Phosphorus and Silicon in Waters, Effluents and Sludges 1992

Methods for the Examination of Waters and Associated Materials

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Phosphorus and Silicon in Waters, Effluents and Sludges 1992

Methods for the Examination of Waters and Associated Materials

This booklet which is an expanded combined second edition of two earlier booklets (Refs 38 and 39) describes two spectrophotometric methods for the determination of phosphorus and silicon, together with an explanatory introduction on the various forms in which these elements may occur in water and associated materials. Methods are described for the conversion of most other forms to the readily determinable form of phosphate and silicate, and an outline of the basic chemistry involved in the formation of heteropoly acids and their related blue complexes is given, which indicates why problems may affect these methods if attention is not paid to detail. Information is also given on the determination by chromatography and emission spectrophotometry and on the automation of such methods.

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About This Series

This booklet is part of a series intended to provide recommended methods for determining the quality of water and associated materials. In addition, short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series is published as a series of booklets on single or related topics, thus allowing for the replacement or addition of methods as quickly as practicable without the need for waiting for the next edition. The rate of publication is also related to the urgency of the requirement for that particular method.

Although ideally, all methods published should be fully tested, this is not often possible without delay in publication. Furthermore, the limit of detection, range, precision and interference effects applying to instrumental methods can depend on the actual instrument used, as well as on sample type, reagent purity and operator skill, etc. Even methods tested in many laboratories have been known to acquire problems, for example, when new products appear (introducing new substances into effluents), when changes in production methods affect reagent quality, or when the method is used to analyse new types of sample (despite apparent similarity to samples already evaluated). As a guide, the following categories have been given to methods:

(i) tested, usually in five or more laboratories
- no grade indicated;

(ii) tested in one to three or four laboratories
- Tentative;

(iii) evaluated, but not fully tested, but publication is urgently required
- Note;

(iv) tested and found to be satisfactory by several laboratories, but in the opinion of experts requires a high degree of skill or has some other difficulty such that the method would be replaced if a better method were discovered.
- Provisional.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the United Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users and senior technical staff, to decide which method to use for the determination in hand. Whilst the attention of users is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has nine working groups, each responsible for one section or aspect of water cycle quality analysis. They are:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage works control methods
9.0 Radiochemical methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and the main committee. The names of those associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. A current list of publications can be obtained from the Secretary.

Every effort is made to prevent errors from occurring in the published text. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically. However, should any errors be found, please notify the Secretary.

Dr D WESTWOOD
Secretary

5 August 1992

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety and COSHH Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text, so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown to be often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', issued by the Royal Society of Chemistry, London: 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Services Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient, give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

Safety while Sampling

Prior consideration must be given, especially when sampling in confined spaces or where access is difficult, to guard against suffocation, drowning, falls and poisoning or infection by ingestion, inhalation or skin contact.

Good Laboratory Practice

The Department of Health issues a booklet entitled: Good Laboratory Practice; the United Kingdom Compliance Programme, 1989. This can be obtained by writing to that Department in London. It deals chiefly with toxicity studies, but much can be applied to analytical chemistry.

Introduction

1. Phosphorus may be present in water, effluent and sewage in a variety of physical and chemical forms. See section D1.

2. For natural waters (fresh and saline) as well as for wastewaters (sewages and effluents) it is usually sufficient to determine the orthophosphate present, as this is the form of phosphorus most readily available to biota. The spectrophotometric method A described in this booklet, which is based on the formation of an antimony containing phosphomolybdenum blue complex, is rapid and well suited to routine analysis and to automation. It should be noted that it is probably more appropriate to refer to the determinand as 'available phosphate' as there is a possibility that the acidity of the reagent may cause hydrolysis of the most labile organic phosphates to produce extra phosphate. Total dissolved phosphorus (inorganic and organic) can also be determined by application of the method following a preliminary decomposition stage (eg peroxodisulphate oxidation or UV photolysis (see Part D for the various pretreatments). Method B is based on the direct spectrophotometric measurement of a coloured phosphovanadomolybdate complex, without prior reduction to the more common blue complex. It is less sensitive than method A and can be used to determine higher concentrations without predilution. Both these methods can also be applied to the determination of total phosphorus in particulates (>0.45 μm), sediments and sewages using digests prepared by preliminary treatment of the samples.

3. Part C outlines the various separations and determinations of phosphorus containing ions which are described in the various methods given in Ref 1.

4. Silicon may also be present in water, effluents and sewage in a variety of physical and chemical forms. See section G1.

5. Often, for natural waters (fresh and saline) and wastewaters it is sufficient to determine the soluble monomeric and dimeric forms of silicon. The spectrophotometric methods described in sections E and F of this booklet, based on the formation of a silicomolybdenum blue complex will determine the sum of both.

6. Method E describes an established method for silicon based on the use of 1-amino-2-naphthol-4 sulphonic acid (ANSA) to achieve the reduction step and is intended for the analysis of clean waters, especially those with low silicon content. The use of Metol as a reductant is also mentioned.

7. Method F describes a relatively new method for silicon based on the use of ascorbic acid to achieve the reduction step. The spectrophotometric measurement is made at 700 nm in 10 mm cells, which makes it particularly suitable for a wide range of silicon concentrations up to 100 mgL^{-1}, although it can easily be modified to increase or decrease the sensitivity.

8. Testing has shown that with slight adaptation, both methods E and F can be interchanged. Analysts wishing to do this are advised to re-check the performance characteristics of the particular method used.

9. Occasionally it may be necessary to determine monomeric, dimeric and polymeric forms of silicon or total silicon. For these purposes a series of pretreatments is described in Part G. Silicones are unlikely to occur. If their presence is known or suspected consult reference 2.

10. Methods A, E and F make use of phosphomolybdenum or silicomolybdenum blue complexes which rely on the initial formation of heteropolymolybdic acids prior to reduction to the blue complex. Method B uses an alternative unreduced phosphorus heteropoly acid. There are a large number of poly and heteropoly acids, formed by molybdates and tungstates with phosphorus (V), arsenic (V), silicon (IV), germanium (IV), titanium (IV), and also with cobalt (II) and (III), nickel (II) and (IV) and vanadium (V). Which acid is formed is highly dependent on the particular molybdate (or tungstate) used (paramolybdate $Mo_7\ O_{24}^{6-}$ is the one usually used for analysis), on the molybdenum concentration and acidity of the solution, the other ions present (such as tartrate or oxalate), and in some instances on the temperature and reaction time. Interconversion can also occur, as with silicomolybdate, the rate being dependent on many variables, including for example, salinity. Molybdenum and other metals such as tungsten, vanadium, titanium and iron form Berthollides (a non-stoichiometric series of compounds), all highly coloured, in which some of the metal atoms in the complex lattice are one valency below (or above) the others, the strong colour being due to the electron charge transfer phenomenon. As molybdenum can form almost continuous series of berthollides from 6 valent to 4 valent, with varying colours and solubilities, it is equally essential for reliable analysis that both the conditions for the initial heteropoly acid formation and the reduction are carefully controlled. The reduction-oxidation potential to a blue complex varies between the various poly and heteropoly acids and a combination of these effects can be used to differentiate between species of hetero atoms, eg phosphorus or silicon. Only a few reducing agents such as ascorbic acid and tin (II) are efficient reductants for phosphomolybdates and large amounts of oxalate and tartrate can cause interferences with the reduction. However most reductants are suitable for reducing silicomolybdates to a molybdenum blue complex. For further information, consult a modern text book on inorganic chemistry (eg Ref 3).

11. Both silicon and phosphorus can be determined by optical emission spectrophotometry and these techniques are covered in Part H.

12. When making trace level determinations, it is essential to consider sources of contamination and to check equipment and materials thoroughly. Both phosphorus and silicon are found in many cleaning materials and glassware. Phosphorus is found in matches and both can occur in dust and ashes. Not all phosphorus or silicon compounds are white. If in doubt, check.

13. Biota in any sample probably contain phosphorus and silicon and consideration should be given as to whether this needs to be determined. If not, care should be taken to avoid contamination from this source.

14. By convention, silicon is normally reported as silica (SiO_2) in water analysis. This procedure has been adopted throughout this booklet. No similar convention exists for phosphorus which is variously reported as the element (P), the pentoxide (P_2O_5), orthophosphate (PO_4^{3-}), or other specified ion. In this booklet, phosphorus is reported as the element. Analysts should make it clear which method of reporting is being used.

Determination of Phosphorus

Methods A, B, C and H plus Pretreatments in D
See also Introduction notes 1–3 and 10–14.

A Phosphorus in Waters, Effluents and Sludges by Spectrophotometry — phosphomolybdenum blue method

A1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see Ref 34)

Note: Throughout this method phosphorus is expressed as the element (P). All data given are based on a 40 ml sample volume.

A1.1	Substance determined	Reactive phosphorus (see section A2).
A1.2	Type of sample	Saline and non saline waters, effluents and sewages (and also digested samples where the phosphorus concentration of the digest is within the range of this method).
A1.3	Basis of method	Reaction with the acidic molybdate reagent in the presence of antimony to form a phosphomolybdenum blue complex, the concentration of which is measured spectrophotometrically.
A1.4	Range of application (a)	0 to 0.40 mgL^{-1} (The range may be extended by dilution, see section A10).
A1.5	Calibration curve (a)	Linear to 0.4 mgL^{-1}.
A1.6	Standard deviation (a)	

Type of sample	Phosphorus concentration (mgL^{-1})	Total standard deviation (mgL^{-1})	Degrees of freedom
Standards (b)	0.00	0.001*	6
Standards (b)	0.04	0.002	14
Standards (b)	0.35	0.003	14
Standards (b)	0.60	0.005	11
River water	0.36	0.004	14
Sea water	0.07	0.002	14
Lime treated sewage	0.32	0.015	14

*within batch standard deviation

A1.7	Limit of detection (a)	0.003–0.006 mgL^{-1} (35 degrees of freedom)
A1.8	Sensitivity (a)	0.25 mgL^{-1} gives an absorbance at 882 nm in a 40 mm cell of approximately 0.6.
A1.9	Bias (a)	No important sources of bias were detected, except where interferences were present.
A1.10	Interferences	See sections A3, D5 and Introduction note 10.
A1.11	Time required for analysis (a)	The total analytical and operator times are virtually the same and for 50 samples are equal to 2 h (4 h if turbidity/colour correction is required).

(a) These data were obtained at the Water Research Centre, the Freshwater Biological Association, Windermere Laboratory and the former Yorkshire Water and North West Water Laboratories. Data obtained using 40 mL of sample.

(b) Deionized water spiked with the stated phosphorus concentration, as KH_2PO_4

A2 Principle

The method described is based on that developed by Murphy and Riley (Ref 4) and experimental work carried out at the Water Research Centre (Ref 5), Stevenage Laboratory and Imperial Chemical Industries Laboratories (Ref 6). Orthophosphate ions in the sample are reacted with a mixed reagent containing molybdate, hydrogen ions, antimony (III) and ascorbic acid. The reaction leads to the formation of two isomeric heteropoly acids, which are subsequently reduced to give a phosphoantimonyl molybdenum blue complex, the absorbance of which is measured.

The acidic conditions used may lead to gradual hydrolysis of condensed phosphates and the more labile organic phosphates, if present. For this reason, the determinand is referred to as reactive phosphorus rather than orthophosphate. This hydrolysis can be minimized by making the photometric measurement within 10–15 min after addition of the mixed reagent.

Tests have shown that, at 20°C, phosphomolybdenum blue formation is usually completed in 3–4 minutes.

A3 Interferences
(see also Ref 12)

A3.1 There is little detailed information about the effects of interferences in the method. Generally, no interference problems, other than those listed below, are likely with unpolluted saline and fresh waters (eg there is no 'salt error'). The possibility of interference should be considered, particularly with polluted samples and at phosphorus levels close to the limit of detection.

A3.1.1 Arsenic

Arsenic present as arsenate is potentially a source of serious error at high As/P ratios as it forms an analogous arsenomolybdenum blue complex. As the rate of formation of the arsenic complex is slow, interference can be greatly reduced by making photometric measurement 6 minutes after the addition of the mixed reagent. At full colour development, 0.025 mgL^{-1} As gives a similar response to 0.015 mgL^{-1} P (40 mL sample volume). Interference from As can be eliminated by reducing it to arsenite (see section D5).

A3.1.2 Silicate

Silicate causes negligible interference in the determination of phosphate at room temperature as the formation of silicomolybdenum blue is inhibited by the high $[H^+]$:[Mo] ratio used (Refs 7 and 8). Koroleff has reported that 5 mgL^{-1} Si gives an absorbance of 0.003 at 880 nm in a 10 cm cuvette after 30 min. Interference from Si can be considerably reduced by carrying out spectrophotometric measurements 6–10 minutes after addition of the mixed reagent as the kinetics of formation of the molybdenum blue complex are relatively slow.

A3.1.3 Chromium

Cr(VI) is reported to interfere at 1 mgL^{-1} level (Ref 9).

A3.1.4 Oxidizing agents

The action of oxidising agents in this method is complex. They may destroy the reducing agent, or subsequently reoxidize the phosphomolybdenum blue complex.

A3.1.5 Nitrite

A concentration of 1 mgL^{-1} N (as nitrite) may be tolerated in the presence of 0.1 mgL^{-1} phosphorus, but the interference of nitrite is both complex and variable and appears to be related to exposure to air.

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salt error—High concentrations of ions not common to a reaction increase the overall ionic strength of the solution and so change the solubility of salts and equilibria of complex formation. For details consult standard works on physical chemistry.

A3.1.6 Nitrate

Nitrate nitrogen may be tolerated up to 20 gL^{-1} (as N) provided that the absorbance is measured within 2 hours of colour development.

A3.1.7 Sulphide

Interference from sulphide is complex, variable and dependent on conditions, reacting with both antimonate and molybdate to form thioacids. Complete removal is advocated (by oxidation to sulphate or by aspiration with nitrogen, see Ref 10).

A3.2 Removal of interferences

There is no single pretreatment that will systematically deal with all interference problems. There are, however, certain processes that will have a selective effect and the analyst must use discretion in their application.

A3.2.1 The effect of oxidizing agents and arsenate may be overcome by treatment with an excess of metabisulphite/thiosulphate in acid solution (Ref 11). The presence of sulphur dioxide has no influence on the final production of molybdenum blue, but the treatment can only be applied to determinations of inorganic phosphorus and total phosphorus, ie where hydrolysis is acceptable, as opposed to the determination of dissolved reactive phosphorus (see section D5).

A3.2.2 A slight excess of sulphamic acid is effective in breaking down nitrite; 100 mg of the acid will deal with a nitrite concentration of 10 mgL^{-1} N in a 40 mL aliquot of test solution.

A4 Hazards

Several reagents, eg antimony potassium tartrate, ammonium molybdate and industrial methylated spirits are poisonous; care should be taken at all times.

Acids and alkalis are used making it advisable to wear protective clothing and suitable eye protection.

A5 Reagents

Analytical reagent grade chemicals are suitable.

A5.1 Water

The water used for blank determinations and for preparing reagent and standard solutions and for dilution purposes, should have a phosphorus content that is negligible compared with the smallest concentrations to be determined in the samples (see section A11.1). Distilled or deionized water is usually suitable.

A5.2 14% V/V Sulphuric acid

Add slowly and cautiously, with stirring, 140 ± 2 mL of sulphuric acid (d_{20} 1.84) to 800 ± 10 mL of water in a 2-litre beaker immersed in cold water, allow to cool and dilute to about 1 litre. Store in a glass bottle.

A5.3 4% m/V Ammonium molybdate

Dissolve 20 ± 0.2 g of finely ground ammonium molybdate $(NH_4)_6Mo_7O_{24}4H_2O$ in water and dilute with water to 500 ± 10 mL. Store in a polyethylene bottle.

A5.4 0.28% m/V Antimony potassium tartrate

Dissolve 0.28 ± 0.01 g of antimony potassium tartrate $(K(Sb0)C_4H_4O_6)$ in water, warming if necessary and dilute with water to 100 ± 1 mL. Store in a borosilicate glass bottle, in a refrigerator.

A5.5 1.76% m/V Ascorbic acid

Dissolve 1.76 ± 0.02 g of ascorbic acid in 100 ± 1 mL water. This solution must be prepared just before use, prepare only as much as is required, scale up if necessary. A full batch of Mixed Reagent (A5.6) requires 150 mL.

A5.6 Mixed reagent

A quantity suitable for 50 determinations may be prepared by mixing together, in the order given, 250 ± 2 mL of 14% V/V sulphuric acid, 75 ± 1 mL of 4% m/V ammonium molybdate, 150 ± 2 mL of 1.76% m/V ascorbic acid and 25 ± 0.5 mL of 0.28% m/V antimony potassium tartrate, mixing after each addition. This reagent should be prepared fresh as required and kept in a refrigerator when not in use. Under these conditions it should be suitable for use during 1 working day, any excess remaining after one day should be discarded. Do not prepare more than is required.

A5.7 Sodium hydroxide solution (M). May be required to neutralize some samples

Cautiously dissolve 20 ± 1 g of sodium hydroxide pellets in about 400 mL of water. Cool and dilute with water to 500 ± 10 mL. Store in a polyethylene bottle.

A5.8 Sulphuric acid (M). May be required to neutralize some samples.

Dilute 20 mL of 14% V/V sulphuric acid with water to 100 ± 10 mL. Store in a glass or polyethylene bottle.

A5.9 0.5% m/V Phenolphthalein solution

Dissolve 0.5 ± 0.05 g of phenolphthalein in 60 ± 1 mL industrial methylated spirit. Add 40 ± 1 mL water. Mix well and store in a glass dropper bottle.

A5.10 Standard phosphate solutions

A5.10.1 Solution A 1 mL is equivalent to 100 µg P

Dissolve 0.4394 ± 0.0005 g of potassium dihydrogen orthophosphate (KH_2PO_4), previously dried at 105°C and cooled in a desiccator, in water and dilute to 1 litre in a calibrated flask. Store in the dark in a borosilicate glass bottle in a refrigerator. This solution is stable for at least 3 months.

A5.10.2 Solution B 1 mL is equivalent to 1 µg P

Dilute 10.00 ± 0.02 mL of solution A with water to 1 litre in a calibrated flask. This solution may be stored in a refrigerator and is stable for up to a few days, however it should be prepared freshly when required.

A6 Apparatus

A6.1 Spectrophotometer

A spectrophotometer for use at 882 nm and capable of accepting 40 mm cells is suitable. If this wavelength is not obtainable, a wavelength of 725 nm may be used with approximately 70% of the sensitivity at 882 nm. Alternatively, a filter photometer with a suitable red filter may be used, but a loss of sensitivity will occur and the results will be less reliable. Linearity of calibration should be checked.

A6.1.1 40 mm optically matched cells

Both sample and reference cells should be kept scrupulously clean and should not be interchanged. They should always be placed in the same position in the holder with the same face towards the light source.

10 mm cells may be used, but a fourfold loss of sensitivity will occur, different standards should be used and the calculation should be adjusted.

For greater sensitivity 100 mm cells can be used with a suitably large capacity spectrophotometer.

A6.2 Glassware

Glassware should be cleaned and allowed to stand overnight filled with sulphuric acid (d_{20} 1.84), then rinsed several times with water and stored filled with water. On no account should glassware be allowed to come into contact with detergents or alkaline liquids.

With reasonable care, the acid hardening treatment need only be repeated occasionally.

A7 Sample Collection and Preservation

A7.1 Phosphate is readily adsorbed on to many plastic surfaces. For this reason, good quality borosilicate glass containers must be used. They should first be conditioned as described in A6.2 and stored in a dark, cool place. The use of plastic containers for samples at higher concentration ranges may be acceptable. Some losses may occur, but they may be small in relation to the phosphate present and at lower concentrations the suitability must be ascertained. Ideally, waters should be analysed as soon as possible after sampling as there is no generally satisfactory method of long term preservation.

Refrigeration (without freezing) is reasonably effective over short periods of storage (eg a few days).

Some samples may require filtering on site to ensure changes do not occur during transit or storage (see also Part D1).

A7.2 Sample pretreatment

The majority of samples encountered probably require no pretreatment at all, except possibly, the need to eliminate interference as given in section A3.

If other forms of phosphorus, in addition to reactive phosphorus, are to be determined consult Part D.

A7.3 As a guide to analysts supporting algology projects, if the major requirement is to measure 'dissolved reactive phosphate' in samples containing less than 25 $\mu g L^{-1}$ P, carry out one of the following procedures:

(a) filter the sample 'on site' through a glass fibre paper, pre-washed with about 500 mL of water (Ref 13), into a suitable bottle and analyse within 3 hours of sampling.

If this is not possible, filter the sample through a pre-washed 0.45 µm membrane filter within 3 hours of sampling and store in the dark at, or close to, 4°C. The delay should be stated.

NOTE. If the pressure drop of the filter is too high or if the sample is allowed to freeze, changes of dissolved phosphate concentrations may result from biological cell rupture and the release of phosphate into the solution.

'On site' filtration may be mandatory depending upon which forms of phosphate are to be measured. The insoluble phosphate content of an unfiltered sample can increase due to the continuation of zooplankton activity to give deposits containing phosphate.

A8 Analytical Procedure

Step	Procedure	Notes
	Analysis of sample (see note a and section A10)	
A8.1	Transfer a suitable volume V mL (not exceeding 40 mL) of the sample to a 50 mL calibrated flask (note b)	(a) The sample may be the original sample or a pretreated sample. If necessary it should be just neutralized to phenolphthalein by addition of sodium hydroxide solution (M) or sulphuric acid (M) as appropriate and its temperature adjusted to 15-25°C.
		(b) See section A10 for suitable sample volume.
	Add sufficient water to produce a volume of 40 ± 1 mL, add 8 ± 0.2 mL of mixed reagent (note c), dilute to the mark with water, stopper and mix well (note d).	(c) An automatic pipette is suitable for dispensing this reagent.
		(d) For shipboard use, it is convenient to substitute the calibrated flask by a conical flask and omit the making up to volume stage.

Step	Procedure	Notes
A8.2	After at least 6 minutes but within 10-15 minutes, (see A2) (note e) measure the absorbance of the solution at 882 nm (note f) using 40 mm cells, the reference cell being filled with water (notes g and h). Let the absorbance = A_s	(e) The colour formed is stable for up to 24 hours, but there is a tendency for some non reactive phosphorus to hydrolyse in the acid solution giving rise to a slow increase in colour intensity when determining reactive phosphorus. (f) The exact wavelength of maximum absorption must be checked for each instrument and used throughout the analytical procedure. This maximum should be checked at regular intervals and after each service. If this wavelength is not achievable see section A6.1 (g) If greater sensitivity is required, longer cells may be used, eg 100 mm with proportional increase in absorption. For higher concentrations 10 mm cells can be used. (h) Matched cells should be used.
	Blank determination	
A8.3	40 ml of water is used in place of the sample through steps A8.1 and A8.2 (note i). Let absorbance be A_b.	(i) If the water used for the blank determination or dilution water contains phosphorus, the blank correction will be falsely high and the results for samples falsely low. It is the responsibility of the user to assess the importance of this error, but the following advice may be useful to users of this method. A reasonable target for the lowest concentration of interest is 1 μgL^{-1} P. This implies that a bias arising from the phosphorus content of the blank water of 0.2 μgL^{-1} P is acceptable (Ref 14). Users should aim to produce consistently, a high quality blank water satisfying this criterion, rather than to estimate the phosphorus content of the blank water. Correction is not advised as it will lower the precision and accuracy of the test results. Therefore, users of the method wanting high accuracy should satisfy themselves that the water used for blank determinations contains less than 0.2 μgL^{-1} P by examining a concentrated sample of blank water (A10.2). The criterion that the concentration technique described in A10.2 should lower the limit of detection of the analytical method means that a concentration factor for testing the blank water should be at least 10 fold greater. This preconcentration is not applicable if a synthetic sea or other mineralized water is used as a blank (in order to correct for refractive index effects). In which case see A12.2.

Step	Procedure	Notes
	Compensation for colour and turbidity (note j)	
A8.4	A separate portion of V mL of the sample is processed through steps A8.1 and A8.2 in exactly the same way as the sample, except that 4 ± 0.1 mL of 14% V/V sulphuric acid is used in place of the mixed reagent in step A8.1. Let the absorbance = A_c.	(j) This step may be omitted when it is known that the colour or turbidity of the sample is not contributing an appreciable fraction of the total absorbance.
	Calculation of results	
A8.5	The absorbance A_p due to phosphorus in the processed sample is given by $A_p = A_s - A_b$ or when a correction for colour and turbidity is made $A_p = A_s - A_b - A_c$	
A8.6	Using the calibration curve and the value of A_p, determine the mass M (in µg P) of phosphorus in the processed sample. (See section A9).	
A8.7	Calculate the phosphorus concentration, C (note k) in the original sample (in mgL^{-1} P) from $C = \frac{M}{V}$	(k) A factor may be required if the sample was diluted or concentrated before analysis or from manipulations during pretreatment stages.

A9 Calibration Graph

The procedure given in this section must be carried out on at least two independent occasions before application of this method to any samples and regularly thereafter. Any significant departure from linearity indicates that the technique is suspect at some stage.

To each of a series of 50 mL calibrated flasks add 0.00, 1.00, 2.00, 5.00, 10.00, 15.00 mL of standard phosphate solution B. The flasks now contain 0.0, 1.0, 2.0, 5.0, 10.0, 15.0 µg P respectively. Subject the solutions to the procedure given in section A8 steps 1-2. Plot the results for $(A_s - A_b)$ against µg P. The calibration curve is linear to at least 15 µg P.

A10 Changing the Concentration Range of the Method

A10.1 Aliquots for higher concentration samples

Suitable aliquots of sample to be used may be estimated from the following table:

Expected concentration (mgL^{-1} P)	Aliquot to be used (mL)
<0.2	40.0
0.2–0.5	20.0
0.5–1	10.00
1–2	5.00
2–5	2.00
5–15	1.00

When high concentrations of phosphorus are likely to be encountered, it is recommended that the samples are diluted to an appropriate concentration and a corresponding multiplication factor be incorporated in the calculation of the results. Alternatively, 10 mm cells could be used, but the calibration standards should be adjusted accordingly

and the linear range of the method must be checked (tests have indicated the limit of linearity under these conditions is 50 µg in a 40 mL sample).

Method B may also be used.

A10.2 Preconcentration for lower concentrations

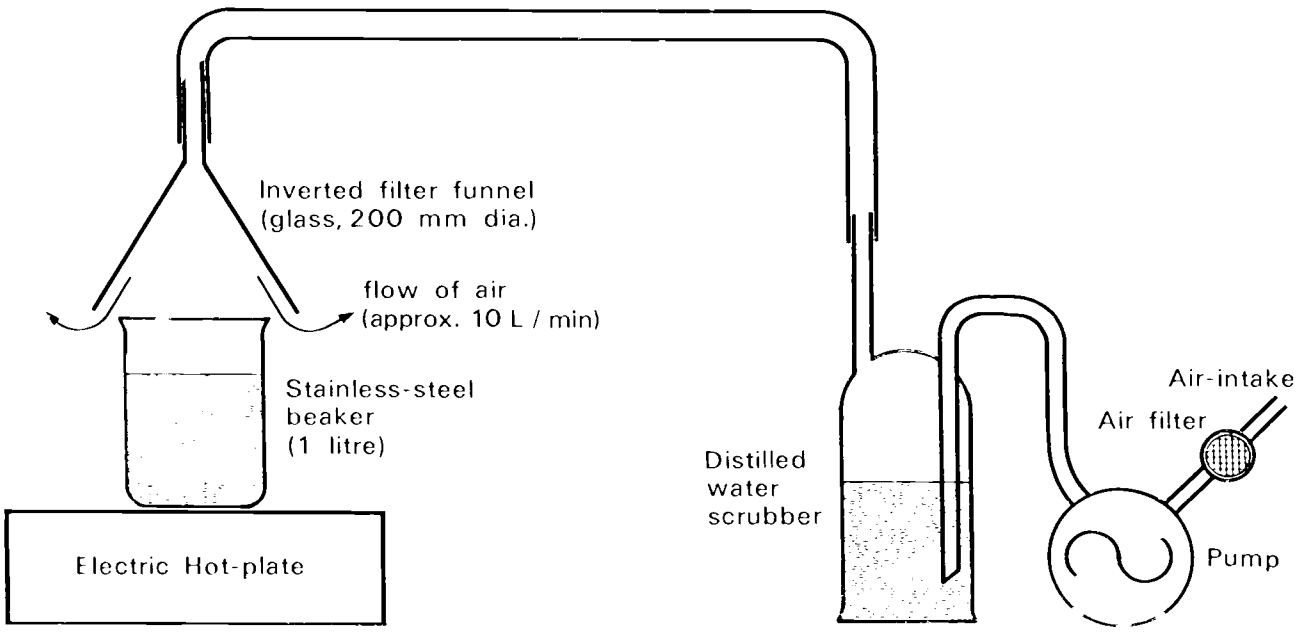
(Not applicable for highly mineralized waters, see A12.2).

Although concentration of water by evaporation is time consuming and subject to the risk of contamination from the atmosphere or equipment used, and to the adsorption of phosphorus on to the container walls, a procedure follows which is suitable for use with this method. This procedure was tested by concentrating samples of both good quality distilled water and a low concentration phosphorus standard (KH_2PO_4) and determining the phosphate content. No gain or loss was found. The test also demonstrates the absence of normally non-reacting phosphorus compounds such as some organophosphates which hydrolyse on boiling.

A10.2.1 Procedure for concentration by evaporation

Step	Procedure
A10.2.1.1	Set aside in clean, borosilicate aspirators sufficient volumes of the distilled water to be used and of any samples concentrated.
A10.2.1.2	Assemble the apparatus shown in Figure 1.
A10.2.1.3	Transfer 1,000 mL of the water or sample to the 1 litre stainless steel beaker placed on the hot plate. Place the inverted funnel in position as shown and start up the air pump. Switch on the hot plate and evaporate the water down to about 180 mL. Control the heating to avoid bumping or splashing.
A10.2.1.4	Allow the concentrated solution to cool and quantitatively transfer to a 200 mL volumetric flask, adjust the volume by the addition of distilled water. Carry out steps A8.1 to A9, to determine the phosphorus content.
A10.2.1.5	Divide the result obtained by 5 to give the concentration of phosphorus in the original water.
A10.2.1.6	This determination should be done in multiplicate and the mean result used.

Figure 1 Evaporation Apparatus



Notes This procedure replaces A8.3 for blank measurement. The procedure is likely to be impracticable for coloured or turbid waters. The criterion of practicability is that the procedure does not cause precipitation or change in the sample absorption wavelength. Blank values must be very low especially, if the sample itself has been pre concentrated.

A11 Sources of Error

A11.1 Phosphorus content of the water used for blank determinations

The reagent blank, although small, is not insignificant for the most accurate work or when less than 0.05 mgL^{-1} P is being determined. When working with samples at or near the limit of detection, a possible bias of this order may be considered important. If this is so, the analyst should preconcentrate (see section A10.2).

A11.2 Colour and turbidity blank

This measurement is rarely required for every sample, but may be an appreciable fraction of the total absorbance for some samples. If the colour and turbidity blank gives an absorbance exceeding 0.05, the suspended matter may adversely affect the method and consideration should be given to determining the filterable phosphorus fraction (see Part D2).

A11.3 Interferences

Attention to detail is essential. See Introduction note 10 and section A3.

A11.4 Determination of different species of phosphorus

See Introduction notes 1 and 2 and Part D.

A11.5 Sampling

If the sample cannot be thoroughly homogenized, the sampling error will exceed the analytical error. This is especially true for solid samples and multiphase samples.

A12 Adaptation to Automatic Analysers

A12.1 The method can be adapted for use on:

i. Air segmented continuous flow analysers (Refs 15 and 16).

ii. Flow injection analysers (Ref 17).

iii. Discrete analysers (Refs 15 and 18).

A12.2 Recent work at the University of Southampton has shown that the limit of detection can be lowered without preconcentration when using flow injection by use of long capillary cells. It is necessary to add a phosphorus-free surfactant to prevent wall plating.

B Phosphorus in Waters and Acidic Digests by Spectrophotometry — phosphovanadomolybdate method

B1 Performance Characteristics of the Method

See also Ref 34

B1.1	Substance determined	Phosphorus as orthophosphate
B1.2	Type of sample	Waters and acidic digests
B1.3	Basis of method*	The sample, acidified with sulphuric acid, is reacted with ammonium molybdate and ammonium vanadate to form a yellow coloured soluble complex with orthophosphate.
B1.4	Range of application	0–0.75% P (= 0–50 mgL^{-1}P in digest solution)
B1.5	Calibration curves	Linear
B1.6	Standard deviations** (within batch)	*Samples of plant material* Concentration — Std Dev 0.20% P — 0.01% P *Orthophosphate concentration* 10 mgL^{-1} P — 0.1 mgL^{-1} P 50 mgL^{-1} P — 0.2 mgL^{-1} P
B1.7	Limit of detection†	1 mgL^{-1} P (= 0.02% P)
B1.8	Sensitivity	50 mgL^{-1} P gives an absorbance at 420 nm of about 0.3 using a 50 mm flow cell
B1.9	Time required for analysis	Analysis time: 40–80 samples per hr. Allow an extra hour if solid samples are to be digested.

Notes

* See section B2

** The standard deviations quoted for standard solutions were obtained by analysis in ascending order of concentration with 9 degrees of freedom and those quoted for the plant material were obtained by successive analysis of replicates also with 9 degrees of freedom.

† The limit of detection was obtained visually from the recorder traces. Data from Water Research Centre Report LR1033 July 1979.

B2 Principle

The acidic solution is analysed for orthophosphate using an automated colorimetric technique which involves the spectrophotometric measurement at 420 nm of the yellow coloured heteropoly acid, formed by the reaction between orthophosphate and ammonium molybdate under acidic conditions in the presence of vanadium.

B3 Interferences

Any substance giving appreciable absorption at 420 nm under the conditions of this method.

B4 Apparatus

An auto-analyser consisting of:

Sampler
Proportioning pump
Colorimeter fitted with flowcell and 420 nm interference filters, or equivalent spectrophotometer
Recorder

Assemble the apparatus as shown diagramatically in Figure 2

B5 Reagents

B5.1 Water

Distilled water should be used when indicated and the phosphate content of this water should be negligible compared with the smallest concentrations to be determined in the samples. See A8 note h.

B5.1.1 Add 1 mL of wetting agent per litre to the water used to supply the wash receptacle on the sampler.

B5.2 Ammonium molybdate solution

Dissolve 25.0 ± 0.1 g ammonium paramolybdate $(NH_4)_6 Mo_7 O_{24} 4H_2O$) in 400 ± 5 mL of water.

B5.3 Acidified ammonium metavanadate solution

Cautiously with continuous stirring add 65.0 ± 0.1 mL of sulphuric acid (d_{20} 1.84) to 500 ± 5 mL of water. Dissolve 1.25 ± 0.01 g of ammonium metavanadate in this acid solution and cool to room temperature.

B5.4 Vanadomolybdate working reagent

Pour solution B.5.2 into solution B.5.3. Mix, cool and make up to 1 litre with water in a calibrated flask. Store in a dark glass bottle. This solution is stable for at least four weeks.

B5.5 Stock standards solution, 1 mL = 0.333 mg P

Dry about 2 g of potassium dihydrogen orthophosphate, KH_2PO_4 at 105°C for 1 hr. Cool in a desiccator. Dissolve 1.4644 ± 0.0002 g of the dried salt in water. Transfer quantitatively to a 1-litre volumetric flask and make up to the mark with water. This solution is stable for at least 4 weeks.

B5.6 Calibration standard solutions

Using grade A burettes, prepare a series of mixed standard solutions by adding 1.50, 3.0, 6.0, 9.0, 12.0 and 15.0 mL of the stock solution respectively to 100-mL volumetric flasks* and making up to the mark with water. These calibration standards, which are stable for at least one week, contain respectively: 5, 10, 20, 30, 40 and 50 mgL^{-1} P.

* The volumetric flasks used to contain the standard solutions are first 'acid-hardened' by soaking them in concentrated sulphuric acid overnight and then rinsing them free of acid with water (see A6.2).

B6 Sample Collection and Preservation

See section A6.

Step	Procedure	Notes
B7.1	Connect the system as shown in the flow-diagram (Figure 2) (note a) and switch on the colorimeter	(a) Follow manufacturer's general operating instructions. See also Ref 15.
B7.2	With the sample probe at rest in the wash-receptacle solution, place all the reagent lines in their respective reagents (note b) and start the pump (note c)	(b) Ensure that there is sufficient of each reagent to avoid refilling during a batch of analyses. (c) Allow the system to equilibrate and during this period check that the bubble pattern and hydraulic behaviour of the system are satisfactory. If not, eliminate difficulties before proceeding to step B7.3
	Initial sensitivity setting	
B7.3	When an acceptably smooth baseline trace is given on the measurement unit, adjust the baseline response to about 5 per cent of full scale (note d) and then transfer the sample probe into a C_M standard solution (note e).	(d) An elevated setting of the baseline allows for any negative drift that may occur. (e) C_M is the greatest concentration that the calibration is intended to cover.
B7.4	When there is a positive stable response at the measurement unit due to the colour produced from the C_M standard solution (note f), adjust this response to read between 90 and 95 per cent of full scale (notes g and h).	(f) The sample probe need only remain in the C_M standard solution for sufficient time to give a stable reading. (g) A setting 5 to 10 per cent below full scale allows for any increase in sensitivity that may occur. (h) This may be directly possible on some measurement units but others may require range expansion facilities.
B7.5	Return the sample probe to rest in the wash position (note i).	(i) First remove any traces of C_M standard solution from the outside of the sample probe.
	Analysis of samples	
B7.6	Load the sample tray in accordance with manufacturer's general instructions (notes j and k): Use calibration standards in ascending order. Blank (distilled water) and samples should be interspersed according to users requirements (see notes l to r)	(j) The tray can be loaded during the initial stabilisation period. (k) The order can be chosen to suit users requirements. (l) At least two portions of the blank solution should be analysed in succession after a C_M standard, since the first portion may give a falsely-high reading. (m) Two portions of the first sample should be taken in succession, since the first portion may give an erroneous result due to instrumental lag if the change in concentration is great. (n) A third portion of the first sample is included in the last sample in each block of samples for quality-control* purposes.

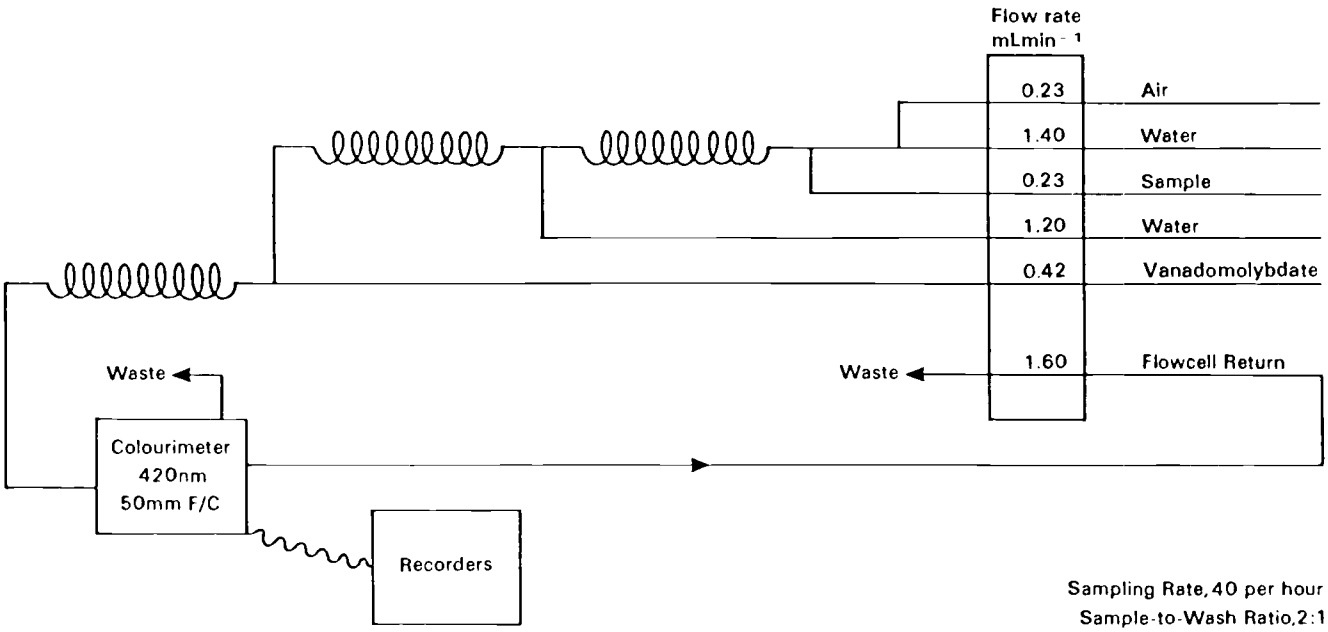
Step	Procedure	Notes
		(o) An 0.5 C_M standard solution is analysed at the end of each block of samples for quality-control* purposes.**
		(p) An 0.8 C_M standard solution is included at the end of each block of samples for calibration checking purposes.
		(q) The calibration curve may be repeated at the end of the batch of analyses if considered necessary.
		(r) If cross-contamination occurs between two samples (visible on the recorder trace as incomplete separation of consecutive plateaux), both samples should be repeated.
B7.7	When a steady baseline is obtained on the measurement unit (after step B7.5) re-adjust the baseline to about 5 per cent of full scale if necessary and start the sampling unit.	
B7.8	When all the system responses due to the processed solutions have appeared on the measurement unit and a final baseline has been obtained, the unit can be switched off. (Note s).	(s) The complete calibration may be checked at the end of the analytical batch if necessary.
	Calculation of results	
B7.9	Plot a calibration curve of measurement unit responses (y axis) against concentration of standard solutions (x axis) (note t).	(t) Providing the blank corrected responses of the calibration standard analysed at the end of each group of samples and those at the end of the turn-table (if used) are all acceptably close to their respective blank corrected initial calibration standard response. If not, refer to reference 15 for suggested procedures to obtain calibration curves.
B7.10	Using the calibration curve, convert the measurement unit responses due to the samples into concentrations of orthophosphate in the samples (note u). The results are expressed as mgl^{-1} P.	(u) The measurement unit responses of the samples must first be corrected for any baseline and sensitivity changes.
	Shut-down procedures	
B7.11	Transfer all reagent lines to water and continue to pump for at least 15 minutes or according to manufacturer's instructions. Switch off pump and detection unit.	

* The control standard and duplicate samples analysed with each block of samples are required to maintain a continuous check of analytical errors of samples and standards. (Ref 14).

** This control standard, 0.5 C_M standard solution, is prepared from a different standard stock solution from that used to prepare the calibration standards, but which has been stored under the same conditions. See note e above.

B8 Sources of Error See Ref 15.

Figure 2 Flow Diagram for Method B



C Ion Chromatographic Methods for the Determination of Phosphorus Compounds

(Silicates are not usually determined by chromatography though it is possible using resin columns)

C1 Introduction

Ion Chromatography is a very useful technique for the speedy determination of phosphorus species, especially phosphates; however because of the often rapid interconversion of polybasic ions with pH, it is essential to tailor the method to the analysis required. The following is a short resume of the problems with, where appropriate, methods which can be found in the companion booklet in this series (Ref 1).

Phosphorus forms a large number of ions, chiefly anions, which can be separated by chromatography. Presumably the phosphonium cations can, if necessary, be separated in the same way as ammonium ions, provided care is taken over their stability.

Phosphorus forms a series of acids, all based on the PO_4^{3-} unit which can form both linear and ring poly acids (such as pyrophosphate $P_2O_7^{4-}$ and cyclic trimetaphosphate $P_3O_9^{3-}$). Interconversion can be slow and in addition, one or two of the oxygen atoms in the PO_4^{3-} group may be replaced by a hydrogen or an organic radical; two phosphorus atoms may even bind directly (similarly to carbon and sulphur). Typical acids of this type are:

Phosphites $(HPO_3)^{2-}$
Monophosphonates $(RPO_3)^{2-}$
Hypophosphites $(H_2PO_2)^-$
Hypophosphates $(O_3PPO_3)^{4-}$

These too, can sometimes disproportionate to form mixtures of other phosphorus compounds. Although the common phosphorus anions are based on the fully oxygenated mono and poly acids, there are industrial uses for some of the others, especially the organophosphonates. However, there is an additional problem in that many of these acids are polybasic, especially orthophosphoric acid itelf, which can therefore occur as free acid H_3PO_4 and 3 different ions: $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} dependent on the pH of the sample, with instantaneous interconversion on change of pH. This means that the location of even the orthophosphate ion in a chromatogram is dependent not only on the pH of the initial sample but even more on the pH of the eluent.

Phosphorus can form permono- and perdi-phosphates (analogous to the permono- and perdi-sulphates) and also a series of fluorophosphates, of which the monofluoro- $(PO_3F)^{2-}$ and hexafluoro- $(PF_6)^-$ are industrially important. Thiophosphates, in which sulphur replaces the oxygen of the orthophosphate ion (and also of phosphonates) and aminophosphorous acids can also occur occasionally. Several methods are therefore necessary for the separation and quantification of the phosphorus containing acids in water and effluents.

C2 Orthophosphate in the presence of other common anions

This is the simplest case where the only problems are due to variation in ionization with pH and possible interferences.

For a detailed method, see the related booklet in this series (Ref 1) 'The Determination of Anions and Cations, Transition Metals, Other Complex Ions and Organic Acids and Bases in Water by Chromatography 1990', Part II, Method 1. Orthophosphates can also be determined by methods 2, 3, 4, 5 and 6 of the same part, depending on the other ions present.

C3 Ortho and polyphosphates, phosphonates and fluorophosphates

These usually require more specialised techniques than Method 1 of Part II in the above reference.

Phosphite can be separated by method 2, while another variant of method 2 will give monofluorophosphate. Method 4 is probably the most useful. Hexafluorophosphates are probably best determined by method 1.

Analysts are advised to consult Part I Chapters 3 and 4 of this reference.

D Pretreatment Methods for Phosphorus Determinations

D1 General Pretreatment Information

In the past, soluble (or dissolved) reactive phosphate (often considered to be orthophosphate) was the most frequently required determinand. However polyphosphates can readily convert into orthophosphate; substituted phosphates, phosphonates, and phosphonium compounds can oxidise or hydrolyse (even if only slowly) and insoluble or encapsulated compounds can be solubilized. Hence, total phosphorus determinations are becoming important. Occasionally, especially when studying sources of pollution, a knowledge of the speciation is required. The pretreatments which follow are designed to separate off particulate matter, to hydrolyse and to oxidise. It is possible to use several in succession.

As several variations in pretreatment are possible with either method, the analyst is recommended to try out the proposed combination of procedures, prior to analysing samples. As given, except for the division into particulate and filtrable, the pretreatment procedures are written for use with method A. Adaptation is necessary for use of these pretreatments with method B.

It must be remembered that as pretreatments do reduce precision and accuracy, analysts should check their performance.

Phosphorus in natural (including saline) and waste waters will almost invariably be present in one or more of the following forms:

(a) orthophosphate (the commonest form found);

(b) condensed phosphates (eg pyro and polyphosphates), these are often referred to as hydrolysable phosphates;

(c) organophosphorus, mainly phosphate esters, but also compounds in which phosphorus is directly bonded to carbon (eg aminophosphonic acids).

Phosphorus in suspended matter may be present as phosphate minerals (such as apatite), be adsorbed onto other solids or be contained in the microbiota. Even free phosphorus has been found following pollution. It is also desirable to make arbitrary distinctions between dissolved and particulate forms (arbitrary because this will depend on the nominal porosity of the filter used for the separation). The fact that no separation of this nature can be truly complete is reflected in the use of the terms 'filtrable' and 'particulate'. Natural waters may be filtered through a cellulose membrane retaining particulate material greater than 0.45 μm in size. On the other hand, glass fibre 'papers'—effective porosity 1 μm having relatively fast filtration speeds would be more suitable for some effluents where the level of suspended matter is high. If appreciable amounts of solid are retained by the filter, this solid may itself sometimes retain particles which might otherwise have passed the filter.

Of the three groups listed above, only (a) will respond directly to the spectrophotometric procedures (described in Methods A and B). The condensed phosphates of group (b) require hydrolysis by boiling in the presence of dilute sulphuric acid to convert them to orthophosphate. Similarly, the organic forms of group (c) must first be broken down by suitable techniques such as oxidation by persulphate. While spectrophotometric procedures described in Sections A and B will measure the orthophosphate content of the sample, the acidic conditions present may concurrently bring about partial hydrolysis of some group (b) compounds, or even the conversion of the more labile group (c) forms if these are present. For this reason, some analysts choose to refer to all the species responding to the molybdenum blue procedure as 'reactive phosphate phosphorus' instead of orthophosphate. Subject to this limitation, group (b) forms are usually

determined by difference after separate analyses of hydrolysed and unhydrolysed samples. Group (c) forms are similarly arrived at, via a determination of total phosphorus.

When studying the phosphorus cycle in water, it may be useful to be able to categorize the phosphorus compounds present into types. By determining 6 fractions [a combination of 2 physical (total and filtrable) and 3 chemical (orthophosphate, inorganic and total)] it is possible to report 15 fractions [a combination of 3 physical (total, filtrable and particulate) and 5 chemical (ortho, inorganic, organic, condensed and total)]. Although strictly speaking these fractions are defined analytically, they may be of practical use to users of the analytical data. The fractions are detailed in Table 1 with information on the relevant sections for pretreatment suggestions and/or calculation steps required. When an accurate determination of a particulate fraction is required, and this fraction is very small compared with the dissolved fraction, filter a large volume of sample and determine by pretreatment of material retained on the filter (see also Ref 19).

Great care is needed if filtering off biota not to rupture cells as this may greatly increase the apparent soluble phosphate value.

Table 1

Approximate composition of fraction	Analytically defined Determinand	Details of Pretreatment	Calculation
Total orthophosphate (TRP)	Total reactive phosphorus	None required.	
Dissolved orthophosphate (DRP)	Filtrable reactive phosphorus	See section D2.	
Particulate orthophosphate (PRP)			(PRP) = (TRP – DRP)
Total inorganic phosphate (TIP)	Total reactive and hydrolysable phosphorus	See section D3.	
Dissolved inorganic phosphate (DIP)	Filtrable reactive and hydrolysable phosphorus	See sections D2 and D3.	
Particulate inorganic phosphate (PIP)			(PIP) = (TIP – DIP)
Total condensed phosphate (TCP)			(TCP) = (TIP – TRP)
Dissolved condensed phosphate (DCP)			(DCP) = (DIP – DRP)
Particulate condensed phosphate (PCP)			(PCP) = (TIP + DRP – TRP – DIP)
Total phosphate (TP)	Total phosphorus	See section D4.	
Total dissolved phosphate (TDP)	Total filtrable phosphorus	See sections D2 and D4.	
Total particulate phosphate (TPP)			(TPP) = (TP – TDP)
Total organic phosphate (TOP)			(TOP) = (TP – TIP)
Dissolved organic phosphate (DOP)			(DOP) = (TDP – DIP)
Particulate organic phosphate (POP)			(POP) = (TP + DIP – TIP – TDP)

The suggestions on pretreatment given in detail in the sections which follow, must be regarded as tentative. Each analyst should make tests to ensure that the appropriate pretreatment is chosen for the particular sample being analysed.

D2 Filtration to separate dissolved from particulate phosphorus

Note: Care is necessary to avoid rupturing the cell walls of biota.

D2.1 Apparatus

A filter assembly suitable for membrane filtration, with 0.45μm or 0.1μm membrane filters as required (which is used should be stated when reporting the analytical results).

D2.2 Procedure

Procedure	Notes
Filter a known volume of sample through the membrane, sufficient to meet the requirements of subsequent analyses (notes a and b).	(a) It is recommended that a blank be carried through this pretreatment procedure. If particulate phosphorus is also to be determined by a section D4 procedure, the blank filters should be treated as the corresponding blank samples.
If the sample contains much suspended matter, a preliminary filtration through an appropriate porosity ashless cellulose filter paper may be added as an initial step. Store the filtrate in a clean bottle for subsequent analysis by method A, B, C or H (see section A7). If, as mentioned in Section D1, direct analysis of the particulate phosphorus fraction is desired, preserve the filters for treatment by one of the Section D4 procedures (note b).	(b) If biota are present, avoid an excessive pressure differential, which may rupture cells.

D3 Acid hydrolysis procedure for the determination of Total Inorganic Phosphorus (TIP) and Dissolved Inorganic Phosphorus (DIP)

(Some labile organophosphorus compounds may be included). Check whether biota cell walls are ruptured, liberating phosphorus. This is best achieved by examining both a small portion of the unfiltered sample and the material on the filter using a suitably powered microscope.

D3.1 Reagents

D3.1.1 Water see A5.1.

D3.1.2 Phenolphthalein see A5.9.

D3.1.3 Sodium hydroxide (M) see A5.7.

D3.1.4 Sulphuric acid (M) see A5.8.

D3.2 Procedure

Step	Procedure	Notes
D3.2.1	Add a suitable volume V ml of the sample (note a) to a 150 ml graduated beaker and dilute to about 40 ml with water. A blank determination must be included with each batch of samples.	(a) For TIP use original sample, for DIP use the filtrate from the pretreatment described in section D2. See section A10.1 for suitable volumes.

Step	Procedure	Notes
D3.2.2	Neutralize to phenolphthalein with sodium hydroxide (M) or sulphuric acid (M) if necessary (note b).	(b) If the sample to be analysed for TIP contains particulate matter, the analyst should first ascertain whether the solid is leached by acid (D3.2.3 to D3.2.5) then decide whether to filter (section D1) and treat as 2 separate samples—liquid and solid.
D3.2.3	Add 10 ± 0.2 mL M sulphuric acid	
D3.2.4	Cover the beaker with a watch glass and heat to boil the solution gently for 25 ± 5 minutes (note c). Allow the solution to cool then neutralize with sodium hydroxide (M). (Note d).	(c) The solution should not be allowed to evaporate to less than 15 mL and may be maintained at 20 ± 5 mL by topping up with water from time to time. (d) If the sample contains metals with phosphates insoluble at neutrality, omit the neutralization; but if necessary, adjust the acidification accordingly, as in the spectrophotometric method. Note that double anion compounds such as $Ca_5(PO_4)_3F$ exist, which may also be precipitated.
D3.2.5	Transfer the solution quantitatively to a 50 mL calibrated flask (note e) and reserve the solution for the colorimetric stage (see section A8).	(e) The quantity of rinse water used should be controlled so that the final volume at this stage does not exceed 40 mL.

D4 Determination of Total Phosphorus (TP) and Total Dissolved Phosphorus (TDP) by oxidation

(Note: oxidative pretreatments will liberate phosphorus from biota).

Introduction

The analyst may be required to determine total phosphorus (or total filtrable phosphorus) or organic phosphorus (or filtrable organic phosphorus) and will require a pretreatment to convert all forms of phosphorus to reactive phosphorus. It will usually be advantageous to use the simplest possible method of pretreatment, consistent with maximum conversion to orthophosphate.

The simplest method, which is described in section D4.1 uses a fixed amount of persulphate to achieve oxidation and is often suitable. However the presence of other organic matter in the sample may cause interference because of the competition for the oxidising agent and the presence of chloride interferes with the oxidation of some organic phosphorus compounds. Variants are described in section D4.2.

Rigorous pretreatments are described in section D4.3. Some of these techniques will convert almost every form of phosphorus compound and even the free element to reactive phosphorus if allowed to react for sufficient periods of time. The important disadvantages are slowness and markedly worsened precision of the analytical results. These disadvantages make it desirable not to employ these procedures for routine analysis if a persulphate pretreatment is suitable.

D4.1 Mild oxidative digestion step for the determination of Total Phosphorus (TP) and Total Dissolved Phosphorus (TDP)

D4.1.1 Reagents (all reagents must be phosphate free)

D4.1.1.1 Water see A5.1.

D4.1.1.2 Sodium hydroxide (M) see A5.7.

D4.1.1.3 Sulphuric acid (M) see A5.8.

D4.1.1.4 Phenolphthalein see A5.9.

D4.1.1.5 Ammonium (Sodium or Potassium) Peroxodisulphate.

D4.1.1.6 Bromocresol green

To a clean hard plastic beaker add 14.4 mL of 0.1M sodium hydroxide solution (prepared by dilution from A5.7). Add 1g of bromocresol green and stir until dissolved. Transfer to a 1 litre plastic volumetric flask and make up to the mark with water.

D4.1.2 Procedure

Step	Procedure	Notes
D4.1.2.1	Transfer a suitable volume V mL of the sample (note a) to a 150 mL graduated beaker. Dilute to about 40 mL with water. A blank determination must be included with each batch of samples.	(a) For TP use original sample, for TDP use filtrate from pretreatment described in section D2. See section A10 for suitable volumes.
D4.1.2.2	Neutralize with sodium hydroxide (M) or sulphuric acid (M) if necessary (note b).	(b) The quantity of sodium hydroxide or sulphuric acid to be added should be determined on a separate portion of V mL of the sample.
D4.1.2.3	Add 0.2 ± 0.01 g ammonium peroxodisulphate and swirl to dissolve.	
D4.1.2.4	Add 10 ± 0.2 mL sulphuric acid (M) and mix well.	
D4.1.2.5	Cover the beaker with a watch glass and heat to boil the solution gently for 25 ± 5 minutes (notes c, d and e). Allow the solution to cool then neutralize with sodium hydroxide (M). (notes f and g)	(c) The solution should not be allowed to evaporate to less than 15 mL and may be maintained at 20 ± 5 mL by topping up with water from time to time. (d) A few organophosphorus compounds are more resistant and may need up to 3 hours boiling, with occasional addition of extra amounts of ammonium peroxodisulphate. This is sometimes preferred to using procedure D4.3. (e) If arsenic was present originally, it will now be present as arsenate and will interfere in the colorimetric stage—see section D5. (f) To the green of bromocresol green is suggested. (g) See also D3.2 note d.
D4.1.2.6	Transfer the solution quantitatively to a 50 mL calibrated flask (note h) and reserve the solution for the colorimetric stage (see section A8).	(h) The quantity of rinse water used should be controlled so that the final volume at this stage does not exceed 40 mL.

D4.2 Variant mild oxidative procedures

Several variant procedures are often used for oxidising organic and reduced phosphorus compounds. The most commonly used methods are described below.

D4.2.1 As Procedure D4.1 but instead of using a glass beaker at step D4.1.2.1 and heating at step D4.1.2.5, a fused silica reaction tube is used and the sample is irradiated with ultraviolet light. For a typical procedure, see reference 20.

D4.2.2 Hydrogen peroxide may be used, with ultraviolet light instead of peroxodisulphate.

A typical procedure is as follows:

Step	Procedure
D4.2.2.1	Place 250 ± 2 mL of sample into a beaker, and, using a pH meter, adjust the pH to 8.1 ± 0.1 (as in D4.1.2.2).
D4.2.2.2	Transfer the solution quantitatively to a silica irradiation tube using the minimum of rinse water. Add 0.5 ± 0.2 ml of 30% w/v hydrogen peroxide. A suitable apparatus is given in reference 20.
D4.2.2.3	Cap the tube loosely (to allow gas to escape) and irradiate with ultraviolet light for 5.0 ± 0.5 hours at 60 ± 3°C. Cool to room temperature. Note the final volume if significantly changed.

D4.2.3 Acid peroxodisulphate pressure digestion procedure
The procedure given in section D4.2.1 may be carried out in a pressure bottle or container at 120 ± 5°C (c 1 bar pressure). Take care to cool the bottle or container before opening. (See Ref 21 for a method and Ref 22 for an alternative pressurised reactor technique).

D4.3 Rigorous oxidative digestion steps

D4.3.1 The procedures given in references 23 and 24 have proved suitable, but the limits of detection tend to be higher than those for Method A on clean samples. This is especially true if the sample is solid or semi-solid. The automated analytical method in Reference 25 has been used with these procedures.

D4.3.2 On rare occasions, free elemental phosphorus and other refractory phosphorus compounds have been encountered in sludge and sediment samples. The following procedure has been suggested for these substances.

D4.3.2.1 Reagents

D4.3.2.1.1 Sulphuric acid (d_{20} 1.84).

D4.3.2.1.2 Nitric acid (d_{20} 1.42).

D4.3.2.1.3 Water see A5.1.

D4.3.2.1.4 Sodium hydroxide (5M).
Dissolve 20 ± 1 g of sodium hydroxide in about 80 mL of water, cool and dilute to 100 ± 1 mL with water. Store in a polyethylene bottle.

D4.3.2.2 Apparatus

200 mL Kjeldahl flasks

Step	Procedure	Notes
	Analysis of Samples (notes a and b)	
D4.3.2.3.1	Digestion	
D4.3.2.3.1.1	For liquid samples	
	Add 50 ± 0.5 mL of sample to a 200 mL Kjeldahl flask. Add cautiously 2.0 ± 0.1 mL of sulphuric acid (d_{20} 1.84) and heat gently until white fumes begin to be evolved. Allow to cool.	(a) For TP use original sample, for TDP use filtrate from pretreatment D2. (b) Great care must be taken during this stage to avoid contamination.
	A water blank determination must be included with each batch of samples.	
D4.3.2.3.1.2	For solid samples (see also D6).	
	After preparation of the diluted acid and before heating, carefully add a known weight of solid, not exceeding 50 mg of P. Swirl to mix, then continue with the heating. If fluoride or fluorapatite is present use a new thick walled flask. Avoid skin contact.	
D4.3.2.3.2	Add cautiously 0.5 ± 0.05 mL of nitric acid (d_{20} 1.42) dropwise and continue heating until brown fumes cease to be evolved.	
D4.3.2.3.3	Repeat step D4.3.2.3.2 at least 3 times (note c). Cool, add 10 ± 1 mL water and heat to fuming. Allow to cool, add a further 10 ± 1 mL of water, heat to fuming and allow to cool.	(c) This step should be repeated until the solution is clear and colourless.
D4.3.2.3.4	Cautiously wash down the sides of the flask with 25 ± 1 mL of water and allow to cool (note d).	(d) If arsenic was present originally, it will now be present as arsenate and will interfere in the colorimetric stage—see D5.
D4.3.2.3.5	Neutralize the solution with sodium hydroxide (5M) (note e).	(e) To the green of bromocresol green is suggested (see D4.1.1.6)
D4.3.2.3.6	(a) If organophosphorus compounds, not attacked by the nitric acid-sulphuric acid treatment but decomposable by peroxodisulphate are also present, proceed to section D4.1 at step D4.1.2.3.	
	(b) Otherwise, transfer the solution quantitatively to a 50 mL calibrated flask and dilute to the mark with water (note f). The colorimetric procedure described in section A8 can now be applied by taking V mL of this solution (note g).	(f) The sample is restored to its original volume. (g) For solid samples amend the calculation accordingly.

D5 Elimination of arsenic interference

Arsenate, but not arsenite, can interfere.

D5.1 If Method A is used and the phosphorus absorbance is read at 6 ± 1 minutes after the mixing at step A8.2, the interference due to moderate amounts of arsenic will be negligible due to a slower rate of reduction to a blue complex. It is essential to standardize the reading time for samples, standards and blanks if using this procedure. Otherwise, if arsenate interference is significant use the reduction procedure D5.2.

D5.2 Reduction of arsenate to arsenite

D5.2.1 Principle

Arsenic (V) is reduced to Arsenic (III) by an acidic mixture of bisulphite and thiosulphate.

D5.2.2 Reagents

Prepare freshly as required. Do not store. See D5.3.

D5.2.2.1 14% V/V Sulphuric acid see A5.2.

D5.2.2.2 10% m/V Sodium metabisulphite.

Dissolve 4.0 ± 0.1 g of sodium metabisulphite in 40 ± 1 mL of water.

D5.2.2.3 1% m/V Sodium thiosulphate.

Dissolve 0.40 ± 0.01 g of sodium thiosulphate pentahydrate in 40 ± 1 mL of water.

D5.2.2.4 Arsenate reducing reagent (prepare only as required for use).

Mix together 20 ± 0.5 mL of 14% V/V sulphuric acid, 40 ± 1 mL of 10% m/V sodium metabisulphite and 40 ± 1 mL of 1% m/V sodium thiosulphate solution.

D5.2.3 Hazard

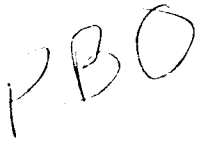
The mixed reagent D5.2.2.4 evolves sulphur dioxide. Use in a fume hood and flush away waste with copious amounts of water to clear sinks, traps and drains.

D5.2.4 Procedure

Step	Procedure	Notes
D5.2.4.1	To the sample, before dilution to volume at the end of the respective pretreatment step, add 5 ± 0.1 ml of the arsenate reducing reagent, mix thoroughly and allow to stand for 15 minutes before diluting to volume as required (note a).	(a) If used in conjunction with section D4, this step is carried out prior to the final neutralization, which must also be carried out prior to volume adjustment.

D6 Sampling Solid Samples

Whilst the majority of analyses will be made on samples sufficiently clean not to require filtration with separate analysis of the solid component, analyses are often required on samples containing much suspended matter, or on those sludges and sediments that are best treated as solids. This raises sample homogeneity problems which lower the standards of accuracy and precision attainable. To improve accuracy, such analyses should always be carried out on at least duplicate, preferably triplicate, or even a higher number of samples and care must be taken to look for obvious anomalies. For advice on these sampling problems see references 25 and 26. For such heterogeneous samples, the sampling error can greatly exceed the analytical error.


PBO

Determination of Silicon

(Methods E, F and H plus
Pretreatments G)
See also Introduction notes 4–14.

E Spectrophotometric Determination of Molybdate Reactive Silicon — 1-amino-2-naphthol-4-sulphonic acid (ANSA) or Metol reduction methods

E1 Performance Characteristics of the Method (a)

(For further information on the determination and definition of performance characteristics see Ref 34)

E1.1	Substance determined	Molybdate reactive silicon—mainly monomeric and dimeric silicic acids and silicate. Fluorosilicate may also be determined as silica.
E1.2	Type of Sample	Clean waters.
E1.3	Basis of method	See E2.2
E1.4	Range of application	Up to 500 μgL^{-1} as SiO_2
E1.5	Calibration curve	Linear to at least 500 μgL^{-1} SiO_2, for a 100 mL sample volume.
E1.6	Total standard deviation	See table below
E1.7	Limit of detection	2 μgL^{-1} SiO_2, when PO_4^{3-} absent. 4 μgL^{-1} SiO_2, when 50 mgL^{-1} PO_4^{3-} present, both estimated with 10 degrees of freedom.
E1.8	Sensitivity	100 μgL^{-1} SiO_2, gives an absorbance of approximately 0.13 in 40 mm cell at 810 nm, for a 100 mL sample volume.
E1.9	Bias	In fresh water, no bias greater than 2% was detected, except when interference occurred.
E1.10	Interferences	Phosphate, arsenate, and germanium may interfere slightly. Ferrous iron, hydrazine and sulphite may also interfere slightly in the presence of phosphate. All these effects will normally be inappreciable, but for full details and the effects of other substances see section E.3.

Type of Sample	SiO_2 concentration μgL^{-1}	Total standard deviation μgL^{-1}
standard solutions (b)	10	0.5
	200	0.8
	500	1.3
distilled water plus 50 mgL^{-1} PO_4^{3-} (b)(d)	10	1.1
	200	1.1
	500	2.0
boiler water (c)	4,800	18

each estimate has 19 degrees of freedom

E1.11	Time required for analysis	The total analytical and operator times are the same. Typical times for 1 and 10 samples are 40 and 60 minutes respectively excluding any pretreatment.

(a) These data are from results obtained at the Central Electricity Research Laboratories
(b) 100 mL sample volume
(c) 10 mL sample volume.
(d) For the slight positive interference due to phosphate see Table 2.

E2 Principle

E2.1 The method is based on the work of Webber and Wilson (Ref 28) and experimental work by Central Electricity Research Laboratories, Leatherhead (Refs 28, 29, 30)

E2.2 Reactive forms of silicon are treated with ammonium molybdate under acid conditions, molybdophosphoric acid, which is formed in the presence of phosphates, is destroyed by addition of tartaric acid. The resultant silicomolybdic acid is reduced by means of 1-amino-2-naphthol-4-sulphonic acid to the heteropolymolybdenum blue complex. Metol (4-methylaminophenol sulphate) may be used as an alternative reductant with similar test data (communication from Prof J P Riley).

E2.3 The method determines only 'molybdate reactive' silicon compounds. Certain silicon containing species may not react with molybdate even though they pass through filters and produce no noticeable turbidity in the solution. The extent to which these forms of silicon exist varies with the type of water and the analyst should decide whether pretreatment by fusing or heating with alkali is required (see Part G).

E3 Interferences

E3.1 If the pH of the sample falls outside the range 2–10 units or if the sample is heavily buffered, the required silicomolybdate complex may not be formed under the conditions of this method. Neutralize the sample to a methyl orange indicator end point with the appropriate acid or alkali (see A5.7 or A5.8) to eliminate this cause of interference.

E3.2 Phosphate, arsenate and germanium may interfere slightly, but in a complex way (Ref 27); the latter two are unlikely to be present in most natural water samples. If their presence is confirmed in effluents, investigate the effect using spiked synthetic standards and samples. Ferrous iron, hydrazine and sulphite may also interfere slightly in the presence of phosphate, although these effects will normally be minimal, details are given in Table 2.

Table 2

Other ion or substance	Concentration of other substances (mgL^{-1}) (in order given in first column if two impurities)	Effect in μgL^{-1} SiO_2, of other substances at silicon concentrations of stated value (μgL^{-1})		
		0	100	500
Iron (III)	5.0	<1		−2
Chromium (III)	5.0	<1		<1
Aluminium (III)	5.0	<1		−1
Copper (II)	5.0	3		−3
Nickel (II)	5.0	<1		−2
Zinc (II)	5.0	<1		<1
Calcium	100	<1		−2
Magnesium	100	<1		−5
Potassium	100	<1		−2
Sodium	100	<1		−3

Other ion or substance	Concentration of other substances (mgL^{-1}) (in order given in first column if two impurities)	Effect in μgL^{-1} SiO_2, of other substances at silicon concentrations of stated value (μgL^{-1})		
		0	100	500
Manganese (II)	0.1			
Cobalt (II)	0.1			
Tin (II)	0.1			
Molybdate (VI)	0.1	<1		2
Vanadate (V)	0.1			
Titanium (IV)	0.1			
Tungstate (VI)	0.1			
Fluoride	10	<1		<1
Cyclohexylamine	10	<1		<1
Morpholine	10	<1		<1
Octadecylamine	1.0	1		<1
Alkyl-aryl-sulphate	5.0	2		2
Fluorescein	5.0	1		2
Orthophosphate	0.1	3	2	0
	5.0	3	3	1
	25.0	4	3	−1
	50.0	5	2	−7
Sulphate + Orthophosphate	50 + 0	<1		<1
	50 + 5	3		2
	50 + 50	4		<1
Iron (II) + Orthophosphate	0.1 + 0	<1		
	0.1 + 0.2	7		
	0.1 + 1.0	7		
	0.1 + 5.0	7		
	0.1 + 25	5		
	0.1 + 50	5		
	0.3 + 0	<1		
	0.3 + 0.2	14		
	0.3 + 1.0	15		
	0.3 + 5.0	11		
	0.3 + 25	9		
	0.3 + 50	7		
	1.0 + 0	<1	<1	<1
	1.0 + 0.2	17	<1	
	1.0 + 1.0	37	12	
	1.0 + 5.0	35	11	<1
	1.0 + 25	20	9	
	1.0 + 50	15	8	<1
Hydrazine + Orthophosphate	0.2 + 50	5		−6
	1.0 + 0	<1		−3
	1.0 + 5	6		3
	1.0 + 50	14		4

If the other substances did not interfere, the effect would be expected (95% confidence) to lie within the following ranges; 0 ± 7 for 0 μgL^{-1} SiO_2, 500 ± 3 for 500 μgL^{-1} SiO_2.

E4 Hazards

Some aromatic amino compounds can cause health problems. No data are available for ANSA or Metol (Reagents E5.4 and E5.4.1 below), but no hazard has so far been reported from their industrial use. It is recommended that either be handled with care to avoid skin contact, inhalation, ingestion or spillage.

E5 Reagents

Analytical reagent grade chemicals are suitable unless otherwise specified.

E5.1 Water

Prepare and store in a polythene bottle, a large batch of water containing not more than 5 $\mu g L^{-1}$ SiO_2. Determine the 'reactive' silicon content of this water by treating it as a sample and analysing it as described in section E8 (ie taking 100 mL aliquot at step E8.1). Use this water to prepare reagent and standard solutions and for diluting samples.

Distilled water from a still, the boiler of which has first been flushed out with fresh tap water and the first litre of distillate rejected; or alternatively, water that has been passed through a laboratory-scale mixed-bed de-ionization unit, have been found to be adequately pure. Precautions should be taken against silica elution from the resin bed. (See section E11.2).

E5.2 Ammonium molybdate – sulphuric acid reagent

Dissolve 89 ± 1 g of finely ground ammonium molybdate. $(NH_4)_6Mo_7O_{24}4H_2O$ in about 800 mL of water at room temperature.

Add slowly and cautiously with stirring 63 ± 0.5 mL of sulphuric acid (d_{20}1.84) to about 100 mL of water in a beaker immersed in cold water, allow to cool. Add the acid solution to the molybdate solution with stirring. Cool and dilute with water to 1000 ± 10 mL. Store in a polyethylene bottle.

This solution is stable for at least 6 months.

If a blue colour appears in the solution it need not be discarded until the absorbance of the blank determination exceeds 0.02 units.

E5.3 28% m/V Tartaric acid solution

Dissolve 280 ± 3 g of tartaric acid, in about 800 mL of water and dilute with water to 1000 ± 10 mL. Store in a polyethylene bottle.

This solution is stable for at least 6 months.

E5.4 0.2%m/V 1-Amino-2-naphthol-4-sulphonic acid* (ANSA)

Dissolve 2.4 ± 0.1 g of sodium sulphite, $Na_2SO_37H_2O$ in about 10 mL of water. Add 0.2 ± 0.002 g of 1-amino-2-naphthol-4-sulphonic acid, $NH_2C_{10}H_5OH.SO_3H$ (purest grade available) and stir to dissolve. Dilute the solution to about 90 mL with water and add 14 ± 0.7 g of potassium metabisulphite, $K_2S_2O_5$. Stir to dissolve and dilute the solution with water to 100 ± 1 mL.

Store in a polyethylene bottle in the dark. Prepare this solution freshly each week.

*also known as 4-amino-3-hydroxy-naphthalene-1-sulphonic acid.

E5.4.1 Metol Reagent

Prepare as ANSA but substitute 0.15 ± 0.002 g of 4-methylaminophenol sulphate (Metol) for the aminonaphthol sulphonic acid. Use analytical grade reagent.

E5.5 Standard silica solutions

E5.5.1 Solution A 1 mL is equivalent to 1000 μg SiO_2

Weight 1000 ± 0.001 g of finely powdered predried (at 105°C) silica, spectrographic grade, into a clean platinum crucible, add 5 ± 0.1 g of anhydrous sodium carbonate and mix intimately with a thin nickel spatual, cover the crucible with a platinum lid. Heat the crucible until the mixture begins to fuse. Careful control of the heating will be required to avoid losses due to spitting as the melt bubbles. When bubbling has subsided heat the crucible strongly to a bright red heat until a clear transparent melt is obtained. Allow the crucible to cool, place it on its side in a 250 mL polyethylene beaker and place the lid in the beaker. Add 150 ± 10 mL of boiling water to the beaker and place it on a steam bath until the melt has dissolved. After rinsing the crucible and lid, remove from the beaker and cool the solution. Transfer the contents of the beaker with washings to a 1 L calibrated flask and dilute to the mark with water, mix well, and transfer the solution to a clean, dry polyethylene bottle. An equivalent amount of dry sodium hydroxide (2.1 ± 0.1 g) may be used instead of sodium carbonate. In which case a silver or nickel crucible must be used and heat only to a dull red heat.

The solution is stable for at least 1 year.

NB. Alternatively, transparent Spectrosil rod, 3 mm diameter, may be used to prepare the solution, instead of the spectrographic grade silica. As supplied by Thermal Syndicate Limited the impurity content is less than 1 mgL^{-1} and the rod does not require to be heated or ignited before use as it is not appreciably hydroscopic. 300 mm of rod weighs about 5 g.

A solution of sodium fluorosilicate containing the appropriate concentration of silicon may be used, provided that the reagent is of suitable quality and that the solution prepared is free from undissolved particles. Silicon standards are available commercially, but may contain polymeric silicates and may therefore be unsuitable.

E5.5.2 Solution B. 1 mL is equivalent to 5 μg SiO_2

Dilute 5.00 ± 0.02 mL of solution A with water to 1 litre in a calibrated flask.

Transfer to a dry polyethylene bottle.

This solution must be freshly prepared on the day of use.

E6 Apparatus

E6.1 Plastic bottles, 125 or 250 mL capacity, eg polyethylene, polypropylene, polycarbonate, or PTFE.

Clean new bottles by washing thoroughly with water.

Before using new bottles check that the effect of contamination is negilgible. When bottles have been shown to be satisfactory they should be reserved for silicon determinations only.

E6.2 Spectrophotometer

A spectrophotometer for use at 810 nm capable of accepting 40 mm cells is suitable. A wavelength of 670 nm may be used or a filter photometer with a suitable filter may be used, but a loss of sensitivity will occur and the results will be less reliable.

E6.3 40 mm optically matched cells

Use as described for 100 mm cells in section E6.2.

E7 Sample Collection and Preservation

Collect a representative sample in a polyethylene or similar plastic bottle and analyse as soon as possible. If storage is unavoidable, maintain the sample at 4 ± 1°C. Do not freeze as experience has shown that freezing can change the form of silica present. If microbiological activity within the sample is suspected, or is of special interest, eg in eutrophication studies, it may be desirable to filter the sample on site at the time of

collection, and also to consider whether either a 0.45 μm or a 0.1 μm membrane should be used. Silicon can occur as a vital natural or impurity constituent in many forms of living matter. Knowledge of the forms in which silicon can occur may be of interest for biological and medical studies (see section G1). Prolonged storage can lead to polymerization of soluble silica, resulting in either precipitation or formation of gels which are very difficult to sample representatively.

E8 Analytical Procedure

Step	Procedure	Notes
	Analysis of sample	
E8.1	Add (100 - V) ± 0.5 mL of water to a clean dry plastic bottle. Add V ± 0.005 V mL of sample at a temperature not below 15°C (Notes a and b) and mix by swirling gently.	(a) The volume of sample taken (V mL) should not contain more than 50 μg silicon (as SiO_2), and not more that 800 μg of phosphorus (as P).
		(b) If the sample is coloured, or if it contains suspended matter, carry out the procedure in section E10.1 using a separate sample.
E8.2	Add 2.5 ± 0.1 mL of ammonium molybdate—sulphuric acid reagent (E5.2). Note the time and immediately mix the contents of the bottle by swirling (note c).	(c) To ensure rapid delivery of reagents, the use of a syringe type pipette is recommended.
E8.3	After 10 ± 1 minutes (step E8.2), add 2.5 ± 0.1 mL of 28% m/V tartaric acid solution. Note the time and swirl the bottle to mix the solution (note c).	
E8.4	After 5 ± 1 minutes (step E8.3), add 2.0 ± 0.1 mL of 20% m/V ANSA solution. Note the time and again mix the contents of the bottle by swirling (notes c and d).	(d) The same volume of Metol solution (E5.4.1) may be used instead of the ANSA solution.
E8.5	Meanwhile set up the spectrophotometer (see section E6.2) according to the manufacturer's instructions. With water in the reference cell, adjust the absorbance to zero (note e).	(e) For double beam instruments, water should be in both cells at this point.
E8.6	After 40 ± 20 minutes (step E8.4) measure the absorbance of the solution in a 40 mm cell at a wavelength of 810 nm against water in the reference cell. Let the absorbance of the sample be A_1 (note f).	(f) Good laboratory temperature control is recommended, see E9.
	Blank determination	
E8.7	A blank must be included with each batch of determinations using the same batch of reagents as for samples. (See Section E10.2). Add 100 ± 0.5 mL of water at a temperature not below 15°C to a plastic bottle (note g).	(g) Strictly, a blank should be analysed for each different sample dilution that is used in the batch of analyses, but see section E10.2.
	Proceed as described in steps E8.2 to E8.6. Let the absorbance of the blank be A_0.	

Step	Procedure	Notes
	Calculation of results	
E8.8	The absorbance due to silicon, A_S in the sample is given by: $A_S = A_1 - A_0$ Determine the concentration of silicon in μgL^{-1} SiO_2 in the processed sample from the calibration graph (see section E9) taking into account the volume, if 100mL of sample is not used.	

E9 Preparation of Calibration Curve

The calibration curve is linear to about 500 μgL^{-1} SiO_2; but the slope of the 810 nm calibration graph decreases by about 0.25% for an increase in temperature of 1°C.

Any significant departure from linearity indicates that the technique is suspect at some stage.

Using a burette, add 100, 98, 96, 94, 92 and 90 mL (all to ± 0.3 mL) of water to a series of dry plastic bottles.

Similarly, add 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mL respectively of standard silicon solution B and swirl to mix. The bottles now contain standard solutions representing 0, 100, 200, 300, 400 and 500 μgL^{-1} SiO_2 respectively. Subject the solutions to the procedure given in section E8.2 to E8.6.

Plot the results of A_S against μgL^{-1} SiO_2.

E10 Sources of error

See also section F11.

E10.1 Turbidity and/or colour correction

If the sample is coloured or turbid, carry out the blank procedure in step E8.7 replacing the final additions of reagents with an equivalent volume of water.

Note the absorbance due to this solution. Let this be A_E.

For such samples $A_S = S_1 - A_0 - A_E$.

E10.2 Silicon content of the water used for blank determinations

The presence of silicon in the water used for blank determinations could lead to an unacceptable bias in results close to the limit of detection. There should be no cause for concern provided the criterial set out in section E5.1 are satisfied and if, when a number of samples requiring different dilutions are analysed, a blank is analysed for each different dilution as described in section E8.7. When suitably prepared water (E5.1) is used for sample and blank analyses, the differences between the different blanks will be small and the error introduced by using a blank at only one dilution may be tolerable. This is for the analyst to decide.

E10.3 Effect of temperature

The temperature of the sample should not be less than 15°C during the determination or the formation of the molybdosilicic acid may be incomplete.

E11 Automated Procedures

Methods E and F for silicon may be readily automated using a continuous flow analysis (Refs 31 & 32), discrete analysis or a flow injection system (Ref 33).

Manufacturers of automatic equipment may provide data sheets for the determination of silicon in water. Note that most silicomolybdate methods give a mixture of an alpha complex and a beta complex. The relative proportion of each complex is dependent on the molybdate/acidity ratio, the overall concentration and the reaction time. Hence some methods may not be strictly comparable with methods E and F. For sea waters, see Refs 42 and 43.

F

Spectrophotometric Determination of Molybdate Reactive Silicon—ascorbic acid reduction method

F1 Performance Characteristics of the Method (a)

(For further information on the determination and definition of performance characteristics see Ref 34)

F1.1	Substance determined	Molybdate reactive silicon—mainly monomeric and dimeric silicic acids and silicate. Fluorosilicates can also be determined directly as silicon.
F1.2	Type of sample	All types of water and effluents, and also solutions from pretreatments (see section G).
F1.3	Basis of method	Reaction with ammonium molybdate under controlled acidic conditions to form a yellow molybdosilicic acid that is reduced in situ with ascorbic acid to yield a silicomolybdenum blue complex which is measured spectrophotometrically.
F1.4	Range of application (b)	Up to 10 mgL^{-1} as SiO_2. (Range may be extended by dilution or by the use of smaller aliquots, see section F10).
F1.5	Calibration curve (b)	Linear to 10 mgL^{-1} SiO_2.
F1.6	Total standard deviation	See table below.
F1.7	Limit of detection	0.03 mgL^{-1} (10 degrees of freedom). Using 10 mm cells. Proportionately lower limits can be obtained by using 40 mm or 100 mm cells.
F1.8	Sensitivity (b)	10 mgL^{-1} SiO_2 gives an absorbance of approximately 0.84 in a 10 mm cell (based on a sample volume of 50 mL).
F1.9	Bias (c)	In saline waters the slope of the calibration curve is changed (see section F11.5 and Ref 40).
F1.10	Interferences (b)	See section F3.

Type of Sample	SiO_2 concentration (mgL^{-1})	Total standard deviation (mgL^{-1})
Standard solution (d)	0	0.01
Standard solution (d)	2.0	0.05
Standard solution (d)	8.0	0.10
Standard solution (d)	50.0	0.32(e)

each estimate has 19 degrees of freedom.

F1.11	Time required for analysis	Typical times for 12 samples are 1.5 hr operator time, 3 hr total time.

(a) Data obtained by Imperial Chemical Industries Limited, Mond Division, Winnington Laboratories.
(b) Confirmed by the former Southern Water Authority, Sussex River and Water Division, including the pretreatment in Section G3.
(c) Confirmed by Imperial Chemical Industries Ltd Brixham Laboratory.
(d) Distilled water spiked with the stated concentration of silicon.
(e) Using a 5.0 mL aliquot. Other data obtained using 50.0 mL aliquots.

F2 Principle

F2.1 The method is based on work carried out at Imperial Chemical Industries, Mond Division, Winnington Laboratories.

F2.2 Monomeric and dimeric silicon in acid solution below pH 2 react with molybdate ions to form a yellow silicomolybdate which is reduced in situ with ascorbic acid to a blue silicomolybdate complex.

F2.3 When it is required to determine total silicon and forms of molybdate unreactive silicon the sample requires pretreatment to convert the unreactive species to a form capable of reacting with molybdate reagent (see appendices).

F3 Interferences

F3.1 There is no detailed information concerning the effects of interfering substances, other than those listed below, on the method described. Generally no important interference problems are likely with unpolluted fresh waters, but the effect of interferences should be considered particularly in polluted samples. The most likely sources of interference are listed below. See also Introduction, note 10.

F3.2 Phosphorus

Initially, phosphorus and silicon compete for the molybdate reagent to form their respective complexes, but the phosphate complex is decomposed under the strongly acidic conditions that prevail at a later stage of the method.

Up to 10 mgL^{-1} of silicon (as SiO_2) can be determined in the presence of 60 mgL^{-1} phosphorus (as P) without interference exceeding 0.01 mgL^{-1} (as SiO_2). Higher levels of phosphorus may prevent complete formation of the silicomolybdate complex and also cause a precipitate to form which persists throughout the procedure and interferes with spectrophotometric measurements.

F3.3 Arsenic (a)

Although arsenic (V) is known to form an arsenomolybdate complex it has been shown that under the conditions of this method no significant interference takes place until the arsenic (V) concentration exceeds 400 mgL^{-1} (as arsenic).

F3.4 Germanium

Germanates interfere by forming a germanomolybdate complex. If present, quantify the effect using spiked standards.

F3.5 Nitrite (a)

The effect of nitrite is both complex and variable. At concentrations greater than 0.5 mgL^{-1} (as N), nitrite appears to catalyse the formation of the silicomolybdenum blue complex. However, where levels exceed 2 mgL^{-1} (as N), colour bleaching may subsequently occur and this appears to be related to the degree of exposure to air.

(a) Based on tests carried out at Southern Water Authority, Sussex River and Water Division.

F4 Hazards

Only normal laboratory hazards are known to occur with this method.

F5 Reagents

Analytical reagent grade chemicals are suitable.

F5.1 Water

The water used for blank determinations, preparing standard and reagent solutions and for dilution purposes, should have a silicon content that is negligible compared with the smallest concentration to be determined in samples.

Distilled water is preferred. Deionized water may be suitable if the water supplied to the ion-exchange unit has a negligible silicon content (see section F11.2).

F5.2 10% m/V Ammonium molybdate solution

Dissolve 25 ± 0.2 g of finely powdered ammonium molybdate $(NH_4)_6Mo_7O_{24}4H_2O$ in about 200 mL water and dilute with water to 250 mL in a measuring cylinder. Store in a polyethylene bottle. Discard if a precipitate forms.

F5.3 25% V/V Sulphuric acid solution

Add slowly and cautiously with stirring 200 ± 2 mL of sulphuric acid (d_{20} 1.84) to 600 ± 10 mL of water in a 2 litre beaker immersed in cold water, allow to cool. Store in a glass or polyethylene bottle.

F5.4 Ascorbic acid

0.2 g of ascorbic acid powder is normally used for each determination of silicon. Alternatively, use an equivalent weight of ascorbic acid in the form of tablets or a freshly prepared 10% aqueous solution. Solutions should be discarded after each determination.

F5.5 Methyl orange indicator solution (0.05% m/V)

Dissolve 0.05 ± 0.01 g of methyl orange in about 100 mL of water.

F5.6 Hydrochloric acid solution (1.00M)

Dilute 90 ± 1 mL of hydrochloric acid (d_{20} 1.18) with water to 1 litre in a calibrated flask.

Standardize this solution as follows:—

Dry 5 ± 1 g of sodium carbonate, anhydrous at 260 ± 10°C for 2 ± 0.25 hours. Allow to cool in a desiccator.

Weigh out accurately 1.330 ± 0.001 g of this dried material into a 250 mL conical flask. Let this mass be W g. Add 50 ± 5 mL of water and swirl to dissolve. Add 2 drops of methyl orange indicator solution and titrate with hydrochloric acid solution just to the appearance of a permanent red colour. Let the titre obtained by T_1 mL.

The molarity of the hydrochloric acid solution is given by

$$\frac{W \times 18.87}{T_1}$$

If necessary, adjust the concentration of hydrochloric acid so that the molarity falls within the range 1.000 ± 0.005 M.

Alternatively, use commercially prepared molar hydrochloric acid solution.

F5.7 Sodium hydroxide solution (1.00M)

Dissolve 40.8 ± 0.3 g of sodium hydroxide in about 800 mL of water in a plastic beaker, cool and dilute with water to 1 litre in a calibrated flask. Store in a polyethylene bottle. Standardize this solution as follows.

Pipette 25.00 ± 0.05 mL of sodium hydroxide solution into a 250 mL conical flask. Add 2 drops of methyl orange indicator solution and titrate with hydrochloric acid solution (1.00M) just to the appearance of a permanent red colour. Let this titre be T_2 mL.

The molarity of the sodium hydroxide solution is given by

$$\frac{T_2 \times M_1}{25.00}$$

where M_1 is the molarity of the hydrochloric acid solution (1.00M)

If necessary adjust the concentration of the sodium hydroxide solution so that the molarity falls within the range 1.000 ± 0.005 M. Alternatively use commercially prepared molar sodium hydroxide solution.

F5.8 Standard silica solutions

F5.8.1 Solution A 1 mL is equivalent to 1000 μg SiO_2

Proceed as detailed in Section E5.5.1

F5.8.2 Solution B 1 mL is equivalent to 50 μg SiO_2

Dilute 25.00 ± 0.05 mL of solution A to 500 mL with water in a calibrated flask.

Store in a polyethylene bottle. This solution is stable for at least one week.

F6 Apparatus

F6.1 Spectrophotometer

A spectrophotometer for use at 700 nm and at 810 nm capable of accepting 10 mm cells is suitable. If greater sensitivity is required 40 mm cells or 100 mm cells may be used. (See section F10.2).

A filter photometer may be used, but a decrease is sensitivity along with possible non linearity of calibration may occur and the results may be less reliable.

F6.2 10 mm optically matched cells

Both sample and reference cells must be kept scrupulously clean and should not be interchanged. They should always be placed in the same position in the holder with the same face towards the lightsource. Alternatively, use 40 or 100 mm cells.

F6.3 Plastic vacuum filtration unit with 0.45 μm membrane filters

A water pump or hand operated piston device is usually satisfactory.

F6.4 Plastic* beakers, 100 mL capacity, plastic* graduated pipettes, plastic* stirring rods and plastic* volumetric flasks.

*eg polyethylene, polypropylene, polycarbonate or PTFE.

F7 Sample Collection and Preservation

See section E7 and Ref 41.

Step	Procedure	Notes
	Analysis of sample	
F8.1	Filter a sufficient quantity of sample through an 0.45 μm membrane filter (note a).	(a) This step may be omitted if the analyst, due to his experience, judges it to be unnecessary. If the initial filtrate is unclear, refilter through the same or a finer filter
F8.2	Using a plastic pipette transfer a suitable volume V (not exceeding 50 mL) of the sample, filtered if necessary, to a 100 mL plastic beaker (note b).	(b) See section F10 for suitable sample volumes.
F8.3	Add a second V mL of (filtered) sample to a 250 mL conical flask. Add 2 drops of methyl orange indicator and, as appropriate titrate with either hydrochloric acid solution (1.00M) or with sodium hydroxide solution (1.00M) to the indicator end point. Note the titre and discard this second sample. Let the titre of hydrochloric acid solution (1.00M) obtained be T_{HA} mL or let the titre of sodium hydroxide solution (1.00M) obtained by T_{SH} mL. If the sample is neutral proceed to step F8.4a. If the sample is alkaline proceed to step F8.4b If the sample is acidic proceed to step F8.4c.	
F8.4	*a. Sample neutral*: Add 10.00 ± 0.05 mL of hydrochloric acid solution (1.00M) to the filtered sample in the polyethylene beaker and stir. *b. Sample alkaline*: Add (10.00 + T_{HA}) ± 0.05 mL of hydrochloric acid solution (1.00M) to the (filtered) sample in the polyethylene beaker and stir. *c. Sample acidic*: Add (10.00 - T_{SH}) ± 0.05 mL of hydrochloric acid solution (1.00M) to the (filtered) sample in the polyethylene beaker and stir.	
F8.5	Add 5.0 ± 0.2 mL of 10% m/V ammonium molybdate solution stir and allow to stand for 12 ± 2 minutes.	
F8.6	Add 20 ± 0.5 mL of 25% V/V sulphuric acid solution stir and allow to stand for 1 to 1.5 minutes.	
F8.7	Add 0.2 ± 0.01 g of ascorbic acid and stir to dissolve.	
F8.8	Transfer the solution quantitatively to a 100 mL calibrated flask and dilute with water to the mark. Stopper the flask, mix well and allow to stand for 60 ± 10 minutes away from bright sunlight, preferably in the dark.	

Step	Procedure	Notes
F8.9	Meanwhile set up the spectrophotometer according to the manufacturer's instructions. Adjust the absorbance reading to zero with water in the reference cell.	
F8.10	Measure the absorbance of the solution at 700 nm using 10 mm cells, against water in the reference cell. Let the absorbance of the sample be A_s. (note c)	(c) 40 or 100 mm cells can be used if greater sensitivity is required
	Blank determination (note d)	
F8.11	A blank must be included with each batch of determinations using the same batch of reagents as for samples. Carry out steps F8.2 to F8.10 using 50 mL water in place of the sample. Let the absorbance of the blank be A_b.	(d) The blank sample should be water if no other pretreatment was required or, if a pretreatment (section G) has been used, the blank solution from a pretreatment described in the section.
	Calculation	
F8.12	The absorbance due to silicon in the sample is given by $A_p = A_s - A_b$ For coloured or turbid samples see also section F11.4. Determine the mass M (in μg SiO_2) of silicon in the processed sample, from the value of A_p and the calibration curve (see section F.9). Calculate the silicon concentration, C, of the original sample (in mgL^{-1} SiO_2) from $C = \frac{M}{V}$	

F9 Preparation of calibration curve

The procedure given in this section must be carried out on at least two independent occasions before the application of this method to any samples and regularly thereafter. Any significant departure from linearity indicates that the technique is suspect at some stage.

To a series of 100 mL plastic volumetric flasks add 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mL of standard silicon solution B. The beakers now contain 0,100, 200, 300, 400 and 500 μg of SiO_2. Dilute each solution to 50 ± 5 mL with water and mix well. Proceed as described in steps F8.5 to F8.10. Plot the results for $(A_s - A_b)$ against μg SiO_2. The calibration graph is linear to at least 500 μg SiO_2.

F10 Concentration ranges of the method

F10.1 Suitable volumes of sample to be used may be estimated from the following table

Table 2

Expected concentration mgL^{-1} SiO_2	Aliquot to be used (mL)
< 10	50
10–20	25
20–50	10
40–100	5

When higher concentrations of silicon are likely to be encountered, it is recommended that the samples are diluted to an appropriate concentration and a corresponding multiplication factor incorporated in the calculation of the results.

F10.2 If greater sensitivity is required measure the absorbance at 700 nm using 40 mm or 100 mm cells.

F10.3 If even greater sensitivity is required measure the absorbance at 810 nm in either 10 mm or 40 mm cells. Even longer cell paths may be used if necessary. See comment under phosphorus earlier.

(At 810 nm, a 40 mm cell offers a sensitivity about 10 times that measured at 700 nm in a 10 mm cell). When using these modifications appropriate calibration graphs must be prepared, and the linear range checked.

F11 Sources of error

The attention which it is necessary to pay to sources of error depends on the accuracy required of the analytical results. The following sub-sections summarize the main sources of error.

F11.1 Contamination

Silicates are ubiquitous. The technique and working conditions should be critically examined and any sources of contamination eliminated or minimised. It is advisable to carry out a preliminary series of blank determinations before analysing any samples. This will ensure that any unduly high and/or variable blank values are detected so that steps can be taken to eliminate the problem.

F11.2 Silicon content of the water used for blank determinations

If the water used for the determinations contains silicon compounds, the results for samples will be falsely low. Clearly the importance of this error depends on the silicon content of the water and the concentrations of interest in the samples. Experience has shown that with good quality reagents, blank values should not exceed 0.02 absorbance units under the conditions used in section F8. Ideally, the silicon content of the water should be less than 1 mg L^{-1} and tests should be made to verify this (see section F10.2). If this concentration is likely to lead to unacceptable bias in the sample results, then method E should be used together with suitably purified water. When preparing deionized water, care should be taken not to run ion exchange beds too near to exhaustion as 'silica breakthrough' could produce very high silicon concentrations without any conductivity increase being found in advance of the appearance of major anions. Such breakthrough is a potential source of blank bias.

F11.3 Interfering substances

See section F3.

F11.4 Colour and Turbidity

Coloured and/or turbid samples may interfere in the spectrophotometric measurement of the silico-molybdenum blue complex.

It may be possible to compensate for such interference by taking the same volume of sample through the colorimetric procedure except that step F8.5, the addition of ammonium molybdate, is omitted and replaced by an equal volume of water.

Note the absorbance due to this solution, let it be A_c

Then $A_p = A_s - A_b - A_c$ and this value of A_p should be used in the calculation step.

F11.5 Saline Waters

Calibration curves in saline waters and distilled water are linear, but differ significantly in slope (eg in sea water of salinity 35 gL^{-1}, 10 mg $SiO_2 = 0.79$ aborbance units in a 10 mm cell). Saline samples determined using a calibration curve prepared with distilled water will be negatively biased. Such saline samples should be analysed by a standard addition procedure, with a distilled water reagent blank. See also Ref 42.

F12 Automated Procedures

See section E11.

F13 Modification to Procedure F8

Under certain conditions, both alpha and beta forms of the silicomolybdic acid can be formed. By careful timing of the reagent mixing processes, including the addition of the reducing agent, reproducible 'blue' production can be achieved. Interference from phosphomolybdate production at high acidities can be minimized by the addition of oxalate or tartrate, in significant amounts.

G Pretreatment Methods to Convert Other Forms of Silicon to Soluble Molybdate Reactive Silicon

As several variations in pretreatment are possible, the analyst is recommended to try out the proposed procedures prior to analysing samples. As given, the pretreatment procedures are written for use with method F but see the Introduction notes 8 and 9. After neutralization, it is often possible to use method E with treated samples.

For very insoluble materials see Ref 22.

G1 General Information

G1.1 Forms of Silicon

Silicon occurs in natural (including saline) and waste waters in one or more of the following forms (see Figure 3 which summarizes the pretreatment options):

(a) Silicates and silicic acid present in true solution in monomeric and dimeric forms. These are the forms that react with ammonium molybdate.

(b) Silicates and silicic acid present in solution in a polymeric form. These may be present in a filtered sample, but do not react with ammonium molybdate unless converted to the reactive form by suitable pretreatment.

(c) Silicon present as submicron particles of 'colloical silica' is difficult to define as many fall somewhere between the polymeric form in solution and the insoluble form.

(d) Insoluble silica that can be retained on a filter. This form can be converted to the soluble molybdate reactive form by suitable pretreatment.

(e) Silicate minerals, usually insoluble.

(f) Organosilicon derivatives.

(g) Some biota such as diatoms, and some grasses etc contain much silica.

Arbitrary distinctions on the basis of particle size distribution are commonly used. In this booklet soluble silicon is defined as that which passes an 0.45 μm filter. Sometimes, however an 0.1 μm filter may be more appropriate.

Insoluble silica cannot always be determined directly since it may not be possible to wash matter retained by a filter without altering its character. Therefore insoluble silica is usually determined as the difference between total silica and total soluble silicon. When this difference is very small, at high total silicon contents, this procedure could lead to unacceptably large errors and it may then be preferable to determine the insoluble silica retained on the filter (Ref 35).

Figure 3 below illustrates this information.

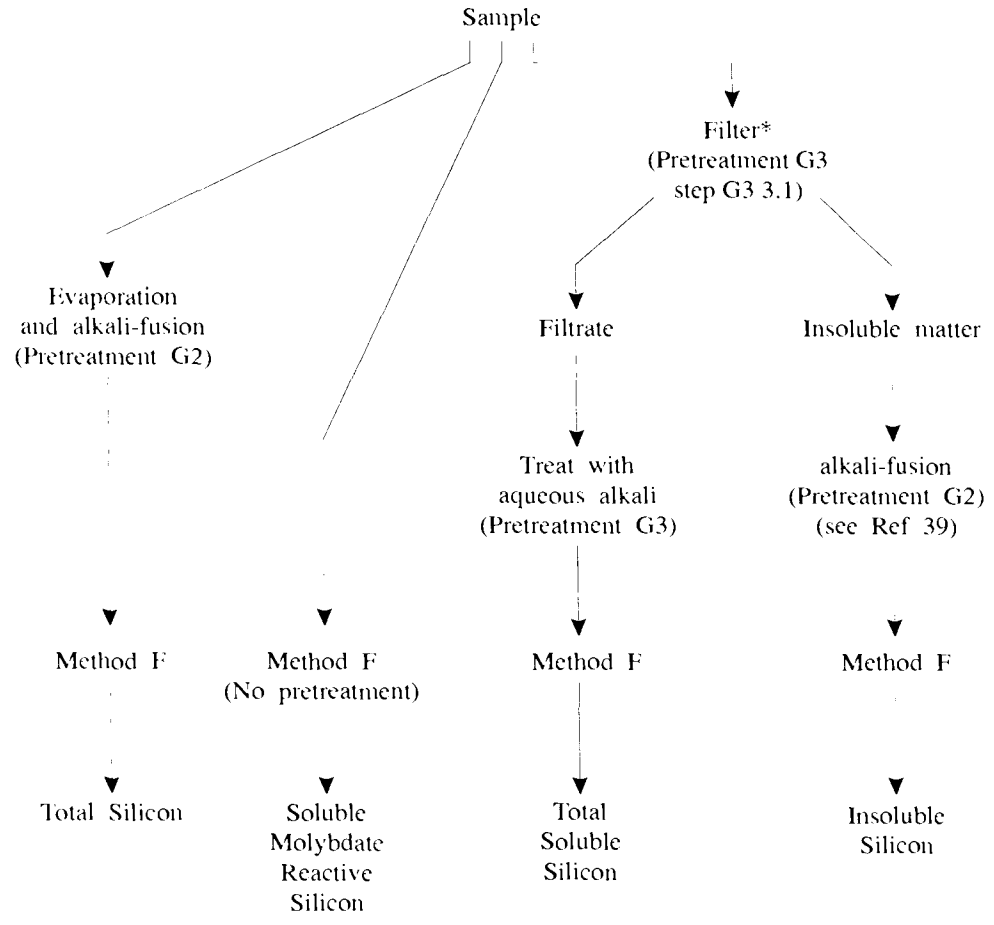
If biota are present decide whether the silica it may contain is relevant to the investigation. Note that the freezing of samples can change the reactivity of dissolved silica. See Ref 41.

G1.2 Sample Collection and Preservation

See section E7.

Figure 3

Forms of silicon obtained using various pretreatments in combination with Method F



*For clean waters in the absence of suspended matter, filtration of the sample is not necessary and total soluble silicon will also be the total silicon.

Polymeric silicon = Total soluble silicon – soluble molybdate reactive silicon.

Insoluble silicon = Total silicon – total soluble silicon

G2 Sample Pretreatment for Converting Total Silicon to Molybdate Reactive Silicon

To determine total silicon it is necessary to pretreat a sample without filtration. Full blank determinations should be carried out before the routine use of pretreatment procedures and also alongside sample analyses. (See also section F11.4).

G2.1 Hazards

Only normal hazards, especially those associated with strong acids and with alkali fusions are known to occur with this method.

G2.2 Reagents

Analytical reagent grade chemicals are suitable.

In addition to the reagents described in section F5, the following will be required.

G2.2.1 20% m/V Sodium carbonate solution

Using plastic apparatus, dissolve 20.0 ± 0.1 g of sodium carbonate anhydrous in about 80 mL of water, dilute to 100 ± 1 mL. Filter and store in a polyethylene bottle.

G2.3 Apparatus

In addition to the apparatus described in section E6 the following will be required.

G2.3.1 Platinum crucibles of about 30 mL capacity fitted with a lid

The usual laboratory precautions for the care of platinum apparatus must be observed, for instance, fusions must not be carried out in the presence of peroxides or hydroxides of the alkali and alkaline earth metals.

Never allow red-hot platinum crucibles to come into contact with base metals. Handle crucibles only with platinum tipped tongs. Always use an oxidizing flame when gas burners are the source of heat since reducing flames, particularly smoky flames, will seriously damage platinum ware.

It is not advisable to heat platinum ware on hotplates.

Crucibles may be cleaned by adding a small quantity of potassium hydrogen sulphate and heating to fusion. Ensure that the molten salt comes into contact with the entire inner surface of the crucible. Cool, dissolve the melt with water and rinse thoroughly with water.

In addition to the above treatment carry out the sodium carbonate fusion procedure given below in steps G2.4.3–5. Leach the crucible with hot water and rinse thoroughly with water. Dry the crucible in a dust free electric oven at 105 °C and store protected from dust.

G2.3.2 **Plastic beakers** 250 mL eg polyethylene, polypropylene, polycarbonate, or PTFE.

G2.3.3 **Muffle furnace** capable of heating a platinum crucible to a temperature sufficient to fuse sodium carbonate (ie intense red heat ca 850°C).

Alternatively, a suitable gas burner may be used.

G2.4 Pretreatment procedure

Step	Procedure	Notes
	Pretreatment of sample	
G2.4.1	Transfer a suitable volume of homogenous sample to a platinum crucible (note a). Let this volume be V_1 mL.	(a) Select the volume of sample as shown in section G2.5. If the volume V_1 exceeds 30 mL, add the sample in small portions and carry out step G2.4.2 until a total of V_1 mL has been evaporated.
G2.4.2	Evaporate the sample to dryness on a steam bath or by using a radiant heater, taking care to avoid spattering (note b).	(b) Take care to avoid loss of contents by spattering and/or frothing. Also, see section G2.3 concerning the care of platinum apparatus.
G2.4.3	Add 10.0 ± 0.1 mL of 20% m/V sodium carbonate solution ensuring that the entire residue is moistened.	
G2.4.4	Cover the crucible with a lid, reduce the solution to low volume, preferably on a steam bath, then evaporate to dryness. Heat gently over a low flame until the contents become quiescent (note b). Transfer the crucible to the muffle furnace and continue this heating until the entire contents appear as a transparent glassy melt. (See G2.3.1 above).	
	Allow the crucible to cool to room temperature in a dust-free atmosphere (note c).	(c) The exterior surface of the crucible must be kept scrupulously clean to minimise contamination.

Step	Procedure	Notes
G2.4.5	Place the crucible on its side together with the lid in a 250 mL plastic beaker. Add 150 ± 10 mL of boiling water and place on a steam bath until the melt has dissolved. Remove the crucible and lid from the beaker using platinum tipped tongs and rinse with water so that the washings are collected in the beaker. Allow the solution to cool to room temperature.	
G2.4.6	Cautiously add 50 ± 1 mL of hydrochloric acid solution (1.00M) (note d). Mix, allow to cool and transfer the solution to a 250 mL calibrated flask, dilute with water to the mark, stopper and mix well.	(d) The acid must be added slowly to prevent frothing and loss of solution.
G2.4.7	Reserve the solution for the determination of molybdate reactive silicon as described in section F steps F8.2 to F8.10 inclusive (note e).	(e) This solution is used in place of the sample. The volume (V mL) to use is given in section G2.5. Pay particular attention to the neutralisation steps given in step F8.3 and note c.
	Blank determination (note f)	
G2.4.8	Add 10.0 ± 0.1 mL of sodium carbonate solution to a platinum crucible. Proceed as described from G2.4.4 to step G2.4.7. Reserve this solution for the blank determination as described in step F8.11 (note g).	(f) Carry out a blank determination with each set of sample determinations. (g) This solution is used in place of water. The volume to use will be the same as that used for the sample solution. Pay particular attention to the neutralisation steps given in steps F8.4 and F8.11 and note c.
	Calculation of results	
G2.4.9	The absorbance due to total silicon in the processed sample is given by $A_p = A_s - A_b$. Determine the mass M_1 (in μg SiO_2) of total silicon in the processed sample from the value of A_p and the calibration curve. Calculate the total silicon concentration, C, in the original sample (in mg L^{-1} SiO_2) from $C = \frac{M_1}{V} \times \frac{250}{V_1}$	

G2.5 Suitable Sample Volumes

Suitable volumes of sample to be used may be estimated from the following table:

Table 3

Expected total silicon content of the sample (mgL^{-1} SiO_2)	Aliquots to be used for pretreatment stage V_1 mL	for colorimetric stage V mL
<10	100	50
10-20	50	50
20-80	25	50
80-200	10	50
200-400	5	50
400-1,000	5	20
1,000-2,000	5	10
2,000-4,000	5	5

G3 Sample Pretreatment for Converting Total Soluble Silicon to Molybdate Reactive Silicon

To determine soluble silicon, it is necessary to pretreat a filtered sample.

G3.1 Reagents

As described in section F5.

G3.2 Apparatus

As described in Section F6.

G3.3 Pretreatment procedure

Step	Procedure	Notes
	Pretreatment of sample	
G3.3.1	Filter about 100 mL of homogenous sample through an 0.45 μm membrane filter to obtain a clear solution (notes a and b).	(a) This step may be omitted if the analyst, due to his experience, judges it to be unnecessary. (b) For some purposes an 0.1 μm membrane may be more suitable.
G3.3.2	Using a plastic pipette transfer a suitable volume V (not exceeding 50 mL) of the filtrate to a 100 mL plastic beaker (note c).	(c) See section F10.1 for suitable volumes. A polyethylene bottle may be used instead of the beaker, in order to reduce the risk of contamination.
G3.3.3	Neutralize with sodium hydroxide solution (1.00M) or hydrochloric acid solution (1.00M) if necessary (note d) and add sufficient water to produce a volume of 50 ± 5 mL if necessary.	(d) The quantity of sodium hydroxide solution (1.00M) or hydrochloric acid solution (1.00M) to be added to neutralise V mL or sample to methyl orange indicator should be determined on a separate V mL portion of the (filtered) sample. (See F8.3).
G3.3.4	Add 5.0 ± 0.1 mL of sodium hydroxide solution (1.00M) and mix well.	
G3.3.5	Heat the solution on a steam bath for 30 ± 5 minutes. Allow the solution to cool to room temperature.	
G3.3.6	Pipette 15.0 ± 0.1 mL of hydrochloric acid solution (1.00M) into the beaker and mix well.	

Step	Procedure	Notes
G3.3.7	Reserve this solution for the determination of silicon as described in section F8 commencing at step F8.5.	
	Blank determination (note e)	
G3.3.8	Transfer 50 ± 5 mL water to a 100 mL plastic beaker. Proceed as described in steps G3.3.4 -6. Reserve this solution for the blank determination described in step F8.11 but omitting steps F8.2 to F8.4	(e) Carry out at least one blank determination with each set of sample determinations.
	Calculation	
G3.3.9	The absorbance due to total soluble silicon in the processed sample is given by $A_p = A_s - A_b$ Determine the mass M_2 (in μg SiO_2) of total soluble silicon in the processed sample from the value of A_p and the calibrated curve. Calculate the total soluble silicon concentration, C_S, in the original sample (in mgL^{-1} SiO_2) (from $C_S = \frac{M_2}{V}$	

H Determination of Phosphorus and Silicon by Emission Spectrophotometry

H1 Inductively Coupled Plasma Emission

Both phosphorus and silicon can be determined by Inductively Coupled Plasma Emission Spectrophotometry (ICPES). If not already in solution, a known weight of sample is brought into solution and made up to a standard volume and analysed according to the instrument maker's instructions. See also the information on ICPES in the booklet on Inductively Coupled Plasma Spectrometry in preparation (Ref 36).

H1.1 Limits of Detection (typical)

Silica 10 $\mu g l.^{-1}$

Phosphorus 40 $\mu g l.^{-1}$ (lower on some instruments)

H1.2 Wavelengths

Many instruments only have set wavelengths (in nm) in which case a choice is not possible.

Silicon	250.69	
	251.61	(usually the most sensitive line)
	288.16	
	390.55	
Phosphorus	177.50 }	using a vacuum path
	178.29 }	
	213.62	
	214.9	
	253.57	

H1.3 Known problems

Silicon

Some instruments develop source problems which must be rectified by cleaning. Regular control standards should be included in each batch of samples as a guard against this. Doubtful analyses should be repeated after rectification of the fault and the calibration has been re-checked.

Chromium, iron, manganese and vanadium may interfere at 251.61 nm and chromium, iron, magnesium and vanadium at 288.16 nm.

Phosphorus

Copper, aluminium, chromium, iron, manganese and titanium may interfere at some wavelengths.

H2 DC Arc Emission

Silicon in solid samples can be determined by DC arc emission spectrophotometry

A weighed sample should be ignited to constant weight at 450°C in a metal crucible, cooled and reweighed. Then weigh out 30 mg portions, mix thoroughly with an equal weight of silicon free spectroscopic graphite, pack into an undercut graphite cup electrode and examine using a complete burn DC arc (10 amp is usual) on a suitable emission spectrograph (see ref 37). Prepare a calibration curve using known amounts of

pure silica as samples. Addition and use of an internal standard to both standards and samples is suggested.

Useful lines are

nm	
212.3	over 5% in solid sample
212.4	over 0.1% in solid sample
250.7	usable down to about 0.001% in solid sample
251.4	
251.6	
288.2	

H3 Phosphorus by Flame Photometry

Phosphorus, especially in organic compounds, is sometimes determined by measurement of either the phosphorus-oxygen band head intensity at 526 nm, or the intensity of the phosphorus-oxygen band at 540.8 nm. An oxy-hydrogen or air-hydrogen flame is used. This is the basis of many of the phosphorus detectors used in chromatography; but, given appropriate standard samples, can be used for simple quantiative analysis.

I Estimation of the Validity of Analytical Results

The analyst should establish an analytical quality control procedure to check the validity of results obtained. Because of the complex nature of phosphorus and silicon in water, analysis in respect of the forms to be determined and the wide range of concentrations likely to be encountered, it is beyond the scope of this booklet to present a scheme that satisfactorily covers all possible parameters. The analyst should follow the guidelines and statistical practices recommended in Refs 14 and 34. Controls should be selected appropriate to the range and conditions that prevail in the chosen overall analytical procedure.

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