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PHOSPHORUS SPECIATION IN WATER AND SEDIMENTS

(Technical Report)

Prepared for publication by
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Phosphorus speciation in water and sediments (Technical Report)

Abstract: Environmentally significant phosphorus species in water and phosphorus fractions in sediments are briefly discussed and the methods for their determination are described. One of the most critical analytical steps is the separation of the different forms which, after conversion into orthophosphates, may be determined by a multitude of various techniques. Spectrophotometric methods are often preferred for routine analysis. Several rapid automatic methods for the separation and determination of orthophosphate, linear polyphosphates, cyclic condensed phosphates and lower oxidation state anions of phosphorus, which may exist in natural and waste waters, have been developed. They are mainly based on the use of flow-injection analysis, high-performance liquid chromatography, including ion chromatography, capillary electrophoresis and a few other techniques. These methods have been described and critically evaluated.

INTRODUCTION

For most inland waters, phosphorus constitutes a limiting nutrient determining its biological productivity. This is due to the role of this element in the binding of carbon in living organic matter

$$106CO_2 + 16NO_3^- \; HPO_4^{2-} + 122H_2O + 18H^+ \underset{R}{\overset{P}{\rightleftarrows}} \; C_{106}H_{263}O_{110}N_{16}P + 138O_2$$

where P stands for photosynthesis and R for respiration [1].

This simply means that, if more phosphorus becomes available to a river, lake or ocean, more biomass is formed, which after its death will remove a corresponding amount of oxygen from the water, thus reducing the amount of oxygen available for higher organisms, such as fish.

Extended phosphorus input by over-fertilization of arable land and/or release from industrial and municipal sources (detergents) causes eutrophication and hence, following massive algal blooms, fish suffocation and other undesired effects.

Phosphorus is introduced into the aquatic environment in a number of different chemical forms, and has been described in general as being present in the aqueous phase as a small fraction of the total and in the solid phase as a large fraction of the total [2]. Each fraction is made up of a large number of different components, most of which may change between their dissolved or particulate state according to a dynamic equilibrium:

$$P_{\text{dissolved}} \rightleftarrows P_{\text{particulate}}$$

The most frequent soluble forms of phosphorus are orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) under the pH conditions normally encountered in natural waters and organic phosphorus compounds.

Orthophosphates are readily available for assimilation by organisms; they may, however, be removed from the dissolved phase by chemical precipitation with Al^{3+}, Fe^{3+} and Ca^{2+}.

According to Stumm and Baccini [1], hydroxyl-apatite and fluor-apatite might be the most stable solid phosphate compounds under aerobic conditions, normal pH (6–9) and a calcium concentration of 1×10^{-3} M.

Particulate and dissolved organic phosphorus forms undergo bacterial decomposition (mineralization) and the phosphorus is transferred into the soluble orthophosphate pool (Fig. 1).

Iron(III) phosphates or Fe(III) complexes, which absorb phosphorus, most obviously play an important role in the cycling of phosphorus in the environment. This is shown by the fact that, under anaerobic (reducing) conditions caused by massive phosphorus-induced algal blooms, phosphorus is released from

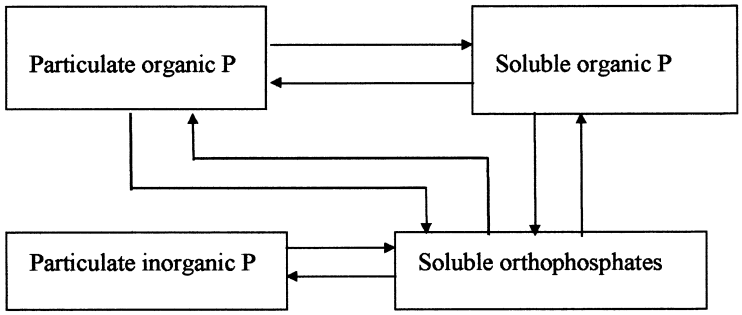

Fig. 1 Transfer cycle of phosphorus in aquatic systems.

the sediment to the overlying water. An accepted model is that phosphorus is mainly associated with amorphous hydrated ferric oxides [3–9]. The reduction of these oxides and the concomitant release of phosphorus accelerates eutrophication processes.

In nutrient-rich shallow lakes, the reduction of external phosphorus sources, for example by waste water treatment, was shown to be compensated by a net phosphorus flux from the sediment.

The extension of these fluxes does not depend simply on the total phosphorus concentration (the higher the phosphorus content, the higher the flux); they also depend on the chemical association between the phosphorus and the sediment.

The major quantity of the phosphorus that enters the aquatic systems does so in particulate form. Rock weathering releases a number of so-called accessory minerals, apatite being one of the most abundant in silicate rocks. Rock weathering also produces as reaction products a number of clay minerals with large adsorptive capacities [10], and orthophosphates as well as some organic phosphorus compounds might be sorbed on the surface of such particles and reach the lake bottom. Similarly, the formation of inorganic compounds such as hydroxyl-apatite upon settling $CaCO_3$ particles has been described [11].

The association of both orthophosphates and organic phosphorus compounds with iron, manganese and aluminum occurs as surface sorption on freshly precipitated oxyhydrate gels of these metals; post-depositional processes (diagenesis), however, produce a large number of well-defined chemical species, the most often encountered ones being vivianite $Fe_3(PO_4)_2 \times 8H_2O$ and ludlamite (Fe,Mn, $Mg)_3(PO_4)_2 \times 4H_2O$ [12,13]; others such as dufrenite $Fe_3^{2+} + Fe_6^{3+}[(OH)_3/PO_4]_4$, graftonite (Fe,Mn, $Ca)_3[PO_4]_2$, phosphoferrite $(Fe,Mn)_3[PO_4]_2 \times 3H_2O$, klinostrengite $FePO_4 \times 2H_2O$ and anapaite Ca_2Fe $[PO_4]_2 \times 4H_2O$ [14] are to be expected as well according to the local redox conditions.

A common feature of these compounds is that they will release their phosphorus content under anaerobic conditions. Strong pH increases have been shown to prompt phosphorus release as well [15]. Organic matter of allogenic or endogenic origin (structural elements of dead settling organisms) contain organic phosphate esters, which are hydrolysed by phosphatases [16] and are a continuous source of phosphorus to the water column (Table 1).

Some semiquantitative characteristics extracted from the pertinent literature suggest that:

- the content of inorganic phosphorus in sediments and water is considerably higher than that of organic phosphorus;
- the inorganic fraction consists mainly of non-apatitic phosphorus, but in sediments from calcareous regions apatitic phosphorus might be dominant;
- diagenetic processes seem to act towards a partial conversion of other phosphorus forms into apatitic phosphorus in deeper sediments, hence reducing phosphorus availability to remobilization through redox processes.

These considerations on the environmental behaviour of phosphorus highlight the need for a series of measurements which go beyond the simple determination of total phosphorus concentration in the water phase and in different solids encountered in aquatic systems.

Numerous methods for preconcentration, separation and determination of inorganic phosphorus

Table 1 Environmentally significant phosphorus fractions in water

Particulate	Total suspended phosphorus
	Suspended reactive phosphorus
	Suspended acid-hydrolysable phosphorus
	Suspended organic phosphorus
Dissolved	Total dissolved phosphorus
	Dissolved reactive phosphorus
	Dissolved acid-hydrolysable phosphorus
	Dissolved organic phosphorus

species have been developed. However, only a few of these techniques are used for phosphorus studies of natural waters [17,18] and sediments [19,20]. As in many other areas of environmental analytical chemistry, operationally defined methods have been developed for phosphorus analysis. These methods differ widely in the complexity of procedures, detection and resolution power, significance of obtained data and possibilities of interpretation.

WATER ANALYSIS

The most frequently determined phosphorus species include orthophosphates (reactive phosphorus) which respond to chemical reactions without previous hydrolysis or oxidation, condensed phosphates such as pyro-, meta- and other polyphosphates (Table 2) and organic phosphorus [21–24]. Tabulated are also some lower oxidation state compounds of phosphorus which may normally be detected only in waste waters.

Table 2 Oxo acids of phosphorus [21,23,24]

Formula	Abbreviation	Name of anion
H_3PO_4	P_1	Orthophosphate
Polyphosphates (linear condensed phosphates) $H_{n+2}P_nO_{3n+1}$		
$H_4P_2O_7$	P_2	Diphosphate
$H_5P_3O_{10}$	P_3	Triphosphate
$H_6P_4O_{13}$	P_4	Tetraphosphate
Cyclic condensed phosphates (metaphosphates) $(HPO_3)_n$		
$H_3P_3O_9$	P_{3m}	Trimetaphosphate
$H_4P_4O_{12}$	P_{4m}	Tetrametaphosphate
Lower oxidation states		
H_3PO_2	P^1	Phosphinate
H_3PO_3	P^3	Phosphonate
$H_4P_2O_6$	P^4-P^4	Hypophosphate
$H_4P_2O_7$	P^3-O-P^3	Diphosphonate
$H_4P_2O_6$	P^3-O-P^5	Isohypophosphate

The first and most critical analytical step is the separation of the different forms. After conversion into orthophosphates, they may be determined by a multitude of different techniques.

The separation between dissolved and particulate fractions is achieved by filtration through 0.45 μm membrane filters. It is now generally accepted that smaller particles do exist in every water sample and the interpretation of such separations should be made with caution. The analysis of the dissolved and particulate phosphorus forms should be made separately.

The conversion of different condensed phosphates, including higher molecular weight species such as hexametaphosphate, into orthophosphates is achieved by acid hydrolysis at 100 °C and the respective fraction of the total phosphorus is called hereafter ''acid-hydrolysable phosphorus'' [25,26]. The

procedure most often proposed [27] employs 1 mL of a maxiture of concentrated nitric acid and sulfuric acid for each 100 mL of water sample and gentle boiling for 90 min. Alternatively, heating for 30 min in an autoclave at 98–137 kPa is recommended [28].

Digestion of organic phosphorus and all other chemical forms that might be present and their subsequent conversion into orthophosphate, i.e. the destruction of all organic matter present in the sample, should be made on filtered samples and three essentially different methods are in use.

- Perchloric acid, heated to white fumes, is considered to be the most severe and effective oxidizing agent and is recommended for particularly organic-rich and inert organic carbon-containing samples. The sample should always be evaporated first with nitric acid; then a mixture of nitric and perchloric acid is added and evaporated to dense white fumes. Special hoods, abating perchloric acid fumes by water showering, are needed to avoid spontaneous reactions of perchloric acid with organic material.
- Nitric–sulfuric acid mineralization is recommended for most samples, evaporating the sample with a mixture of concentrated sulfuric and nitric acid (1:5, v/v), adding more nitric acid to the sample and evaporating excess nitric acid.
- Persulfate oxidation of sulfuric acid-treated samples is gaining much appreciation. The sample is acidified with 1 mL of sulfuric acid (volume fraction of 30%) for each 50 mL sample and 0.5 g of solid potassium or ammonium persulfate is added. The mixture is boiled for 40 min until a final volume of 10 mL is reached. Alternatively, heating the mixture in an autoclave at 98–137 kPa for 30 min is recommended [28].

Figures 2 and 3 present operationally defined schemes for the determination of several phosphorus species groups in water.

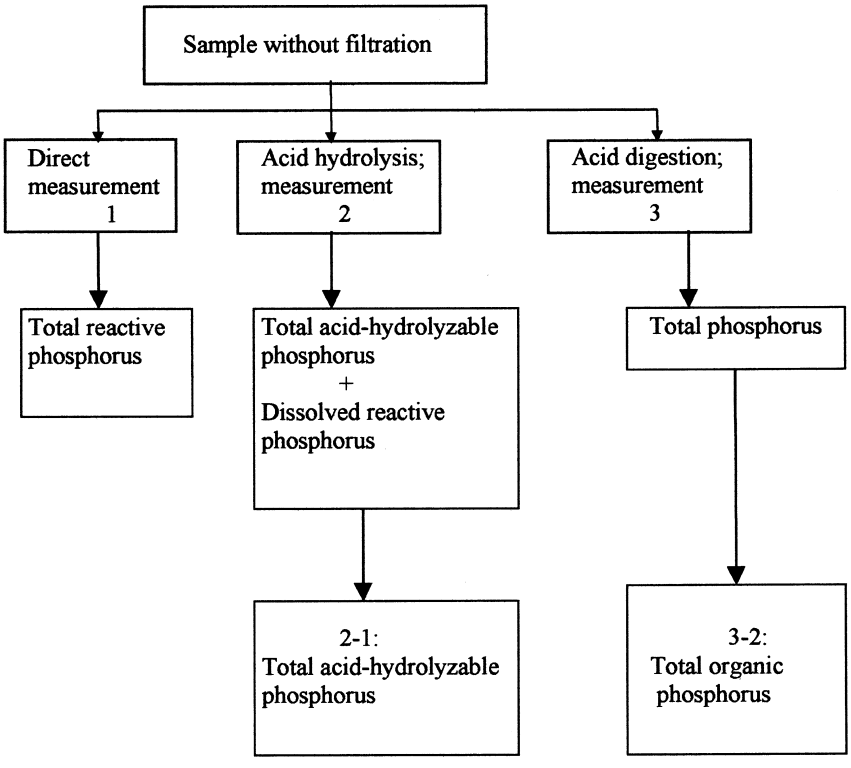

Fig. 2 Analytical scheme for phosphorus species determination in water (without sample filtration).

The final determination of orthophosphate in any digested or undigested sample can be performed by a wide choice of analytical techniques (Table 3). Spectrophotometric methods are usually preferred for routine analysis [18].

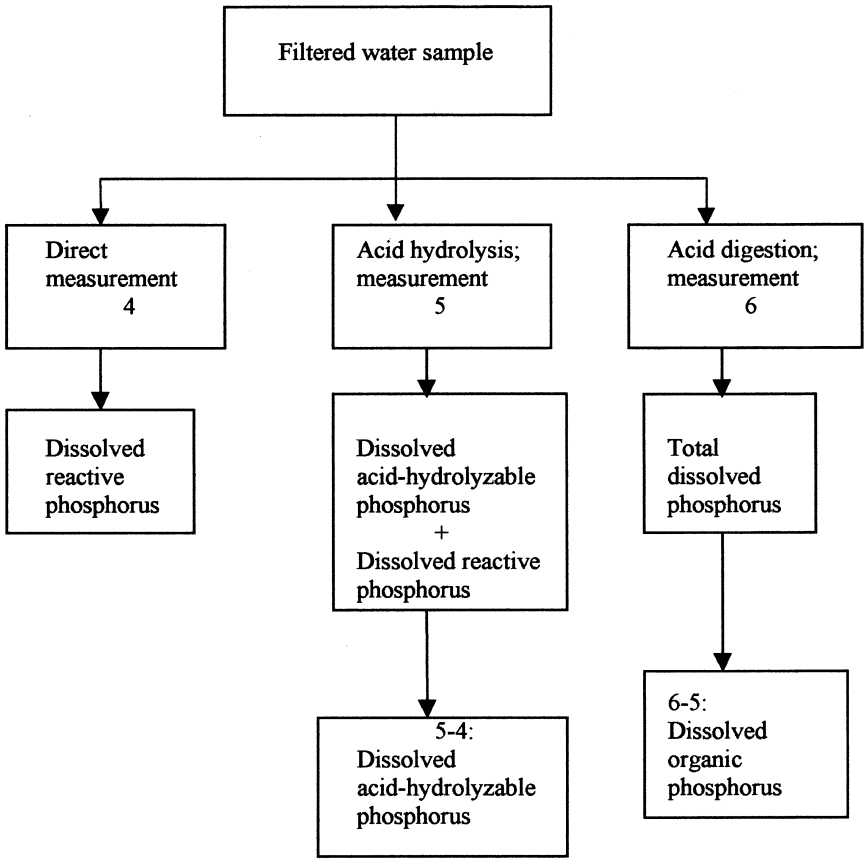

Fig. 3 Analytical scheme for phosphorus species determination in water (with sample filtration).

The most often used spectrophotometric techniques are the vanadomolybdophosphoric acid, the stannous chloride and the ascorbic acid techniques. All three methods are based upon the formation of heteropoly species of phosphoric acid with ammonium molybdate, the colour of which can be enhanced by adding vanadate (vanadomolybdophosphoric acid method), or by reducing the molybdophosphoric acid either by stannous chloride or ascorbic acid in the presence of potassium antimonyl tartrate (stannous chloride and ascorbic acid methods, respectively). The sensitivities of the three methods differ considerably (Table 4).

If increased sensitivity is needed or special interferences must be eliminated, the extraction of the molybdophosphoric acid into a benzene–isobutanol mixture or into another organic solvent is recommended [27].

Combinations of different digestion and final determination methods have been tested in interlaboratory trials (Tables 5–7).

Orthophosphate, linear polyphosphates, cyclic condensed phosphates and lower oxidation state anions of phosphorus, which may exist in natural and waste waters at detectable levels, can be separated by normal pressure ion exchange chromatography, paper, thin-layer or gel chromatography before determination [23,24,56].

During the last decade, several rapid automatic methods for the separation and determination of phosphorus species have been developed which meet the requirements of the environmental analyst of today. They are mainly based on the use of flow-injection analysis (FIA), ion chromatography (IC), high-performance liquid chromatography (HPLC) and a few other techniques. The methods mentioned and evaluated below have been successfully used for the speciation studies of water samples or can be recommended for the purpose.

In most cases, spectrophotometric detection of different heteropoly complexes of orthophosphate has

Table 3 Detection limits for phosphorus in the analysis of water samples

Technique	Detection limit (μg/L)	Sample	Reference
Spectrophotometry (SP)	1–100	River water	[29–31]
	2–5	Tap water	[30,32]
	0.005*	Sea and lake waters	[30]
	0.1–400	Natural and tap waters	[33–37]
X-ray fluorescence analysis (XRFA)	0.03*	River water	[38]
	0.006*	Natural waters	[39]
Flame photometry	30	Tap, river and lake waters	[40]
Voltammetry	1–3	Natural and waste waters	[30,40]
Amperometric titration	2000	Natural waters	[41]
Potentiometric titration	40	Natural waters	[42]
Inductively coupled plasma atomic emission spectrometry (ICP-AES)	1–20	Natural waters	[31,43,44]
	0.5–0.005*	River and sea waters	[30,45]
Inductively coupled plasma mass spectrometry (ICP-MS)	40	Natural waters	[46]
Electrothermal vaporization ICP-MS	0.3	Natural waters	[46,47]
Molecular fluorimetry	2–20	Natural and tap waters	[48,49]
Ion chromatography	10	Natural and tap waters	[44]
Liquid chromatography-SP	10	Natural waters	[50]
Flow-injection analysis (FIA)-SP	0.1–12	Natural waters	[30,51–55]

* After preconcentration.

Table 4 Concentration ranges for spectrophotometric methods

Method	Concentration range (μg/L)
Vanadomolybdophosphoric acid	0.1–20
Stannous chloride	0.007–2.0
Ascorbic acid	0.01–2.0

Table 5 Interlaboratory testing of spectrophotometric orthophosphate determination methods [28]

Method	Orthophosphate (μg/L)	No. of laboratories	Relative standard deviation (%)
Vanadomolybdophosphoric acid	0.1	45	75
	0.6	43	20
	7.0	44	9
Stannous chloride	0.1	45	26
	0.6	44	14
	7.0	45	8
Ascorbic acid	0.1	3	9
	0.6	3	4
	7.0	3	5

Table 6 Interlaboratory testing of polyphosphate analysis in water [28]

Method	Polyphosphate (μg/L)	No. of laboratories	Relative standard deviation (%)
Acid hydrolysis + vanadomolybdophosphoric acid	80	37	107
	300	38	67
	3000	37	36
Acid hydrolysis + stannous chloride	80	39	60
	300	36	48
	3000	38	37

Table 7 Interlaboratory testing of total phosphorus determination in water [28]

Method	Total phosphorus (μg/L)	No. of laboratories	Relative standard deviation (%)
Persulfate + vanadomolybdophosphoric acid	210	32	56
	990	32	24
	10230	31	7
Sulfuric–nitric acids + vanadomolybdophosphoric acid	210	23	66
	990	22	47
	10230	20	7
Perchloric acid + vanadomolybdophosphoric acid	210	4	34
	990	5	20
	10230	6	12
Persulfate + stannous chloride	210	29	28
	990	30	15
	10230	29	12
Sulfuric–nitric acids + stannous chloride	210	20	21
	990	17	9
	10230	19	8

been applied. Before detection, other phosphorus forms are converted to orthophosphate by hydrolysis, oxidation or other pretreatment processes.

The continuous flow analysis of P_1, P_2 and P_3 (see abbreviations in Table 2) by an air-segmented technique using a Technicon Auto Analyzer has been reported [57]. Although the air-segmented method was satisfactory with respect to reproducibility and sensitivity, there was a serious drawback in that the sample had to remain in the reaction coil for about 18 min to achieve the hydrolysis of P_n to P_1 and the colour reaction of the resultant P_1 with a molydenum reagent. Therefore, the authors have developed an FIA method [88] for the determination of P_1 and P_n (P_2 and P_3) using a strongly acidic solution containing Mo(V) and Mo(VI) as the carrier so that hydrolysis of polyphosphates and colour development of the resultant P_1 can be achieved simultaneously. Total amounts of inorganic polyphosphates can be determined at a rate of 45 samples per hour with a relative standard deviation of less than 1%.

An FIA system was also developed for the rapid analysis of lower oxidation state forms, such as P^1 and P^3 [59]. A sodium hydrogen sulfite solution was used as an oxidizing agent for P^1 and P^3, and a strongly acidic solution containing Mo(V) and Mo(VI) was used as a colour-forming reagent for the resultant orthophosphate. Lower oxidation state forms of phosphorus can be determined at a sampling rate of 60 samples per hour with a relative standard deviation of less than 1%. The system can be recommended as a post-column reaction detector for HPLC of P_1 and reduced forms of phosphorus. A parallel detection flow injection system was designed for the simultaneous determination of P(V) and P(III) oxo anions [60]. In the first analytical line, a sample containing P_1 and P_n anions was mixed with an Mo(V)–Mo(VI) reagent to

hydrolyse P_n to P_1 and to form a heteropoly blue complex of the resultant P_1. In the second line, the sample was mixed with the same reagent solution containing sulfite to oxidize the reduced forms for measuring the total phosphorus content. Separate determination of P_1, P_2, P_3 and different P(III) species is possible if the detector is combined with HPLC separation [61].

A method has been proposed for the spectrophotometric determination of P_1, P_2 and P_3 in the form of molybdenum blue species of P_1. P_2 is determined after the hydrolysis by pyrophosphohydrolase at 25 °C and pH 7, and P_3 is hydrolysed to form P_1 in 6 M HCl at 100 °C [62]. A Technicon Auto Analyzer has been used for the determination of P_1, P_2 and organic phosphorus in water samples [39]. Orthophosphate is determined by a molybdenum blue procedure. The total amount of inorganic phosphates is determined after sample hydrolysis and total phosphorus contents after UV sample photolysis and subsequent acid hydrolysis to form P_1. A combined method is recommended for the differential determination of phosphates and total phosphorus contents of waste waters using a Tecator FIA Analyzer for determining phosphates as a molybdovanadium complex and inductively coupled plasma atomic emission spectrometry (ICP-AES) for total phosphorus quantification [43].

The possibility was shown of determining P_1 and P_3 by FIA with a lead ion-selective electrode as detector [63]. A two-channel FIA system, in which the reagent stream contains lead perchlorate in aqueous ammonium acetate solution adjusted to pH 8, was used. Under these conditions, the predominant ions are HPO_4^{2-} in the case of orthophosphate, and $P_3O_{10}^{5-}$ and $HP_3O_{10}^{4-}$ in the case of triphosphate. P_3 and P_2 ions can also be determined in the presence of a large excess of P_1 ion by using a copper metal indicator electrode in an FIA system [64]. However, such a system has to be combined with a pre-separation unit.

A phosphorus-specific detection system was developed for IC determinations of polyphosphates (P_1, P_2, P_3) and a number of organophosphonates [65]. After the hydrolysis of P_n or oxidative decomposition of the phosphonates, the resultant P_1 is converted to the yellow vanadomolybdophosphate and measured photometrically. With KCl–ethylenediaminetetraacetic acid (EDTA) eluents, the resolving power is very high, which is of importance for speciation assessment studies. A single-column IC system with indirect UV detection was tested and used for the determination of condensed linear phosphates, P_2 to P_{13}, under isocratic conditions [66]. A method for the separation and detection of condensed phosphates in domestic waste waters using unsuppressed IC coupled with post-column FIA was reported [67]. The advantages of this technique over previously reported methods are its short analysis time (12 min per sample) and low detection limits (10, 20 and 20 mg/L for P_1, P_2 and P_3, respectively).

An IC method using suppressed condition detection was established for the simultaneous determination of orthophosphate, condensed phosphates P_2–P_4 and trimetaphosphate [68]. A method was developed for IC determination of $H_2PO_2^-$, HPO_3^{2-} and PO_4^{3-} in concentrations from 1 mg/L up to 10 mg/L in the presence of Cl^-, NO_3^- and SO_4^{2-} ions using mixtures of Na_2CO_3 and $NaHCO_3$ as eluents [44]. The method was tested on samples of raw water used for preparation of drinking water, river and rain waters. The water samples were filtered through membrane filters of 0.45 μm pore size and stored at 4 °C. Comparison of the results obtained with measurements using ICP-AES has shown the method reliability and its applicability to speciation studies. IC enables the determination of P^1, P^2 and P^3 along with Cl^-, NO_3^- and SO_4^{2-} ions using indirect UV detection [69]. The determination is performed in a flow of 4-amino-2-hydroxybenzoic acid solution with detection limits of 1.5 mg/L. A refractometric detector may also be used in IC studies of phosphorus species [70].

Phosphonate and orthophosphonate can be determined after separation by HPLC using a post-column reaction with a vanadomolybdate (the detection limits are about 4 ng calculated as phosphorus) [71]. The separation of P_1, P_2, P_3, mixtures of polyphosphates with average degrees of polymerization of 5 and 10; and P_{3m}, P_{4m}, P_{6m} and other linear and cyclic condensed phosphate ions by HPLC combined with an automatic phosphate analyser was described [72]. The detection system was composed of a heating bath for the hydrolysis of condensed phosphate, a heating bath for the colour development of molybdophosphate heteropoly blue and a colorimeter. More than 30 kinds of condensed phosphates were separated by use of a Hitachi 2630 ion-exchange resin, sodium chloride solutions containing Na_4EDTA as eluent and a gradient elution technique.

If a sample to be analysed contains a complex mixture of polyphosphates, it is usually recommended that gradient elution, by which more than 20 components can be separated, is employed. On the other

hand, more rapid isocratic elution becomes advantageous in the routine analysis of not too complex samples [73]. An isocratic elution technique was developed for the analysis of P_1, P_2, P_3 and P_4 by HPLC with an anion-exchange column and a post-column detector. The technique was applied to the investigation of phosphorus compounds, but it can also be recommended for the analysis of samples from industrial, biological and environmental sources [73]. Six polyphosphates (from P_1 to P_6) can be effectively separated and detected at an elevated column temperature using a similar technique [74]. More complicated mixtures should be analysed under gradient elution conditions. The possibility was also shown of using size-exclusion chromatography for the separation of P_1, P_2, P_3 and other phosphorus oxo acids [75].

Spectrophotometric detectors have proved to be efficient in the described HPLC studies of phosphorus species. A phosphorus-sensitive molecular emission cavity analysis (MECA) detector for HPLC is also of interest [76]. The detection of phosphorus from the emission of HPO molecules in a hydrogen–nitrogen flame, applied to the determination of organophosphorus substances, may be used for the speciation studies of inorganic oxo compounds as well.

Oxo anions of phosphorus can be separated by capillary isotachophoresis [23]. For example, P_1, P_2 and some cyclic phosphates [77] and various reduced phosphorus forms containing two phosphorus atoms (P^2, P^3-O-P^5, P^3-O-P^3) [78] were separated and determined for about 20 min. Twelve polyphosphates and some polyphosphonates were separated and detected photometrically by capillary electrophoresis using ribonucleotide electrolytes with the detection limits quite comparable to those obtained with commonly used HPLC methods [79]. Capillary gel electrophoresis with indirect photometric detection can also be used for the separation of orthophosphate and condensed phosphates [80]. However, further investigations seem to be required before these techniques may be recommended for speciation studies of water samples. First of all, interferences from metal cations, which can complex phosphorus anions in water and compensate their charge, and therefore affect the mobilities, should be examined.

Metal cations and some anions forming mixed heteropoly species (dichromate, silicate, arsenate, etc.) can also interfere with the spectrophotometric detection of orthophosphate [24]. The interferences can be minimized by using a chromatographic phase which allows both the pre-separation of the species to be determined from other elements and their separation from each other. Such a phase may be based on organotin extractants (e.g. dioctyltin or dinonyltin dichloride) which are very selective to phosphate ions. Extraction–chromatographic separation of P_1 and P_2 anions on an inert support modified with an organotin extractant was used for their subsequent determination in a flow-injection system [55]. The proposed method was tested on synthetic solutions containing Cu(II), Fe(III), Si(IV), As(V), Cr(VI) and used for the analysis of river water samples. Only comparable amounts of arsenate interfered with the determination. Countercurrent chromatography with the same reagents combined with a flow-injection detector can also be applied to the determination of phosphate ions [81].

Direct speciation analysis of phosphorus is possible by Fourier transform ^{31}P nuclear magnetic resonance (NMR) studies. The technique was used for the determination of P_1, P_2 and P_3 species in water [82]. The possibility was also shown of determining P_1, P_2, P_{3m} and P_{4m} [83]. Unfortunately, the method is not sensitive enough for trace analysis of environmental samples.

The methods for determining orthophosphate and total phosphorus at mg/mL and mg/L levels are well established and successfully applied to water samples. Simultaneous determination of orthophosphate, total linear and cyclic condensed anions of P(V), total reduced species and total organic phosphorus is also no serious problem. FIA with photometric detection seems to be the most developed technique for the purpose. Further progress may be connected with the use of atomic emission, molecular emission and other spectroscopic detection systems.

The speciation of individual phosphorus compounds requires the application of HPLC, IC and other kinds of liquid chromatography. Unfortunately, only a few papers have referred to the analysis of real samples using chromatographic techniques. Further studies are necessary to check their applicability to the speciation of phosphorus compounds in water.

PARTICULATE PHOSPHORUS FORMS

Although for some special purposes unfiltered water samples are analysed, filtration through 0.45 μm membrane filters should be the rule. Highly complicated analytical schemes start with the analysis

without filtration and, after the determination of total reactive, total acid-hydrolysable and total organic phosphorus, proceed with the determination of the corresponding species in filtered samples in order to obtain the concentration of suspended reactive, suspended acid-hydrolysable and suspended organic phosphorus by difference. Such schemes suffer from the usual problems associated with subtracting small values from large ones, each carrying different systematic and random errors.

The often poor representativeness of subsampling ''particle suspensions in water'' adds further uncertainty, and independent analysis of either filter-deposited or centrifuged solids is therefore preferred.

The determination of total suspended phosphorus can be easily achieved after the mineralization of the particles and the filter together, provided that the blank values of the filters are sufficiently low. Mineralization can be achieved using either the nitric–sulfuric acid mixture (kjeldahlization) or the persulfate method, preferentially performed in autoclaves.

A valid alternative is the mineralization of particle-loaded cellulose filters using the nitric acid pressure-digestion technique [84]. The determination of further suspended phosphorus fractions requires additional filters and, for the determination of both the acid-hydrolysable and suspended organic phosphorus, the use of glass fibre filters is recommended.

It should be noted that, depending on the phosphorus forms present and their quantitative proportions, acid hydrolysis will to some extent result in mobilization of some phosphorus belonging to the ''organic'' fraction.

DETERMINATION OF PHOSPHORUS FRACTIONS IN SEDIMENTS

The phosphorus fractions of environmental interest (Table 8) present in sediments and similar materials are normally determined following operationally defined procedures. These were most often developed in the context of eutrophication studies and generally suffer from a lack of validation. They range from simple one-step procedures to rather complex sequential leaching schemes.

Table 8 Phosphorus fractions in sediments and their significance

Phosphorus fraction	Significance
1. Total phosphorus	Indicative of total phosphorus burden
2. Adsorbed phosphorus	Easily available fraction
3. Non-apatitic phosphorus	Available phosphorus fraction in oxygen absence
4. Apatitic phosphorus	Relatively stable and inert phosphorus fraction
5. Organic phosphorus	Slowly but continuously available fraction
6. Residual phosphorus	Not available under environmental conditions

The simple sulfuric acid dissolution procedure (0.5 M H_2SO_4, 16 h at 25 °C) releases, besides carbonate-associated and easily available surface-bound forms, variable amounts of iron/manganese/aluminum-bound phosphorus depending on the ageing of the respective compounds.

Metha *et al*. [85] used sequential leaching by hydrochloric acid and sodium hydroxide (Fig. 4). The method has been found to mobilize large amounts of organic phosphorus up to 60%, ascribed to saponification of phospholipids at 85 °C, [88], as already suspected by Frink [87].

More complex and correspondingly sensitive to analytical errors is the scheme proposed by Williams *et al*. [88–90] and shown in Fig. 5. Phosphorus associated with amorphous iron oxyhydrates is mobilized by a mildly reducing agent such as sodium dithionite (solution 1); in the subsequent step, distinct iron, manganese and aluminum phosphate phases are destroyed and orthophosphate ions set free (solution 2) and the sequence is concluded by dissolving apatitic phosphorus (solution 3). Solutions 1, 2 and 3 are analysed for their orthophosphate content, and the total phosphorus obtained from solutions 1 and 2 yields the concentration of non-apatitic inorganic phosphorus (NAI-P).

The sum of apatitic and non-apatitic inorganic phosphorus is subtracted from the total phosphorus concentration in order to obtain the organic phosphorus concentration.

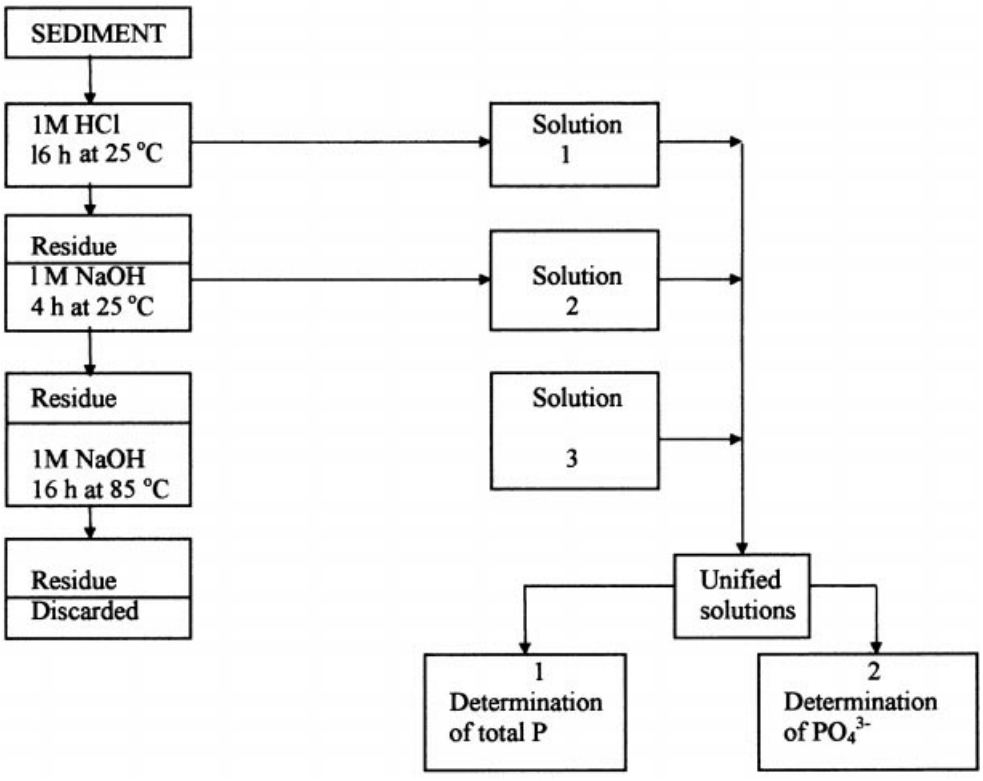

Fig. 4 Sequential leaching scheme for the determination of phosphorus species groups in sediment [85]. Fractions 1–2 = organic phosphorus fraction.

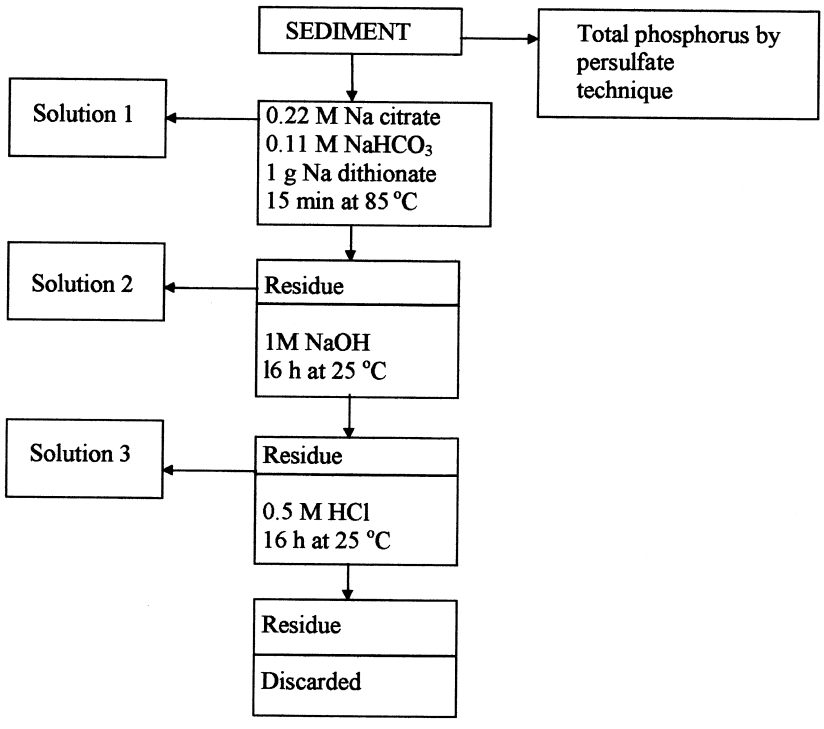

Fig. 5 Sequential leaching scheme for the determination of phosphorus species groups in sediment [88].

The direct comparison of the methods of Metha and Williams shows an acceptable agreement for total inorganic phosphorus, but organic phosphorus data differ largely [27].

Hieltjes and Lijklema [91] use a scheme similar to that of Williams, except that the sediment is shaken with ammonium chloride solution (0.1 M) in order to obtain an estimate of ''very loosely bound'' sorbed phosphorus.

The work under the auspices of the Commission of the European Communities led quite recently to the harmonization of extraction techniques for the analysis of metal fractions in sediments and soil [92]. Many different techniques at present in use were scrutinized and a commonly agreed procedure, thoroughly tested in interlaboratory trials, was agreed upon. This work will be backed up in the future by appropriate certified sediment reference materials.

The method, although developed for metals, appears also to be convenient for phosphorus speciation assessment, hitting essentially the same target fractions of the sediment. It has been tested with quite satisfactory results [93].

The procedure (Fig. 6) consists of extracting the sediment by acetic acid (0.11 M) yielding an estimate of surface-bound and carbonate-associated phosphorus; this is followed by reducing iron(III) compounds by hydroxylammonium chloride at pH 2 (hydrochloric acid); the organic matter of the sediment is destroyed in the next step and the associated phosphorus set free. The whole procedure is completed by an independent measurement of the total phosphorus concentration by a non-destructive method, e.g. X-ray fluorescence spectrometry, which allows the determination of significant amounts of inert rock-derived phosphates should they exist.

There is some overlap possible between fractions 2 and 3, some of the apatitic crystals remaining intact during step 2 and being dissolved along with the organic matter. The procedure is faster than those of Metha, Williams and Hieltjes, so that more samples may be processed per time unit, thereby enhancing the representativeness of the obtained data with respect to the area.

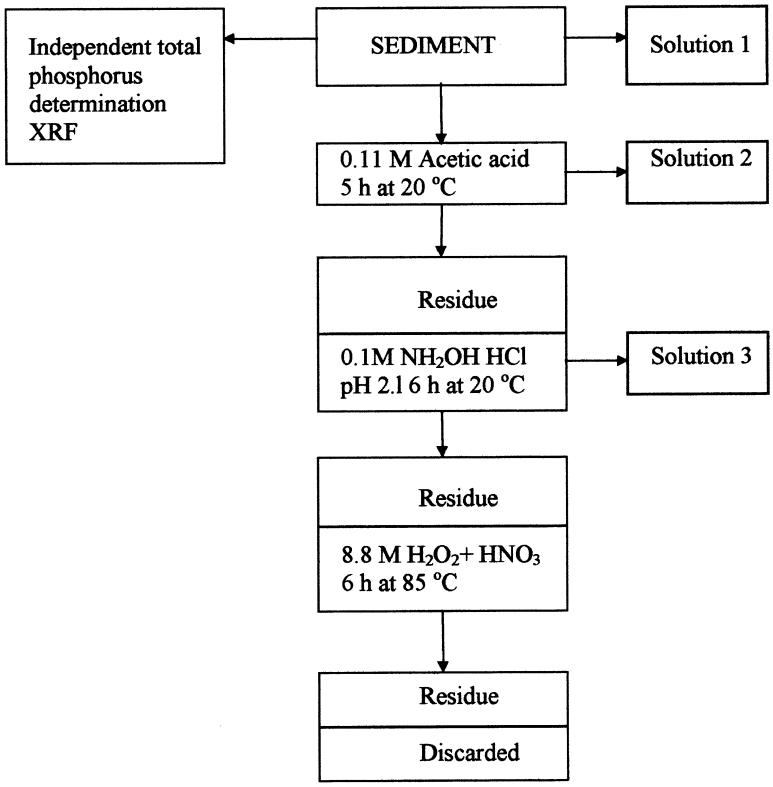

Fig. 6 Sequential leaching scheme ''CEC Common Procedures'' [92].

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