TR 136

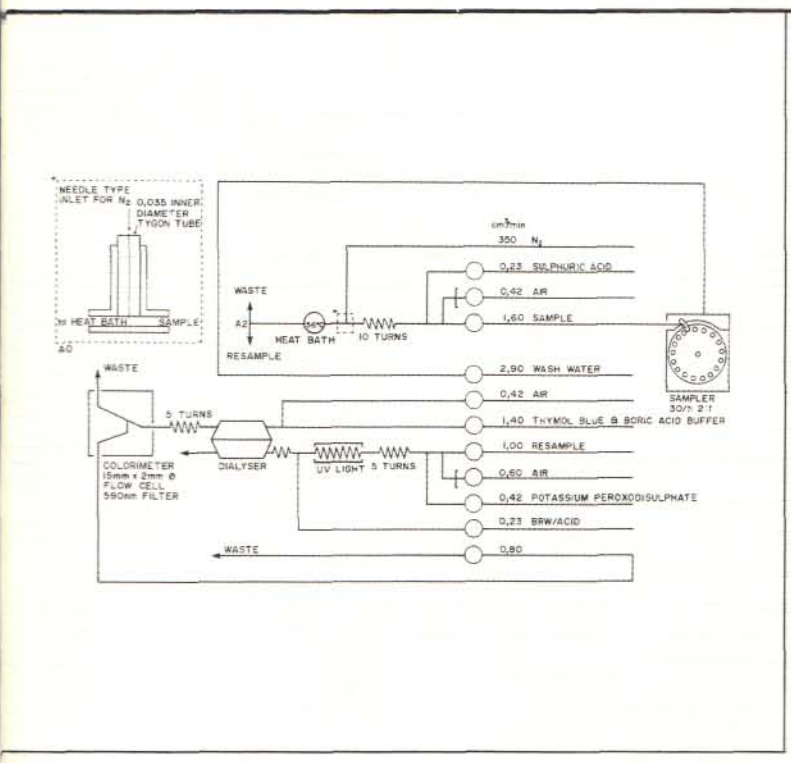
REPUBLIC OF SOUTH AFRICA

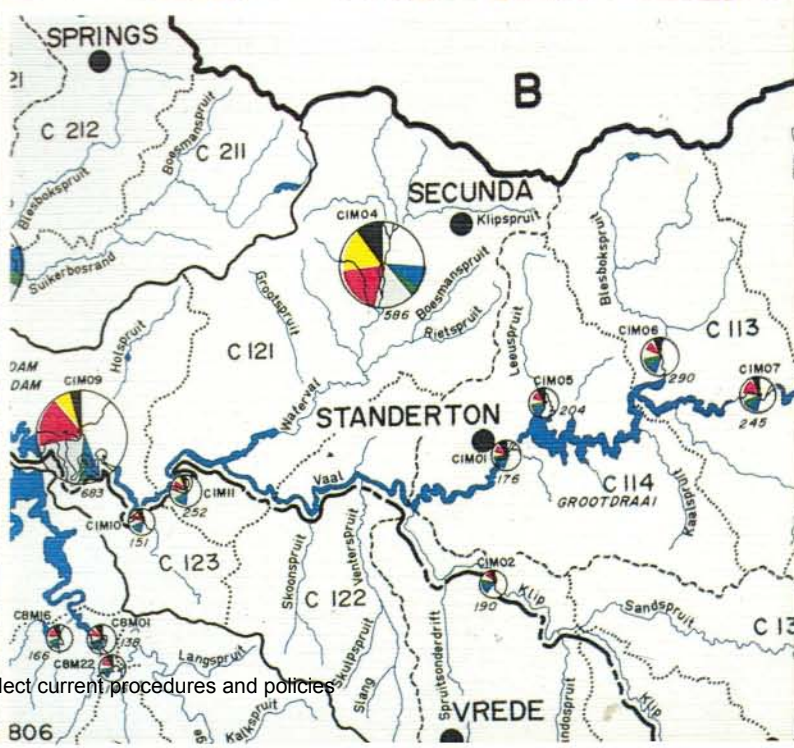
DEPARTMENT OF WATER AFFAIRS

ANALYTICAL METHODS MANUAL

H. R. VAN VLIET
D. P. SARTORY
I. J. SCHOONRAAD

D. L. KEMPSTER
F. A. GERBER





DEPARTMENT OF WATER AFFAIRS
BRANCH: SCIENTIFIC SERVICES
HYDROLOGICAL RESEARCH INSTITUTE

TR 136

ANALYTICAL METHODS MANUAL, HYDROLOGICAL RESEARCH INSTITUTE, DEPARTMENT OF WATER AFFAIRS

Methods for:

- Inorganic determinands
- Biological and microbiological determinands
- Soil dispersivity evaluation

H R van Vliet
P L Kempster
D P Sartory
F A Gerber
I J Schoonraad

March 1988

Department of Water Affairs
Private Bag X313
Pretoria
0001

ACKNOWLEDGEMENTS

The methods used for the analysis of water samples at the Hydrological Research Institute have been developed and refined over many years, and have received inputs from a very large number of people, both present and past staff of the laboratory having played major roles in developing and improving the analytical service to its present state. In addition, some of the methods are new, and others are methods which already existed but have been improved upon.

Despite the indebtedness to a number of people, there are however, specific individuals who should be thanked. Firstly, Miss J. Whitmore, Mr. L.H.W. Verhoef and Mr. S.A. Engelbrecht, who played a major role in the initial creation of the analytical service in the early seventies. Secondly, Dr. W.H.J. Hattingh, who played a major role in the growth of the analytical service, especially in his continual encouragement to improve methods. Thirdly, Mr. E. Braune, who has seen the laboratories through many a difficult period, when staff and or funding restrictions have taxed the ingenuity to the utmost.

Special thanks also go to Mr. Gravelèt-Blondin, who was closely involved with the development of analytical methods in the past. Special appreciation must also be extended to Mrs. A. Kolbe for preparing the figures, compiling the draft manuscript and for spending many hours on editorial work. Thanks must also go to the typist, Mrs. B. Sutton for her input.

CONTENTS

INTRODUCTION

Over the past decade many analytical procedures have been developed and adapted within the laboratories of the Hydrological Research Institute, but have not been formally documented. The primary purpose of this volume is to provide detailed descriptions of all the automated and manual inorganic analytical procedures as well as the biological and bacteriological methods routinely used in the Institute's laboratories. In addition test procedures used for the classification of dispersivity of soils are also included. These methods were taken from many sources, some have been modified and others developed in the Institute's laboratories.

A second objective of this report is to serve as a reference document for the analytical procedures employed in the Department of Water Affairs' water quality monitoring programmes. To this end each determinand/method combination has been assigned a 7 digit determinand code. These codes will, amongst other things, also serve as links between the methods described in this manual and the data archived on the National Water Quality Database. This method manual will consequently be updated from time to time as procedures of analyses employed are either changed or additional determinands are included in monitoring programmes.

The report is divided into three sections. A brief discussion on the contents of each section is presented in the following paragraphs.

Section 1 contains descriptions of the continuous flow procedures used for the analysis of the major inorganic ions and nutrients. In addition some manual methods are also given. A high premium is placed on the quality of the data produced by the automated laboratories. In keeping with this policy these particular laboratories participate annually in a number of inter-laboratory quality control exercises and as a result the methods described in this section have been evaluated extensively and their accuracies sufficiently established.

Section 2 provides descriptions of a range of biological and bacteriological analytical methods, ranging from relatively simple spectrophotometric procedures to more sophisticated High Performance Liquid Chromatographic techniques for the analysis of chlorophyllous pigments. Brief discussions on sampling and sampling techniques are also included in the section.

In section 3 procedures developed at the Institute for the classification of dispersive soils are described. Many chemical and physical factors influence the stability of earth structures such as embankment dams. One of the soil properties that influence the erodibility and susceptibility to piping of embankment dams is soil dispersivity. The classification of soil dispersivity is important not only in the determination of the suitability of soils for the construction of embankment dams but also in the assessment of the risk of total failure of existing dams.

SECTION 1: INORGANIC ANALYTICAL METHODS

1.1 ALKALINITY - TOTAL

1.1.1 Introduction

The alkalinity of water is defined as the capacity that some substances have to take up protons, in other words, to react with an equivalent quantity of a strong acid. Examples of such substances are hydroxide ions and anions of weak acids, e.g. bicarbonate, carbonate, phosphate and silicate. The equivalent quantity of strong acid required to neutralize these ions is equal to the total alkalinity.

The alkalinity of surface water with a pH of less than 8,3 can generally be attributed to the bicarbonates of calcium and magnesium. Water with a pH greater than 8,3 contains bicarbonate, carbonate and hydroxide ions. The alkalinity fraction which is equivalent to the quantity of acid required to lower the pH to 8,3 is the phenolphthalein alkalinity.

Phenolphthalein alkalinity and total alkalinity can therefore be determined by titrating to an endpoint pH of 8,3 and 4,5 respectively. The endpoint pH for total alkalinity must be between 4 and 5 and it is dependent on the alkalinity and the free carbon dioxide concentration. An endpoint pH of 4,5 gives sufficiently accurate results.

Two methods are described here, the automated bromocresol green (pH 4,2) method which is applicable in the 4 to 400 mg $CaCO_3$/ℓ concentration range and a titrimetric method which is suitable for all alkalinity concentration ranges. The latter method is generally used for samples containing colour and/or alkalinity concentrations higher than 400 mg/ℓ as calcium carbonate ($CaCO_3$).

1.1.2 Total Alkalinity, Automated, Bromocresol Green - 0100601

Optimum concentration range: 4 to 400 mg/ℓ as calcium carbonate.

Application of method

The method is applicable to surface, ground and drinking water.

Principle of automated method

Bromocresol green is used in the method as the acid-base indicator because the pH range (3,6 to 5,2) corresponds with the equivalence point of total alkalinity and it has a definite colour change (yellow to blue) which can be measured colorimetrically. The bromocresol green is dissolved in a buffer (pH 4,2) where the buffer capacity is so chosen that the addition of alkalinity causes a small change in the pH and this results in a change in the colour of the acid-base indicator.

Sample preservation

Preserve the samples with 10 mg/ℓ mercury(II) chloride. Sample bottles must be filled completely and must not be opened before analysis.

Interferences

Colour and turbidity interfere in this method. The flow system includes a blank channel which to some degree compensates for colour and turbidity. However, high turbidity must be removed by means of filtration through a 0,45 µm membrane filter. Coloured samples should preferably be analysed using the titrimetric method.

Reagents

(i) Sodium hydroxide solution. Dissolve 6,6 g sodium hydroxide (NaOH) in deionized water and dilute to 500 mℓ. Store the solution in a polyethylene container.

(ii) Bromocresol green stock solution. Add 14,5 mℓ NaOH solution to 500 mℓ deionized water. Dissolve 3 g bromocresol green in this solution, stir for one hour and filter immediately. Dilute to 1 ℓ. Store the solution in a dark glass container at 4°C. It remains stable for 5 days.

(iii) Buffer/Bromocresol green solution. Successively dissolve 4 g calcium chloride dihydrate ($CaCl_2.2H_2O$) and 20 g potassium hydrogen phthalate ($C_8H_5O_4K$) in 1 ℓ deionized water. Add 100 mℓ bromocresol green stock solution and 1 mℓ Brij-35 wetting agent. Adjust the pH to 4,0 with a 1 mol/ℓ HCl solution. Dilute to 2 ℓ. This solution is stable for two days.

(iv) Blank reagent. Successively dissolve 4 g $CaCl_2.2H_2O$ and 20 g $C_8H_5O_4K$ in 1 ℓ deionized water. Add 1 mℓ Brij-35 wetting agent and dilute to 2 ℓ.

(v) Stock bicarbonate solution. Dissolve 6,731 g (dependent on purity of the reagent used) sodium bicarbonate ($NaHCO_3$) (dried for 1 h at 60°C) in deionized water and dilute quantitatively to 1 ℓ. The solution contains 4 mg $CaCO_3$/mℓ).

(vi) Standard bicarbonate solutions. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ $CaCO_3$
10,0	40
30,0	120
50,0	200
80,0	320
100,0	400

Flow system

A schematic diagram of the flow system is shown in Figure 1.1.

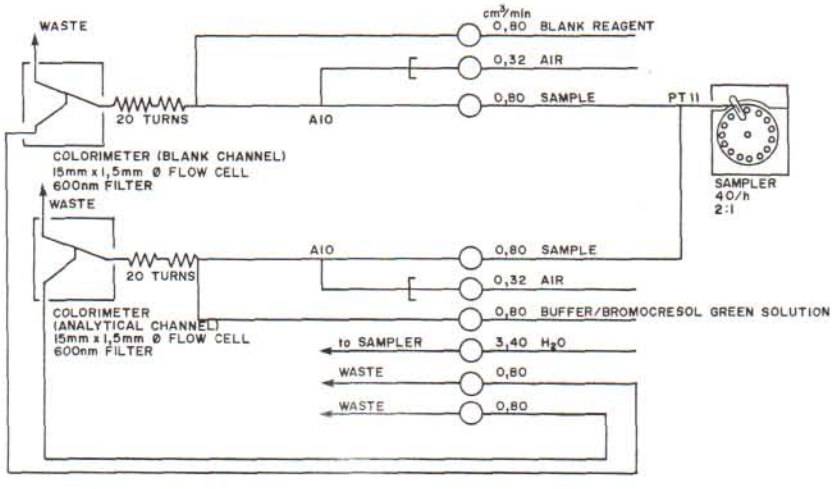


Figure 1.1: Schematic flow diagram for total alkalinity (bromocresol green).

1.1.3 Total Alkalinity, Titrimetric, Mixed Indicator - 0100602

Optimum concentration range: Concentrations greater than 400 mg/ℓ as calcium carbonate.

Application of method

This method is applicable to coloured samples and/or concentration ranges above 400 mg/ℓ (as $CaCO_3$).

Titrimetric determination of alkalinity

The alkalinity is determined by means of the titration of the sample with a standard solution of a strong acid. A description of both the acid-base indicator (Vogel, 1978) and the potentiometric method is given.

The visual method, which makes use of an acid-base indicator is satisfactory for routine applications. In cases where greater accuracy is required, the potentiometric method is more suitable. The latter method must also be used where there is colour, turbidity or suspended material in the samples.

Indicator method

Reagents

(i) Sodium carbonate solution (0,05 mol/ℓ). Dissolve 5,3 g anhydrous sodium carbonate (Na_2CO_3) (dried for 1 h at 250°C) in deionized water and dilute quantitatively to 1 ℓ.

(ii) Hydrochloric acid solution (0,1 mol/ℓ). Dilute 10 mℓ hydrochloric acid (HCl) to 1 ℓ with deionized water. Standardize the HCl solution against the 0,05 mol/ℓ carbonate solution by using the mixed indicator.

(iii) Phenolphthalein indicator. Dissolve 0,5 g phenolphthalein in 50 mℓ ethanol and add 50 mℓ deionized water.

(iv) Mixed indicator solution. Dissolve 1 g methyl orange and 2,5 g indigo carmine in deionized water and dilute to 1 ℓ.

Procedure

Over a white surface, add 2 to 3 drops phenolphthalein indicator to 25 mℓ of sample in a conical flask. If the sample remains colourless the phenolphthalein alkalinity is nil. If the sample turns pink the alkalinity can be determined by titrating with the standard acid until the pink colour disappears.

Add a few drops of the mixed indicator to the same solution. If the sample changes to the colour purple, then the total alkalinity is nil. If the sample changes to green, then the total alkalinity is determined by titrating with the standard acid until the colour changes noticeably to grey/grey-purple.

Potentiometric method

Apparatus

(i) pH meter equipped with a combined pH electrode.

(ii) Magnetic stirrer.

Reagents

Hydrochloric acid solution (0,1 mol/ℓ). As described above.

Procedure

Transfer 100 mℓ of sample to a beaker and place it on the magnetic stirrer. Place the electrode and the point of the burette in the sample. Titrate the solution with the standard acid solution to a pH of 8,3. Note the volume which corresponds with the phenolphthalein alkalinity. Continue titrating to a pH of 4,5. The total volume of acid solution gives the total alkalinity.

Calculations

$$\text{Phenolphthalein alkalinity} = \frac{5 \times A \times M \times 10^4}{V} \text{ mg/ℓ } CaCO_3$$

$$\text{Total alkalinity} = \frac{5 \times B \times M \times 10^4}{V} \text{ mg/ℓ } CaCO_3$$

Where

A = mℓ standard acid solution required for the phenolphthalein endpoint of pH 8,3.

B = mℓ standard acid solution required for the mixed acid indicator endpoint of pH 4,5.

M = the concentration acid in mol/ℓ.

V = mℓ sample.

1.2 BORON - DISSOLVED, AUTOMATED, AZOMETHINE - 0005101

Optimum concentration range: 0,05 to 2,0 mg/ℓ boron.

Application of method

This method is applicable to surface, ground and drinking water.

Principle of method

The method is based on the formation of a coloured complex between boron and azomethine-H at a pH of 6,6 (Basson, Böhmer and Stanton, 1969). EDTA is added to the samples before the addition of the azomethine-H solution to prevent interferences from zinc, copper, iron and aluminium.

Reagents

(i) Azomethine-H solution. Dissolve 1 g azomethine-H and 4 g ascorbic acid ($C_6H_8O_8$) in 200 mℓ deionized water. Filter the solution through a 0,45 µm membrane filter and leave it to stand for at least an hour before use. The solution must be freshly prepared daily. Store in a polyethylene container.

(ii) Buffer solution. Dissolve 120 g ammonium acetate ($H_3CCOONH_4$) and 15 g EDTA ($C_{10}H_{14}N_2Na_2O_8.2H_2O$) in 800 mℓ deionized water. Dilute to 1 ℓ. Adjust the pH to 6,6 with a 2 mol/ℓ sulphuric acid (H_2SO_4) solution. Store in a polyethylene container.

(iii) Preparation of azomethine-H. Dissolve 18 g 8-amino-1-napthol-3,6 disulfonic acid in 1 ℓ deionized water. Filter if necessary. Adjust the pH to 7,0 with a mol/ℓ potassium hydroxide (KOH) solution. Add concentrated hydrochloric acid (HCl) until the pH is 1,5. To this solution add 20 mℓ salicylaldehyde ($C_7H_6O_2$) and shake vigorously. Allow to stand for 16 h and then filter. Wash the azomethine-H 5 times with ethanol and dry for 3 h at 100°C.

(iv) Boron stock solution. Dissolve 0,2288 g boric acid (H_3BO_3) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The solution contains 0,04 mg B/mℓ.

(v) Boron standard solutions. Prepare the following series of standards in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ B
5,0	0,2
15,0	0,6
25,0	1,0
35,0	1,4
50,0	2,0

Store the standard solutions in polyethylene containers.

Flow system and general observations

(i) A schematic diagram of the flow system is given in Figure 1.2.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

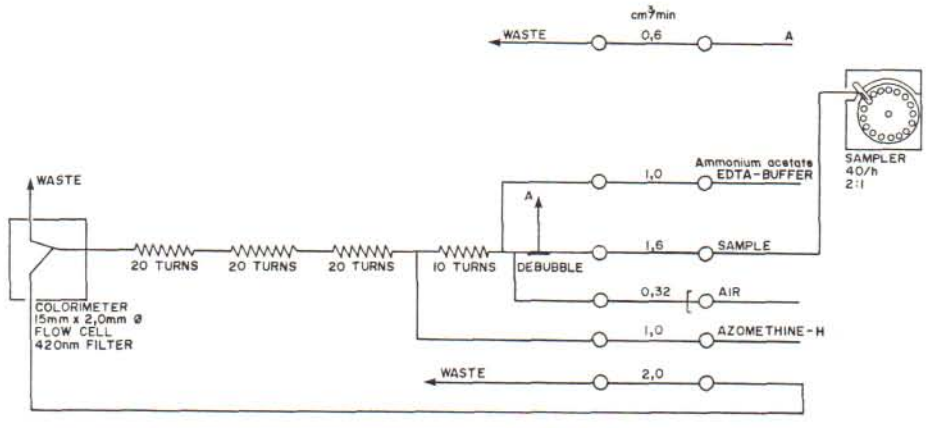


Figure 1.2: Schematic flow diagram for Boron.

1.3 CALCIUM - DISSOLVED, AUTOMATED, ATOMIC ABSORPTION - 0020101

Optimum concentration range: 8 to 200 mg/ℓ calcium.

Application of method

This method is applicable to the analysis of surface, ground and waste waters.

Principle of method

Calcium is determined by atomic absorption spectrometry. Lanthanum is used to prevent the interference of phosphate while the addition of potassium serves as an ionization buffer.

Apparatus

Atomic absorption spectrophotometer equipped with a high solids air-acetylene burner.

Reagents

(i) Hydrochloric acid solution. Add 10 mℓ concentrated hydrochloric acid (HCl) to 500 mℓ deionized water and dilute to 2 ℓ.

(ii) Potassium/lanthanum solution. Dissolve 5 g potassium chloride and 20 g lanthanum chloride in deionized water and dilute to 2 ℓ.

(iii) Standard solutions. Suspend 4,995 g calcium carbonate ($CaCO_3$) (dried for 1 h at 180°C in deionized water and dissolve carefully with a minimum quantity of concentrated hydrochloric acid. Dilute quantitatively to 1 ℓ with deionized water. 1 mℓ contains 2,0 mg Ca^{2+}. Alternatively a Titrisol calcium standard (obtainable from Merck Chemicals) may be used.

(iv) Standard solutions. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume of stock solution (mℓ)	mg/ℓ Ca
Series 1	
4,0	8
16,0	32
30,0	60
40,0	80
50,0	100
Series 2	
10,0	20
30,0	60
50,0	100
70,0	140
100,0	200

The concentration range of the first series of standard solutions is suitable for the analysis of surface water whilst the second series of standard solutions is generally used for groundwater analyses.

Flow system and general observations

(i) Flow diagram for the flow system is represented in Figure 1.3.

(ii) The instrument parameters are given in Table 1.1.

(iii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

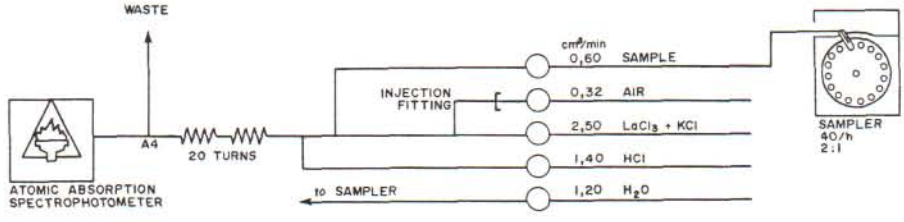


Figure 1.3: Schematic flow diagram of flow system for calcium.

Table 1.1: Instrument parameters for the atomic absorption analysis of calcium.

Wavelength	422,7 nm
Inflammable gas	Acetylene
Oxidant	Air
Flame	Reducing
Current (mA)	5
Slit width (nm)	2,0

1.4 CARBON

1.4.1 Dissolved Organic Carbon, Automated, UV oxidation - 0006101

Optimum concentration range: 0,5 to 40,0 mg/ℓ carbon.

Application of method

This method is applicable to surface, ground and drinking waters.

Principle of method

The sample stream is segmented with CO_2 free air, acidified, heated and sparged with nitrogen gas to remove the inorganic carbon. After resampling and air segmentation, persulphate and mercury(II) nitrate solutions are added and the mixed liquid stream is pumped through a quartz coil where it is exposed to a low intensity ultra violet light. This process oxidizes the dissolved organic carbon to CO_2 which by means of a gas permeable silicon membrane is dissolved in a weakly buffered thymol blue indicator solution. The colour change of the acid-base indicator is measured at 590 nm and is proportional to the concentration of dissolved organic carbon in a water sample (Gravelèt-Blondin, van Vliet and Mynhardt, 1980).

Reagents

(i) Sulphuric acid solution (2,5 mol/ℓ). Add 140 mℓ concentrated H_2SO_4 to 600 mℓ deionized water and dilute to 1 ℓ.

(ii) Buffer solution (pH 9,6). Add 500 mℓ boric acid (6,2 g/ℓ H_3BO_3 in deionized water) to 370 mℓ sodium hydroxide solution (4 g/ℓ NaOH). If necessary adjust the pH to 9,6 with diluted hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions.

(iii) Thymol blue indicator solution. Dissolve 1 g thymol blue in 43 mℓ 0,05 mol/ℓ NaOH solution and dilute it to 1 ℓ with deionized water.

(iv) Stock wetting agent. Dilute 30 mℓ Brij-35 to 1 ℓ with deionized water.

(v) Wetting agent. Add 5 mℓ of solution (iv) to 100 mℓ 0,5 mol/ℓ H_2SO_4 (3 mℓ) dilute to 100 mℓ with deionized water.

(vi) Buffer/indicator solution. Mix the following volumes of the above mentioned solutions and dilute to 500 mℓ with deionized water; 6 mℓ solution (ii), 10 mℓ solution (iii), and 5 mℓ solution (v). Store the solution in a polyethylene container.

(vii) Persulphate solution. Dissolve 4 g potassium persulphate ($K_2S_2O_8$) and 5 g mercury(II) nitrate ($Hg(NO_3)_2$) and dilute to 100 mℓ with 1 mol/ℓ H_2SO_4 solution. This solution must be made up 12 h before use and it remains stable for a further 12 h. Add 30 mℓ solution (iii) to 30 mℓ H_2SO_4 (0,5 mol/ℓ) and dilute to 300 mℓ with solution (iv).

(viii) Stock standard solution. Dissolve 2,1254 g potassium hydrogen phthalate ($C_8H_5O_4K$) (dried for 1 h at 105°C) in deionized water and dilute it quantitatively to 1 ℓ. This solution contains 1 mg/mℓ carbon.

(ix) Standard solutions. Prepare the following series of standards in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ C
2,0	2
5,0	5
10,0	10
15,0	15
20,0	20

Preserve by adding 1 mℓ of a 6 mg/ℓ Hg(II) solution to each standard.

Flow system and general observations

(i) A schematic diagram of the flow system is shown in Figure 1.4.

(ii) The ultra violet light source must be placed in a well ventilated area.

(iii) Samples are filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

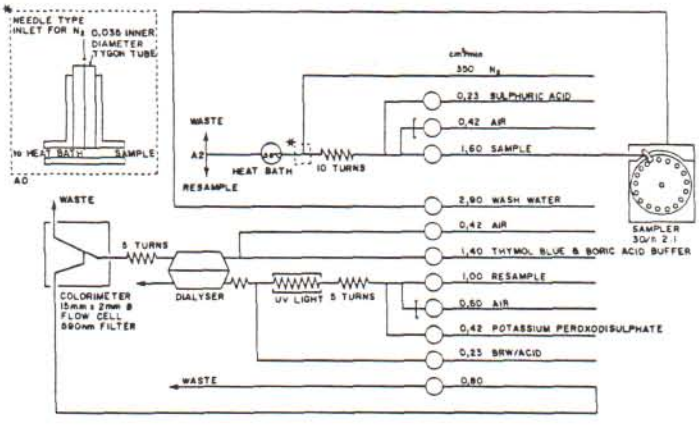


Figure 1.4: Schematic flow diagram for dissolved organic carbon.

1.4.2 Inorganic carbon, Automated - 0006102

Optimum concentration range: 50 to 500 mg/ℓ HCO_3 which is approximately 10 to 100 mg/ℓ carbon.

Application of method

This method is applicable to the analysis of fresh water samples.

Principle of method

The sample stream is segmented with CO_2 free air, acidified and heated which releases CO_2 from dissolved organic carbon. The released CO_2 is introduced into a weakly buffered thymol blue indicator solution by means of a gas permeable silicon membrane. The colour change is then measured at 590 nm and is proportional to the inorganic carbon content of the water (Gravelèt-Blondin, van Vliet and Schoones, 1980).

Reagents

(i) Sulphuric acid solution. 1 ℓ sulphuric acid (0,05 mol/ℓ) wetted with 1 mℓ Brij-35. (Solution A).

(ii) Buffer solution (pH 9,6). Add 500 mℓ boric acid solution (6,2 g/ℓ H_3BO_3 in water) to 370 mℓ of a sodium hydroxide solution (4,0 g/ℓ NaOH in water). If necessary adjust the pH to 9,6 with NaOH or hydrochloric acid (HCl). (Solution B).

(iii) Thymol blue indicator. Dissolve 1 g thymol blue in 43 mℓ of 0,05 mol/ℓ NaOH and dilute to 1 ℓ with water. Stir mixture for 30 min. and filter if necessary. (Solution C).

(iv) Dilute Brij-35 solution. Dilute 30 mℓ commercial concentrate with 1 ℓ deionized water. (Solution D).

(v) Working buffered acid-base indicator. Add together the following and make up to 100 mℓ with water; 5 mℓ solution B, plus 2 mℓ solution D. (Solution E).

(vi) Stock standard solution. Dissolve 1,3768 g $NaHCO_3$ (dried for 1 h at 105°C) in deionized water and make up to 1 ℓ with deionized water. This gives a solution containing 1 000 mg/ℓ HCO_3 (conc. 197 mg/ℓ C). Dilute as necessary for standards.

Both the wash water and acid-base indicator are kept in bottles equipped with CO_2 traps.

Flow system

The flow diagram is shown in Figure 1.5.

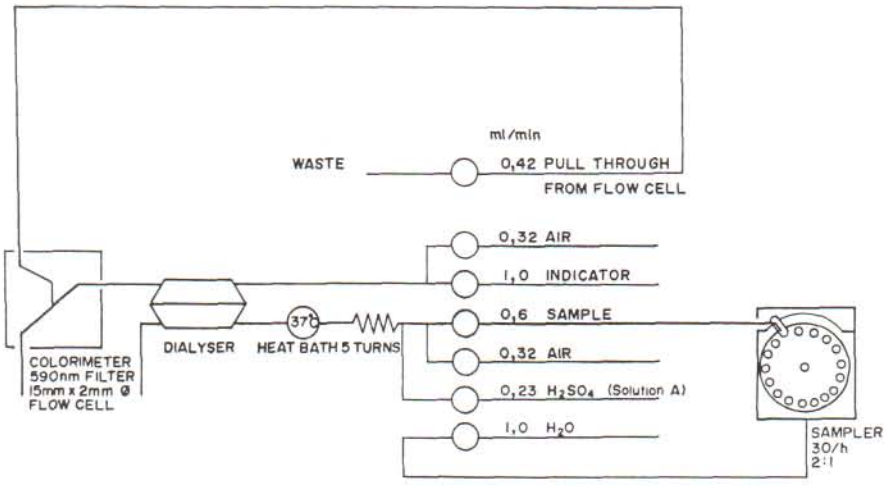


Figure 1.5: Schematic flow diagram of flow system for inorganic carbon.

1.5 CHLORIDE - DISSOLVED, AUTOMATED, FERRIC THIOCYANATE - 0017101

Optimum concentration range: Two series of standards are used for the determination of chloride. The first series determines chloride concentrations of 3 to 300 mg/ℓ and the second series of standards determines chloride in concentration ranges of 50 to 500 mg/ℓ.

Application of method

This method is applicable to surface, ground and waste water.

Principle of method

This automated method for the analysis of chloride in water is based on the reaction between mercury(II) thiocyanate and chloride ions to form low solubility mercury(II) chloride. In the presence of iron(III) the liberated thiocyanate forms an intensely coloured iron(III) thiocyanate complex which is colorimetrically measured at 480 nm.

Sample preservation

There are no specific preservation measures required for chloride.

Reagents

(i) Iron(III) nitrate stock solution. Dissolve 202 g iron(III) nitrate ($Fe(NO_3)_3$) in 500 mℓ deionized water. Dilute the solution to 1 ℓ with concentrated nitric acid (HNO_3).

(ii) Iron(III) nitrate working solution. Combine 100 mℓ concentrated HNO_3 and 150 mℓ $Fe(NO_3)_3$ stock solution and dilute to 1 ℓ with deionized water.

(iii) Mercury(II) thiocyanate stock solution. Add 4,17 g mercury(II) thiocyanate ($Hg(SCN)_2$) to 500 mℓ ethanol (C_2H_5OH, 95%). Dissolve the $Hg(SCN)_2$ by heating the mixture to 50°C. Leave to cool and dilute to 1 ℓ with ethanol.

(iv) Mercury(II) thiocyanate working solution. Add 500 mℓ ethanol to 150 mℓ stock $Hg(SCN)_2$ solution and dilute to 1 ℓ with deionized water.

(v) Brij dilution water. Dilute 5 mℓ Brij-35 wetting agent to 1 ℓ with deionized water.

(vi) Chloride stock solution. Dissolve 8,242 g sodium chloride (NaCl) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The solution contains 5 mg/mℓ Cl.

(vii) Chloride standard solution. Prepare the following two series of standard solutions in volumetric flasks. The concentration range of the series 1 standard solutions are suitable for surface water, whilst the series 2 standard solutions are generally used for ground and waste water analyses.

Volume of stock solution (mℓ)	mg/ℓ Cl
Series 1	
6,0	30
16,0	80
30,0	150
44,0	220
60,0	300
Series 2	
10,0	50
20,0	100
40,0	200
70,0	350
100,0	500

Flow system and general observations

(i) The flow system is shown schematically in Figure 1.6.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

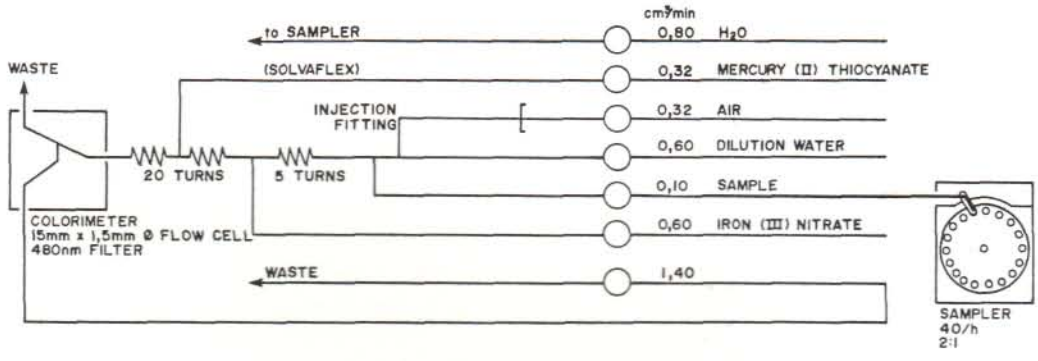


Figure 1.6: Schematic flow diagram for chloride.

1.6 ELECTRICAL CONDUCTIVITY - AUTOMATED - 0101601

Measurement range: 0,1 to 20 000 mS/m.

Application of method

Applicable to all types of water samples.

Principle of method

The method makes use of a micro-flow conductivity cell, with two platinum wire electrodes. The aspirated sample is first pumped through a jacketed capillary coil, through which water thermostated at 25°C is pumped. The sample, at a temperature of 25°C, then passes through the micro-flow conductivity cell. This cell is also jacketed and kept at 25°C. Conductivity measurements are made using a good quality conductivity meter attached to the micro-flow conductivity cell. The range selection for the appropriate conductivity value is done automatically. Calibration is done using 0,01 mol/ℓ potassium chloride (KCl) (Verhoef and Engelbrecht, 1977).

In our laboratory conductivity is used as a guide in preparing dilutions prior to the analyses of calcium, magnesium, sodium, sulphate and chloride. These dilutions are made to ensure as far as possible that the concentration of these determinands are within the calibration range of the respective automated analytical methods. The relevant dilution factors are as follows:

Conductivity (mS/m)	Dilution factor
less than 260	no dilution
from 260 to 459	5x
from 460 to 899	10x
from 900 to 4999	40x
5 000 and greater	100x

Reagents

Potassium chloride (KCl) solution. Dissolve 1,49852 g KCl in 2 ℓ deionized water. The conductivity of this solution is 141,3 mS/m at 25°C.

Flow system

The flow system is shown schematically in Figure 1.7.

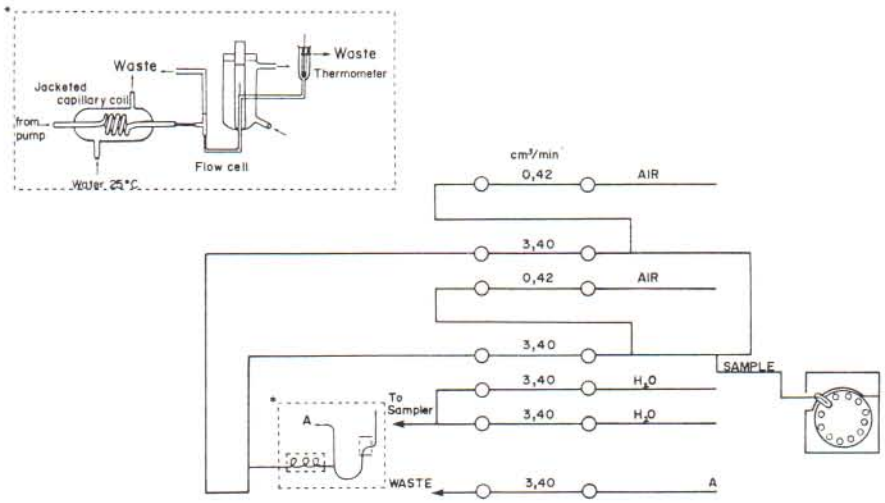


Figure 1.7: Flow diagram of flow system for electrical conductivity.

1.7 FLUORIDE

1.7.1 Fluoride, Dissolved, Automated, Ion Selective Electrode - 0009101

Optimum concentration range: 0,1 to 5,0 mg/ℓ fluoride.

Application of method

This method is applicable to the determination of fluoride in surface, ground and drinking water.

Principle of method

The fluoride electrode consists of a single lanthanum fluoride crystal which is internally in contact with a constant fluoride ion concentration and an internal reference electrode. When the external electrode surface comes in contact with a solution (standard or unknown sample) a potential difference originates over the crystal which is dependent on the different fluoride concentrations in contact with the crystal surfaces. An external reference electrode (calomel) which allows the measurement of the membrane or crystal potential, completes the circuit. Variations in the ionic strength between samples and standards must be prevented. Since only the free fluoride ion activity gives a response, the formation of un-dissociated hydrogen fluoride must be prevented. The automated procedure is designed to reduce these problems. (Technicon AutoAnalyzer methodology).

Interferences

Polyvalent cations such as for example, Al(III), Fe(III), Si(IV) will remove free fluoride ions out of a solution by the formation of soluble complexes. Similarly, the pH must be greater than 5, seeing that the presence of molecular hydrogen fluoride lowers the free fluoride ion concentration. In this method the cyclohexylene-diamine-tetraacetic acid (CDTA) must be used as a complexing agent, whilst an excess of sodium chloride ensures a constant ionic strength.

The pH of the sample solution must be lower than 8 since hydroxide ions make a significant contribution to the electrode response.

Sample preservation

(i) No specific preservation measures are required.

(ii) Samples must be collected and stored in polyethylene containers. Glass bottles must preferably be avoided.

Apparatus

Technicon ion selective electrode module equipped with the following electrodes:

Fluoride electrode no. 94-09-00, Orion Research Inc.
Reference electrode no. 90-01-00, Orion Research Inc.

Reagents

(i) Fluoride stock solution A. Dissolve 1,105 g sodium fluoride (NaF) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The solution contains 0,5 mg/mℓ F. Dilute 100 mℓ of stock solution A quantitatively to 1 ℓ. This solution contains 0,05 mg/mℓ F.

(ii) Buffer solution. Dissolve 120 g sodium acetate ($NaCH_3COO$) in 1 ℓ deionized water. Add to this solution 7,5 g CDTA (1,2-cyclohexane-diamine-tetraacetic acid monohydrate), plus 100 mℓ acetic acid (CH_3COOH), together with 18 g sodium chloride (NaCl) and 0,2 mℓ Brij-35 wetting agent, and 3mℓ of the fluoride stock solution A. Stir to dissolve and adjust pH to 5,8 with sodium hydroxide (NaOH). Make up to 2 ℓ with deionized water.

(iii) Fluoride standard solutions. Prepare the following series of fluoride standard solutions in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ F
10,0	0,5
30,0	1,5
50,0	2,5
80,0	4,0
100,0	5,0

Flow system and general observations

(i) A schematic diagram of the flow system is shown in Figure 1.8.

(ii) The calomel reference electrode must always be full and kept in deionized water. The electrode may not dry out.

(iii) At the end of a work day the electrodes are lifted out of the temperature controlled compartment. The buffer feeding tube is placed in deionized water which is then pumped through for a period of 15 min, both drainage tubes from the electrodes are clamped and after this the pump platten is lifted.

(iv) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

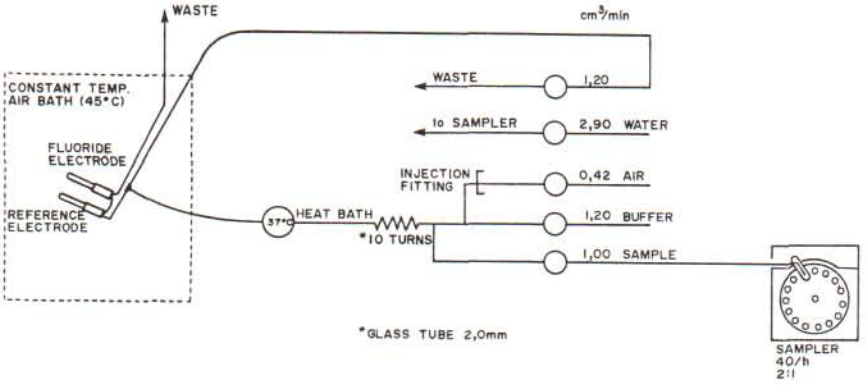


Figure 1.8: Schematic flow diagram for fluoride (ion electrode).

1.7.2 Fluoride, Dissolved, Automated, SPADNS - 0009102

Optimum concentration range: 0,05 to 5,00 mg/ℓ fluoride.

Application of method

This method is applicable to the determination of fluoride in surface, ground and waste water.

Principle of method

In the inverse colorimetric method, the fluoride ion concentration is directly proportional to the extent of discolouration of the dye formed from zirconium and 2-(sulphophenylazo)-1,8-dihydroxy-naphthalene-3,6 disulfonic acid (SPADNS).

There are interferences present in this method which cannot be controlled in a direct analysis technique. Fluoride is therefore selectively removed by means of distillation. Distillation of both fluorosilicic acid and hydrogen fluoride from an acid solution with a high boiling point (sulphuric acid), separates fluoride from the sample.

Apparatus

Special continuous flow distillation unit which is shown schematically in Figure 1.9.

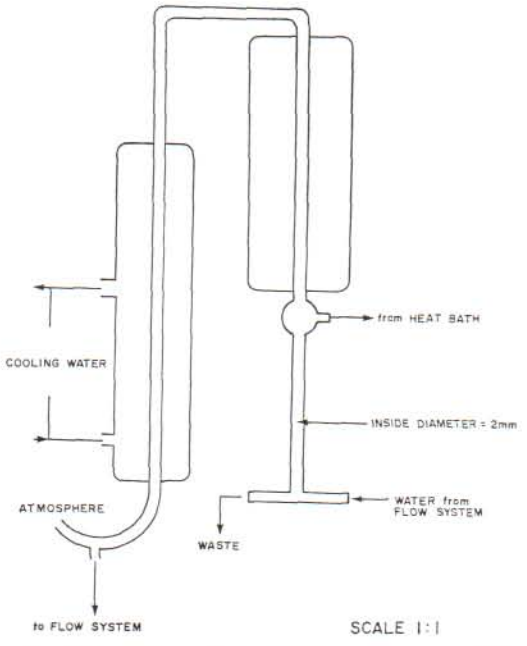


Figure 1.9: Schematic diagram of the continuous flow distillation unit.

Reagents

(i) Sulphuric acid solution. Carefully add 2 ℓ concentrated sulphuric acid (H_2SO_4) to 2 ℓ deionized water. Leave to cool and dilute to 5 ℓ.

(ii) SPADNS reagent. Dissolve 0,133 g zirconyl chloride-octahydrate ($ZrOCl_2.8H_2O$) in approximately 500 mℓ deionized water. Add 350 mℓ concentrated hydrochloric acid (HCl) and dissolve 0,958 g SPADNS in this solution. Add 1 mℓ Brij-35 wetting agent and dilute to 1 ℓ. Filter the solution before use. This solution remains stable provided it is stored in a dark container and can be used for an indefinite length of time.

(iii) Stock fluoride Solution A. Dissolve 1,105 g sodium fluoride (NaF) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The solution contains 0,5 mg F/mℓ. Dilute 100 mℓ of stock Solution A quantitatively to 1 ℓ. This solution contains 0,05 mg/mℓ F.

(iv) Standard fluoride solution. Prepare the following series of standard fluoride solutions in volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ F
10,0	0,5
30,0	1,5
50,0	2,5
80,0	4,0
100,0	5,0

Flow system and general observations

(i) A schematic diagram of the flow system is shown in Figure 1.10.

(ii) Due to the accumulation of salts and carbonized organic material in the distillation coil, the coil must be cleaned at least monthly by removing it from the heating bath, rinsing it with concentrated hydrogen fluoride (HF) solution and rinsing it thoroughly with water.

(iii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

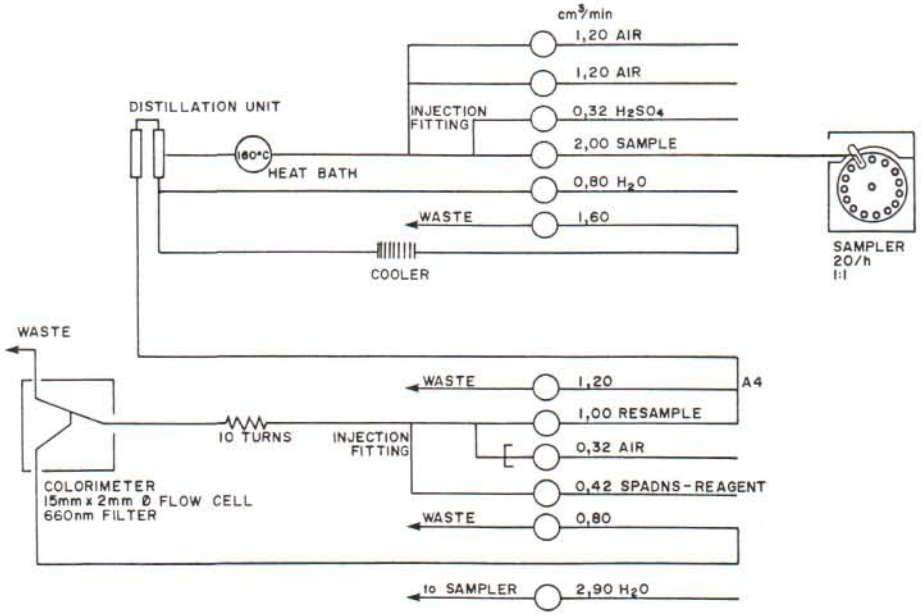


Figure 1.10: Schematic diagram of flow system for fluoride (distillation SPADNS).

1.8 MAGNESIUM - DISSOLVED, AUTOMATED, ATOMIC ABSORPTION - 0012101

Optimum concentration range: 6 to 150 mg/ℓ magnesium whereby one series of standards is used for concentrations between 6 and 75 mg/ℓ magnesium and a second series of standards is used for determinations in the concentration range of 15 to 150 mg/ℓ Mg.

Application of method

This method is applicable for the determination of magnesium in surface, ground and waste water.

Principle of method

Magnesium is determined by atomic absorption spectrometry. Lanthanum is used to prevent the interference of phosphate while the addition of potassium serves as an ionization buffer.

Apparatus

Atomic absorption spectrophotometer equipped with a high solids air-acetylene burner.

Reagents

(i) Hydrochloric acid solution. Add 10 mℓ concentrated hydrochloric acid (HCl) to 500 mℓ deionized water and dilute to 2 ℓ.

(ii) Potassium/lanthanum solution. Dissolve 5 g potassium chloride (KCl) and 20 g lanthanum chloride ($LaCl_3$) in deionized water and dilute to 2 ℓ.

(iii) Standard solutions. Dissolve 2,487 g magnesium oxide (MgO) (dried for 1 h at 180°C) in 10 mℓ nitric acid (HNO_3) and dilute quantitatively to 1 ℓ with deionized water. One mℓ contains 1,5 mg Mg^{2+}. Alternatively a Titrisol (Merck Chemicals) standard may be used.

(iv) Standard solutions. Prepare the following 2 series of standard solutions in 1 ℓ volumetric flasks.

Volume of stock solution (mℓ)	mg/ℓ Mg
Series 1	
4	6,0
16	24,0
30	45,0
44	60,6
50	75,0
Series 2	
10	15,0
20	45,0
50	75,0
70	105,0
100	150,0

The concentration range of the first series of standard solutions is suitable for the analysis of surface water whilst the second series of standard solutions is generally used for ground and waste water analyses.

Flow system and general observations

(i) Flow diagram for the flow system is shown in Figure 1.11.

(ii) The instrument parameters are given in Table 1.2.

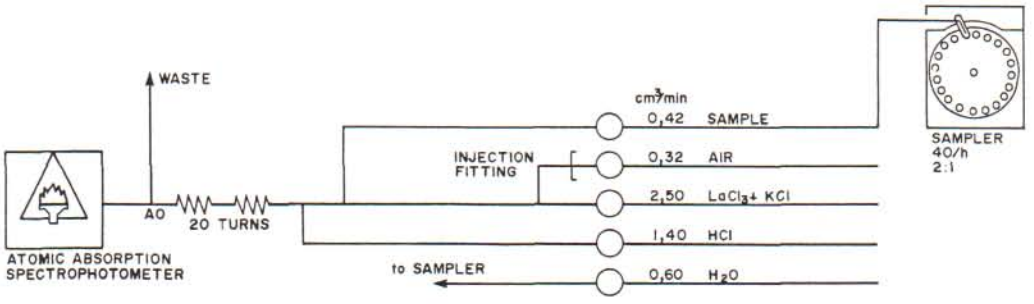


Figure 1.11: Schematic flow diagram for magnesium.

Table 1.2: Instrument parameters for the atomic absorption analysis of magnesium.

Wavelength	215,2 nm
Fuel	Acetylene
Oxidant	Air
Flame	Oxidizing
Current (mA)	3
Slit width (nm)	0,5

1.9 NITROGEN

1.9.1 Nitrate, Dissolved, Automated, Cadmium Reduction - 0007101

Optimum concentration range: 0,1 to 2,0 mg/ℓ nitrogen.

Application of method

This method is applicable to the determination of nitrate in surface, ground and drinking water.

Principle of method

This method is based on the reduction of nitrate to nitrite and the subsequent colorimetric determination of nitrite. The reduction takes place in a copper coated cadmium tube. The conditions for the reduction process are set up such that the nitrate can be quantitatively reduced to nitrite. The effectiveness of the reduction of nitrate to nitrite is dependent on the metal of the reductor, the pH of the solution and the activity of the metal surfaces. Copper coated cadmium is suitable for this heterogeneous reduction but in a neutral medium the cadmium ions, which are produced in the reduction process, form a precipitate with hydroxide ions. In addition the reduction potential is a function of the hydrogen ion concentration with the result that the pH, especially on the surface of the metal, changes if the solution is not buffered. Since the buffering capacity of the surface water is not sufficient, ammonium chloride is added, which serves as both a complexing agent and a buffer.

The colorimetric determination of the formed nitrite is based on the reaction of nitrite with an aromatic amine (sulphanyl-amide-hydrochloride) which results in the formation of a diazonium compound which is coupled to a second aromatic amine (n-1-naphthylethylene-diamine-dihydrochloride). The reaction results in the formation of an azo-dye.

The absorbence of the dye is measured colorimetrically at 520 nm. Separate nitrate and nitrite values can be obtained through analysis without the initial reduction step (van Vliet, 1974).

Sample preservation

Preservation of the samples by the addition of 20 mg/ℓ Hg(II) is essential.

Interferences

The concentration of oxidation and reduction agents and potential interfering metal ions is usually low in the surface water. Metal ions which are present at high concentration, can lead to positive errors.

Reagents

(i) Colour reagent. Add 50 mℓ concentrated phosphoric acid (H_3PO_4) to 400 mℓ deionized water. Dissolve 20 g sulphanilamide ($C_6H_8N_2O_2S$) and 1 g N-1-naphthylethylenediamine dihydrochloride ($C_{10}H_7NHCH_2NH_2.2HCl$) in this solution and dilute to 500 mℓ with deionized water. Store in a dark glass bottle. This solution is stable for one week.

(ii) Buffer solution. Dissolve 30 g ammonium chloride and 0,2 g EDTA ($C_{10}H_{14}N_2Na_2O_8.2H_2O$) in 800 mℓ deionized water. Add 0,5 mℓ Brij-35 wetting agent and dilute to 1 ℓ. Adjust the pH to 6,6 with diluted NH_3 solution (10 mℓ concentrated NH_3 solution diluted to 100 mℓ with deionized water).

(iii) Nitrate stock solution A. Dissolve 0,7218 g potassium nitrate (KNO_3) (dried for 1 h at 105°C) and dilute to 1 ℓ with deionized water. Preserve the stock solution with 20 mg/ℓ Hg(II). This solution contains 0,1 mg/mℓ NO_3-N. Stock solution B. Dissolve 2,888 g dried KNO_3, as described for stock solution A. This solution contains 0,4 mg/mℓ NO_3-N.

(iv) Nitrate standard solution. Prepare the following series of standard solutions in 1 ℓ volumetric flasks by appropriate quantitative dilution of the stock solutions.

Volume of stock solution (mℓ)	mg/ℓ NO_3-N
Series 1	
4,0	0,4
10,0	1,0
20,0	2,0
30,0	3,0
40,0	4,0
Volume stock solution B (mℓ)	mg/ℓ NO_3-N
Series 2	
5,0	2,0
10,0	4,0
25,0	10,0
35,0	14,0
50,0	20,0

Preserve the series of standard solutions with 20 mg/ℓ Hg(II) and store in polyethylene containers at 4°C.

(v) Preparation of the reductor tube. The reductor coil is made out of a 1 m long cadmium tube (Reactor Experiments Inc. California, USA) with an internal diameter of 1 mm. With the use of a syringe, 10 mℓ of a 2 mol/ℓ HCl solution is injected through the tube and that is followed by deionized water. The reductor is then connected to the flow system, and, with all the reagents flowing the highest standard solution is aspirated until constant peak heights are obtained.

Flow system and general observations

(i) The flow system is schematically represented in Figure 1.12.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

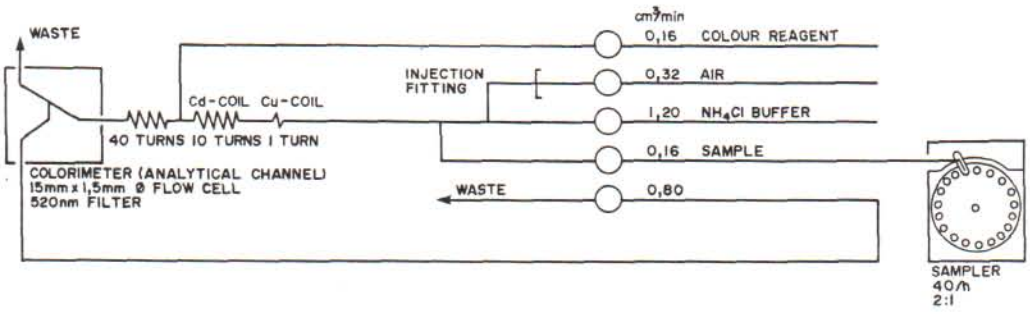


Figure 1.12: Schematic diagram of flow system for nitrate (cadmium reduction).

1.9.2 Kjeldahl Nitrogen, Dissolved, Automated - 0007003

Optimum concentration range: 0,05 to 4,00 mg/ℓ nitrogen.

Application of method

This method is applicable to the determination of Kjeldahl nitrogen in surface, ground, waste and drinking water.

Principle of method

The sample is dried and digested with concentrated sulphuric acid, which converts the organic nitrogen to ammonium ions. Inorganic nitrogen compounds which include amongst others, nitrate, nitrite, organic oximes and azides are not reduced. For most surface waters it is accepted that the method determines the sum of the ammonium originally present and the organic nitrogen. The digestion is accelerated in two ways: by the addition of Mercury(II) as a catalyst and potassium sulphate.

After digestion of the sample, the ammonium ions are determined using the indophenol-blue method (van Vliet, 1974).

Reagents

(i) Digestion mixture. Dissolve 2 g mercury oxide (HgO) in a 25 mℓ 3mol/ℓ sulphuric acid (H_2SO_4) solution. Add 200 mℓ concentrated H_2SO_4 to 500 mℓ deionized water, and whilst it is still warm add 134 g potassium sulphate (K_2SO_4) to the solution. Combine the two above mentioned solutions, leave to cool and dilute to 1 ℓ with deionized water. As K_2SO_4 has the tendency to crystalyze out at <20°C the solution must be stored at about 25°C.

(ii) Sodium nitroprusside solution. Dissolve 0,25 g sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H_2O$) in 400 mℓ deionized water and make up to 500 mℓ. Allow to stand overnight before use.

(iii) Phenol working reagent. Dissolve 83 g phenol and 36 g sodium hydroxide in 900 mℓ deionized water, and dilute to 1 ℓ with deionized water.

(iv) Sodium hydroxide stock solution. Dissolve 8,75 g sodium hydroxide in 400 mℓ deionized water, and make up to 500 mℓ.

(v) Complexing buffer. Dissolve 33 g potassium sodium tartrate ($C_4H_4KNaO_6.4H_2O$) and 24 g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in deionized water and make up to 1 ℓ. Add 3 drops Brij-35 wetting agent. Replace this solution every third day.

(vi) Sodium hypochlorite (NaOCl) solution. Dilute 150 mℓ commercial bleach solution (Javel) to 200 mℓ with deionized water.

(vii) Wash water. Add 100 mℓ of the digestion mixture to 500 mℓ deionized water.

Digestion procedure

(i) Pipette 10 mℓ sample into a digestion tube. Add 2 mℓ digestion mixture with the aid of an automatic injection pipette (Zipette, Holpro, Johannesburg) and 3 carborundum boiling chips.

(ii) Place the digestion tubes in a pre-heated (150°C) digestion block. After 20 min increase the temperature of the block to 380°C. Digest the samples for 35 ± 5 min after the temperature of 380°C is reached.

(iii) Immediately remove the tubes from the block, leave to cool and add 10 mℓ deionized water. Shake well to dissolve any precipitate; a Vortex mixer (Whirlimixer, Labotec) is suitable for this purpose.

(iv) Each group of samples digested per digestion block, must include a set of standards and a blank solution.

Flow system and general observations

(i) The flow system is shown schematically in Figure 1.13.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C equivalent).

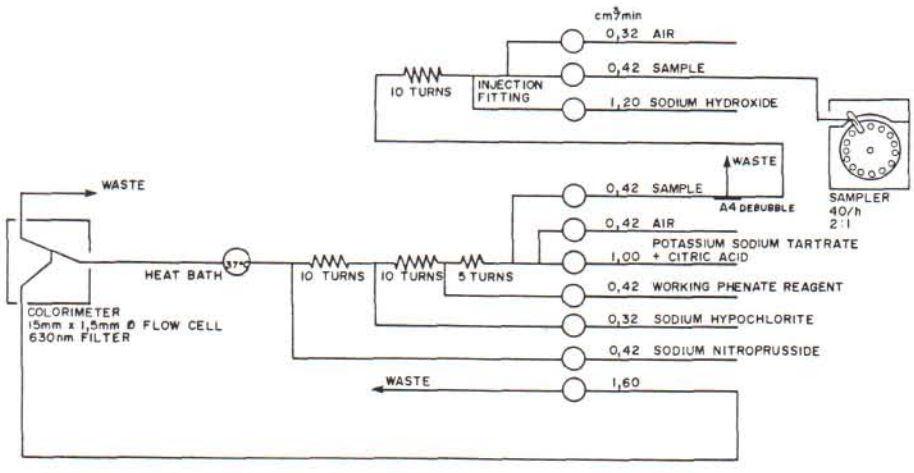


Figure 1.13: Schematic diagram of flow system for Kjeldahl nitrogen.

1.9.3 Ammonium, Dissolved, Automated, Indophenol-blue - 0007102

Optimum concentration range: 0,02 to 2,00 mg/ℓ ammonium.

Application of method

This method is applicable to the determination of ammonium in surface, ground and drinking water.

Principle of method

Ammonium reacts in a mildly alkaline medium with hypochlorite to form monochloramine, which forms indophenol-blue in the presence of phenol, catalytical quantities of nitroprusside and an excess of hypochlorite. The forming of monochloramine requires a pH between 8 and 11,5. At higher pH values incomplete oxidation of ammonium to nitrite occurs.

Precipitates of calcium and magnesium hydroxide are formed at pH values higher than 9,6 and a complexing agent (citrate) is added to prevent precipitation.

Reagents

(i) Sodium nitroprusside solution. Dissolve 1 g sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H_2O$) in 800 mℓ deionized water and dilute to 1 ℓ.

(ii) Phenol stock solution. Dissolve 300 g phenol (C_6H_5OH) in 500 mℓ ethanol.

(iii) Sodium hydroxide stock solution. Dissolve 400 g sodium hydroxide (NaOH) in 1 ℓ deionized water.

(iv) Complexing buffer. Dissolve 200 g potassium sodium tartrate ($C_4H_4KNaO_6.4H_2O$) and 25 g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and dilute to 1 ℓ in deionized water.

(v) Phenol working solution. Add 50 mℓ phenol stock solution to 100 mℓ NaOH stock solution and dilute to 1 ℓ with deionized water.

(vi) Sodium hypochlorite (NaOCl) solution. Dilute 60 mℓ commercial bleach solution (Javel) to 200 mℓ with deionized water.

(vii) Ammonium stock solution. Dissolve 0,1911 g ammonium chloride (NH_4Cl) (dried for 2 h at 105°C) and dilute quantitatively to 1 ℓ with deionized water.

(viii) Ammonium standard solutions. Prepare in 500 mℓ volumetric flasks the following series of ammonium standards by appropriate quantitative dilution of the concentrated stock solution which contains 0,05 mg/mℓ NH_4-N.

Volume stock solution (mℓ)	mg/ℓ NH_4-N
4,0	0,2
10,0	0,5
20,0	1,0
30,0	1,5
40,0	2,0

Preserve the standard solutions with 20mg/ℓ Hg(II) and store in polyethylene containers at 4°C.

Flow system and general observations

(i) The flow system is represented in Figure 1.14. To avoid contamination of the system by ammonium which may be present in the atmosphere of the laboratory, the segmentation air must be pumped through a 2,5 mol/ℓ H_2SO_4 solution.

(ii) The flow system must be rinsed periodically with diluted sulphuric acid to prevent the accumulation of hydroxide precipitates.

(iii) As a result of the complex forming of Hg(II) with ammonium ions, mercury chloride gives a negative interference. This can be overcome by adding the same quantity of Hg(II) to the standard used for the calibration curves and the samples.

(iv) The colour intensity is pH dependent. If samples are preserved with acid, both the standard solution and the washing water must contain the same quantity of acid.

(v) The ammonium concentration in the standard changes with time. Therefore it is necessary to prepare fresh standard solutions at least once a week.

(vi) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C equivalent).

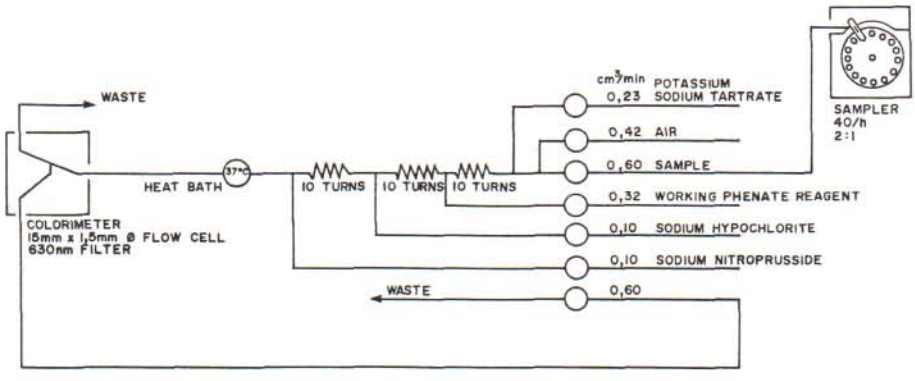


Figure 1.14: Schematic flow diagram for ammonium (indophenol-blue).

1.10 pH - AUTOMATED - 0001601

Measurement range: 2,5 to 12 pH units.

Application of method

Applicable to all water samples.

Principle of method

The sample is pumped past a micro-pH electrode, connected to a suitable pH meter (Verhoef and Engelbrecht, 1977). Calibration is done with pH 7 and pH 11 buffers.

Reagents

(i) Hydrochloric acid (HCl) solution: Add HCl to 10 ℓ deionized water until a pH of 2,3 is reached. Preserve the solution with 0,5 mℓ $HgCl_2$.

(ii) Buffer solution (pH 7). Add commercially available Titrisol buffer of pH 7 (Merck Chemicals) to 500 mℓ deionized water.

(iii) Buffer solution (pH 11). Add commercially available Titrisol buffer of pH 11 (Merck Chemicals) to 500 mℓ deionized water.

Flow system

Flow diagram is shown schematically in Figure 1.15.

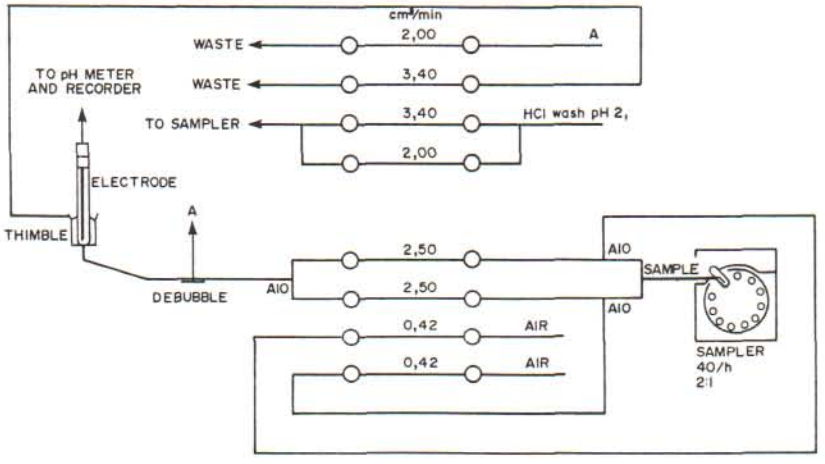


Figure 1.15: Schematic flow diagram of flow system for pH.

1.11 PHOSPHORUS

1.11.1 Introduction

The analytical methods described here are based on the reactions which are specific for the orthophosphate ion. Different phosphorus compounds are present in surface waters and depending on the preliminary sample treatment, the different forms may be determined. The presence of the different forms and the corresponding analytical procedures for the determination of these forms is given in the following classification (Table 1.3).

Table 1.3: Classification of the different forms of phosphorus in water with the corresponding preliminary sample treatment

Chemical form	Preliminary sample treatment
Total phosphate-phosphorus	Acid-oxidation
Orthophosphate	Filtration
Hydrolyzable phosphates	Acid hydrolysis (sulphuric acid)
Organo-phosphate compounds	Acid-oxidation

Application of method

The method is applicable to the determination of phosphate in a water sample or a digested sample.

Principle of method

This method is based on the reaction of the phosphate ion in an acid medium with molybdate to form a molybdophosphate complex, which is reduced to an intensely blue coloured compound. A noticeably high increase in the formation rate of the heteropoly-acid is obtained in the presence of trivalent antimony ions. Ascorbic acid is used as the reducing agent (Murphy and Riley, 1962).

Interferences

(i) No interferences are caused by metal concentrations which are usually present in water. High iron concentrations can however precipitate phosphate. The interference of mercury(II), which is used for sample preservation, is eliminated by the addition of chloride ions.

(ii) Arsenate in concentrations higher than that present in sea water does not interfere, (Murphy and Riley, 1962).

(iii) The influence of silica which also forms a blue complex, is dependent on both the pH and the reaction time. In general it can be accepted that silica does not cause any interferences provided the pH is between 0,8 and 1,0 and the reaction time is not longer than 10 min. Even for longer reaction times silica interferences are usually negligible. For the method described here, 20 mg/ℓ silicon gives an apparent concentration of <0,002 mg/ℓ phosphorus.

Sample preservation

If the samples are not analysed within 2 h after being taken, they must be preserved with 20 mg/ℓ Hg(II).

1.11.2 Phosphorus, Dissolved, Orthophosphate, Automated, Phosphomolybdate - 0015101

Optimum concentration range: 0,05 to 0,5 mg/ℓ phosphorus.

Application of method

This method is applicable to the determination of orthophosphate in filtered surface, ground and waste water samples.

Reagents

(i) Sulphuric acid solution. Add 234 mℓ concentrated sulphuric acid (H_2SO_4) to 700 mℓ deionized water and dilute to 1 ℓ.

(ii) Sampler wash solution. Deionized water.

(iii) SLS solution. Dissolve 15 g sodium lauryl sulphate in 85 mℓ deionized water. (Commercially available at Merck Chemicals as dodecyl hydrogen sulphate sodium salt).

(iv) Sodium chloride solution. Dissolve 5 g sodium chloride (NaCl) in deionized water and dilute to 1 ℓ. Add 2 mℓ SLS solution.

(v) Ammonium molybdate solution. Dissolve 25 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in deionized water and dilute to 1 ℓ.

(vi) Antimony potassium tartrate solution. Dissolve 2,5 g antimony potassium tartrate ($K(SbO)C_6H_4O_6$) in deionized water and dilute to 1 ℓ. Store in a glass container. This solution can be used for as long as it remains clear.

(vii) Combined reagents. Combine gradually and mix thoroughly 200 mℓ ammonium molybdate solution, 250 mℓ H_2SO_4 solution and 50 mℓ antimony potassium tartrate solution. Provided the solution is stored dust free in a dark glass container, it remains stable for several months.

(viii) Ascorbic acid solution. Dissolve 25 g ascorbic acid ($C_6H_8O_3$) in deionized water and dilute to 1 ℓ. Provided the solution is stored in a dark glass container at 4°C, it remains stable for at least a week.

(ix) Phosphate stock solution A. Dissolve 0,5492 g potassium hydrogen phosphate (KH_2PO_4) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The stock solution contains 0,125 mg/mℓ PO_4-P.

(x) Phosphate stock solution B. Dilute 100 mℓ of stock solution A quantitatively to 1 ℓ. This solution contains 0,0125mg/mℓ PO_4-P.

(xi) Phosphate standard solutions. Prepare the following series of standard solutions by quantitative dilution of stock solution B in volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ P
4,0	0,050
10,0	0,125
20,0	0,250
30,0	0,375
40,0	0,500

The above mentioned stock and standard series must be preserved with 20 mg/ℓ Hg(II) solution. Provided the solution is stored at 4°C, it remains stable for several months.

Flow system and general observations

(i) The flow system is schematically shown in Figure 1.16.

(ii) The presence of suspended material in the sample generally leads to a positive bias. Therefore if there is any observable sediment and/or algae in the sample it must be removed by filtration through a prewashed 0,45 µm membrane filter. (Whatman GF/C or equivalent).

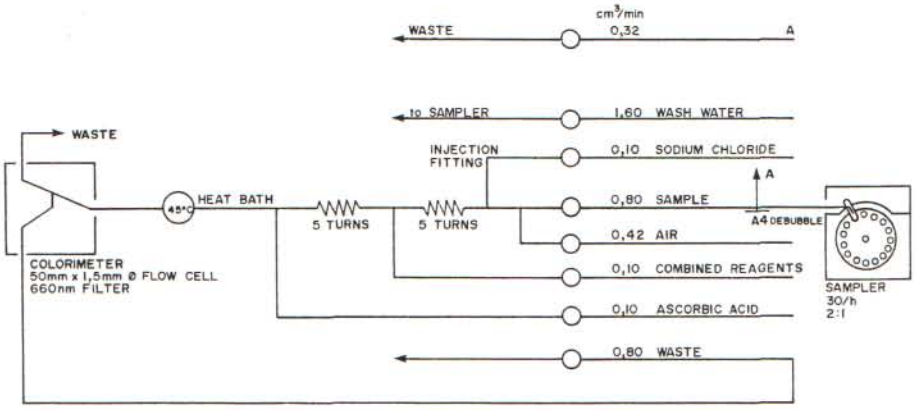


Figure 1.16: Schematic flow diagram for orthophosphate.

1.11.3 Phosphorus, Total, Dissolved, Automated, Phosphomolybdate - 0015002

Optimum concentration range: 0,05 to 0,500 mg/ℓ phosphorus.

Application of method

This method is applicable to the determination of total phosphorus in surface, ground and waste water.

Reagents

(i) Digestion mixture. Add 20 mℓ sulphuric acid (H_2SO_4) to 100 mℓ deionized water. Dissolve 32 g ammonium peroxydisulphate ($(NH_4)_2S_2O_8$) in the above solution and dilute to 200 mℓ and store in a polyethylene container. This solution is stable for 12 h.

(ii) Sampler wash solution. Add 40 mℓ digestion mixture to 800 mℓ deionized water. Boil the mixture under reflux for 20 min and leave to cool.

(iii) SLS solution. Dissolve 15 g sodium lauryl sulphate in 85 mℓ deionized water. (Commercially available at Merck Chemicals as dodecyl hydrogen sulphate sodium salt).

(vi) Sodium chloride solution. Dissolve 15 g sodium chloride (NaCl) in deionized water and dilute to 1 ℓ. Add 2 mℓ SLS solution.

(v) Ammonium molybdate solution. Dissolve 25 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in deionized water and dilute to 1 ℓ.

(vi) Sulphuric acid solution. Add 152 mℓ concentrated H_2SO_4 to 800 mℓ deionized water and dilute to 1ℓ.

(vii) Antimony potassium tartrate solution. Dissolve 2,5 g antimony potassium tartrate ($K(SbO)C_6H_4O_6$) in deionized water and dilute to 1 ℓ. Store in a glass container. This solution can be used for as long as it remains clear.

(viii) Combined reagents. Combine gradually and mix thoroughly 200 mℓ ammonium molybdate solution, 250 mℓ H_2SO_4 solution and 50 mℓ tartrate solution. Provided the solution is stored dust free in a dark glass container, it remains stable for several months.

(ix) Ascorbic acid solution. Dissolve 25 g ascorbic acid ($C_6H_8O_3$) in deionized water and dilute to 1 ℓ. Provided the solution is stored in a dark glass container at 4°C, it remains stable for at least a week.

(x) Phosphate stock solution A. Dissolve 0,5492 g potassium di-hydrogen phosphate (KH_2PO_4) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. The stock solution contains 0,125 mg/mℓ PO_4-P.

(xi) Phosphate stock solution B. Dilute 100 mℓ of stock solution A quantitatively to 1 ℓ. This solution contains 0,0125 mg mℓ PO_4-P.

(xii) Phosphate standard solutions. Prepare the following series of standard solutions by quantitative dilution of stock solution B in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ P
4,0	0,050
10,0	0,125
20,0	0,250
30,0	0,375
40,0	0,500

The above mentioned stock and standard series must be preserved with 20 mg/ℓ Hg(II) solution. Provided the solution is stored at 4°C, it remains stable for several months.

Digestion procedure

The acidified samples are autoclaved in closed containers with peroxodisulphate. Potential interferences from the released chlorine can be eliminated by adding ascorbic acid to the samples in the flow system, before the molybdate reagent is added.

The oxidation takes place in 30 mℓ pyrex test tubes which are equipped with screw tops with teflon linings. An ordinary kitchen pressure cooker can be used as an alternative to an autoclave.

Analysis of samples

Pipette 20 mℓ of sample in the test tube and add 2 mℓ digestion mixture. Screw the cap closed and autoclave for 30 min at 103 kPa. The volume of the samples must remain unchanged. The total phosphate is determined with the flow system in Figure 1.17.

If a pressure cooker is used the digestion should take place for 30 min at 103 kPa.

Analysis of standards and blanks

Take 20 mℓ of each standard solution or 20 mℓ deionized water in the case of the blank, and conduct the digestion process in the same way as is described for samples in the digestion process mentioned earlier. A series of standards and two blanks must be included with each group of samples to be digested.

Flow system and general observations

(i) The flow system is shown schematically in Figure 1.17.

(ii) Glass apparatus. All glassware used in the analysis must be washed with a 5 mol/ℓ HCl solution and this must be followed with deionized water. The glassware must preferably be reserved only for phosphate determinations. After the glassware has been washed it should be covered with deionized water. If this procedure is followed, the hydrochloric acid treatment is only required periodically.

(iii) Commercial washing agents must not be used under any circumstances. If necessary only phosphate free washing agents should be used.

(iv) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

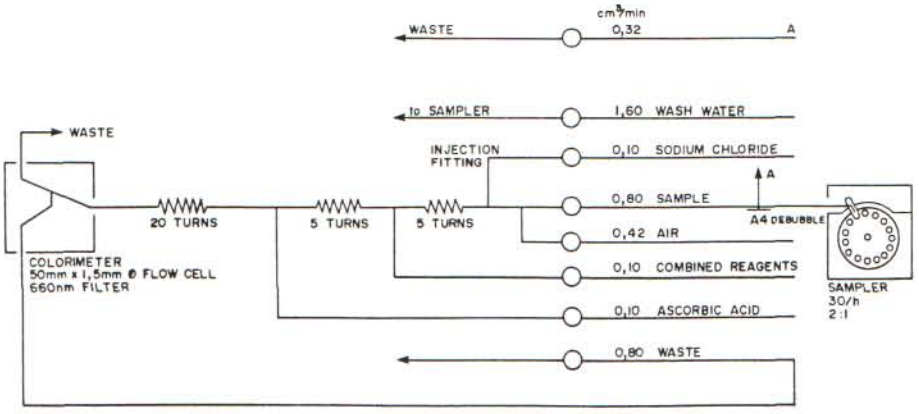


Figure 1.17: Schematic flow diagram of flow system for total phosphate.

1.12 POTASSIUM, DISSOLVED, AUTOMATED, FLAME EMISSION - 0019101

Optimum concentration range: 2 to 50 mg/ℓ potassium.

Application of method

This method is applicable to the determination of potassium in surface, ground and waste water.

Principle of method

Potassium is determined by flame emission spectroscopy. Lithium is used as an internal standard.

Apparatus

Technicon dual channel flame photometer, model no. 110-A005-03.

Reagents

(i) Internal standard solution. Dissolve 2,38 g lithium nitrate (LiNO3) in 1 ℓ deionized water and dilute to 2 ℓ.

(ii) Standard solutions. Dissolve 1,9066 g potassium chloride (KCl) (dried for 1 h at 140°C) in deionized water and dilute quantitatively to 2 ℓ. 1 mℓ contains 0,5 mg K.

(iii) Standard solutions. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume of stock solution (mℓ)	mg/ℓ K
Series 1	
4,0	2
16,0	8
30,0	15
40,0	20
50,0	25
Series 2	
10,0	5
30,0	15
50,0	25
70,0	35
100,0	50

Flow system and general obersevations

(i) A schematic diagram of the flow system is given in Figure 1.18.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

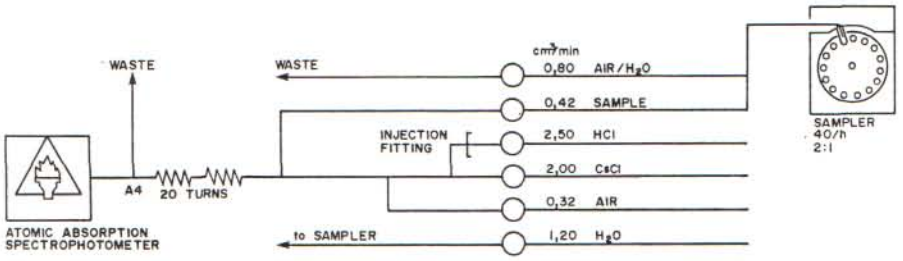


Figure 1.18: Schematic flow diagram for potassium.

1.13 SILICA - DISSOLVED, AUTOMATED, MOLYBDATE BLUE - 0014101

Optimum concentration range: 0,5 to 40,0 mg/ℓ silica.

Application of method

This method is applicable to the determination of silica in surface, ground and drinking water.

Principle of method

The automated determination of silica in the ortho-silicate form in natural water is based on the formation of β-1:12 molybdosilicic acid and the partial reduction of that to a blue heteropoly acid. The β-isomer of the 1:12 molybdosilicic acid. is unstable and is spontaneously converted to the α-isomer. This isomerization is dependent on temperature, pH and the dissolved salt concentration, and requires a relatively long period for completion.

By setting the pH at 2 the conditions for the forming of the β-complex are optimized (Truesdale, Smith and Smith, 1979). The addition of oxalic acid prevents the forming of the phosphorus complex by reacting with the excess molybdate. The reduction of the β-isomer with ascorbic acid produces a heteropoly acid which is measured colorimetrically at 660 nm.

Sample preservation

(i) Samples for silica analysis must preferably not come in contact with glass and should be stored in polyethylene containers.

(ii) Preserve the samples with 20 mg/ℓ Hg(II) per sample.

(iii) Silica has the tendency to polimerize at low temperatures so the samples must not be frozen.

Reagents

(i) Ammonium molybdate solution. Dissolve 5 g ammonium molybdate ($(NH_4)_6MoO_{24}.4H_2O$) in deionized water. Add 1,4 mℓ sulphuric acid (H_2SO_4) and dilute to 1 ℓ.

(ii) Oxalic acid solution. Dissolve 50 g oxalic acid ($C_2H_2O_4.2H_2O$) in 800 mℓ deionized water. Add 2,5 mℓ Contrad (Merck Chemicals) and dilute to 1 ℓ.

(iii) Ascorbic acid solution. Dissolve 17,6 g ascorbic acid ($C_6H_8O_6$) in deionized water and dilute to 1 ℓ. Provided the solution is stored at 4°C in a dark glass container, it is stable for at least a week.

(iv) Silica stock solution. By making use of a commercially available silica concentrate (Titrisol, Merck Chemicals) prepare a stock solution that contains 1000 mg/ℓ silicon.

(v) Silica standard solution. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume stock solution (mℓ)	mg/ℓ Si
4,0	4
10,0	10
20,0	20
30,0	30
40,0	40

Preserve the series of standard solutions with 20 mg/ℓ Hg(II) solution and store in a polyethylene container.

Flow system and general observations

(i) The flow system is shown schematically in Figure 1.19.

(ii) The samples must be free of any observable suspended matter. If there is suspended material present it can be removed by filtration through a prewashed 0,45 µm membrane filter. (Whatman GF/C or equivalent)

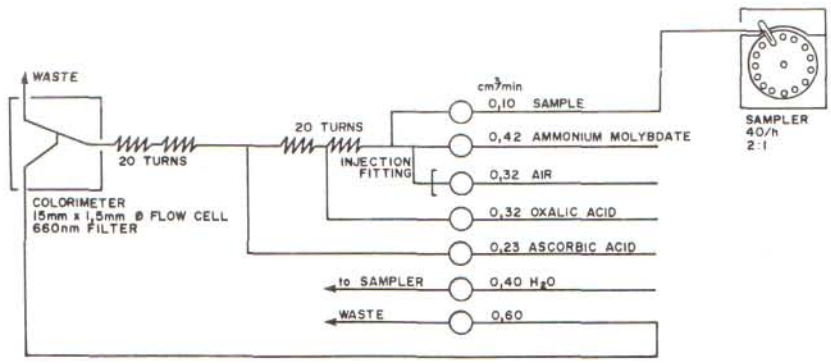


Figure 1.19: Schematic flow diagram for silica.

1.14 SODIUM - DISSOLVED, AUTOMATED, FLAME EMISSION - 0011101

Optimum concentration range: 16 to 400 mg/ℓ sodium.

Application of method

This method is applicable to the determination of sodium in surface, ground and waste water.

Principle of method

Sodium is determined by means of flame emission spectroscopy. Lithium is used as an internal standard.

Apparatus

Technicon dual channel flame photometer.

Reagents

(i) Internal standard solution. Dissolve 2,38 g lithium nitrate ($LiNO_3$) in 1 ℓ deionized water and dilute to 2 ℓ.

(ii) Standard solutions. Dissolve 20,336 g sodium chloride (NaCl) (dried for 1 h at 140°C) in deionized water and dilute quantitatively to 2 ℓ, 1 mℓ contains 4,0 mg Na.

(iii) Standard solutions. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume of stock solution (mℓ)	mg/ℓ Na
Series 1	
4,0	16
16,0	64
30,0	120
40,0	160
50,0	200
Series 2	
10,0	40
30,0	120
50,0	200
70,0	280
100,0	400

Flow system and general observations

(i) A schematic diagram of the flow system is given in Figure 1.20.

(ii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

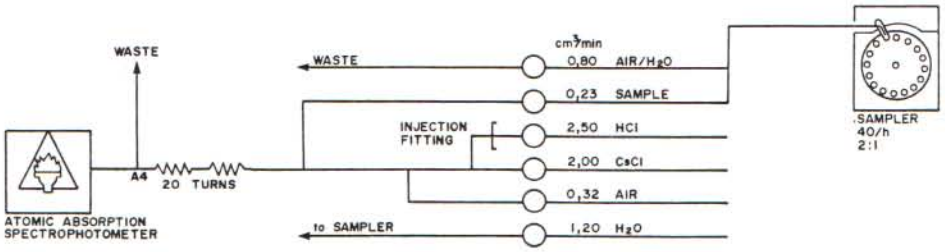


Figure 1.20: Schematic diagram of flow system for sodium.

1.15 SULPHATE - DISSOLVED, AUTOMATED, TURBIDIMETRIC - 0016101

Optimum concentration range: 15 to 250 mg/ℓ sulphate.

Application of method

This method is applicable to the determination of sulphate in surface, ground and drinking water.

Principle of method

The method is based on the formation of a low solubility barium sulphate suspension (Ksp of $BaSO_4$ = 9,2 x 10^{-11}) in a gelatine medium. The degree of suspension of the $BaSO_4$ is dependent on the reaction conditions and it is necessary to ensure that the conditions and requirements with regard to the reagents must be strictly followed. The addition of hydrochloric acid prevents the forming of precipitates of sulphite, carbonate, chromate and phosphate with barium. The turbidity of the $BaSO_4$ suspension is determined colorimetrically at 405 nm.

High sulphate concentrations lead to the forming of an excess $BaSO_4$ which accumulates on the walls of the flow cell and of the tubes. The addition of EDTA to a large extent prevents this accumulation.

Interferences

(i) Suspended material and colour cause interferences in the determination. Precautions must be taken to ensure the minimum turbidity or colour in the samples. Although the flow system includes a blank channel, it does not compensate fully for relatively high turbidities or colour. It is therefore essential that turbid samples must be filtered or centrifuged.

Reagents

(i) Dilute hydrochloric acid solution. 50 mℓ hydrochloric acid (HCl) dissolved in 2 ℓ deionized water.

(ii) Buffer solution. 60 g EDTA (tetra sodium) and 10 g sodium hydroxide (NaOH) dissolved in 2 ℓ deionized water.

(iii) Barium chloride solution. Heat 500 mℓ deionized water (80°C) in a 2 ℓ beaker. Dissolve 0,05 g thymol crystals in this solution and add 1,5l deionized water. Slowly dissolve 4 g gelatine (carboxyl methyl cellulose) in this. After everything is dissolved add 25 g barium chloride ($BaCl_2$).

(iv) Gelatine solution. The same procedure is followed as above except that the barium chloride ($BaCl_2$) is left out.

(v) Sulphate stock solution. Dissolve 5,9146 g anhydrous sodium sulphate (Na_2SO_4) (dried for 1 h at 105°C) in deionized water and dilute quantitatively to 1 ℓ. Store at 4°C in a polyethylene container. The solution contains 4,0 mg SO_4/mℓ.

(vi) Sulphate standard solutions. Prepare the following series of standard solutions in 1 ℓ volumetric flasks.

Volume of stock solution (mℓ)	mg/ℓ SO_4
Series 1	
6,0	24
16,0	64
30,0	120
44,0	176
60,0	240
Series 2	
10,0	40
20,0	80
40,0	160
70,0	280
100,0	400

The standard solutions are stored at 4°C in polyethylene containers. The concentration range of the first series of standards is used for ground and waste water analyses.

Flow system and general observations

(i) The flow system is shown schematically in Figure 1.21. The sampler is equipped with two probe tubes; one is used for the normal sampling process and the second is used for addition of the barium chloride into the flow system. The latter probe tube goes back to the buffer solution during the wash cycle of the sampler. The barium chloride is pumped to a small glass container which is mounted onto the sampler in such a way that the second probe tube is in the sampler during the sampling cycle (Basson and Bõhmer, 1972).

(ii) To obtain acceptable peaks it is essential that the blank and analytical channels are properly synchronized. The sample flow in the blank channel must be one liquid segment in advance of the sample flow in the analytical channel. Similarly the barium chloride solution in the analytical channel must reach the injection fitting two segments after the sample. Both synchronization procedures can be carried out by making use of a coloured solution in a sample container.

(iii) Samples are to be filtered through a prewashed 0,45 µm membrane filter (Whatman GF/C or equivalent).

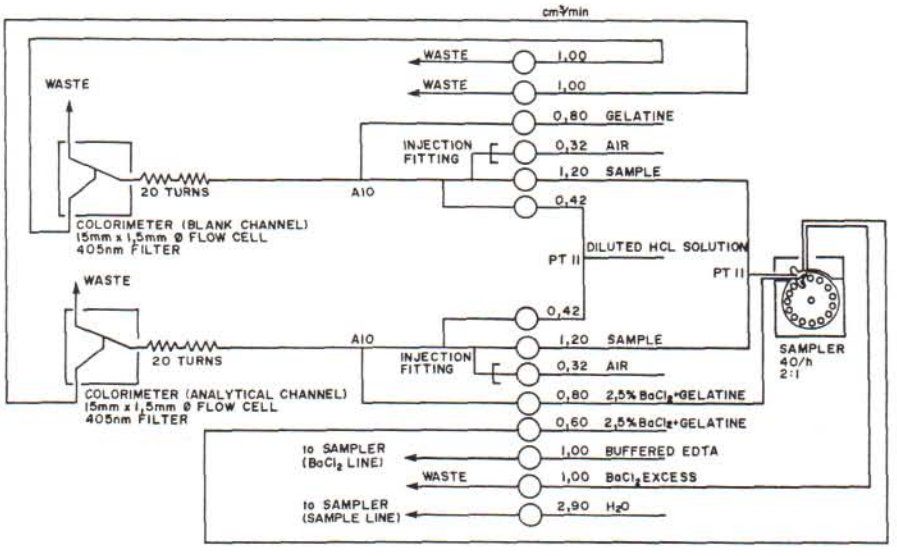


Figure 1.21: Schematic diagram of flow system for sulphate.

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SECTION 2: BIOLOGICAL AND BACTERIOLOGICAL SAMPLING AND ANALYTICAL METHODS

2.1 BIOLOGICAL, SAMPLING AND ANALYTICAL METHODS

2.1.1 Choice of sampling site

Biological sampling

If the area being sampled is an impoundment for eutrophication or other limnological studies it should be noted that algal populations are often very patchy and false impressions of the trophic state of the impoundment may be gained by collection of samples in areas of artificially high or low phytoplankton density. Before sampling begins, therefore, cognizance should be taken of such factors as wind direction, flow, point sources and spatial distribution of nutrients.

2.1.2 Plankton sampling method

The plankton of an impoundment is the free floating aquatic biota. Samples are most commonly taken using either a 5 m hose-pipe or a van Dorn sampler. The hose-pipe sampler allows collection of 5 m depth integrated sample and consists of a 5 m length of standard garden hose-pipe (~19 mm I.D.), tied at both ends of a 6 to 7 m length of cord and weighted at one end with lead. The weighted end is lowered into the impoundment and the pipe slowly lowered in a vertical position until the unweighted end is just below the surface. The weighted end is then hauled in, bending the pipe into a U-shape. When the two ends are together the sampler is removed and the contents emptied into a suitable container. This type of sampler gives about 1,4 ℓ of sample. A van Dorn sampler or equivalent is used to collect discrete samples at selected depths.

Samples for algal cell counts are preserved with 1 mℓ of Lugol's solution (Weber, 1968) per 100 mℓ of sample and stored in the dark. Samples for chlorophyll a, ATP and other cellular constituents should be stored as close to field conditions as possible and analysed within 6 hours. Depending upon phytoplankton density, between 100 mℓ and 1 ℓ of sample is needed for cell counts, and samples of up to 3 ℓ are required for each of the other analyses.

2.1.3 Phytoplankton cell enumeration - Inverted microscope method - 2000001

Scope

In many instances a knowledge of the groups and genera present in a phytoplankton assemblage is necessary in the study of aquatic ecosystems. The impact of abundant growth of algae on water quality and aesthetic characteristics of an impoundment is often dependent upon the species present, particularly upon whether the dominant forms are green algae (Chlorophyta) or blue-green algae (Cyanophyta). The inverted microscope method allows

quantitative numerical assessment of phytoplankton composition from a wide range of densities and is applicable for all phytoplankton of greater than 5 µm in size.

Summary of Method

An aliquot of preserved sample is poured into a suitable sized chamber and the algae allowed to settle onto a cover glass which forms the bottom of the chamber (Utermöhl, 1958; Lund, Kipling and Le Cren, 1958). The chamber is then carefully placed on an inverted microscope and examined from below. This procedure allows the use of high power objectives without disturbance of the settled cells. Counting is aided by a graticule placed in the eyepiece.

Interferences

Interferences can be caused by obscuring of the cells by very large populations of zoöplankton or high sediment/detritus concentrations. Thorough cleaning of sample bottles and sedimentation chambers is necessary to avoid carry-over of cells to the next sample. Underestimates of cell numbers may occur due to adherence of algal cells to sedimentation chamber walls. Problems may also be encountered when the phytoplankton population is dominated by loosely packed colonial forms (e.g. Microcystis aeruginosa).

Apparatus

(i) An inverted microscope (Zeiss Invertoscope D or equivalent).

(ii) An ocular micrometer graticule (Whipple grid or equivalent).

(iii) Plankton sedimentation chambers with cover glasses and replacement base cover glass. A range of cell volumes of 5, 10 and 25 mℓ is advisable and compound chambers up to 100 mℓ may be required for low density populations (Zeiss Invertoscope chambers or equivalent).

Reagents

Lugol's iodine preservative solution: 15 g iodine crystals and 50 g potassium iodide are dissolved in 50 mℓ glacial acetic acid. Distilled water is then added to make the volume up to 500 mℓ. One millilitre of this solution is added per 100 mℓ of sample and the samples are stored in the dark. This preservative is effective for at least one year (Weber, 1968).

Analysis

(i) Prior to analysis the ocular Whipple grid is calibrated against a stage micrometer at the various magnifications to be used. The areas covered by a counting square are calculated. The area of the base of the sedimentation chamber is also calculated.

(ii) Depending upon the phytoplankton density a suitable volume chamber is selected. The preserved sample is then shaken very well but not vigorously, and the chamber filled. The chamber is then covered with a cover glass (to avoid contamination or evaporation) and left on a flat surface to settle. Three hours per centimetre depth should be allowed for settling of the algae. For a standard 10 mℓ Zeiss chamber the minimum settling time is 6 h. If extended settling times are needed, the chambers should be placed in the dark. Dense populations may be diluted with distilled water, shaken well (but not over vigorously as this may cause colony breakdown or cell damage) and an aliquot added to the chamber. With sample volumes of 20 mℓ and 100 mℓ in compound chambers, the excess water is carefully siphoned off so as not to disturb the settled algae, and the sedimentation tube removed. If the phytoplankton population is very sparse, large volumes (500 mℓ to 2 ℓ) may be settled in plastic measuring cylinders fitted with small taps about 2 cm from the base through which the excess water can be drained after settling. The algae are then resuspended in the remaining water and decanted into a suitable volume sedimentation chamber for resettling and analysis. When filling sedimentation chambers, care must be taken to avoid air bubbles adhering to the side of the chambers.

Avoid storing the chambers during settling in places with large temperature fluctuations, as convection currents set up under such conditions will affect uniform settling of the cells.

The sedimentation chamber is placed onto the inverted microscope stage and checked for uniform settling using low power magnification. If settling is uneven the cells should be resuspended and resettled. If the sample is uniformly settled, select a magnification of X200-300 and count and identify algae in randomly selected grid fields. At least 20 fields should be counted if there are high numbers of algae, more if the population is less dense (so as to at least achieve a count of 100 for the dominant phytoplankton). Count all cells wholely within the grid. Where cells lie on the edge of the outer square, count those on the upper and right hand "count" edges but not those on the lower and left hand "no-count" edges. Any cells on the "no-count" edges should not be counted, even if 90%+ of the cell is within the counting square.

When counting filamentous algae, count the number of cells of the filament wholely within the grid or on the "count" edges. Similarly for colonial forms. If the colony is a small tightly compact form of a known number of cells, then all colonies within the square or on the "count" edges are recorded and multiplied by the cell number, and all colonies on the "no-count" edges are disregarded even if there are cells wholely within the grid. If the colonial forms present are loosely packed and of variable cell number (e.g. Microcystis aeruginosa), then the number of cells within the grid on "count" edges are tallied. If the number of colonies is very high then dilution is recommended as cell number to

colony size is extremely variable, although Reynolds (1973) was able to produce approximate estimates of Microcystis aeruginosa using a derived relationship between cell number and colony average diameter.

Calculations

Calculate cell numbers of individual species, genera or groups using the following formula:

$$\text{cells } m\ell^{-1} = \frac{T \times a_s}{t_f \times a_f \times V \times c_p}$$

where

T = Number of cells of species, genus or group counted
a_s = Area of the sedimentation chamber base (mm^2)
t_f = Number of squares that were counted
a_f = Area of a counting square (mm^2)
V = Volume of sample used
c_p = Correction factor for addition of preservative

(The addition of Lugol's iodine preservative is not usually corrected for. Some other preservatives require the addition of large volumes, however, which must be corrected for).

Where concentration or dilution procedures have been employed the results must be corrected by the appropriate factor.

Comments

(i) Sedimentation chambers should be rinsed well with distilled water prior to detergent washing, to avoid possible precipitation of an iodine complex which may be difficult to remove.

(ii) Cell counts can give a distorted impression of the relative contribution of various species or genera of phytoplankton biomass, as a wide range of cell volumes per cell is encountered amongst phytoplankton (e.g. a Chlorella vulgaris cell may occupy 200 μm^3, a Scenedesmus quadricauda cell 1 000 μm^3 and an Anabaena flos-aquae colony 80 000 μm^3; Vollenweider, 1974).

A method used by the Hydrological Research Institute gives results of relative contribution of phytoplankton to the biomass. The method uses the estimation of the percentage area covered in each square by the phytoplankton in relation to all the phytoplankton present. Mucilaginous material of some colonial forms and filamentous algae are included in the estimation of area covered. The average percentage "phytoplankton cover" from the number of squares examined is taken to indicate percentage contribution to phytoplankton biomass.

Alternatively conversion of cell numbers to volume cells occupied using the calculated cell volumes for phytoplankton published by Nauwerk (1963), Vollenweider (1974) and others may be tested.

2.1.4 Seston dry weight - 2010001

Scope

The seston of an impoundment is the total suspended matter. It includes dead and alive phyto- and zoō-plankton, suspended solids and detritus. The amount of seston present influences the optical properties and ecology of the aquatic environment.

Summary of method

A known volume of water is passed through a preweighed glass-fibre filter and the retained matter and filter are dried at 105°C. The increase in weight upon reweighing is a measure of the seston dry weight.

Interferences

Very small particles such as nanoplankton may be lost during vacuum filtration. This can be minimized by using the minimum of suction pressure. High suction pressures can also cause cell disruption with consequent loss of cell contents from some fragile organisms. Thorough cleaning of sample bottles is necessary to avoid carry-over of cells to the next sample. The drying temperature of 105°C may result in the loss of some volatile cell components with the consequent underestimation of seston mass.

Apparatus

(i) Good quality glass-fibre filters (Whatman GF/C grade or equivalent), 47 mm diameter.

(ii) Filter apparatus consisting of 1 ℓ capacity stainless steel filter funnels and receiving flasks (e.g. Millipore or equivalent). Alternatively a 3 or 6 place manifold with 1 ℓ stainless steel filter funnels connected to a 10 or 15 ℓ collecting reservoir.

(iii) Source of vacuum - either a water-aspirator, a hand held pump or an electric pump. If the manifold and reservoir is used an electric pump is needed.

(iv) Good quality balance capable of weighing to at least 0,1 mg.

(v) Drying oven, thermostatically controlled, capable of maintaining a temperature of 105°C ± 1°C.

(vi) Desiccator, smooth tipped stainless steel forceps and graduated cylinders.

Analysis

(i) Samples should be analysed as soon after collection as possible. If an extended delay is expected 1 ℓ samples can be preserved with 1 mℓ of a 55 g ℓ^{-1} aqueous solution of $HgCl_2$ (Greeson et al. 1977). Preserved samples should still be analysed within 7 d.

(ii) Prior to analysis the glass-fibre filters are numbered with a graphite pencil and then left to soak in distilled water overnight. About 100 filters are soaked in 1 ℓ of water. After soaking, the filters are removed and the excess water drawn off using the filter apparatus. The filters are then dried at 105°C for two hours prior to storage in a desiccator containing silica gel. Before analysis begins cool dry filters are weighed to the nearest 0,1 mg.

(iii) Place a preweighed filter on the filtration apparatus. Use smooth tipped forceps in order to avoid damage and loss of fibres from the filter. Shake the sample well but not vigorously and filter a known volume of sample. The volume of sample filtered will depend upon the amount of suspended matter present. Water with very high suspended matter will only require 100 to 500 mℓ for filtration while clear waters may require up to 3 ℓ or more. The vacuum pressure should not exceed 46,5 kPa (about 360 mm Hg) (Greeson et al. 1977). After filtering the sample, and with the vacuum still on, wash the sides of the funnel with 10 to 20 mℓ distilled water and allow the filter to be sucked "dry" of excess water. Switch off the vacuum and allow the system to return to room pressure.

(iv) Remove the filter using smooth tipped forceps and place in the oven at 105°C for 2 h. Include a control through which a similar volume of distilled water as that of samples has been filtered.

(v) After drying remove the filters and allow to cool in a desiccator. Reweigh the filters to the nearest 0,1 mg.

Calculations

Calculate the Seston dry weight as follows:

$$\text{Dry weight (mg } \ell^{-1}) = \frac{Wfs - Wf}{V}$$

where

Wfs = Weight of filter plus suspended matter
Wf = Weight of filter prior to filtration
V = Volume of sample filtered (in litres).

Should the control filters show a weight change the results must be corrected accordingly. Good quality filters should not give blank corrections of greater than ± 0,2 mg.

2.1.5 Chlorophyll a - Spectrophotometric method - 2020001

Scope

Chlorophyll a is the primary photosynthetic pigment and is present in all algae. The determination of chlorophyll a is used to give an estimation of the phytoplankton biomass present. Chlorophyll a constitutes an average 1% to 2% of algal dry weight (APHA, 1980) although the range is 0,1% to 9,7% fresh weight (Nicholls and Dillon, 1978). The higher values are rare and maxima are more frequently in the order of 3% to 4% of fresh weight (Tolstoy, 1977). The ratio of the degradation products, the phaeopigments, to chlorophyll a gives an indication of the physiological state of the algae.

Summary of method

The photosynthetic pigments are extracted from the algae, which have been entrapped on a glass-fibre filter, using boiling 90% ethanol. The concentration of chlorophyll a and phaeopigments are determined by measuring the absorbance, before and after acidification of the extract, at 665,5 nm against a 90% ethanol blank. Ethanol has been shown to be a more superior extraction agent than acetone or dimethyl sulphoxide (DMSO) (Sartory, 1982; Sartory and Grobbelaar 1984).

Interferences

Extracts should be centrifuged prior to spectrophotometry as suspended particles interfere with absorbance measurements. Overestimation of phytoplankton chlorophyll will occur if large populations of photosynthetic bacteria are present (Hussaing, 1973). The presence of carotenoids will also interfere with chlorophyll a determinations (Sartory, 1982).

Apparatus

(i) Spectrophotometer, with narrow band pass (1 to 2 nm) allowing absorbance readings to 0,001 absorbance units in the visible range. Matched cuvettes of 10 mm light pathlength should be used. Spectrophotometers fitted with grid monochromators are preferred and wavelength settings should be regularly checked by calibration on the hydrogen line at 656,3 nm (Sartory, 1982).

(ii) Centrifuge fitted with a swing-out head capable of holding 15 mℓ tubes and of speeds of 3 000 to 4 000 rpm.

(iii) Filter apparatus consisting of 1 ℓ capacity stainless steel filter funnels and receiving flasks (e.g. Millipore or equivalent). Alternatively a 3 to 6 place manifold with 1 ℓ stainless steel filter funnels connected to a 10 or 15 ℓ collecting reservoir.

(iv) Source of vacuum - either a water-aspirator, a hand-held pump or an electric pump. If the manifold and reservoir are used an electric pump is needed.

(v) Good quality glass-fibre filters (Whatman GF/C grade or equivalent), 47 mm diameter.

(vi) 15 mℓ centrifuge tubes and 15 mℓ screw capped glass tubes.

(vii) A 100 µℓ microsyringe (Hamilton or equivalent).

(viii) A water bath with a thermostatically controlled heater capable of maintaining a water temperature of 78°C ± 1°C.

Reagents

(i) 91,8% aqueous ethanol. Use spectrophotometric grade ethanol and distilled water.

(ii) 0,3 mol ℓ^{-1} HCl solution.

Analysis

(i) Samples should be analysed as soon as possible after collection. If there is to be an appreciable delay between collection and analysis then the first steps (filtration and placing the filter and plankton in ethanol) may be carried out in the field. The tubes in this case should be stored in the dark in cooler bags (or equivalent) at less than 10°C. Analysis should be conducted within 48 h.

(ii) Place a glass-fibre filter on the filtration apparatus and filter a known volume of sample. The volume of sample filtered will depend upon the amount of plankton present. Waters with very high algal populations will require only 100 to 500 mℓ for analysis while clear waters may require up to 3 ℓ. The vacuum pressure should not exceed 68 kPa (about 500 mm Hg) (Sartory, 1982). After filtering the sample, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ distilled water and allow the filter to be sucked "dry" of excess water. Release the vacuum and allow the system to return to room pressure.

(iii) Carefully remove the filter with forceps without damaging the filter. Loosely roll the filter up with the entrapped algae on the inside. Take care not to remove any of the algae with your fingers. Place the rolled filter in a 15 mℓ screw-capped tube and add 9,8 mℓ of a 91,8% ethanol solution. As the filters have been found to retain, on average, 0,2 mℓ water the final volume is 10 mℓ of 90% ethanol (Sartory, 1982). Mark the final volume level so that, should evaporation occur during the next

step, it may be corrected for after cooling. Screw on the tube cap, not overtightly or too loosely, place the tube in the water bath at 78°C and allow the contents to gently boil for 5 min.

(iv) After boiling remove the tube and place in the dark at room temperature for 1 h to cool and allow extraction to be completed. After 1 h add additional 90% ethanol, should any loss have occurred during the boiling, and invert the tube a few times to ensure homogeneity. Decant the extract into a centrifuge tube and centrifuge at 4000 rpm for 10 min.

(v) Decant 4 mℓ into a 10 mm pathlength cuvette and read the absorbance at 665,5 nm and 750 nm (turbidity blank). These readings are $E_{665}1$ and $E_{750}1$. Acidify the extract in the cuvette with 100 µℓ of a 0,3 mol $ℓ^{-1}$ HCl solution, mix the contents and allow to stand for 2 min. Reread the absorbance at 665,5 nm and 750 nm. These readings are $E_{665}2$ and $E_{750}2$. The initial E_{665} readings should be within the range 0,1 to 0,8 absorbance units. If the E_{665} reading is greater than 0,8 absorbance units, dilute the extract so that it falls within the 0,1 to 0,8 absorbance units range.

Calculations

(i) Subtract the E_{750} readings from their respective E_{665} readings:

$E_{665}1 - E_{750}1 = E_{665}b$ (i.e. before acidification)
$E_{665}2 - E_{750}2 = E_{665}a$ (i.e. after acidification)

(ii) Using the following equations (Lorenzen, 1967) calculate the chlorophyll a and phaeopigment concentrations in the extract.

$$\text{chlorophyll a (mg } ℓ^{-1}) = \frac{(E_{665}b - E_{665}a) \times (R/R-1) \times k}{L}$$

$$\text{phaeopigments (mg } ℓ^{-1}) = \frac{(R\,[E_{665}a] - E_{665}b) \times (R/R-1) \times k}{L}$$

where

$E_{665}b$ = absorbance at 665,5 nm before acidification (corrected for absorbance at 750 nm)

$E_{665}a$ = absorbance at 665,5 nm after acidification (corrected for absorbance at 750 nm)

R = acid ratio (maximum ratio at $E_{665}b$: $E_{665}a$, i.e. for an extract containing no phaeopigments)

K = absorbance coefficient of chlorophyll a in 90% ethanol (= 1 000 x reciprocal of the specific absorption coefficient)

L = Pathlength of cuvette in centimetres (= 1 cm).

The acid ratio "R" for chlorophyll a in ethanol is 1,72 (Sartory, 1982) and the specific absorption coefficient is 83,4 $\ell\ g^{-1}\ cm^{-1}$ (Wintermans and de Mots, 1965), giving an absorbance coefficient "k" of 11,99. Thus the above equation may be simplified to:

Chlorophyll a (mg ℓ^{-1}) = $(E_{665}b - E_{665}a) \times 28,66$

Phaeopigments (mg ℓ^{-1}) = $(1,72\ [E_{665}a] - E_{665}b) \times 28,66$

The concentrations of chlorophyll a and phaeopigments in the original sample are calculated as follows:

$$\text{pigment concentration } (\mu g/\ell^{-1}) = \frac{Pc \times v}{V}$$

where

Pc = pigment concentration in the extract in mg ℓ^{-1}

v = volume of extract in millilitres (in this case 10 mℓ)

V = volume of original sample in litres.

Report results to the first decimal place in µg ℓ^{-1} sample.

Values of less than 1 µg ℓ^{-1} should be reported as "<1 µg ℓ^{-1}".

2.1.6 Chlorophyllous pigments - Liquid chromatography method - 2020002

Scope

The range of chlorophyllous pigment present is determined by the genera of algae present. The ratios between chlorophylls a and b may give information on the taxonomic composition of the phytoplankton population. With the increased awareness of problems in spectrophotometric determinations due to carotenoid interference (Riemann, 1978) and degradation products (Sartory, 1982) the use of high performance liquid chromatography (HPLC) is being increasingly recommended. HPLC methods allow a detailed investigation of pigment complexes.

Summary of method

The photosynthetic pigments are extracted from the algae and the carotenoids separated. The pigments are then chromatographed on a C_{18} reverse-phase HPLC column using a methanol-acetone-water solvent system and are detected using a fluorometer. Quantification is by comparison with authentic standards. This method allows quantification of chlorophyll a, chlorophyll b, phaeophytin a and phaeophytin b.

Interferences

This method eliminates interference problems encountered in spectrophotometry, due to the presence of carotenoids, bacteriochlorophylls, etc. (Sartory, 1985).

Apparatus

(i) HPLC system with a binary solvent system capable of gradient solvent programmes and fitted with a 10 µℓ sample loop and loading syringe.

(ii) A 15 cm x 3,9 mm Resolve C_{18} 5 µm particle reverse phase HPLC column (Waters Associates).

(iii) Filter fluorometer for HPLC. The excitation filters are a WBS 410 (International Light) and a Corning CS4-76 providing an excitation bandpass of 350 to 550 nm with λ_{max} at 430 nm. The emission filter is a single SCS 610 filter (International Light) allowing transmission of light above 600 nm.

(iv) Single-pen 1 mV/Full scale Recorder and an integrator/computing facility.

(v) Filtration apparatus consisting of 1 ℓ capacity stainless steel filter funnels (e.g. Millipore or equivalent). Alternatively a 3 or 6 place manifold with 1 ℓ stainless steel filter funnels connected to a 10 or 15 ℓ collecting reservoir.

(vi) A source of vacuum - either a water aspirator, a hand held pump or an electric pump. If the manifold and reservoir are used an electric pump is needed.

(vii) A water bath with a thermostatically controlled heater capable of maintaining a water temperature of 78°C ± 1°C.

(viii) Good quality glass-fibre filters (Whatman GF/C grade or equivalent), 47 mm diameter.

(ix) Sep-Pak C_{18} Bondapak cartridges (Waters Associates).

(x) 15 mℓ screw-capped tubes and 20 mℓ glass syringes.

Reagents

(i) HPLC grade acetone, methanol and ethanol (Merck Chemicals or equivalent).

(ii) "Organic free" water prepared by passing water from a Millipore Milli-Q system (or equivalent) through a Millipore Norganic cartridge followed by pumping through a 30 cm x 3,9 mm µBondapak C_{18} column (Waters Associates). The "organic free" water should be stored in well sealed chromic acid washed amber glass bottles.

(iii) From the reagents in (i) and (ii) prepare the following solutions:

(i) 97% Methanol
(ii) 97% Acetone
(iii) 70% Ethanol
(iv) 91% Ethanol
(v) 90% Methanol
(vi) 100% Methanol

(iv) Purified chlorophyll a and chlorophyll b standards (Sigma Chemicals, product Nos. C-5753 and C-5878). Quantitative stock solutions are made up in 100% acetone and stored at -4°C. Phaeophytins a and b are quantitatively prepared from their parent chlorophylls by acidification of acetone standards to 3×10^{-3} mol ℓ^{-1} HCl. After mixing and standing for 5 min the solution is neutralized with an equivolume of an equimolar solution of NaOH.

(v) Details of how to qualitatively prepare the other alteration products of chlorophyll a and b (the C_{10} epimers, epimeric phaeophytins, hydroxy-chlorophylls, chlorophyllides and phaeophorbides) are given in Sartory (1985).

Analysis

(i) Samples should be analysed as soon as possible after collection. If there is to be an appreciable delay between collection and analysis then the first steps (filtration and placing the filters and plankton in ethanol) may be carried out in the field. The tubes in this case should be stored in the dark in cooler bags (or equivalent) at less than 10°C. Analysis should be conducted within 48 h.

(ii) Place a glass-fibre filter on the filtration apparatus and filter a known volume of sample. The volume of sample filtered will depend upon the amount of plankton present. Waters with very high algal populations will require only 100 to 500 mℓ for analysis while clear waters may require up to 3 ℓ. The vacuum pressure should not exceed 68 kPa (about 500 mm Hg) (Sartory, 1982). After filtering the sample, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ distilled water and allow the filter to be sucked "dry" of excess water. Release the vacuum and allow the system to return to room pressure.

(iii) Carefully remove the filter with forceps without damaging the filter. Loosely roll the filter up with the entrapped algae on the inside. Take care not to remove any of the algae with your fingers. Place the rolled filter in a 15 mℓ screw-capped tube and add 9,8 mℓ of a 91,8% ethanol solution. As the filters have been shown to retain, on average, 0,2 mℓ water the final volume is 10 mℓ of 90% ethanol (Sartory, 1982). Mark the final volume level so that, should evaporation occur during the next

step, it may be corrected for after cooling. Screw on the tube cap, not overtightly or too loosely, place the tube in the water bath at 78°C and allow the contents to gently boil for 5 min.

(iv) After boiling remove the tube and place in the dark at room temperature for 1 h. Should any loss of solvent have occurred, make up the volume to the mark with 90% ethanol. Ensure homogeneity of the solution by inverting the tube a few times.

(v) Decant the extractant, squeezing the filter with forceps to obtain as much of the extract as possible. Dilute the 90% ethanol extract to approximately 70% ethanol by adding 3 mℓ "organic free" water to 10 mℓ extract. Remove the plunger from a 20 mℓ syringe and fit a Sep-Pak C_{18} cartridge to the syringe body. Flush the cartridge with 20 mℓ 70% ethanol and then flush through the extract. The pigments are visibly retained in the cartridge. Flush 10 mℓ 90% methanol through the cartridge to remove the carotenoids. The chlorophyllous pigments are eluted off with 10 mℓ 100% methanol, this fraction being collected in a screw-cap tube. The extract, of which 10 µℓ is injected, is ready for chromatography.

(vi) The conditions for HPLC are (Sartory, 1985)
Solvent system: Solvent A = 97% methanol
Solvent B = 97% acetone

Solvent delivery programmed as 100% Solvent A at 1,0 mℓ min^{-1} for the first 15 min after injection, then a linear gradient to 77% Solvent A: 23% Solvent B (i.e. 75% methanol: 22% acetone: 3% water) at 20 min and this mix is maintained until the elution of the phaeophytin *a* peak, after which the solvent is returned to 100%

Solvent A via a linear gradient of 5 min. An example of a typical chromatogram is shown in Figure 2.1.

(vii) Before analytical runs are conducted at least three calibration runs, using a mixed standard containing known concentrations of chlorophyll *a*, chlorophyll *b*, phaeophytin *a* and phaeophytin *b*, are made.

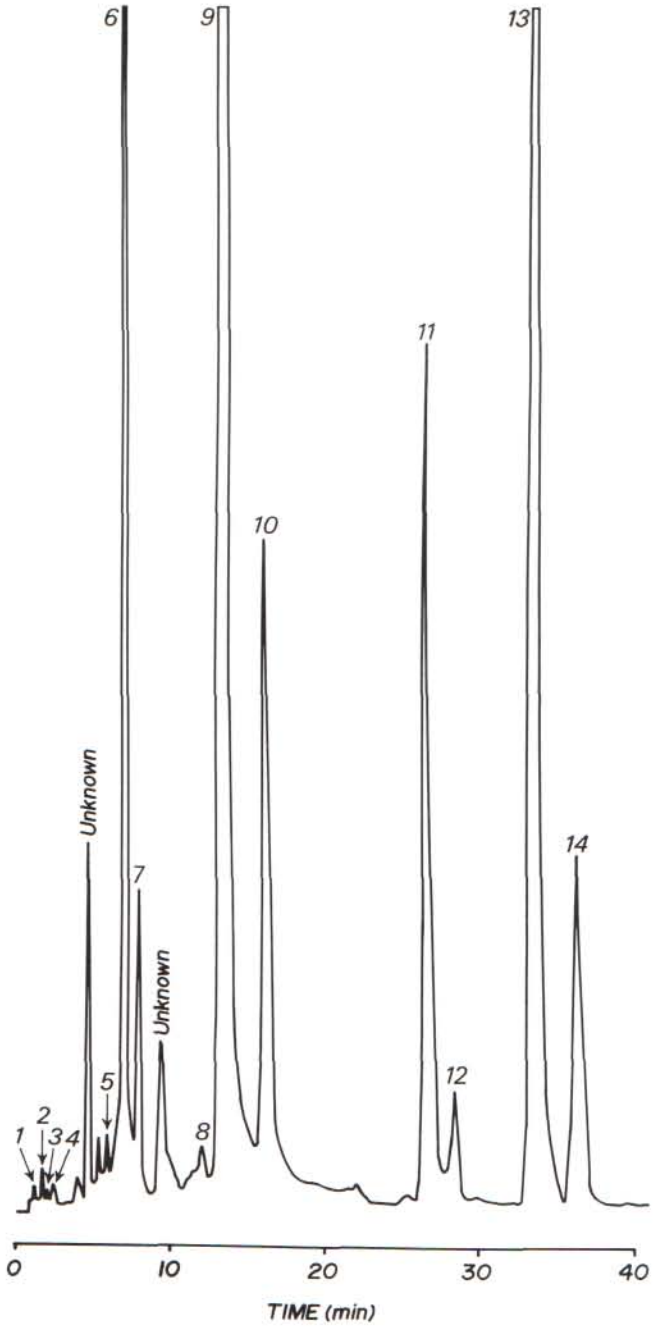

Figure 2.1: HPLC chromatogram of an extract from Scenedesmus bijugatus after Sep-Pak clean-up.
1=chlorophyllide b; 2=chlorophyllide a; 3=phaeophorbide b;
4=phaeophorbide a; 5=10-hydroxyclorophyll b; 6=chlorophyll b;
7=chlorophyll b'; 8=10-hydroxychlorophyll a; 9=chlorophyll a;
10=chlorophyll a'; 11=phaeophytin b; 12=phaeophytin b';
13=phaeophytin a; 14=phaeophytin a'.

Calculations

(i) It is preferable to quantify the pigments by comparison of peak areas to those of the standards. This however, requires an integrator or computer facilities. If these are not available then comparison of peak heights may be used. If the peak areas or heights exceed the linear range of the system then the sample will have to be diluted and rerun.

(ii) The detection limits are in the order of 10 to 20 pg pigment injected. This is equivalent to 10 to 20 ng pigment ℓ^{-1} sample.

(iii) The concentration obtained from HPLC is in µg mℓ extract^{-1} (= mg ℓ^{-1}) and the concentrations of pigments in the original sample are calculated as follows:

$$\text{pigment concentration } (\mu g\ \ell^{-1}) = \frac{P_c \times \nu}{V}$$

where

P_c = pigment concentration in extract in mg ℓ^{-1}

ν = volume of extract in millilitres (= 10 mℓ)

V = volume of original sample in litres.

2.1.7 Adenosine triphosphate - Luminescence method - 2030001

Scope

Adenosine triphosphate (ATP) is found in all living cells and is rapidly degraded upon death of the cell (Holm-Hansen and Booth, 1966). The amount of ATP present in freshwater algae, bacteria and zoöplankton is said to be relatively constant during the exponential growth phase (Holm-Hansen, 1970; 1973). ATP is, therefore, taken as an estimator of total viable biomass present.

Summary of method

The ATP is extracted from plankton, which has been entrapped on a filter, using boiling Tris-buffer solution. The ATP content of the extract is determined by the luminescence output from a chemical reaction involving luciferin luciferase (Firefly lantern extract). The method described is a modification of that of Holm-Hansen (1973).

Interferences

The use of Tris-buffer obviates interferences associated with other extractants such as incomplete extraction or luminescence quenching (Holm-Hansen and Booth, 1966).

Apparatus

(i) ATP Luminometer (LKB Wallac Model 1250 or equivalent).

(ii) Filter apparatus consisting of 1 ℓ capacity stainless steel filter funnels and receiving flasks (e.g. Millipore or equivalent). Alternatively a 3 or 6 place manifold with 1 ℓ stainless steel filter funnels connected to a 10 or 15 ℓ collecting reservoir.

(iii) Source of vacuum - either a water-aspirator, a hand-held pump or an electric pump. If the manifold and reservoir are used an electric pump is needed.

(iv) Good quality 0,45 µm pore size membrane filters (Millipore type HA or equivalent), 47 mm diameter.

(v) A heating block capable of maintaining a temperature of 120°C ± 1°C (Labline Multiblok Heater Model 2091 or equivalent).

(vi) 15 mℓ screw-capped tubes.

(vii) Micropipettor, capable of delivering 400 µℓ, with disposable tips.

(viii) Smooth tipped long handled forceps.

(ix) Disposable cuvettes for luminometer.

(x) If luminometer is not equipped with an automatic reagent delivering system a 100 µℓ syringe (Hamilton or equivalent) is required.

Reagents

(i) Tris-buffer, prepared by dissolving 6,06 g tris (hydroxymethyl)-aminomethane (Merck Chemicals, Product No. 8382) and 0,372 g ethylenediaminetetraacetic acid (disodium salt) in 400 mℓ distilled water. The pH is adjusted to 7,75 ± 0,05 using a 50% aqueous acetic acid solution. The buffer is then made up to 500 mℓ using distilled water and stored for up to 12 months in a refrigerator.

(ii) Luciferin luciferase (Firefly lantern extract) reagent (LKB Wallac Monitoring Reagent or equivalent) stored dehydrated at -18°C. The reagent should be rehydrated and stored according to the manufacturers instructions.

(iii) ATP standards (Sigma Chemicals, Product No. A6144 or equivalent). Prepare a set of ATP standards in Tris-buffer or double distilled, deionized water to give a set of solutions of 25, 125, 250, 500 and 1 250 ng $m\ell^{-1}$.

After mixing 400 µℓ of standard with 100 µℓ of monitoring reagent these standards are equivalent to 20, 100, 200, 400 and 1 000 ng $mℓ^{-1}$. The solutions are used to prepare a linear calibration graph. ATP luminescence is linearly correlated with concentration over the range 1 ng $ℓ^{-1}$ to 1 mg $ℓ^{-1}$. Standards should be prepared daily.

Analysis

(i) Samples should be analysed as soon as possible after collection. If there is to be a delay between sample collection and analysis then the samples may be filtered, placed in 50 mm petri dishes with tightly fitting lids (Millipore PD10 047 00 or equivalent) and frozen immediately. Frozen filters may be stored for up to 3 months. Frozen filters must not be allowed to thaw prior to extraction but plunged immediately into the boiling extracting agent.

(ii) Place a membrane filter on the filtration apparatus and filter a known volume of sample. The volume filtered will depend upon the amount of plankton present. Analysis of samples from eutrophic impoundments rich in phytoplankton will require between 5 and 30 mℓ, mesotrophic waters up to 200 mℓ, while for clear waters between 500 mℓ and 1 ℓ needs to be filtered. The vacuum pressure should not exceed 33 kPa (about 250 mm Hg) (Greeson et al. 1977). Before the last of the water has been drawn through, the vacuum source is turned off and the remaining water allowed to drain. The filter is then removed and either frozen immediately for later analysis or extracted and analysed immediately.

(iii) The filter is loosely rolled up and plunged into 4,8 mℓ boiling Tris-buffer (pH 7,75) in screw-capped tubes held at 120°C. As the filters are not sucked dry an average 0,2 mℓ is allowed for the residual water, making the final extraction volume 5 mℓ. The filters are boiled at 120°C for 3 min. After extraction the tubes are removed from the heating block and allowed to stand for 4 h at room temperature to cool.

(iv) 400 µℓ of the extract is placed in a luminometer cuvette and 100 µℓ monitoring agent added and mixed by gentle swirling for 5 s. The sample is placed in the luminometer and the maximum luminescence is recorded. Readings are corrected by the subtraction of a blank reading of a 5 mℓ tris-buffer extract of a control filter. ATP standards should be rerun after every 20 samples. These should be kept on ice during the day but warmed to room temperature prior to analysis.

Calculations

From the luminescence output of the ATP standards prepare a calibration graph and derive a linear regression equation. From this calculate the ATP concentrations in the extracts based on their luminescence output. The ATP concentration of the extract will be in ng $m\ell^{-1}$ extract. Calculate the ATP concentration in the original sample as follows:

$$ATP\ (ng\ \ell^{-1}) = \frac{ATP_c \times v}{V}$$

where

ATP_c = concentrated ATP in extract in ng $m\ell^{-1}$

v = volume of extract in millilitres (= 5 mℓ)

V = volume of original sample in litres.

Report results above 0,10 µg ℓ^{-1} to the nearest 0,01 µg and results between 1 ng and 100 ng to the nearest 1 ng. Values of less than 1 ng ℓ^{-1} should be reported as "< 1 ng ℓ^{-1}".

2.2 BACTERIOLOGICAL SAMPLING AND ANALYTICAL METHODS

2.2.1 Choice of sampling site

(i) Bacteriological sampling of impoundments

Similarly for bacteriological investigations of impoundments the investigator should not only be aware of major point sources (e.g. sewage works effluent) but also look out for watering places for cattle and other sources of pathogenic bacteria (e.g. dumped carcasses).

(ii) Bacteriological sampling of rivers and streams

Sampling of rivers and streams for bacteriological analysis requires a different approach from that of impoundments. Small streams are generally sampled for background levels and should be sampled close to the stream confluence with a river. Care should be taken on the selection of stream sampling sites so as to avoid the possibility of collecting from stagnating areas or a point where water from the river may have been pushed up. Should there be one, a weir is a suitable site, but care must be taken in interpreting the results if there has been no over-the-wall-flow for an extended period.

Before sampling a river it is advisable to gain an impression of the flow patterns and mixing areas. Point discharges may not mix well with the main body of the river for long distances downstream, and it is generally advisable to take a series of samples transecting the river when sampling downstream of a discharge. Samples taken with the aim of appraising the general bacteriological quality should be taken from a mid-stream position, which is often not the mid-point of a river. Avoid taking samples from the inside bank of a bend in a river as the flow at this point is much slower and the quality may not be representative of that of the main channel, which will be situated nearer the outside edge. When sampling discharges from an impoundment, establish whether the impoundment is stratified or not, and if it is, whether the discharge is hypolimnetic or epilimnetic. The anaerobic hypolimentic water will often contain higher numbers of some bacteria (e.g. Escherichia coli, Clostridium perfringens) than the aerobic epilimnetic waters.

2.2.2 Bacteriological sampling methods

(i) Environmental and effluent samples.

The majority of bacteriological samples from raw and waste water are grab samples. Sample bottles are cleaned, loosely capped, and sterilized prior to sampling by autoclaving for 20 min at 121°C at a pressure of 103,4 kPa (15 psi). The bottle caps should be tightened after sterilization. At the sampling point the bottle is held at its base and the cap removed in

such a fashion so as to ensure that the interior surface of the cap is not touched. The bottle is then plunged neck down into the river to a depth of approximately 200 to 300 mm and then tilted up so the neck of the bottle faces the flow of river. If there is no flow, then the bottle is moved forward so as to fill the bottle, and have any flow pass the neck of the bottle and then the samplers hand thereby preventing contamination of the sample. After collection of the sample and before capping, ensure that there is an air space at the top of the bottle. This facilitates mixing of the sample prior to analysis. Samples should be analysed as soon as possible after collection and should be kept below 10°C, but not frozen, during transportation to the laboratory (SABS, 1984).

Uncooled samples must be analysed within 6 h and cooled samples analysed within 24 h (SABS, 1984). Discrete depth related samples may be collected using a Zobell J-Z Sampler (Zobell, 1941) or equivalent.

(ii) Drinking water samples

The preparation of sample bottles for the sampling of drinking water supplies is the same as above. If, however, the supply is chlorinated it is necessary to add a dechlorinating agent to the bottle. The dechlorinating agent employed is a 100 g ℓ^{-1} solution of sodium thiosulphate, of which 0,1 mℓ is added for every 100 mℓ sample volume collected. The dechlorinating agent is added to the sample bottles prior to sterilization. This procedure should also be followed when chlorinated effluents are sampled. Prior to sampling from a tap, the tap should be turned on and the water allowed to flush out for at least 2 min (SABS, 1984). Should the tap be situated at the end of a long distribution pipe which has not been run for a long time, or from a deep borehole, an extended flushing time will be required. After the pipe has been flushed of standing water, the tap is adjusted to allow a gentle flow and the sample bottle filled and stored as outlined above. Avoid taking samples from encrusted or badly corroded taps. Residual chlorine measurements (e.g. by the DPD Palin test) should be made at the time of collection of the sample. At least 120 mℓ of sample is required for bacteriological testing of drinking water.

2.2.3 Total coliform bacteria - Membrane filter method - 1000001

Scope

The total coliform test gives an indication of the sanitary quality of water. The test is primarily applied to drinking water supplies but may be applied to freshwater and brackish water systems. The total coliform bacteria are defined as aerobic or facultative anaerobic gram-negative asporogenous rod-shaped bacteria which ferment lactose with gas formation within 24 h at 35°C. The principal genera covered by this definition are Escherichia, Klebsiella, Enterobacter and Citrobacter. In sanitary microbiology it has become

increasingly acceptable to define total coliform bacteria as all those bacteria which produce colonies with a golden-green metallic sheen within 24 h when incubated at 35°C on m-Endo medium. For the purposes of this method, this second definition is implied when the term total coliform is used. Apart from encompassing the genera of bacteria listed above this definition also allows the inclusion of some strains of Aeromonas hydrophila which, although not a coliform as delineated in the first definition, is of health significance and whose possible presence is of concern.

Summary of method

A water sample is filtered through a membrane filter upon which bacteria are entrapped. The filter is then placed on a selective growth medium and incubated at 35°C for 24 h, after which colonies characteristic of coliforms are counted.

Interferences

High sediment load may lead to poor growth of colonies. High numbers of some non-coliform bacteria have been reported to inhibit coliform colony formation (Burlingame et al. 1984).

Apparatus

(i) Incubator capable of maintaining a temperature of 35°C ± 0,5°C.

(ii) Filtration apparatus, sterile, preferably Hydrosol type (Millipore or equivalent) and source of vacuum.

(iii) Sterile, white, gridded, 47 mm diameter membrane filters, either 0,45 µm pore-size (Millipore type HAWG or equivalent) or 0,7 µm pore-size (Millipore HCWG or equivalent) and sterile absorbent pads (Millipore AP 10 047 or equivalent).

(iv) 50 mm x 12 mm plastic petri dishes with tightly fitting lids, sterile (Millipore PD10 047 or equivalent).

(v) Smooth tipped forceps.

(vi) Selection of sterile 10 mℓ and 1 mℓ pipettes.

Reagents

(i) m-Endo broth (Fifield and Schauffus, 1958): This medium may be obtained commercially (e.g. Difco product No. 0749 or equivalent) and reconstituted according to manufacturers instructions or prepared as follows:

Yeast extract	1,5 g
Casitone (or Trypticase)	5,0 g
Thiopeptone (or Thiotone)	5,0 g
Tryptose (or Polypeptone)	10,0 g
Lactose	12,5 g
Sodium chloride	5,0 g
Dipotassium hydrogen phosphate	4,375 g
Potassium dihydrogen phosphate	1,375 g
Sodium lauryl sulphate	0,05 g
Sodium desoxycholate	0,1 g
Sodium sulphite	2,1 g
Basic fuchsin	1,05 g

Suspend the ingredients in 1 ℓ distilled water containing 20 mℓ ethanol. Bring to the boil, stirring to aid dissolution and allow to cool. The final pH should be 7,2 ± 0,1. The cooled medium should be used on the day of preparation but may be stored in a capped flask in the refrigerator for up to 3 d.

Some bacteriologists prefer to use an agar medium as there are claims that absorbent pads may contain inhibitory substances (Dutka, 1978). The agar medium may be prepared by adding 15 g agar per litre of the above medium before boiling. After heating to boiling point the medium is cooled to about 45°C and dispensed into 50 mm diameter petri-dishes to a depth of about 2,5 mm and allowed to solidify. Agar plates may be stored in the refrigerator for up to 2 weeks.

(ii) Ringer's dilution solution (quarter strength) may be obtained commercially (e.g. Oxoid product No. BR52 or equivalent) and reconstituted according to manufacturers instructions or prepared by dissolving the following constituents in 1 ℓ distilled water:

Sodium chloride	2,25 g
Potassium chloride	0,105 g
Calcium chloride	0,12 g
Sodium bicarbonate	0,05 g

The solution is dispensed in 99, 90 and 9 mℓ aliquots into dilution bottles and sterilized by autoclaving at 121°C for 20 min.

Analysis

(i) If m-Endo broth is being used, prepare the petri dishes prior to analysis by aseptically placing an absorbent pad into each petri dish (using either a dispenser (e.g. Millipore XX62 00014) or flame sterilized forceps. (Sterilize forceps by dipping the tips in ethanol, passing them through a

flame and allowing the ethanol to burn off). To each pad pipette 2,0 mℓ m-Endo broth, allow the medium to soak into the absorbent pad for 5 min and then drain off the excess medium.

(ii) The volume of sample, or dilution thereof, filtered will depend upon the source of the sample. Suggested sample volumes (and dilutions) are:

Water Source	Volume filtered (mℓ)							
	100	50	10	1*	0,1*	0,01*	0,001*	0,0001*
Domestic supply	X							
Groundwater supply	X	X	X					
Impoundments	X		X	X				
River water			X	X	X	X		
Polluted small river				X	X	X	X	
Chlorinated sewage				X	X	X		
Unchlorinated sewage					X	X	X	X

*Do not attempt to filter volumes of less than 10 mℓ directly. Prepare the lower filtration volumes using quarter strength Ringer's dilution solution as follows:

Sample filtration volume	Sample volume	+	diluent volume	Volume of dilution filtered
1 mℓ	1 mℓ		9 mℓ	10 mℓ
0,1 mℓ	1 mℓ		99 mℓ (= Dilution A)	10 mℓ
0,01 mℓ	10 mℓ of Dilution A		90 mℓ	10 mℓ
0,001 mℓ	1 mℓ of Dilution A		99 mℓ (= Dilution B)	10 mℓ
0,0001 mℓ	10 mℓ of Dilution B		90 mℓ	10 mℓ

Before filtering a sample or dilution, and during the preparation of the dilutions, shake the bottle or tube vigorously to ensure homogeneous distribution of the bacterial cells.

(iii) Assemble the filtration apparatus and, using flame sterilized forceps place a membrane filter, grid side up, on the apparatus. Filter the desired volume of sample or dilution. After filtration, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ sterile quarter strength Ringer's solution, allowing the rinsings to pass through the filter. Switch off the source of vacuum and taking care not to damage the membrane remove the filter using flame sterilized forceps.

Interferences

High sediment loads may lead to poor growth of colonies.

Apparatus

(i) Incubator capable of maintaining a temperature of 44,5°C ± 0,2°C.

(ii) Filtration apparatus, sterile, preferably Hydrosol type (Millipore or equivalent) and source of vacuum.

(iii) Sterile, white, gridded, 47 mm diameter membrane filters, either 0,45 µm pore-size (Millipore type HAWG or equivalent) or 0,7 µm pore-size (Millipore type HCWG or equivalent) and sterile absorbent pads (Millipore AP10 047 or equivalent).

(iv) 50 mm x 12 mm plastic petri dishes, with tightly fitting lids, sterile (Millipore PD10 047 or equivalent).

(v) Smooth tipped forceps.

(vi) Selection of sterile 10 mℓ and 1 ℓ pipettes.

Reagents

(i) m-FC broth without rosolic acid (Presswood and Strong 1978; Sartory, 1980); m-FC(-RA) broth.

This medium may be obtained commercially (e.g Difco m-FC broth base, product No. 0883 or equivalent) and reconstituted according to manufacturers instructions or prepared as follows:

Yeast extract	3,0 g
Tryptose (or Biosate peptone)	10,0 g
Proteose peptone No. 3 (or Polypeptone)	5,0 g
Lactose	12,5 g
Bile salts No. 3 (or Bile salts mixture)	1,5 g
Sodium chloride	5,0 g
Aniline blue	0,1 g

Suspend the ingredients in 1 ℓ distilled water. Bring to the boil, stirring to aid dissolution and allow to cool. The final pH should be 7,4 ± 0,2. The cooled medium should be used on the day of preparation but may be stored in a capped flask in a refrigerator for up to 3 d.

Some bacteriologists prefer to use an agar medium as there are claims that absorbent pads may contain inhibitory substances (Dutka, 1978). The agar medium may be prepared by adding 15 g agar per litre of the above medium

before boiling. After heating to boiling point the medium is cooled to about 45°C and dispensed into 50 mm diameter petri dishes to a depth of about 2,5 mm and allowed to solidify. Agar plates may be stored in the refrigerator for up to 2 weeks.

(ii) Ringer's dilution solution (quarter strength) may be obtained commercially (e.g. Oxoid product No. BR52 or equivalent) and reconstituted according to manufacturers instructions or prepared by dissolving the following constituents in 1 ℓ distilled water:

Sodium chloride	2,25 g
Potassium chloride	0,105 g
Calcium chloride	0,12 g
Sodium bicarbonate	0,05 g

The solution is dispensed in 99, 90 and 9 mℓ aliquots into dilution bottles and sterilized by autoclaving at 121°C for 20 min.

Analysis

(i) If m-FC(-RA) broth is being used, prepare the petri dishes prior to analysis by aseptically placing an absorbent pad into each petri dish (using either a dispenser (e.g Millipore XX62 000 14) or flame sterilized forceps. (Sterilize forceps by dipping the tips in ethanol, passing them through a flame and allowing the ethanol to burn off). To each pad pipette 2,0 mℓ m-FC(-RA) broth, allow the medium to soak into the absorbent pad for 5 min and then drain off the excess medium.

(ii) The volume of sample, or dilution thereof, filtered will depend upon the source of the sample. Suggested sample volumes (and dilutions) are:

Water Source	Volume filtered (mℓ)							
	100	50	10	1*	0,1*	0,01*	0,001*	0,0001*
Groundwater supply	X	X	X					
Impoundments	X	X	X					
River water	X		X	X	X			
Polluted small river			X	X	X	X		
Chlorinated sewage				X	X	X		
Unchlorinated sewage					X	X	X	X

*Do not attempt to filter volumes of less than 10 mℓ directly. Prepare the lower filtration volumes using quarter strength Ringer's dilution solution as follows:

Sample filtration volume	Sample volume	+	diluent volume	Volume of dilution filtered
1 mℓ	1 mℓ		9 mℓ	10 mℓ
0,1 mℓ	1 mℓ		99 mℓ (= Dilution A)	10 mℓ
0,01 mℓ	10 mℓ of Dilution A		90 mℓ	10 mℓ
0,001 mℓ	1 mℓ of Dilution A		99 mℓ (= Dilution B)	10 mℓ
0,0001 mℓ	10 mℓ of Dilution B		90 mℓ	10 mℓ

Before filtering a sample or dilution, and during the preparation of the dilution, shake the bottle or tube vigorously to ensure homogeneous distribution of the bacterial cells.

(iii) Assemble the filtration apparatus and, using flame sterilized forceps, place a membrane filter, grid side up, on the apparatus. Filter the desired volume of sample or dilution. After filtration, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ sterile quarter strength Ringer's solution, allowing the rinsings to pass through the filter. Switch off the source of vacuum and, taking care not to damage the membrane, remove the filter using flame sterilized forceps.

(iv) Transfer the membrane filter to a petri dish containing m-FC(-RA) agar or an absorbent pad soaked with m-FC(-RA). Place the membrane on the growth medium, grid side up, with a rolling action so as to avoid trapping air bubbles between the medium and the membrane. Replace the lid of the petri dish and clearly mark it with sample identification and sample volume. Incubate the filters, upside down, at 44,5°C $\pm$ 0,2°C for 20 to 24 h.

(v) After incubation, count all the colonies that have a blue colour. If possible choose to count a plate that gives a count of 20 to 80 faecal coliform colonies per filter. Samples from groundwater supplies and unpolluted impoundments will often have less than 20 colonies per filter. To aid counting, a low-power binocular dissecting microscope and fluorescent lamp may be used. Ensure adequate destruction of plates once the bacterial numbers have been enumerated.

Calculations

Faecal coliform bacteria numbers are quoted as the number of colonies per 100 mℓ. Calculate the faecal coliform count as follows:

$$\text{Faecal coliforms } 100\ \text{m}\ell^{-1} = \frac{\text{No. of colonies} \times 100}{\text{Vol of sample filtered (m}\ell\text{)}}$$

Comments

Colonies may be picked off and confirmed as faecal coliforms or Escherichia coli using the schemes of DHSS (1969) or Bordner et al. (1978).

2.2.5 Faecal streptococci - Membrane filter method - 1010001

Scope

The presence of faecal streptococci is taken to indicate faecal contamination by warm blooded animals. By definition the term faecal streptococci is taken to mean the streptococci that form Lancefield's Group D (i.e. Streptococcus faecalis and its variants liquifaciens and zymogenes, S. faecium, S. durans, S. bovis and S. equinus). By this definition the oral streptococci, S. mitis and S. salivarius are excluded. The ratio of faecal coliforms to faecal streptococci is taken to be indicative of the source of contamination. Ratios of 4,0 or greater are indicative of human faecal pollution while ratios of less than 0,7 are indicative of animal faecal pollution (Geldreich and Kenner, 1969). The ratios, however, are only valid for 24 h after the pollution has been discharged (Geldreich and Kenner, 1969) and are not applicable to chlorinated effluents (Rosser and Sartory, 1982). For the purpose of this method, faecal streptococci are defined as those bacteria which produce deep red or maroon colonies on m-Enterococcus agar after 48 h incubation at 35°C. Although this method would allow limited growth of S. mitis and S. salivarius (Stanfield et al. 1978) experience at the H.R.I. laboratories has shown that these species rarely grow on this medium.

Summary of Method

A water sample is filtered through a membrane filter upon which bacteria are entrapped. The filter is then placed on a selective growth medium and incubated at 35°C for 48 h after which colonies characteristic of faecal streptococci are counted.

Interferences

High sediment loads may lead to poor development of colonies.

Apparatus

(i) Incubator capable of maintaining a temperature of 35°C ± 0,5°C.

(ii) Filtration apparatus, sterile, preferably Hydrosol type (Millipore or equivalent) and source of vacuum.

(iii) Sterile, white, gridded 47 mm diameter membrane filters, 0,45 µm pore-size (Millipore type HAWG or equivalent).

(iv) 50 mm x 12 mm plastic petri dishes, with tightly fitting lids, sterile (Millipore PD10 047 or equivalent).

(v) Smooth tipped forceps.

(vi) Selection of sterile 10 mℓ and 1 mℓ pipettes.

Reagents

(i) m-Enterococcus agar (Slanetz and Bartley, 1957). This medium may be obtained commercially (e.g. Merck product No. 5262 or equivalent) and reconstituted according to manufacturers instructions or prepared as follows:

Yeast extract	5,0 g
Tryptose	20,0 g
Dextrose	2,0 g
Dipotassium hydrogen phosphate	4,0 g
Sodium azide	0,4 g
2,3,5 Triphenyl tetrazolium chloride	0,1 g
Agar	10,0 g

Suspend the ingredients in 1 ℓ distilled water and heat just enough, with stirring, to dissolve all the ingredients. Do not overheat the medium. Allow the medium to cool before dispensing into 50 mm diameter petri dishes to a depth of about 2,5 mm. The final pH of the medium should be 7,2 $\pm$ 0,1. If sealed and protected from light the plates may be stored in a refrigerator for up to 6 months.

(ii) Ringer's dilution solution (quarter strength) may be obtained commercially (e.g. Oxoid product No. BR52 or equivalent) and reconstituted according to manufacturers instructions or prepared by dissolving the following constituents in 1 ℓ distilled water:

Sodium chloride	2,25 g
Potassium chloride	0,105 g
Calcium chloride	0,12 g
Sodium bicarbonate	0,05 g

The solution is dispensed in 99, 90 and 9 mℓ aliquots into dilution bottles and sterilized by autoclaving at 121°C for 20 min.

Analysis

(i) The volume of sample, or dilution thereof, filtered will depend upon the source of the sample. Suggested sample volumes (and dilutions) are:

Water Source	Volume filtered (mℓ) 100	50	10	1*	0,1*	0,01*	0,001*	0,0001*
Groundwater supply	X	X						
Impoundments	X		X					
River water			X	X	X			
Polluted small river			X	X	X	X		
Chlorinated sewage				X	X	X		
Unchlorinated sewage					X	X	X	X

*Do not attempt to filter volumes of less than 10 mℓ directly. Prepare the lower filtration volumes using quarter strength Ringer's dilution solution as follows:

Sample filtration volume	Sample volume	+	diluent volume	Volume of dilution filtered
1 mℓ	1 mℓ		9 mℓ	10 mℓ
0,1 mℓ	1 mℓ		99 mℓ (= Dilution A)	10 mℓ
0,01 mℓ	10 mℓ of Dilution A		90 mℓ	10 mℓ
0,001 mℓ	1 mℓ of Dilution A		99 mℓ (= Dilution B)	10 mℓ
0,0001 mℓ	10 mℓ of Dilution B		90 mℓ	10 mℓ

Before filtering a sample or dilution, and during the preparation of the dilutions, shake the bottle or tube vigorously to ensure homogeneous distribution of the bacterial cells.

(ii) Assemble the filtration apparatus and, using flame sterilized forceps, place a membrane filter, grid side up, on the apparatus. (Sterilize forceps by dipping the tips in ethanol, passing them through a flame and allowing the ethanol to burn off). Filter the desired volume of sample or dilution. After filtration, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ sterile quarter strength Ringer's solution, allowing the rinsings to pass through the filter. Switch off the source of vacuum and taking care not to damage the membrane remove the filter using flame sterilized forceps.

(iii) Transfer the membrane filter to a petri dish containing m-Enterococcus agar and place the filter on the agar, grid side up, with a rolling action so as not to trap any air bubbles between the agar and the filter. Replace the lid of the petri dish and mark it with sample identification and sample volume. Incubate the filters, upside down, at 35°C ± 0,5°C for 44 to 48 h.

(iv) After incubation, count all colonies that are deep red or maroon in colour. If possible choose to count a plate that gives a count of 20 to 100 faecal streptococci colonies per filter. Samples from groundwater supplies and unpolluted rivers and impoundments will often have less than 20 colonies per filter. To aid counting, a low-power binocular dissecting microscope and fluorescent lamp may be used. Ensure adequate destruction of plates once the bacterial numbers have been enumerated.

Calculations

Faecal streptococci numbers are quoted as the number of colonies per 100 mℓ. Calculate the faecal streptococci count as follows:

$$\text{Faecal streptococci } 100\ m\ell^{-1} = \frac{\text{No of colonies} \times 100}{\text{Vol of sample filtered } (m\ell)}$$

2.2.6 Faecal clostridia - Membrane filter method - 1020001

Scope

The presence of Clostridium perfringens is taken as conclusive proof of faecal contamination of water. Due to the resistant nature of its spores it is "useful in detecting remote or intermittent pollution especially in shallow well waters" (DHSS, 1969). The method described here allows good recovery of C. perfringens, but it has been shown that other sulphite reducing clostridia (predominantly C. ghoni and C. perenne) are able to grow on this medium (Burger et al. 1984). As these other clostridia are primarily isolated from faeces and gastro-intenstinal and urinogenital tracts their presence should also be considered indicative of faecal contamination. For this reason the test name is faecal clostridia, although the predominant species present would be Clostridium perfringens. For the purpose of this method, faecal clostridia are defined as sulphite reducing anaerobic bacteria that produce typical black colonies when incubated on tryptose-sulphite-cycloserine agar under anaerobic conditions for 24 h at 45°C.

Summary of method

A water sample is filtered through a membrane filter upon which the bacteria are retained. The filter is then placed on a selective growth medium and incubated under anaerobic conditions at 45°C for 24 h, after which all colonies characteristic of faecal clostridia are enumerated.

Interferences

High sediment loads may cause excessive spreading of the colonies with subsequent difficulty in counting.

Apparatus

(i) Incubator capable of maintaining a temperature of 45°C ± 0,5°C.

(ii) Filtration apparatus, sterile, preferably Hydrosol type (Millipore or equivalent) and source of vacuum.

(iii) Sterile white, gridded 47 mm diameter membrane filters 0,45 µm pore size (Millipore type HAWG or equivalent).

(iv) 50 mm x 12 mm plastic petri dishes with tightly fitting lids, sterile (Millipore PD10 047 or equivalent).

(v) Anaerobic jars (Oxoid HP11 or equivalent) with anaerobic gas generating kit (Oxoid BR38 or equivalent) and anaerobic indicator strips (Oxoid BR55 or equivalent).

(vi) Smooth tipped forceps.

(vii) Selection of sterile 10 mℓ and 1 mℓ pipettes.

Reagents

(i) Tryptose-Sulphite-Cycloserine agar without egg yolk supplement (TSC-EY) (Hauschild and Hilsheimer, 1974) may be obtained commercially (e.g. Oxoid perfringens Agar Base CM587 and TSC Supplement SR88 or equivalent) and reconstituted according to manufacturers instructions or prepared as follows:

Yeast extract	5 g
Tryptose	15 g
Soya peptone	5 g
Sodium metabisulphite	1 g
Ferric ammonium citrate	1 g
Agar	14 g

Suspend the ingredients in 1 ℓ distilled water and dissolve by heating and stirring. Sterilize by autoclaving at 121°C for 15 min. Allow to cool to 50°C and add 4 mℓ of filter-sterilized solution of 100 mg $mℓ^{-1}$ D-cycloserine in distilled water. Mix well and dispense into 50 mm diameter petri dishes to a depth of about 2,5 mm. The final pH of the medium should be 7,6 ± 0,2. The prepared medium may be stored anaerobically in a refrigerator for at least 1 month. The plates must be well dried prior to use.

(ii) Reducing Ringer's dilution solution (quarter strength) may be prepared by adding 0,3 g cysteine hydrochloride to 1 ℓ of reconstituted commercial Ringer's solution (e.g. Oxoid product No. BR52 or equivalent) or prepared by dissolving the following constituents in 1 ℓ distilled water.

Sodium chloride	2,25 g
Potassium chloride	0,105 g
Calcium chloride	0,12 g
Sodium bicarbonate	0,05 g
Cysteine hydrochloride	0,3 g

The solution is dispensed in 99, 90 and 9 mℓ aliquots into dilution bottles and sterilized by autoclaving at 121°C for 20 min.

Analysis

(i) The volume of sample, or dilution filtered will depend upon the source of the sample. Suggested sample volumes (and dilutions) are as follows:

Water Source	Volume filtered (mℓ) 100	50	10	1*	0,1*	0,01*	0,001*	0,0001*
Groundwater supply	X							
Impoundments	X	X	X					
River water	X		X	X				
Polluted small river			X	X	X	X		
Chlorinated sewage		X		X	X	X		
Unchlorinated sewage				X	X	X	X	

*Do not attempt to filter volumes of less than 10 mℓ directly. Prepare the lower filtration volumes using quarter strength Ringer's dilution solution as follows:

Sample filtration volume	Sample volume	+	diluent volume	Volume of dilution filtered
1 mℓ	1 mℓ		9 mℓ	10 mℓ
0,1 mℓ	1 mℓ		99 mℓ (= Dilution A)	10 mℓ
0,01 mℓ	10 mℓ of Dilution A		90 mℓ	10 mℓ
0,001 mℓ	1 mℓ of Dilution A		99 mℓ (= Dilution B)	10 mℓ
0,0001 mℓ	10 mℓ of Dilution B		90 mℓ	10 mℓ

Before filtering a sample or dilution, and during the preparation of the dilutions, shake the bottle or tube vigorously to ensure homogeneous distribution of the bacterial cells and spores.

(ii) Assemble the filtration apparatus and using flame sterilized forceps, place a membrane filter, grid side up, on the apparatus. (Sterilize forceps by dipping the tips in ethanol, passing them through a flame, and allowing the ethanol to burn off). Filter the desired volume of sample or dilution. After filtration, and with the vacuum still on, rinse the sides of the filter funnel with 10 to 20 mℓ sterile quarter strength reducing Ringer's solution, allowing the rinsings to pass through the filter. Switch off the source of vacuum, and taking care not to damage the membrane remove the filter using flame sterilized forceps.

(iii) Transfer the membrane filter to a petri dish containing TSC-EY agar and place the filter on the agar, grid side up, with a rolling action so as not to trap any air bubbles between the agar and the filter. Replace the lid and mark it with sample identification and sample volume. Incubate the filters in an anaerobic jar containing an atmosphere of approximately 90% hydrogen and 10% carbon dioxide, at 45°C ± 0,20°C for 20 to 24 h.

(iv) After incubation count all black colonies. If possible choose to count a plate that gives a count of 20 to 80 faecal clostridia. To aid counting, a low-power binocular microscope and fluorescent lamp may be used. Ensure adequate destruction of plates once the bacterial numbers have been enumerated.

Calculations

Faecal clostridia are quoted as the number of colonies per 100 mℓ. Calculate the faecal clostridia count as follows:

$$\text{Faecal clostridia } 100\ m\ell^{-1} = \frac{\text{No of colonies} \times 100}{\text{Vol of sample filtered (m}\ell\text{)}}$$

Comments

Colonies can be picked off and confirmed as *Clostridium perfringens* by testing for gram-positive spore-forming anaerobic bacilli that are non-motile, nitrate-reducing, can liquify 12% gelatin and ferment lactose. Details of these tests are given in Hauschild and Hilsheimer (1974).

2.2.7 Standard plate count - Pour plate method - 1030001

Scope

The standard plate count provides a count of the viable aerobic and facultative anaerobic baacteria present in drinking water supplies. It does not represent the total viable bacteria present, but gives an indication of the general bacteriological quality of the water and the efficacy of water treatment processes.

Summary of method

A 1 mℓ aliquot of sample, or dilution of sample, is mixed with 15 to 20 mℓ non-selective nutrient enriched agar medium and incubated for 48 h at 35°C, after which all colonies are counted.

Interferences

Spreading surface growth of some species of bacteria may hinder counting of colonies.

Apparatus

(i) Incubator capable of maintaining a temperature of 35°C ± 0,5°C.

(ii) Sterile 90 mm petri dishes.

(iii) Sterile 1 mℓ pipettes.

(iv) Colony counter with Quebec grid (Chiltern Scientific Model CC30 or equivalent).

Reagents

(i) Standard Plate Count Agar (Tryptone Glucose Yeast Agar (APHA, 1980)). This medium may be obtained commercially (e.g. Oxoid product No. CM463 or equivalent) and reconstituted according to manufacturers instructions or prepared as follows:

Yeast extract	2,5 g
Tryptone	5,0 g
Glucose	1.0 g
Agar	15,0 g

Suspend the ingredients in 1 ℓ distilled water and boil to dissolve constituents. Dispense in 15 to 20 mℓ aliquots into screw-capped tubes and sterilize by autoclaving at 121°C for 25 min. The final pH of the medium should be 7,0 ± 0,2. The medium may be stored in the refrigerator, but should be used within 3 months.

Alternatively similar media such as Yeast Extract Agar (DHSS, 1969) may also be used.

(ii) Ringer's dilution solution (quarter strength) may be obtained commercially (e.g. Oxoid product No. BR52 or equivalent) and reconstituted according to manufacturers instructions or prepared by dissolving the following constituents in 1 ℓ distilled water:

Sodium chloride	2,25 g
Potassium chloride	0,105 g
Calcium chloride	0,12 g
Sodium bicarbonate	0,05 g

The solution is dispensed in 9 mℓ aliquots into dilution bottles and sterilized by autoclaving at 121°C for 20 min.

Analysis

(i) Prior to analysis select the number of tubes required and melt the agar by placing the tube in boiling water. Once melted, place the tubes in an incubator or water bath at 45°C to temper. The agar should only be melted once, so any unused tubes should be discarded or used as quality control checks (i.e. poured into petri dishes uninoculated and incubated with the inoculated plates).

(ii) For most drinking water samples a 1 mℓ subsample is adequate. If, however, elevated counts are expected then decimal serial dilutions using sterile quarter strength Ringer's solution are required and prepared as follows:

Volume sample added to agar	Sample volume	+	Diluent volume	Volume pipetted into petri dish
1 mℓ	1 mℓ		-	1 mℓ
0,1 mℓ	1 mℓ		9 mℓ (= Dilution A)	1 mℓ
0,01 mℓ	1 mℓ of Dilution A		9 mℓ (= Dilution B)	1 mℓ
0,001 mℓ	1 mℓ of Dilution B		9 mℓ (= Dilution C)	1 mℓ
etc.				

Before taking subsamples and during the preparation of the dilutions, shake the sample and dilutions vigorously to ensure homogeneous suspension of the bacterial cells.

(iii) Aseptically pipette 1 mℓ of the sample, or dilution thereof, into a petri dish and add 15 to 20 mℓ molten tempered standard plate count agar. Replace the petri dish lid and carefully move the petri dish in a series of to-and-fro and circular movements to mix the inoculum and agar without splashing. Leave the plate undisturbed until the agar has set. Clearly mark the dish with the sample identification and volume of actual sample pipetted. The plates are then inverted and incubated at 35°C ± 0,5°C for 44 to 48 h. Two plates should be prepared for each sample or dilution thereof.

(iv) After incubation, if possible select plates that will give a count of between 30 and 300 colonies per plate. With good quality domestic supplies, counts of less than 30 per 1 mℓ original sample may be encountered. Place the plate on the colony counter and tally the number of colonies. The Quebec grid will help when there are spreading colonies, or a plate with a very high count has to be used. In such cases a representative area (number of squares) is counted and the count related to the whole plate. For bacterial densities of less than 10 colonies cm^{-2} count the number of colonies in 13 grid squares and multiply the total by 5 for the mumber of colonies per plate. If the density is greater than 10 colonies cm^{-2}, count 4 representative squares, find the average per square and multiply that by 65 to give the count per plate.

Calculations

Standard plate count is quoted in terms of the number of colonies per millilitre. Calculate the count by dividing the mean of the two counts by the volume in millilitres of actual sample pipetted into the dish.

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SECTION 3: IDENTIFICATION OF DISPERSIVE SOILS

3.1 INTRODUCTION

Many soil properties are important when soils are used as construction material for reservoirs. One of these properties is the dispersion potential of a soil. Soils with a high sodium content are potentially dispersive. Chemical assessment of the dispersion phenomenon may be obtained using a classification method based on the measurement of exchangeable sodium percentage (ESP) and cation exchange capacity (CEC), together with data on the clay content (Gerber and Grobler, 1980; Gerber and Harmse, 1986). The clay content may be determined either by use of a sedigraph, or by means of the double hydrometer test (Anderson, 1951; Sherard et al. 1972).

For information on other tests for dispersion such as the erosion ratio, dispersion index, crumb test, slake test and pinhole test, the literature list at the end of section 3 should be consulted.

3.2 METHODS FOR IDENTIFICATION OF DISPERSIVE SOILS

3.2.1 Determination of the cation exchange capacity (CEC) - 7000301

Apparatus

(i) Volumetric flasks (100 mℓ)

(ii) Pipette (30 mℓ) or pipettor

(iii) Plastic bottles (100 mℓ)

Reagents

(i) 1 mol sodium acetate solution (pH 7). Dissolve 136,1 g sodium acetate (NaAc) in approximately 900 mℓ deionized water and determine the pH. If the pH is below 7 add a few drops of a sodium hydroxide (NaOH) solution, and if it is above 7 add a few drops of acetic acid (CH_3COOH).

(ii) 1 mol ammonium acetate solution (pH 7). Dissolve 77,1 g ammonium acetate (NH_4Ac) in approximately 900 mℓ deionized water and determine the pH. If it is below 7 add a few drops of ammonia, and if it is above 7 add a few drops acetic acid (CH_3COOH).

(iii) Ethanol (95%).

(iv) Ethanol (50%).

Method (Sodium saturation and exchange)

(i) Weigh off 5 g (± 0,01 g) soil in a plastic centrifuge bottle.

(ii) Add 30 mℓ 1 mol sodium acetate (NaAc) at pH 7, shake for 15 min, centrifuge for 5 min at 2 000 rpm and decant the supernatant.

(iii) Repeat step (ii) four times.

(iv) Add 30 mℓ 50% ethanol shake for 15 min, centrifuge for 5 min at 2 000 rpm and decant the supernatant.

(v) Repeat step (iv) twice.

(vi) Add 30 mℓ 95% ethanol, shake, centrifuge and decant as in step (iv) above.

(vii) Add 30 mℓ 1 mol ammonium acetate (NH_4Ac) shake for 15 min, centrifuge for 5 min at 2 000 rpm and filter the supernatant using Whatman No. 42 filter paper into a 100 mℓ volumetric flask.

(viii) Repeat step (vii) three times.

(ix) Fill the volumetric flask up to the 100 mℓ mark.

The filter paper must be washed during this process. Use the 1 mol NH_4Ac solution for this purpose.

(x) Determine the concentration sodium using a flame photometer. (This value is expressed in me/100 g soil and represents the CEC value of the soil).

3.2.2 Determination of the percentage adsorbed sodium - 7000101

Apparatus

(i) Volumetric flasks (100 mℓ)

(ii) Pipette (30 mℓ) or pipettor

(iii) Plastic bottles (100 mℓ)

Reagents

(i) 1 mol sodium acetate solution (pH 7). Dissolve 136,1 g sodium acetate (NaAc) in approximately 900 mℓ deionized water and determine the pH. If the pH is below 7 add a few drops of a sodium hydroxide (NaOH) solution, and if it is above 7 add a few drops of acetic acid (CH_3COOH).

(ii) 1 mol ammonium acetate solution (pH 7). Dissolve 77,1 g ammonium acetate (NH_4Ac) in approximately 900 mℓ deionized water and determine the pH. If it is below 7 add a few drops of ammonia, and if it is above 7 add a few drops acetic acid (CH_3COOH).

(iii) Ethanol (95%).

(iv) Ethanol (50%).

Method (Sodium saturation and exchange)

(i) Weigh off 5 g (± 0,01 g) soil in a plastic centrifuge bottle.

(ii) Add 30 mℓ 50% ethanol, shake for 15 min, centrifuge for 5 min at 2 000 rpm and decant the supernatant.

(iii) Repeat step (ii) twice.

(iv) Add 30 mℓ 95% ethanol, shake for 15 min, centrifuge at 2 000 rpm for 5 min and decant the supernatant.

(v) Add 30 mℓ 1 mol ammonium acetate (NH_4Ac), shake for 15 min, centrifuge for 5 min at 2 000 rpm and filter the supernatant using Whatman No. 42 filter paper into a 100 mℓ volumetric flask.

(vi) Repeat step (v) three times.

(vii) Fill the volumetric flask up to the 100 mℓ mark.

The filter paper must be washed during this process. Use the 1 mol NH_4Ac solution for this purpose.

(viii) Determine the concentration sodium using a flame photometer. (This value represents the concentration adsorbed sodium in the soil).

Calculations

The exchangeable sodium percentage (ESP) value is calculated as follows:

$$ESP = \frac{(Na)_1}{(Na)_2} \times 100$$

where

$(Na)_1$ = concentration adsorbed sodium as obtained from 3.2.2.

$(Na)_2$ = concentration sodium as obtained from 3.2.1.

Thus

$$ESP = \frac{\text{Sodium (adsorbed)}}{\text{CEC}} \times 100$$

3.2.3 Determination of the clay content and the percentage dispersion of the soil - 7000201

Two methods can be followed:

(i) Hydrometer method, or

(ii) method using the sedigraph 5000D apparatus.

As a sedigraph is not always available and the procedures are given in the manual of the apparatus, only the hydrometer method is discussed.

Apparatus

(i) Sedimentation cylinder.

(ii) Hydrometer (152 H).

(iii) Thermometer.

(iv) Pipettes (100 mℓ and 25 mℓ).

Reagents

Sodium hexa-metaphosphate $(NaPO_3)_6$, 20% solution. Weigh off 200 g of $(NaPO_3)_6$ in 1 ℓ of water. The solution can be heated to aid the dissolving. The solution used for the experiment remains stable for about 30 d.

Method

(i) Place about 300 g of soil in a drying oven and dry at 80°C for 24 h. Break all the clods (do not crush the soil) and sieve it using a 2000 µm sieve.

(ii) Weigh off exactly 50 g of the oven-dried (2 000 µm) soil. (Use 100 g of soil if the soil contains less than 10% material finer than 75 µm).

(iii) Wash the soil through a 75 µm sieve using deionized water and a brush, into a sedimentation cylinder. The total volume water should not exceed 1 ℓ.

(iv) Three sedimentation cylinders are now used:

one with 1 ℓ deionized water;
one with 15 mℓ Calgon (20%) solution filled to 1 000 mℓ with deionized water, and
one with the material finer than 75 µm filled to 1 ℓ.

(v) Shake the cylinder with the soil-water suspension by turning it upside down and back 30 times in 60 s.

(vi) Take a temperature reading, the temperature in the three cylinders should be within 1°C.

(vii) Place the hydrometer (152H) in the control cylinder (cylinder with the 15 mℓ Calgon) and the cylinder containing the deionized water and take a meniscus reading on the hydrometer. The difference between these readings is the zero-correction and is used for further calculations.

(viii) Take a hydrometer reading in the soil suspension after 60 min has elapsed. (This reading represents the fraction of soil particles finer than 5 µm in water).

(ix) Add 15 mℓ Calgon to the soil suspension and shake as previously described. Take another reading after 60 min.

(x) Shake the soil suspension again and take a reading after 10 h. This reading represents the clay fraction (2 µm) in the suspension. If the temperature has changed repeat (vii) above.

Calculations

(i) $Rc = Ra - Zo + C_T$

where

Ra = actual hydrometer reading,

Rc = corrected hydrometer reading,

Zo = zero-correction = the difference between the reading in the cylinder filled with deionized water and the control cylinder; and

C_T = the temperature correction (Table 3.1).

Table 3.1: Correction factor C_T which is dependent on the temperature.

Temperature (°C)	C_T
15	-1,10
16	-0,90
17	-0,70
18	-0,50
19	-0,30
20	0,00
21	+0,20
22	+0,40
23	+0,70
24	+1,00
25	+1,30
26	+1,65
27	+2,00
28	+2,50
29	+3,80
30	+3,80

Table 3.2: Correction factor (a) for the unit weight of soil.

Unit weight in g/mℓ	Correction factor (a)
2,85	0,96
2,80	0,97
2,75	0,98
2,70	0,99
2,65	1,00
2,60	1,01
2,55	1,02
2,50	1,04

(ii) Percentage finer = $\frac{Rc(a)}{Ws}$

(a) = the correction factor for the unit weight of the soil (Table 3.2) 2,70 is generally used and

Ws = the mass (50 g) of soil used.

Percentage finer =

the percentage of a fraction finer than a certain particle size (2 or 5 µm etc.).

After the above calculations have been done the percentage ≤5 µm in water and in Calgon (dispersion medium), and the percentage clay in the soil sample becomes known.

(iii) The percentage dispersion is calculated as follows:

$$\text{Percentage dispersion} = \frac{\%\ \leq 5\ \mu m\ \text{in water}}{\%\ \leq 5\ \mu m\ \text{in Calgon}} \times 100$$

3.3 DETERMINATION OF THE DISPERSION POTENTIAL - 7000302

3.3.1 Results

(i) The CEC (cation adsorption capacity) value of the soil.

(ii) The concentration adsorbed sodium in the soil.

(iii) The percentage clay.

(iv) The percentage silt plus clay in Calgon and the percentage silt in water.

3.3.2 Calculations

In order to classify the soil in the categories (dispersive, marginal or non-dispersive) according to Figure 3.1 the following calculations must be made:

(i) Cation adsorption capacity in me/100 g clay

$$(CEC)/100\ g\ clay = \frac{CEC\ value \times 8{,}7}{\%\ clay\ content}$$

(ii) Exchangeable sodium percentage (ESP), see 3.2.2. calculations.

(iii) Percentage dispersion, see 3.2.3. calculations.

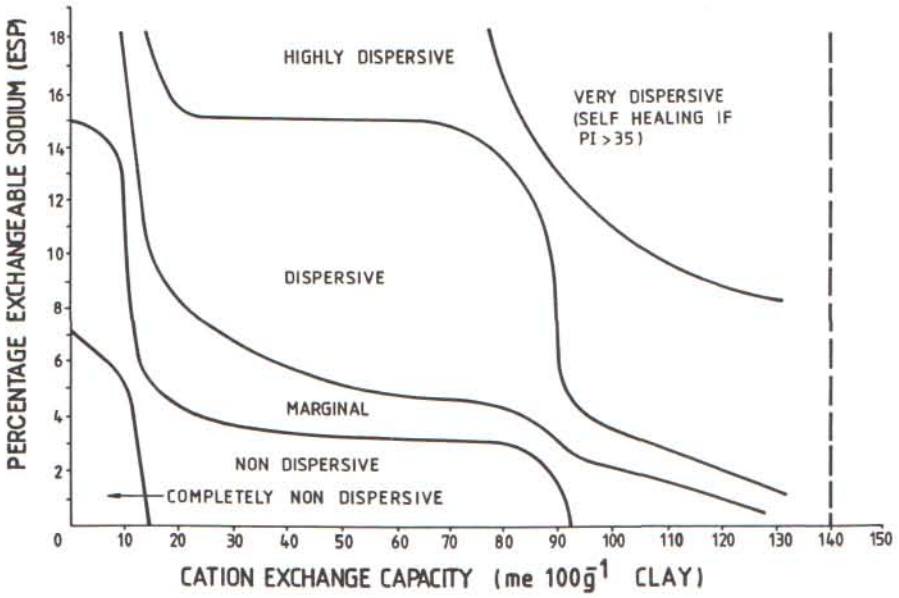

Figure 3.1: Diagram for determination of dispersion potential as a function of ESP and CEC 100 g^{-1} clay.

3.3.3 Classification of soil according to the dispersion potential

By using Figure 3.1 the final classification of the dispersion potential can be made. If a sample, when plotted on Figure 3.1, lies near a line that is for example a border between marginal and dispersive it is necessary that the percentage dispersion value be taken into account. If this value is 60% and above it is advisable to classify the soil in the next category. For example from non-dispersive to marginal or from marginal to dispersive. This evaluation may be used in all cases where soil is to be used as construction material.

3.4 DETERMINATION OF THE REACTION OF THE SOIL AS INFLUENCED BY THE CHEMICAL QUALITY OF THE WATER IN CONTACT WITH THE SOIL

As soil dispersion is influenced by the quality of the water in contact with the soil it is advisable that this water is analysed. To do the evaluation the total concentration cations (TCC in me/ℓ) and the sodium adsorption ratio (SAR) must be obtained, where:

$$SAR = \frac{Na}{\frac{Ca + Mg}{2}}^{1/2} \quad \text{(values in me/ℓ) and}$$

TCC = Na + Ca + Mg + K (in me/ℓ)

Seeing that the dominant clay mineral also plays a role during dispersion, three curves reflecting this are given in Figure 3.2, namely;

CEC/100 g clay = 5-20, 20-80 and 80-140 me*.

Soil (CEC between 20-80 me, Figure 3.2) which classifies as dispersive will tend to flocculate in water of SAR=10 and of TCC = 8 me/ℓ.

If the quality of the water improves, dispersion will occur. This explains why rain water disperses a potentially dispersive soil.

In Figure 3.3 the relationship between the SAR of water and the equilibrium ESP of soil can be seen. From this the expected ESP value of a soil in equilibrium with the water can be obtained and emphasizes the fact that water with high sodium content can make a soil potentially dispersive.

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* me = milli-equivalents

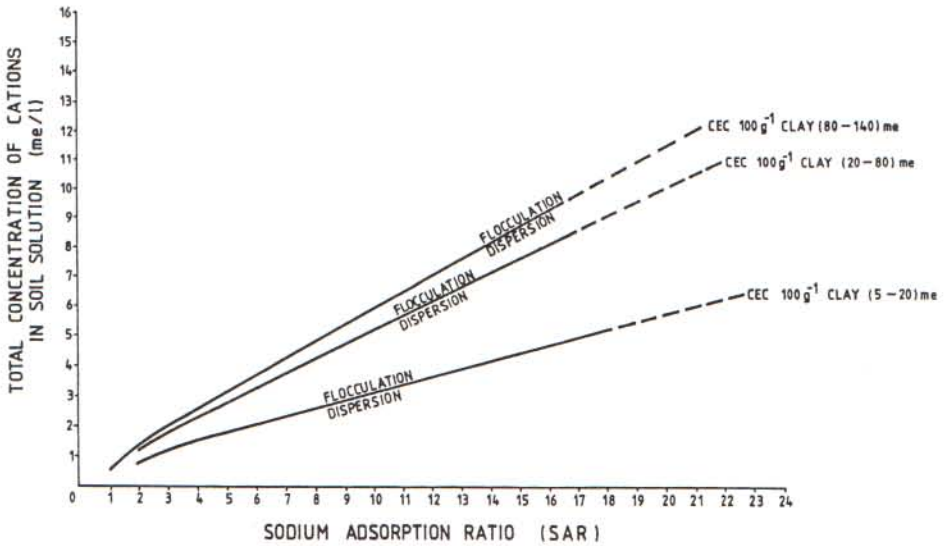

Figure 3.2: The influence of free salts on flocculation and dispersion as influenced by the colloidal composition of soils.

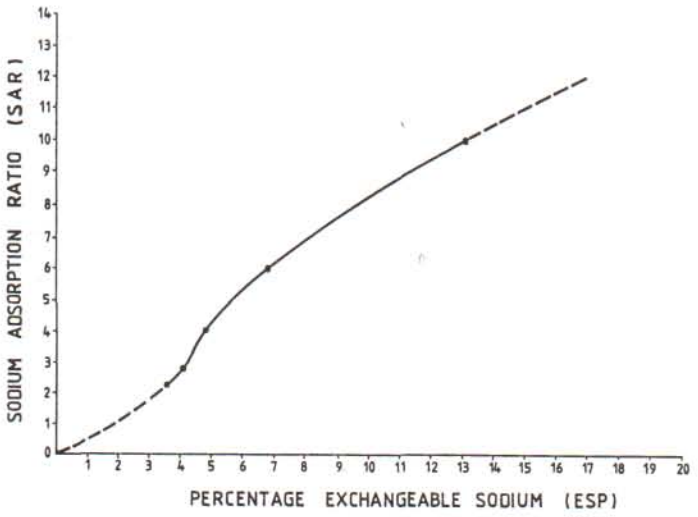

Figure 3.3: The relationship between the ESP of soil and the SAR of water in equilibrium with the soil.

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