbances (corrected for the blank value) versus the standard concentrations. Both curves should be linear over the entire range of concentrations.

### 9.7.3 Analysis of the sample

Transfer 25 cm<sup>3</sup> of the sample into the 100 cm<sup>3</sup> reaction flask, add 25 cm<sup>3</sup> of the buffer solution (reagent 9.4.1) and mix well. If nitrate concentrations of more than about 15 μmol.dm<sup>-3</sup> are expected, 25 cm<sup>3</sup> of the sample must be diluted with 75 cm<sup>3</sup> of the buffer solution.

Pass about 20 cm<sup>3</sup> of the mixture through the reduction column in order to rinse the system and to adjust the time of passage (3 - 5 min). Discard this fraction. Then pass (at unchanged speed) another fraction through the column until the level in the Erlenmeyer flask has reached the 25 cm<sup>3</sup> mark.

Stop the collection of the reduced sample and add 0.5 cm<sup>3</sup> of the sulphanilamide reagent (9.4.2) and 0.5 cm<sup>3</sup> of the diamine solution (9.4.4) in the same way as described for the analysis of nitrite (see 9.7.2). Determine the azo dye colour within about 1 hour (at 540 nm) in 1 cm or 5 cm cells against distilled water as reference.

## 9.8 CALCULATION OF RESULTS

Calculate the slope  $b$  of the calibration curves described above (separately for the low and high concentration levels and for the individual cell lengths used) from the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

The concentration of nitrate is then calculated according to

$$C(\mu\text{mol.dm}^{-3}) = \frac{(A_s - A_b)}{b} - C_{\text{NO}_2^-}$$

where  $A_s$  and  $A_b$  are the absorbances of the sample and of the 'blank sample', and  $C_{\text{NO}_2^-}$  is the nitrite concentration (in μmol.dm<sup>-3</sup>) observed in the same sample.

## 9.9 ESTIMATION OF PRECISION AND ACCURACY

In routine analysis the precision (standard deviation) of the methods with one and the same reduction column is about  $\pm 0.05 \mu\text{mol.dm}^{-3}$  for nitrate concentrations of  $< 5 \mu\text{mol.dm}^{-3}$ ,  $\pm 0.2 \mu\text{mol.dm}^{-3}$  in the range 5 - 10 μmol.dm<sup>-3</sup> and about  $\pm 0.5 \mu\text{mol.dm}^{-3}$  in the higher concentration range. If different reduction columns are used, the deviation of results depend, of course, strongly on the reduction efficiency of the columns.

The accuracy depends on the reliability of the standard used for the calibration procedure, on the quality of sampling, and/or, if necessary, on the procedure of storage.

## 10. SIMULTANEOUS PERSULPHATE OXIDATION FOR THE DETERMINATION OF TOTAL NITROGEN AND PHOSPHORUS COMPOUNDS

### 10.1 SCOPE AND FIELD OF APPLICATION

The method describes a simultaneous oxidation by persulphate for the determination of the total concentrations of nitrogen and phosphorus in the same seawater sample. This method was introduced by Koroleff in 1977 and published in a slightly modified version by Valderrama in 1981. The procedure outlined here follows mainly the recent publication by Koroleff (1983) and is suitable for total carbon and nitrogen contents up to about 10 and 2 mg.dm<sup>-3</sup>, respectively.

It must, however, be emphasized that in this method the yield of total nitrogen (i.e., organic nitrogen compounds) seems to be very much dependent on the chemical structure of the organic material. This problem has been investigated and discussed in a recent paper by Suzuki, Sugimura and Itoh (1985) who used a high-temperature catalytic oxidation method. The results obtained by their method were considerably higher (ranging from 20 to 90%) than those by the wet persulphate oxidation procedure. Although the nature of most organic matter dissolved in seawater is unknown, from the investigation cited above it seems that aromatic nitrogen compounds (or macro-molecular matter) are highly resistant to the persulphate oxidation method. These discrepancies, however, need to be further investigated. Results on total nitrogen in seawaters should, therefore, be accompanied by an exact description of the applied analytical procedure. Further information on the results of recent intercomparison studies of dissolved organic can be found in a report by Williams (1991).

In contrast to organic nitrogen, organic phosphorus compounds are easily broken down to inorganic compounds by treating them with peroxodisulphate solution.

### 10.2 REFERENCES

- KOROLEFF, F. (1983): Simultaneous oxidation of nitrogen and phosphorus compounds by persulphate. In: GRASSHOFF, K., EHRHARDT, M., KREMLING, K. (editors), *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, 168-169.
- SUZUKI, Y., SUGIMURA, Y. and ITOH, T. (1985): A catalytic oxidation method for the determination of total nitrogen dissolved in seawater. *Marine Chemistry*, 16, 83-97.
- VALDERRAMA, J.C. (1981): The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry*, 10, 109-122.
- WILLIAMS, P.M. (1991): Scientists and industry representatives attend workshop on measuring DOC and DON in natural waters. *U.S. JGOFS Newsletter*, 3(1), 1-11.



### 10.3 PRINCIPLE

Organically bound phosphorus and (wet oxidizable) organic nitrogen compounds can be quantitatively converted into phosphate and nitrate with peroxodisulphate in an alkaline medium. In the simultaneous wet oxidation, the reaction starts at pH 9.7 and finishes at pH 4-5. These conditions are established by a boric acid-sodium hydroxide buffer system. The digestion is performed in a pressure cooker (autoclave) at about 110°C and should last not longer than 30 minutes. The free chlorine formed in seawater samples is reduced by adding ascorbic acid before the analysis is started. The precipitate, which is formed at elevated temperatures, dissolves as oxidation proceeds.

After completion of the oxidation step, the nitrogen and phosphorus compounds are determined as nitrate and inorganic phosphate according to the procedures outlined in Sections 9 and 5, respectively.

### 10.4 REAGENTS

In addition to the reagents listed for the determination of inorganic phosphate (Section 5) and nitrate (Section 9), the following reagents are required.

#### 10.4.1 Sodium hydroxide (0.375 mol.dm<sup>-3</sup>)

15.0 g sodium hydroxide (NaOH, a.g.) is dissolved in distilled water containing <0.001% nitrogen and diluted to 1000 cm<sup>3</sup>. The solution should be stored in a tightly stoppered polyethylene bottle.

#### 10.4.2 Oxidation reagent

5 g of pure potassium peroxodisulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) and 3 g boric acid (H<sub>3</sub>BO<sub>3</sub>) are dissolved in 100 cm<sup>3</sup> of the 0.375 mol.dm<sup>-3</sup> NaOH (reagent 10.4.1). The reagent should be stored in a tightly stoppered dark glass bottle and is stable for at least a week.

NOTE: The peroxodisulphate must have a very low nitrogen content. A suitable reagent is the product from Merck (No. 5092) with a max. content of 0.001 %N. Products of less quality can be improved to the above level after one or two recrystallisations. For this purpose 16 g K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> is dissolved in 100 cm<sup>3</sup> redistilled water at 70 - 80 °C. The solution is then cooled to near zero and filtered. The recrystallized salt is dried in a desiccator over anhydrous CaCl<sub>2</sub>. The recovery should be about 80%.

#### 10.4.3 Standard stock solution

4.505 g dried glycine (NH<sub>2</sub>CH<sub>2</sub>COOH, a.g.) and 1.361 g potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>, a.g.), previously dried at 110°C (and kept in a desiccator) are dissolved in distilled water and diluted to 1000 cm<sup>3</sup> in a volumetric flask. The standard solution contains 60.0 μmol.cm<sup>-3</sup> N and 10.0 μmol.cm<sup>-3</sup> P and should be stored in the refrigerator.

#### 10.4.4 Standard working solution

Exactly 10.6 cm<sup>3</sup> of the stock solution (11.4.3) are transferred to a volumetric flask and diluted to 1000 cm<sup>3</sup>. This standard solution contains 0.60 μmol.cm<sup>-3</sup> N and 0.10 μmol.cm<sup>-3</sup> P and must be renewed daily.

### 10.5 APPARATUS

In addition to the equipment listed for the determination of inorganic phosphate (Section 6) and nitrate (Section 10) the following items are needed.

10.5.1 Oxidation bottles (of ca. 50 to 100 cm<sup>3</sup> volume), preferably made of alkali resistant glass (or plastic bottles or polypropylene or teflon) with screw caps of non-nitrogen containing material (Graduated Sovirel bottles are suitable from which, however, the screw caps have to be replaced by nitrogen-free polypropylene caps).

Before use, the bottles should be cleaned with distilled water several times.

NOTE: Between determinations it is recommended to store the bottles filled with ca. 0.1 mol.dm<sup>-3</sup> HCl.

#### 10.5.2 Autoclave

An ordinary kitchen pressure cooker of stainless steel may be used as autoclave, maintaining an internal temperature of about 110°C.

NOTE: The water used in the cooker should be free of ammonia.

### 10.6 SAMPLING

In general, the sampling and storage procedures outlined in connection with analysis of the inorganic phosphate and nitrogen compounds should be followed (see paragraphs 5.6, 7.6, 7.6, and 8.6).

For a preservation of the total nitrogen and phosphorus compounds Valderrama (1981) proposed to add the oxidation reagent and to boil the sample soon after sampling and then to store the sample in oxidized form instead of storing the original sample (the bottles should not be opened until the analysis of nitrate and phosphate is started).

### 10.7 METHOD

#### 10.7.1 Calibration

Prepare a series of working standards from the diluted standard solution (10.4.4). To 100 cm<sup>3</sup> volumetric flasks add the following volumes and fill up with redistilled water to the 100 cm<sup>3</sup> mark. Then the resulting standard concentrations are:

<u>cm<sup>3</sup> standard working solution (10.4.4)</u>	<u>μmol.dm<sup>-3</sup> N</u>	<u>μmol.dm<sup>-3</sup> P</u>
1.0	6.0	1.0
2.0	12.0	2.0
5.0	30.0	5.0
10.0	60.0	10.0

To 50 cm<sup>3</sup> of these working standards add 5 cm<sup>3</sup> of the oxidation reagent and follow the procedure outlined below for analysis of the sample (10.7.2). In addition, determine a blank of the oxidation reagent by boiling a "blank sample" consisting of only 5 cm<sup>3</sup> reagent (with no extra distilled water). This reagent blank must be repeated for every new batch of oxidant. (Note that for the calibration curve the "blank sample" must contain 50 cm<sup>3</sup> of distilled water with 5 cm<sup>3</sup> of the oxidation reagent added.)

Plot the measured absorbance (corrected for the blank) versus the standard concentrations as described previously for phosphate and nitrate (see paragraphs 5.7 and 9.7, respectively).

NOTE: Although the oxidized nitrogen sample is diluted tenfold from 5 cm<sup>3</sup> to 50 cm<sup>3</sup> in the final stage (see 10.7.2), this is partly compensated by using a 5 cm cell for the photometric measurement compared to the 1 cm cell that is (usually) used for nitrate analysis.

#### 10.7.2 Analysis of the sample

Transfer 50 cm<sup>3</sup> of the sample (with a graduated cylinder) into the oxidation bottle and add 5 cm<sup>3</sup> of the oxidizing reagent (10.4.2). Close the bottle immediately and boil the mixture for 30 min (at 110 - 115°C) in the pressure cooker. Cool the autoclave to room temperature before opening. Take out the bottles and swirl gently to promote dissolution of any small precipitate on the bottom. Release any over-pressure and cool the bottles to room temperature.

From the total volume of 55 cm<sup>3</sup> (of the oxidized sample), take a 5.0 cm<sup>3</sup> portion for the determination of total nitrogen (as nitrate), dilute it to 50 cm<sup>3</sup> with distilled water and continue with the analysis of nitrate as described in Section 10.

The remaining 50 cm<sup>3</sup> of the sample is used for the determination of total phosphorus as phosphate. Add 1 cm<sup>3</sup> of the ascorbic acid solution (reagent 5.4.5), mix well and wait for 1 to 2 min. so that free chlorine, that was liberated during the oxidation, is completely destroyed. Then follow the procedure for the analysis of inorganic phosphate as described in paragraph 5.

### 10.8 CALCULATION OF RESULTS

Calculate the slopes  $b_p$  and  $b_N$  of the calibration curves (for total phosphorus as phosphate and total nitrogen as nitrate, respectively) from the equation:

$$\text{Absorbance} = b \cdot \text{Concentration}$$

Then calculate the concentrations of total phosphorus and total nitrogen from the expressions:

$$\text{Total P } (\mu\text{mol.dm}^{-3}) = (A_{S,P} - A_{ox,P})/b_P$$

$$\text{Total N } (\mu\text{mol.dm}^{-3}) = (A_{S,N} - A_{ox,N})/b_N$$

where  $A_{S,P}$  and  $A_{S,N}$  are the absorbances of the total phosphorus and total nitrogen samples, respectively, and  $A_{ox,P}$  and  $A_{ox,N}$  are the corresponding blanks from the oxidation reagent.

The results give the total amounts of phosphorus and (wet oxidizable) nitrogen. By subtracting the concentrations of inorganic phosphate and the concentrations of ammonia, nitrite and nitrate, respectively, which are determined separately, the amounts of organically bound phosphorus and nitrogen are obtained.

## 10.9 ESTIMATION OF PRECISION AND ACCURACY

The reproducibility (coefficient of variation) of the method can be considered as being about 5% for both components. Considerable systematic errors in the determination of total nitrogen, however, may be introduced by a possible inadequate oxidation capacity of the procedure with respect to the digestion of aromatic and/or high molecular compounds. This problem has already been discussed in paragraph 10.1 and should be considered, especially in waters with potentially high amounts of organic nitrogen compounds.