Phosphorus in Waters, Effluents and Sewages, 1980

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £2.80 net

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Methods for the Examination of Waters and Associated Materials

This booklet describes two spectrophotometric methods for the determination of orthophosphate and four appendices describing the conversion of various other forms of phosphorus to soluble orthophosphate and one appendix on arsenic interference removal.

Contents

London: Her Majesty's Stationery Office

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 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. Lone working, whether in the laboratory or field, should be discouraged. 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.

There are numerous handbooks on first-aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; and 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards.

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. 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.

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 are 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. 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.

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About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. 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 will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. 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 – the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst attention of the user 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 one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis
9.0 Radiochemical methods

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occuring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

TA DICK
Chairman

LR PITTWELL
Secretary

4 December 1980

0. About this method

0.1 Phosphorus may be present in water, effluents and sewage in a wide variety of physical and chemical forms. For example it may be present in solution, in suspension, or adsorbed on to particulate matter as orthophosphate, condensed phosphates (eg pyro and poly phosphates) or as organophosphorus compounds. Methods suitable for the determination of phosphorus in sludges, slurries, sediments and plant materials will be given in another booklet in this series.

0.2 Often for natural waters (fresh and saline) and waste waters (sewages and effluents) it is sufficient to determine the orthophosphate present, that being the form in which phosphorus is readily available for biological processes. The spectrophotometric method described in section A of this booklet, based on the formation of a molybdenum blue complex is rapid and well suited to the routine analysis of aqueous samples. The method will also serve as a useful final step in the determination of other phosphorus forms after an appropriate pretreatment to convert the forms of interest into the orthophosphate form.

0.3 Occasionally some analysts, for example those supporting algology projects, may need to determine very low concentrations of phosphorus. For this purpose a higher sensitivity spectrophotometric method based on the extraction of a molybdenum blue complex into an organic solvent, is described in section B of this booklet.

0.4 Although the two methods described in the main part of this booklet will be suitable for most purposes there may be occasions when it will be necessary to differentiate between the different physical and chemical forms of phosphorus. This may necessitate additional pretreatments, which are described in a series of appendices.

Method A Phosphorus in Waters, Effluents and Sewages by Spectrophotometry (1980 Version)

A1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see General Principles of Sampling and Accuracy of Results, also published in this series).

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 acidic molybdate reagents to form a reduced phosphomolybdenum blue complex whose concentration is measured spectrophotometrically.
A1.4	Range of application (a)	0 to 0.40 mg/l (Range may be extended by dilution see Section A10).
A1.5	Calibration curve (a)	Linear to 0.4 mg/l.
A1.6	Standard deviation (a)	

Type of sample	Phosphorus concentration (mg/l)	Total standard deviation (mg/l)	Degrees of freedom
Standards (b)	0.00	0.001*	6
,,	0.04	0.002	14
,,	0.35	0.003	14
,,	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
Sewage effluents (c)	11	0.11–0.17	14

* within batch standard deviation

A1.7	Limit of detection (a)	0.003–0.006 mg/l. (35 degrees of freedom).
A1.8	Sensitivity (a)	0.25 mg/l gives an absorbance at 882nm 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	Interference	See section A3.
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 (Stevenage Laboratory), the Freshwater Biological Association, Windemere Laboratory, Yorkshire Water Authority and North West Water Authority.
(b) Deionized water spiked with the stated phosphorus concentration, as KH_2PO_4.
(c) Determinations made using 1 ml of sample, all other data obtained using 40 ml of sample.

A2 Principle

The method described is based on that of Murphy and Riley[1] and experimental work carried out by the Water Research Centre, Stevenage Laboratory[2] and Imperial Chemical Industries Laboratories[3]. Orthophosphate ions react with a solution containing molybdic acid, ascorbic acid, trivalent antimony ions and hydrogen ions, to form a 12-molybdophosphoric acid, which is reduced in situ to a blue heteropoly compound (phosphomolybdenum blue) in which antimony is incorporated.

The acid conditions used may cause partial hydrolysis of condensed phosphates, and/or some of the more labile organic phosphates, if present. For this reason the determinand is referred to as reactive phosphorus instead of orthophosphate[4].

A3 Interferences

A3.1 There is little detailed information concerning the effect of interfering substances on the method. Generally, no important interference problems are likely with unpolluted saline and fresh waters; but the effect of interferences should be considered particularly in polluted samples and at very low phosphate levels. The most likely sources of interferences and implications are discussed below with references, and by Burton[5] who also highlights other problems associated with the analysis of phosphorus compounds.

A3.1.1 Arsenic

Arsenic present as arsenate causes serious interference in the method. The extent of interference is time dependent because the colour of the arsenomolybdate develops slowly. However, as a guide 0.025 mg/l As may give a response equivalent to about 0.015 mg/l P (40 ml sample volume).

A3.1.2 Silicon

The method will tolerate 10 mg/l of silicon present as silicate[1] but high silicon/phosphorus ratios in samples where the phosphate level is very low may cause significant errors.

A3.1.3 Chromium

Cr^{VI} is reported to interfere at 1 mg/l level[6].

A3.1.4 Oxidizing agents

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

A3.1.5 Nitrite

A concentration of 1 mg/l N (as nitrite) may be tolerated in the presence of 0.1 mg/l phosphorus but the interference of nitrite is both complex and variable and appears to be related to exposure to the air.

A3.1.6 Nitrate

Nitrate nitrogen may be tolerated up to 20 g/l (as N) provided that the absorbance is measured within two hours of colour development.

A3.1.7 Sulphide

Interference from sulphide is complex, variable, and, dependent on conditions, reacting with both antimonate and molybdate. Complete removal is advocated (by oxidation to sulphate or by aspiration with nitrogen)[14].

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 his 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[7]. 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 Appendix V).

A3.2.2 A slight excess of sulphamic acid[3,8] is effective in breaking down nitrite; 100 mg of the acid will deal with a nitrite concentration of 10 mg/l N in a 40 ml aliquot of test solution.

A3.2.3 A solvent extraction procedure[9] that is also effective in reducing certain interferences by virtue of the dilution effect is described in method B.

A4 Hazards

Several reagents eg antimony potassium tartrate and ammonium molybdate are poisonous; do not pipette by mouth.

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 $(K(SbO)C_4H_4O_6)$ 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 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 1 day should be discarded. Do not prepare more than is required.

A5.7 Sodium hydroxide solution (N). 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 (N). 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 anhydrous potassium dihydrogen orthophosphate 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 a few days, however it should be prepared freshly when required.

A6 Apparatus

A6.1 Spectrophotometer

A spectrophotometer for use at 882 nm 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 or 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.

A6.1.1 40-mm optically matched cells

Both sample and reference cells should be kept scrupulously clean, the same cells should be used for sample and reference solutions respectively. 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 loss of sensitivity will occur, different standards should be used and the calculation should be adjusted.

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 is only repeated occasionally.

A7 Sample Collection and Preservation

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

Refrigeration (but not freezing) is reasonably effective over short periods of storage (eg a few days).

Some samples for determinations may require filtering on site to ensure changes are not occurring during transit or storage (see also Appendix I).

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 the Appendices.

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). 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).	(a) The sample may be the original sample or a pretreated sample. If necessary it should be neutralized to phenolphthalein by addition of sodium hydroxide solution (N) or sulphuric acid (N) and the temperature adjusted to 15–25°C. (b) See Section A10 for suitable sample volume. (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.
A8.2	After at least 10 minutes (note e) measure the absorbance of the solution at 882 nm (note f) using 40-mm cells, the reference cell being filled with water (note g). 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 obtainable see Section A6.1. (g) 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. Let absorbance be A_b.	
	Compensation for Colour and Turbidity (note h)	
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 $= A_c$.	(h) 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 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	Determine the mass M (in μg P) of phosphorus in the processed sample, from the value of A_p and the calibration curve. (See Section A9)	
A8.7	Calculate the phosphorus concentration C in the original sample (in mg P/l) from $C = \frac{M}{V}$ (note i)	(i) A multiplication factor may be required if the sample was diluted before analysis or arising out of manipulations during pretreatment stages.

A9 Caliabration 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 Concentration Range of the Method

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

Expected concentration (mg P/l)	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 being 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).

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 mg/l 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 apply the low level extraction method given in Section B (see also Section B11.2).

A11.2 Colour and turbidity blank

This measurement is rarely required for every sample, but may be a very 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 filtrable phosphorus fraction (see Appendix II).

A12 Checking the Validity of the Analytical Results

A standard solution containing 0.3 mg l/P is suitable as a regular control standard for control chart purposes[10].

Method B Phosphorus in Oligotrophic Waters by Extraction and Spectrophotometry Tentative Method (1980 version)

B1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see General Principles of Sampling and Accuracy of Results, also published in this series).

Note: Throughout this method phosphorus is expressed as the element (P).

B1.1	Substance determined	Reactive phosphorus (see Section B2).
B1.2	Type of sample	Saline and non-saline waters containing less than 25 µg/l P particularly bodies of water with oligotrophic and borderline eutrophic characteristics.
B1.3	Basis of method	Reaction with acidic molybdate reagents to form a reduced phosphomolybdenum blue complex which is extracted into hexan-1-ol prior to spectrophotometric determination.
B1.4	Range of application (a)	Up to 25 µg/l P.
B1.5	Calibration curve (a)	Linear to at least 25 µg/l P.
B1.6	Standard deviation (a)	

Type of sample	Phosphorus concentration (µg/l)	Total standard deviation (µg/l)	Degrees of freedom
Standard Solutions (d)	0	0.1–0.14*	25 (b) (c)
	6.0	0.3	11 (a)
	10.0	0.50*	8 (c)
	22.5	1.5	6 (b)
River (soft water)	8.1	0.9*	5 (b)
Borehole (chalk water)	11.9	0.78*	8 (c)
Reservoir water	2.4	0.23*	9 (b)

B1.7	Limit of detection (a) (b) (c)	0.5–0.7 µg/l P (25 degrees of freedom).
B1.8	Sensitivity (a) (b) (c)	10 µg/l P gives an absorbance of approximately 0.27.
B1.9	Bias (b) (c)	No statistically significant bias was detected except when interferences were present (recovery of 10 µg/l P added to river water and borehole water containing 8–12 µg/l P was 97.5%–102.2%) (b) (c).
B1.10	Interferences	The major chemical interference effects on the method are covered in Section B3. At the low phosphate concentrations covered by this method the possible effects of microbiological activity should be considered.

B1.11	Time required for analysis (a) (b) (c)	12 samples per 2–3 hours of which 1.5–2 hours is operator time, including one blank and one control standard, but excluding any pretreatment time.

* Within-batch standard deviations.
(a) Data supplied by Freshwater Biological Association, Windermere Laboratory.
(b) Data supplied by North West Water Authority, Denton Laboratory.
(c) Data supplied by Southern Water Authority, Winchester Laboratory.
(d) Distilled water spiked with potassium dihydrogen orthophosphate.

B2 Principle

The method described is based on that of Murphy and Riley[1] using the extraction modification proposed by Murphy[11] and experimental work by the Freshwater Biological Association Windermere Laboratory[9].

Orthophosphate ions react with an acidic solution containing molybdic acid, ascorbic acid and trivalent antimony to form a reduced phosphomolybdenum blue complex which is extracted into hexan-1-ol.

B3 Interferences

(see also Section A3)

There is little detailed information on the effect of interfering substances on the method described. The effect of potential interferences should be considered particularly in samples for which this solvent extraction technique is appropriate, ie at very low phosphate levels. However, it is unlikely that such samples will contain significant concentrations of such interferences as arsenate, silicate, chromium(VI) or nitrate. Test data indicating the effect of interferences on this method are given in Table I[12]. The possible effects of microbiological activity (either absorption or excretion) should be considered at these low phosphate levels.

TABLE I: Effect of other substances

Other substance	Concentration of other substances (mg/l)	Effect in µg/l P of other substances at phosphorus concentration of:		
		0 µg/l P	5 µg/l P	10 µg/l P (e)
Silicate as SiO_2	1.1	<0.5	<0.5	<0.5
	4.3	0.8	1.0	1.3
Arsenate as As	0.005	<0.5	0.7	—
	0.05	1.4	5.9	—
	0.10	4.5	11.7	—
Germanium as Ge	0.1	<0.5	<0.5	0.9
	1.0	1.2	—	1.3
Chromate as Cr	0.1	<0.5	<0.5	<0.5
	1.0	<0.5	0.6	0.6
Nitrate as N	1.0	<0.5	<0.5	<0.5
	10.0	<0.5	<0.5	−1.2
Nitrite as N	0.1	<0.5	<0.5	<0.5
	0.2	<0.5	−1.5	−1.5
	0.5	<0.5	−3.5	−1.5
	1	—	—	−10
HCO_3^-	10	<0.5	<0.5	<0.5
	200	0.6	0.5	−1.0

(e) If the other substance did not interfere, the results would be expected (95% confidence) to lie within the ranges:
0.00±0.32 µg/l P, 5.00±0.53 µg/l P, 10.00±1.15 µg/l P at concentrations of 0.00, 5.00 and 10.00 µg/l P respectively.

At such low levels of phosphorus, care is essential. Contamination of test solutions from reagents used to prepare the interferent test additions can readily confound interpretation of the test results.

B4 Hazards

(see also Section A4)

Organic solvents such as hexan-1-ol and propan-2-ol are highly flammable and have irritant vapours that are harmful to the eyes, and by inhalation, and by skin absorption. Always use in a well ventilated area away from flames and other sources of heat.

Iodine should not be allowed to come into contact with the eyes or skin, nor be inhaled.

B5 Reagents

Analytical reagent grade quality should be used wherever possible.

B5.1 Water

The water used in this procedure should have a phosphorus content that is negligible compared with the smallest concentration to be determined in samples. Distilled or deionized water is suitable (see Section B11).

B5.2 14% V/V Sulphuric acid

Add slowly and cautiously with stirring 140 ± 2 ml of sulphuric acid (d_{20} 1.84) to about 800 ml of water in a 2-litre beaker partially immersed in cold water, allow to cool and dilute with water to 1000 ± 20 ml. Store in a borosilicate glass bottle.

B5.3 3% m/V Ammonium molybdate

Dissolve 30 ± 0.1 g of finely ground ammonium molybdate, $(NH_4)_6Mo_7O_{24}$, $4H_2O$ in water and dilute with water to 1000 ± 20 ml. Store in a polyethylene bottle. Discard if a precipitate forms.

B5.4 0.34% m/V Antimony potassium tartrate

Dissolve 0.68 ± 0.01 g of antimony potassium tartrate, K(SbO) $C_4H_4O_6$ in water and dilute with water to 200 ± 5 ml. Store in a borosilicate glass bottle in a refrigerator.

B5.5 5.4% m/V Ascorbic acid

Dissolve 5.4 ± 0.1 g of ascorbic acid in 100 ± 1 ml of water ($< 10°C$). This reagent should be prepared just before use in making the mixed reagent B5.6.

B5.6 Mixed reagent

A quantity suitable for about 20 determinations is prepared by mixing in the order given: 250 ± 2 ml of 14% V/V sulphuric acid, 100 ± 2 ml of 3% m/V ammonium molybdate solution, 100 ± 1 ml of 5.4% m/V ascorbic acid and 50 ± 1 ml of 0.34% m/V antimony potassium tartrate mixing after each addition.

This reagent should be prepared as required and should be kept in a refrigerator when not in use. Under these conditions it should be suitable for use during one working day, any excess remaining after 1 day should be discarded.

B5.7 Standard phosphate solutions

B5.7.1 Solution A. 1 ml is equivalent to 100 µg P

Dissolve 0.4394 ± 0.0005 g of anhydrous potassium dihydrogen orthophosphate 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.

B5.7.2 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 should be stored in a refrigerator; it should be prepared freshly each day.

B5.8 Hexan-1-ol, Redistilled

Distil 1000 ml of hexan-1-ol with adequate precautions, including the use of a distillation tray, rejecting the first 100 ml of distillate.

Note: Some batches of hexan-1-ol may not need redistillation.

B5.9 Propan-2-ol

B5.10 10% V/V Sulphuric acid

Add slowly and cautiously with stirring 100±2 ml of sulphuric acid (d_{20} 1.84) to 800±50 ml of water in a 2 litre beaker immersed in cold water, allow to cool, and dilute with water to about 1 litre and mix well. Store in a glass or polyethylene bottle.

B5.11 2% V/V Sulphuric acid

Add slowly and cautiously with stirring 20±0.5 ml of sulphuric acid (d_{20} 1.84) to 800±50 ml of water in a 2 litre beaker immersed in cold water, allow to cool, and dilute with water to about 1 litre and mix well. Store in a glass or polyethylene bottle.

B5.12 Iodine

B5.13 Ethanol or 95% industrial methylated spirit

B6 Apparatus

B6.1 Spectrophotometer

A spectrophotometer for use at 680 nm and capable of accepting 40-mm cells is suitable.

B6.1.1 40-mm Optically matched cells

It is advisable to retain a separate pair of matched cells with lids for this method involving an organic solvent. The cells should be washed with hexan-1-ol, methylated spirits, and acetone after use and then blown dry with a stream of air. Both sample and reference cells should be kept scrupulously clean and the same cells should be used for sample and reference solutions respectively. They should always be placed in the same position in the holder with the same face towards the light source.

B6.2 Glassware

Glassware must not be cleaned with detergents or alkaline liquids. Fill the glassware with sulphuric acid (d_{20} 1.84), allow to stand overnight, then empty out the acid and rinse several times with water. Store in a dark place, filled with water.

B6.3 Iodized plastic bottles (for sampling)

Low density polyethylene or nylon (babies feeding type) bottles are suitable but high density polyethylene or polypropylene bottles are not. The plastic bottles must be treated with iodine to prevent the adsorption of phosphorus which can lead to significant errors.

B6.3.1 Iodization procedure[13]

Remove any rubber washers from the bottle caps and place a small quantity of iodine (about 0.1 g) in the bottle. Replace the cap and store at 60°C for long enough to ensure uniform absorption of iodine (usually 24 hours) during which time the bottle should be rotated and more iodine added if necessary.

Place the bottles in a well ventilated fume cupboard and remove the screw caps. Expel iodine vapour by squeezing the bottle gently. Rinse the bottle with a little ethanol and drain.

Wash the bottle by repeated filling with water at daily intervals until the iodine content of the water is reduced to the extent that the absorbance of the water at 680 nm is not greater than that of water stored in untreated bottles.

When not in use store the bottles in the dark filled with water.

B6.4 Solvent extraction apparatus

B6.4.1 Extraction bottle (not iodized)

250-ml flexible walled polyethylene bottles with polyethylene plugs and wadless caps should be cleaned by overnight soaking with 10% V/V sulphuric acid and then thoroughly washed with water. After use, the polyethylene bottles should be washed well with water and about 100 ml sulphuric acid solution (2% V/V) added to each bottle. The bottles should be plugged and capped, shaken well and stored in that condition. Discard acid before use.

B6.4.2 25-ml graduated pipette

B6.4.3 Silicone rubber bung

The dimensions of the bung should be such that it fits snugly onto the neck of the extraction bottles, and should be bored with a hole to accommodate the tip of the 25-ml graduated pipette. (See figure 1).

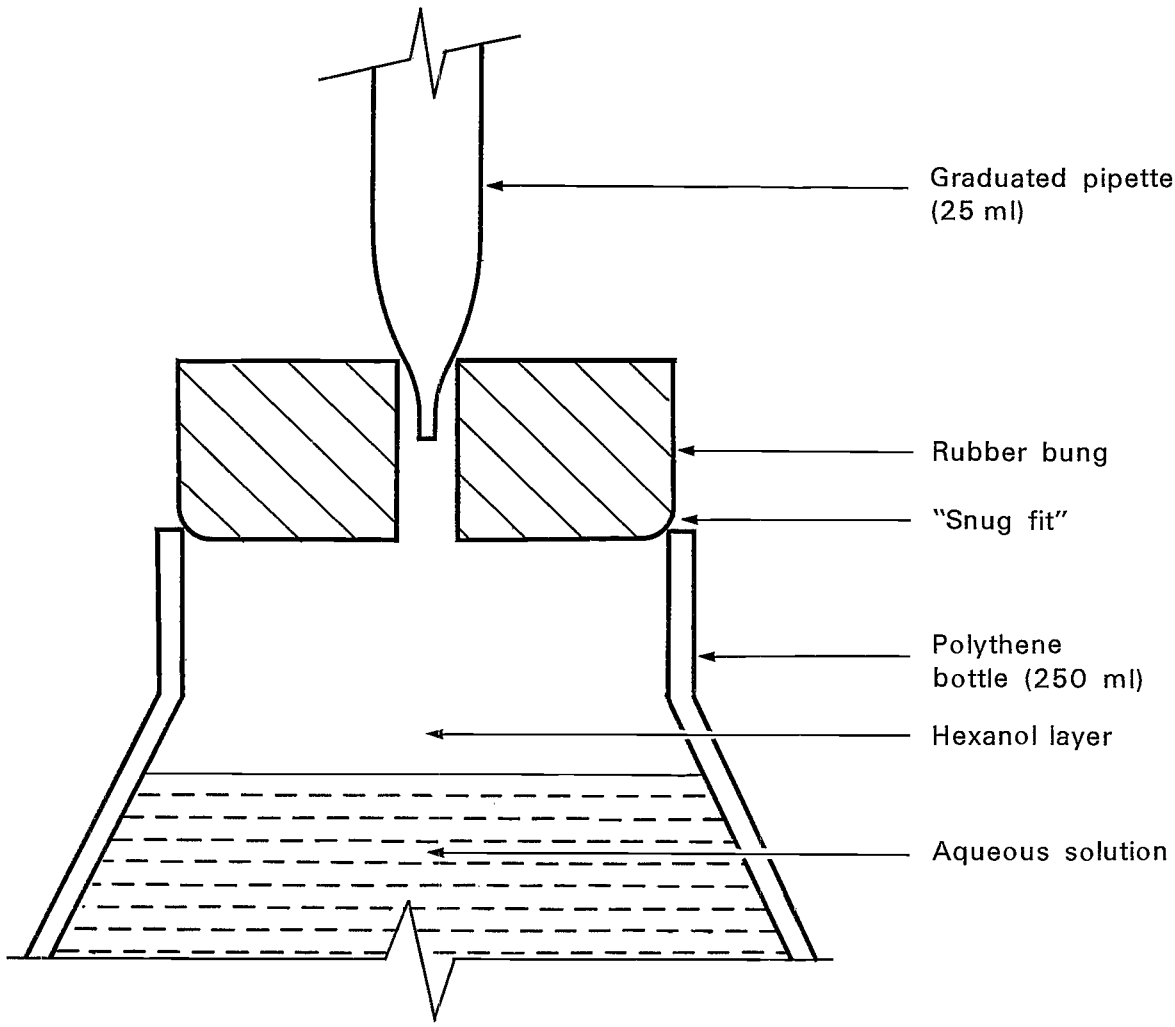


Fig 1 PIPETTING DETAILS

B6.5 A mechanical shaker (for consistent results the same shaker must be used for samples and standards).

B6.6 Bench centrifuge capable of 3000 rev/min

B6.7 15-ml centrifuge tubes

B7 Sample Collection and Preservation

As a guide to analysts supporting algology projects where the major requirement is to measure "dissolved reactive phosphate" in samples containing less than 25 µg/l P either:

(a) filter the sample "on site" through a glass fibre paper, pre-washed with about 500 ml of water [13], into a plastic bottle and analyse within 3 hours of sampling; or if this is not possible—

(b) filter the sample through a pre-washed glass fibre paper[13] within 3 hours of sampling into an iodized bottle and store in the dark at, or close to, 4°C.

NOTE. If the filtration pressure is too high or if the sample is allowed to freeze, changes of dissolved phosphate concentration 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. In this respect it should be noted that the insoluble phosphate content of an unfiltered sample can increase due to the continuation of zooplankton activity to give deposits containing phosphate in the sample.

B8 Sample Pretreatment

In addition to filtration discussed in B7 it may be necessary to consider whether the special requirements of freshwaters with an oligotrophic nature indicate the need for digestion of low levels of "total phosphorus" (see Appendix IV).

B9 Analytical Procedure

Read Section B4 on Hazards before starting this procedure

Step	Procedure	Notes
	Check of matching and cleaning of cells (note a)	
B9.1	Fill both sample and reference cells with hexan-1-ol and measure the absorbance difference at 680 nm using 40-mm cells (note b). This should not exceed 0.015 absorbance units. If so, clean the cells and repeat until a satisfactory match is obtained. If repeated cleaning does not reduce the difference discard the cells. Let the absorbance = A_0.	(a) Do this check on the cells before beginning the colorimetric measurements of any sample or batch of samples (ie before step B9.7). (b) If the absorbance difference is negative reverse the cells.
	Analysis of samples	
B9.2	Transfer 200±2 ml of the sample to a 250-ml extraction bottle (note c), add 20±0.5 ml of mixed reagent, and mix well by swirling.	(c) The volume taken should contain less than 5 µg P. If not, reduce the sample volume and make up to 200±2 ml with water.
B9.3	After 10 minutes add 15±0.1 ml of hexan-1-ol from a burette (note d), insert the plug and cap the bottle.	(d) Certain forms of automatic dispenser are also suitable.
B9.4	Shake for 10±2 min in a mechanical shaker, remove the cap and plug and allow the solvent layer to separate.	
B9.5	Insert the tip of a 25-ml graduated pipette into the hole in the silicone rubber bung. Place the bung with pipette onto the bottle neck and gently squeeze the bottle to eject at least 11 ml of the hexan-1-ol extract into the pipette.	
B9.6	Transfer 11±0.1 ml of the hexan-1-ol layer into a dry 15-ml centrifuge tube, add 1±0.1 ml of propan-2-ol and mix. Centrifuge for 1±0.5 min at 3000 rev/min (note e).	(e) This is necessary to separate any residual water that may be present.
B9.7	Withdraw the clear supernatant liquor and transfer to a 40-mm cell. Measure the absorbance at 680 nm against hexan-1-ol in the reference cell (note f). Let absorbance = A_s.	(f) The colour is stable for at least 2 hours; the wavelength of maximum absorption must be checked for each instrument. It should be checked at regular intervals and after each service.
	Reagent blank determination	
B9.8	A blank must be run with each batch of determinations using the same batch of reagents as for the samples. Proceed through the above stages B9.2 to B9.7 using 200±2 ml of water in place of the sample. Let the absorbance = A_b (note g).	(g) The corrected reagent blank ($A_b - A_0$) should not exceed 0.03 absorbance units (see Section B11.2).

Step	Procedure	Notes
	Sample colour blank	
B9.9	Proceed through the above stages B9.2 to B9.7 with the sample but using 10±0.5 ml of 14% V/V sulphuric acid in place of the mixed reagent in step B9.2. Let the absorbance = A_c (note h).	(h) This step may be omitted when it is known that samples do not contain coloured matter which is extractable by hexan-1-ol.
	Control standard (see Section B12)	
B9.10	(At least one control standard should be run with each batch of determinations) 200±2 ml of a standard phosphate solution containing 10 µg/l P should be taken through stages B9.2 to B9.7 in place of the sample (note i).	(i) The concentration found in this standard should be calculated and may be plotted on a control chart, to demonstrate the achievement of adequate precision.
	Calculation stage	
B9.11	The absorbance due to phosphorus in the processed sample is given by: $A_p = A_s - A_b$ or when a correction for colour is made: $A_p = A_s - A_b - A_c + A_o$	
B9.12	Determine the concentration of phosphorus in the sample, using A_p with reference to the calibration graph prepared on the basis of a sample volume of 200 ml (notes j and k).	(j) Apply a suitable correction if the sample volume was different from 200 ml. (k) Regression analysis and a factor may be used.

B10 Calibration Graph

The procedure given in this section should be carried out on at least three independent occasions before application of the method and regularly thereafter. Any significant deviation from linearity indicates that the technique is suspect at some stage.

Add 200, 199, 198, 197, 196 and 195 ml of water to each of a series of 250-ml polyethylene bottles. Then add 0.00, 1.00, 2.00, 3.00, 4.00, 5.00 ml of standard phosphate solution B to the bottles respectively. The bottles contain 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 µg/l P and each should be taken through the procedure steps B9.2 to B9.7 using each solution in place of the sample. Plot the results ($A_s - A_b$) against µg/l P.

The calibration graph is linear to at least 25 µg/l P.

B11 Sources of Error

This analytical method can be applied to a wide range of samples and the attention which it is necessary to pay to sources of error depends upon the accuracy required.

B11.1 Correction for sample colour

The presence of coloured material in the samples which is extractable by hexan-1-ol may cause falsely high results. The procedure given in step B9.9 allows a correction to be made if required.

B11.2 Phosphorus Content of the Blank Water

If the water used for the blank determination 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 to algologists is 1 µg/l P. This implies that a bias arising from the phosphorus content of the blank water of 0.2 µg/l P is

acceptable[10]. 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 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 µg/l P by examining a concentrated sample of blank water. The criterion that the concentration technique should lower the limit of detection of the analytical method five fold, means that a concentration factor of at least 10 fold must be applied. The concentrate so obtained is then analysed as in Section B9.

Although concentration of distilled water by evaporation is time consuming and subject to the risk of contamination from the atmosphere or the equipment, and adsorption of phosphorus on to the container walls, a tentative procedure is outlined in Section B11.2.1 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.

B11.2.1 Procedure for concentration by evaporation

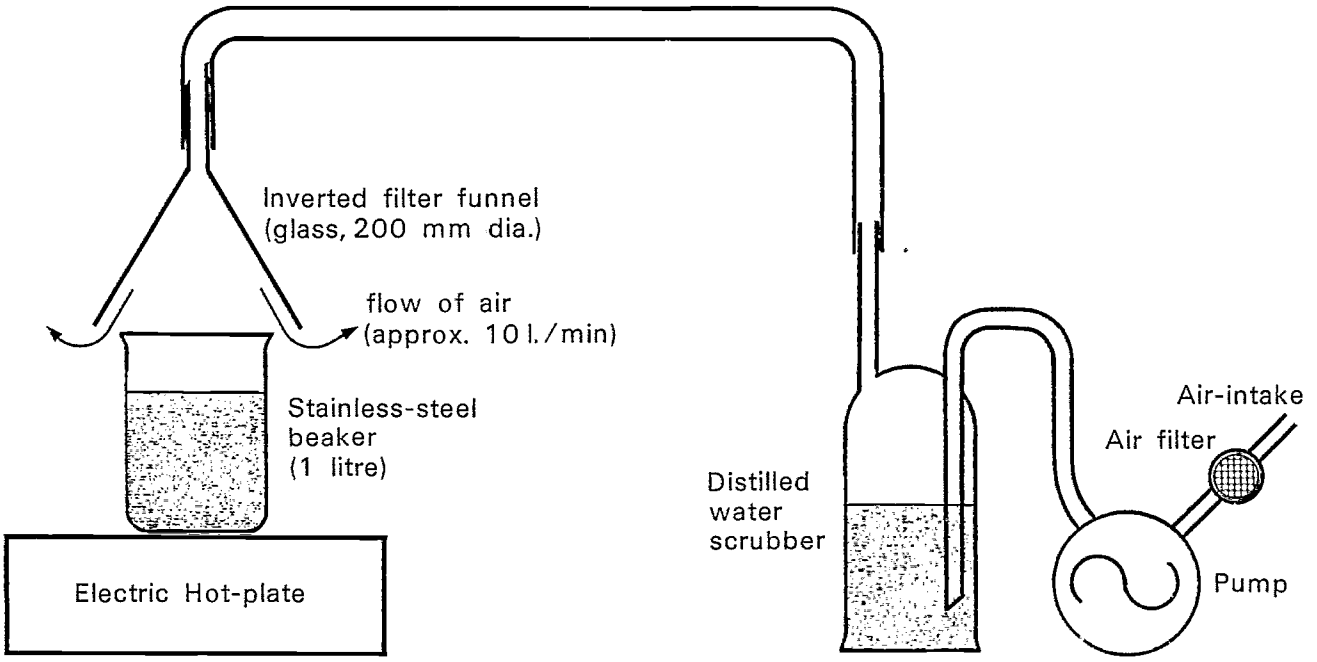


Fig. 2 EVAPORATION APPARATUS

Step	Procedure
B11.2.1.1	Set aside in a clean, polyethylene aspirator a sufficient volume (10–15 l) of the distilled water dependent on the concentration factor required.
B11.2.1.2	Assemble the apparatus shown in figure 2.
B11.2.1.3	Transfer about 800 ml of the distilled water to the 1 litre stainless steel beaker placed on the hot plate. Place the inverted funnel in position as shown above and start up the air pump. Switch on the hot plate and evaporate the water down to about 100 ml before adding further portions of distilled water until 2000 ml have been evaporated down to about 200 ml.

Step	Procedure
B11.2.1.4	Allow the 200 ml residue to cool, transfer to a 250 ml polyethylene bottle for phosphorus determination as in Section B9, adjusting the volume to 200±5 ml by the addition of distilled water if required. Carry out steps B9.2 to B9.12.
B11.2.1.5	Divide the result obtained by 10 to give the concentration of phosphorus in µg/l P in the original distilled water.
B11.2.1.6	This determination should be done in multiplicate.

B12 Checking the Validity of the Analytical Results

A control standard concentration of 10 µg/l P has already been recommended (Section B9.10) and a control chart technique should be instituted[10].

Appendix Pretreatment Methods

As several variations in pretreatment are possible with either method, the analyst is 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 with method B.

1 General Information

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

(a) Orthophosphate;
(b) Condensed phosphates (eg pyro and polyphosphates) these are often referred to as hydrolysable phosphates;
(c) Organo-phosphates.

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 Sections 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 oxidization by persulphate. Whilst the 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, condensed, total, organic)]. 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 II and in Fig 3, with information on the relevant sections for pretreatment suggestions and/or calculation steps required. However it should be noted that on some occasions, such as when an accurate determination of a particulate fraction is required, and this fraction is very small compared with the dissolved fraction, it may be more appropriate to filter a large volume of sample and determine by pretreatment of material retained on the filter (see also Ref 4).

TABLE II

Approximate composition of fraction	Analytically defined Determinand	Details of Pretreatment	Calculation
Total orthophosphate (TRP)	Total reactive phosphorus	None	
Dissolved orthophosphate (DRP)	Filtrable reactive phosphorus	Appendix II	
Particulate orthophosphate (PRP)			(PRP) = (TRP − DRP)
Total inorganic phosphate (TIP)	Total reactive and hydrolysable phosphorus	Appendix III	
Dissolved inorganic phosphate (DIP)	Filtrable reactive and hydrolysable phosphorus	Appendices II & III	
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	Appendix IV	
Total dissolved phosphate (TDP)	Total filtrable phosphorus	Appendices II & IV	
Total particulate phosphate (TPP)			(TPP) = (TP − TDP)
Total organic phosphate (TOP)			(TOP) = (TP − TIP)
Dissolved organic phosphate (DOP)			(DOP) = (DTP − DIP)
Particulate organic phosphate (POP)			(POP) = (TP + DIP − TIP − DTP)

The suggestions on pretreatment given in detail in the Appendices must be regarded as tentative. Each analyst should make any experimental tests he thinks necessary to ensure that a pretreatment appropriate for his particular samples is chosen.

II Filtration to Separate Dissolved from Particulate Phosphorus

II.1 Apparatus

A filter assembly suitable for membrane filtration, with 0.45-μm or 0.1-μm membrane filters as required.

II.2 Procedure

Procedure	Notes
Filter a known volume of sample sufficient to meet the requirements of subsequent analyses through the membrane. If the sample contains much suspended matter, a preliminary filtration through an approriate porosity ashless cellulose filter paper may be added as an initial step. Store the filtrate in a clean bottle for subsequent analysis by either method (see Sections A7 and B7).	
If, as mentioned in Appendix I, direct analysis of the particulate phosphorus fraction is desired, preserve the filters for treatment by an Appendix IV procedure (note a).	(a) It is recommended that a blank be carried through this pretreatment procedure. If particulate phosphorus is also to be determined by an Appendix IV procedure, the blank filters should be treated as the corresponding blank samples.

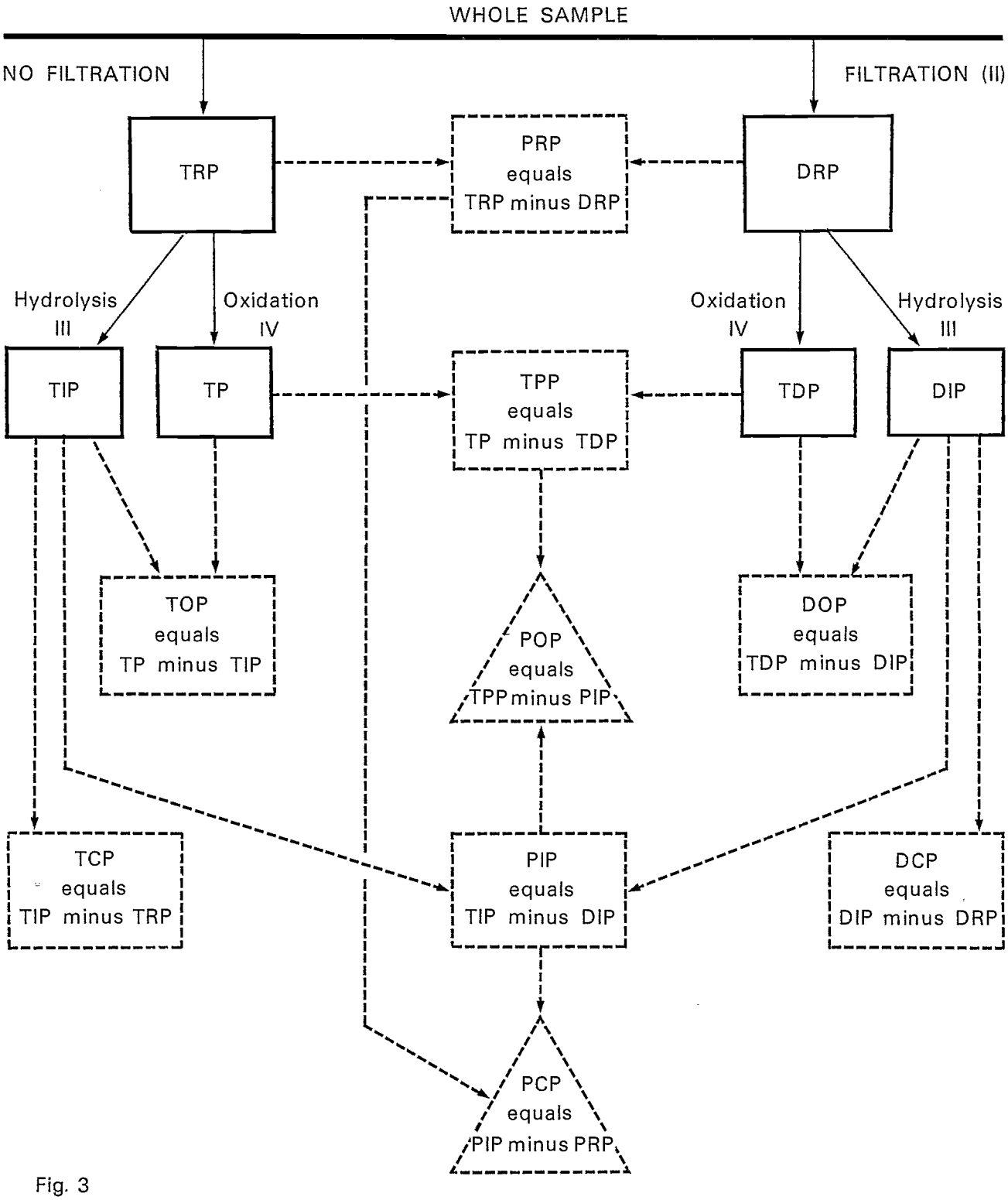
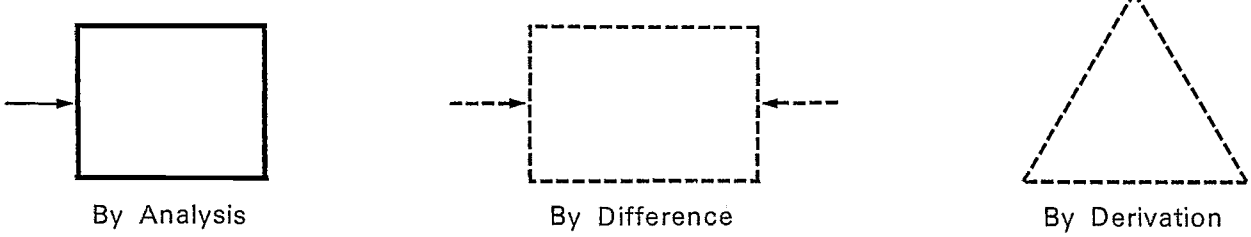


Fig. 3

RELATIONSHIP BETWEEN THE VARIOUS PHOSPHORUS DETERMINATIONS IN TABLE II



For abbreviations see Table II

Pretreatment procedure numbers in Roman type

III Acid Hydrolysis Procedure for the Determination of Total Inorganic Phosphorus (TIP) and Dissolved Inorganic Phosphorus (DIP)

(some labile organophosphorus compounds may be included).

III.1 Reagents

III.1.1 Water see A5.1.

III.1.2 Phenolphthalein see A5.9.

III.1.3 Sodium hydroxide (N) see A5.7.

III.1.4 Sulphuric acid (N) see A5.8.

III.2 Procedure

Step	Procedure	Notes
III.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 Appendix II. See section A10 for suitable volumes.
III.2.2	Neutralize to phenolphthalein with sodium hydroxide (N) or sulphuric acid (N) if necessary.	
III.2.3	Add 10 ± 0.2 ml sulphuric acid (N).	
III.2.4	Cover the beaker with a watch glass and heat to boil the solution gently for 25 ± 5 minutes (note b). Allow the solution to cool then neutralize with sodium hydroxide (N).	(b) 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.
III.2.5	Transfer the solution quantitatively to a 50-ml calibrated flask (note c) and reserve the solution for the colorimetric stage (see Section A8).	(c) The quantity of rinse water used should be controlled so that the final volume at this stage does not exceed 40 ml.

IV Determination of Total Phosphorus (TP) and Total Dissolved Phosphorus (TDP)

IV.1 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 IV.2 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.

A rigorous pretreatment is described in Section IV.3. This technique will convert almost every form of phosphorus compound and even the free element to reactive phosphorus if carried on long enough. It is suitable as a reference method to compare the efficiencies of the less rigorous techniques, but this treatment has three important disadvantages. It is slow, requires almost continuous attention from the analyst, and markedly worsens the precision of the analytical results. These disadvantages make it desirable not to employ this procedure for routine analysis if the persulphate pretreatment (Section IV.2) alone is suitable.

The special requirements of freshwaters with an oligotrophic nature indicate a need for the determination of low levels of "total phosphorus". Consideration should be given to a pressure digestion technique with sulphuric acid and potassium persulphate using either pressure bottles or aluminium foil capped flasks in an autoclave at about 1 bar (15 psi). The method in use at the FBA, Windermere Laboratory makes convenient use of potassium persulphate in tablet form and has been published elsewhere[9]. It should be noted however that the persulphate tablets contain potassium nitrate as a binding agent and the digest may not therefore be used for the simultaneous determination of nitrogen.

IV.2 Mild Digestion Step for the Determination of Total Phosphorus (TP) and Total Dissolved Phosphorus (TDP)

IV.2.1 Reagents

IV.2.1.1 Water see A5.1.

IV.2.1.2 Sodium hydroxide (N) see A5.7.

IV.2.1.3 Sulphuric acid (N) see A5.8.

IV.2.1.4 Phenolphthalein see A5.9.

IV.2.1.5 Ammonium (or Potassium) Persulphate

IV.2.2 Procedure

Step	Procedure	Notes
IV.2.2.1	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 TOP use filtrate from pretreatment described in Appendix II. See Section A10 for suitable volumes.

Step	Procedure	Notes
IV.2.2.2	Neutralize with sodium hydroxide (N) or sulphuric acid (N) 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.
IV.2.2.3	Add 0.2±0.01 g ammonium persulphate and swirl to dissolve.	
IV.2.2.4	Add 10 ; 0.2 ml sulphuric acid (N) and mix well.	
IV.2.2.5	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 (N).	(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 persulphate. This is sometimes preferred to using procedure IV.3. (e) If arsenic was present originally, it will now be present as arsenate and will interfere in the colorimetric stage—see Appendix V.
IV.2.2.6	Transfer the solution quantitatively to a 50-ml calibrated flask (note f) and reserve the solution for the colorimetric stage (see Section A8).	(f) The quantity of rinse water used should be controlled so that the final volume at this stage does not exceed 40-ml.

IV.3 Rigorous Digestion Step for the Determination of Total Phosphorus (TP) and Total Dissolved Phosphorus (TDP)

IV.3.1 Reagents

IV.3.1.1 Sulphuric acid (d_{20} 1.84)

IV.3.1.2 Nitric acid (d_{20} 1.42)

IV.3.1.3 Water see A5.1.

IV.3.1.4 Sodium hydroxide (5N)

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.

IV.3.2 Apparatus

IV.3.2.1 200-ml Kjeldahl flasks

IV.3.3 Analytical Procedure

Step	Procedure	Notes
	Analysis of Samples (notes a, b)	
IV.3.3.1	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 blank determination must be included with each batch of samples.	(a) For TP use original sample, for TDP use filtrate from pretreatment Appendix II. (b) Great care must be taken during this stage to avoid contamination. Kjeldahl nitrogen digestion solutions and the related hydrogen peroxide total nitrogen digestion solutions can sometimes be substituted for this procedure (see the appropriate method in this series).
IV.3.3.2	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.	

Step	Procedure	Notes
IV.3.3.3	Repeat step 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.
IV.3.3.4	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 Appendix V.
IV.3.3.5	Neutralize the solution with sodium hydroxide (5N).	
IV.3.3.6	(a) If organophosphorus compounds not attacked by the nitric acid-sulphuric acid treatment but decomposable by persulphate are also present, proceed to section IV.2 at step IV.2.2.3.	
	(b) Otherwise transfer the solution quantitatively to a 50ml calibrated flask and dilute to the mark with water (note e). The colorimetric procedure described in Section A8 can now be applied exactly by taking V ml of this solution.	(e) The sample is restored to its original volume.

V Elimination of Arsenic Interference

V1. Introduction

When interfering substances are present or are formed during a pretreatment process (for instance, arsenite may be oxidised to arsenate) a suitable additional pretreatment should be incorporated at an appropriate stage of the analysis to eliminate or suppress the interference.

V2. Reduction of Arsenate to Arsenite

V2.1 Reagents

Prepare freshly as required. Do not store. See V2.2.

V2.1.1 14% V/V Sulphuric acid see A5.2.

V2.1.2 10% m/V Sodium metabisulphite

Dissolve 4.0±0.1 g of sodium metabisulphite in 40±1 ml of water. Use below.

V2.1.3 1% m/V Sodium thiosulphate

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

V2.1.4 Arsenate reducing reagent

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

V2.2 Hazard

The mixed reagent V2.1.4 evolves sulphur dioxide. Use in a fume hood, and flush away waste with copious amounts of water to clear sinks, traps and drains.

V2.3 Procedure

Step	Procedure	Notes
V2.3.1	To the sample, before dilution to volume in the respective pretreatment step, add 5 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 Appendix IV, this step is carried out prior to the final neutralization, which must also be carried out prior to volume adjustment.

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