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Review

Ion chromatographic separations of phosphorus species: a review

V. Ruiz-Calero, M.T. Galceran *

Departament de Química Analítica, Universitat de Barcelona, Martí i Franqués 1-11, 08028 Barcelona, Spain

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Abstract

The aim of this paper is to review recent literature regarding the determination of phosphorus species by ion chromatography (IC), and describe the implementation of new developments in sample treatment and ion chromatography methodology for the analysis of these compounds. Ion-exchange methods using both carbonate/hydrogencarbonate and hydroxide selective columns in combination with self-regenerating membrane and solid-phase-based suppressors enable determination of phosphate down to ppb levels. New technology, particularly on-line electrolytic hydroxide generators and electrolytic self-regenerating suppressor devices, has allowed the use of elution gradients in both carbonate/hydrogencarbonate and hydroxide selective systems, improving sensitivity and reducing total analysis time for samples containing phosphate together with other inorganic anions. In addition to a review of these developments, optimization and application of chromatographic methods using reversed stationary phases and cationic and/or zwitterionic surfactants is also discussed.

The objective of most of the IC methods developed for phosphorus species is the determination of phosphate and total phosphorus. Therefore, sample treatment and separation conditions specifically developed for this purpose are also described. In addition, application of IC to the analysis of other inorganic (reduced and condensed) and organic (phytates, alkyl phosphate, and phosphonates) phosphorus species is discussed along with methodology and relevant applications in water analysis and other miscellaneous fields.

Keywords: Ion chromatography; Phosphorus; Phosphate determination

Contents

Abbreviations: AES, anion electrolytic suppressor; AMMS, anion micromembrane suppressor; ANN, artificial neural network; ASRS, anion self-regenerating suppressor; CTA, cetyltrimethylammonium; DBP, dibutyl phosphate; DDA, didodecyldimethylammonium; EIC, electrostatic ion chromatography; ERIS, electrochemically-regenerated ion suppressor; ESI-MS, electrospray ionization-mass spectrometry; EVB/DBV, ethylvinylbenzene/divinylbenzene; FIA, flow injection analysis; FID-IC, flow injection dialysis-ion chromatography; IC, ion chromatography; ICP-MS, inductively coupled plasma-mass spectrometry; InsP6, phytate; InsP6–InsP1; IP, inositol hexakis- to mono-phosphates; LOD, limit of detection; LOQ, limit of quantitation; MBP, monobutyl phosphate; MES, 2-(*N*-morpholino)ethanesulfonic acid; MFP, monofluorophosphate; MOPS, 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid; MPA, molybdophosphoric acid; MTA, myristyltrimethylammonium; PGC, porous graphitized carbon; SCAN, sample concentration and neutralization processor; SPME, solid phase microextraction; TBA, tetrabutylammonium; TBP, tributyl phosphate; TRIS, tris(hydroxymethyl)aminomethane; TTA, tetradecyltrimethylammonium; VPD, vapour phase digestion

* Corresponding author. Tel.: +34 93 4021275; fax: +34 93 4021233.
E-mail address: galceran@apolo.qui.ub.es (M.T. Galceran).

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1. Introduction

Phosphorus, a key nutrient in certain living organisms, such as plants and microorganisms, is involved in several biological and environmental processes. Phosphates [P(V)], the most abundant form of phosphorus in the environment, are readily available for assimilation, and for this reason, they have traditionally been used as fertilisers. In addition, phosphate and other phosphorus compounds are widely employed as detergents and food additives, among other uses [1,2]. Extensive input of phosphorus by overfertilization, and by industrial and domestic wastewater pollution result in overabundance in aquatic media [3]. Consequently, monitoring of phosphorus content in natural and wastewaters is essential to control and avoid eutrophication of the aquatic environment. Although, in addition to orthophosphate, several phosphorus species, such as polymeric inorganic and organic phosphorus compounds, can be found in the environment, analysis is commonly performed as phosphate [4,5]. Acid hydrolysis using sulfuric-nitric acid mixtures and digestion procedures, mainly by means of persulfate oxidation, transform condensed and organic phosphorus species to orthophosphate, allowing determination of total phosphorus content. The final step, which involves determination of orthophosphate, may be performed using various techniques, commonly by spectrometry and ion chromatography (IC). In addition to environmental studies, determination of phosphates and total phosphorus content is also important in other areas, including food and plants analysis. For this purpose, ion chromatography has been extensively applied [6–9].

Since its introduction by Small et al. [10], ion chromatography has become the method of choice for the analysis of anions in a wide variety of samples. Since then, the term 'ion chromatography', which was initially used to define a chromatographic method for ion analysis using ion-exchange columns and suppressed conductivity detection, has evolved substantially. Nowadays, ion chromatography is also performed using non-suppressed conductivity detection. Non-suppressed IC was developed by Gjerde et al. as an alternative approach to ion analysis using low conductivity eluents [11]. Currently, in addition to ion-exchange, 'ion chromatography' encompasses various chromatographic systems for ion determination, such as ion-exclusion, ion-interaction, and the novel electrostatic ion chromatography (EIC). Moreover, in addition to conductimetry, which is by far the most common detection technique in IC, other techniques, such as UV (indirect or post-column), refractive index, evaporative laser light scattering, and inductively coupled plasma and atmospheric pressure ionisation mass spectrometry, have also been combined with IC separations [12].

Although IC methodology for routine phosphate determination is well established, and has even been included in regulatory and standard methods, research in this field remains active [13,14]. The implementation of recent advances, such as the development of new suppressors, on-line eluent generators, and special columns, to the development of new IC methods for the determination of phosphate and other common anions has been directed towards the improvement of sensitivity and selectivity in ion chromatography. Thus, various papers deal with the optimization and comparison of anion separations using new technology, and its further application to complex samples, enabling the resolution of compromised separations with enhanced detection limits. Recent technical advances and applications of ion chromatography in various analytical fields have been periodically reviewed. Most of these reviews have been published in the yearly special issues of the *Journal of Chromatography* devoted to the International Ion Chromatography Congress. In particular, reviews focused on new developments in ion chromatography [12,15], particularly in suppressor [16] and column technology [17], and applications in different fields [6–9] are highly recommended.

This review surveys ion chromatography methods for the determination of phosphorus species, addressing methods and applications published since 1997. The focus of the revision is centered on the determination of phosphate and total phosphorus, which is the objective of most of the IC methods developed for phosphorus species. However, IC applications

for the analysis of other inorganic and organic phosphorus compounds will also be discussed. In addition, new developments in sample treatment procedures aimed at avoiding interference and converting phosphorus species into phosphate, and methodology for the determination of phosphate based on anion-exchange in both suppressed and non-suppressed conductivity conditions, and in modified reversed phase columns have been reviewed. Recent research in the field of electrostatic ion chromatography is also included. Finally, applications of IC to the determination of phosphate in complex mixtures, such as environmental and food matrices, are addressed along with IC methods for the analysis of other natural and anthropogenic phosphorus species.

2. Sample treatment

Although this review is focused on the separation of phosphorus species by ion chromatography, some comments about classical and recently developed sample treatment procedures are included, since optimal separation conditions frequently depend on the complexity of the injected sample. The general objective of most sample treatment procedures is to simplify the separation and also to improve conditions for detection. Nevertheless, it must be mentioned that with the latest advances in ion chromatography technology, particularly in columns and suppressors, preseparation operations are now simpler or even unnecessary.

In the analysis of phosphorus species, the sample treatment to be applied frequently depends on the purpose of the determination. For instance, for the determination of inorganic non-condensed phosphorus species, sample treatment procedures are commonly orientated towards removal of interfering anions and preconcentration of phosphate [9]. In contrast, for total phosphorus determination, digestion is necessary in order to convert organic and inorganic condensed phosphorus to orthophosphate [4,18,19]. In addition, and depending on the digestion procedure, further treatment may be necessary.

2.1. Sample digestion for total phosphorus analysis

One of the more popular applications of ion chromatography for phosphate analysis is the determination of total phosphorus in environmental, food, and plant samples. Since phosphorus is currently analysed as orthophosphate, a prechromatographic step is required to transform all forms of phosphorus into this species before injection into the chromatographic system. The standard methods for phosphorus speciation [5] indicate that condensed inorganic phosphorus and small fractions of organic phosphorus can be hydrolysed by mixtures of sulfuric–nitric acid. However, digestion with oxidising reagents is required for the quantitative determination of organic phosphorus forms [4,5].

Thermal acidic persulfate digestion is the oxidation procedure that has been traditionally used as the initial step in the determination of total phosphorus [18]. Nevertheless, during the digestion process decomposition of persulfate occurs, producing high concentrations of sulfate that interfere with the separation of phosphate by ion chromatography, and as a consequence, elimination of sulfate by means of on-line or off-line strategies is required. Persulfate digestion is frequently used as a pretreatment if it is necessary to perform simultaneous determination of total nitrogen and total phosphorus. One of the advantages of using ion chromatography for this kind of determination is that a combination of one digestion and one chromatographic separation method is sufficient for the analysis. Digestion under alkaline conditions is commonly performed because under these conditions oxidation of chloride to chlorate, which interferes with nitrate determination by IC, especially in sea water samples, is minimised. However, the ratio of hydroxide to persulfate must be controlled to achieve the acidic conditions during the digestion process that ensure quantitative conversion of phosphorus species to orthophosphate [20,21]. Even with the use of optimal digestion conditions for the simultaneous determination of both total nitrogen and phosphorus, the use of an adequate chromatographic column, such as the IonPac AS9-HC, is mandatory in order to obtain a satisfactory resolution between the interfering chlorate and the nitrate [21].

Some recently published methods use microwaves for the digestion, taking advantage of the improved performance of this technology over that of conventional heating procedures. Microwaves heat the solution directly, reducing temperature gradients and accelerating the speed of heating. Microwave-assisted persulfate digestion has been applied to the simultaneous determination of phosphorus and nitrogen in wastewater, with a total digestion time of only 30 min. In this case, to remove the sulfate generated in the digestion, a column switching system was developed [22]. Other agents, such as hydrogen peroxide (22%, v/v) under acidic conditions, have also been proposed for the oxidation of phosphorus, nitrogen, and sulfur species to their respective oxoanions using microwave-assisted digestion. For instance, Colina and Gardiner [23], using two 40-min digestion cycles, obtained recoveries in the range of 88–117% for nitrogen, phosphorus, and sulfur species in a sediment reference material. The combination of microwave and UV photoradiation has proved to be an advantageous approach for complete photo-oxidation of organic phosphate species without the use of chemical oxidizing reagents, although to our knowledge, this combination has not been applied to the treatment of samples analysed by IC. One advantage of this technique is that a rapid photo-decomposition of organophosphate compounds occurs; only 3–5 min are needed using a microwave-excited UV lamp. However, this approach was not efficient in converting condensed phosphorus species to phosphate, and an additional acid hydrolysis was required [24]. Without the use of microwave heating, UV photolysis in conjunction with an oxidizing reagent (22% hydrogen peroxide) has also been proposed for the removal of the organic matrix from various

samples (plant extracts, edible vegetal oils, fats, and milk) [25–27]. A digestion procedure based on contact of the sample with Pt pieces has been developed for the analysis of traces of phosphate and other inorganic anions in hydrogen peroxide matrices [28]. This platinum decomposition procedure reduced the peroxide level to ca. 50–200 ppm, enabling determination of the trace anions with satisfactory recoveries. In the case of phosphate, a recovery of 98% was reported [28].

2.2. Elimination of interference

The analysis of phosphate in real samples by IC has posed a significant challenge, due to the presence of high concentrations of interfering anions, such as chloride, nitrate, and sulfate. In order to eliminate or reduce the content of these interfering anions in the chromatographic separation and improve phosphate determination, several off-line and on-line approaches have been developed.

During the early 1990s, the development of off-line methodology to accomplish these objectives was an important focus of ion chromatography research. Ion exchange resins that were funtionalized with Ag^+ and Ba^{2+} were successfully used for the removal of high concentrations of chloride and sulfate, respectively [29]. Although these strategies are currently in use, recent methodology is now based principally on on-line systems [9]. Nevertheless, it is worth mentioning that with the increasing development of high capacity (HC) anion exchange columns and the possibility of applying gradient elution to IC, an improvement of the resolution between compounds in compromised separations can be accomplished in single column systems without the need for additional elimination of interfering ions.

2.2.1. Off-line procedures

Off-line operations are performed before IC separations. For instance, filtration of liquid samples or extracts to remove particulate matter and high-molecular mass organic compounds is a mandatory step. Moreover, several approaches are frequently applied to reduce interference arising from the presence of high amounts of other anions. The simplest one is sample dilution. In this case, although the concentration ratio is not affected, the risk of column capacity overloading is reduced, and hence, resolution is improved at the expense of higher detection limits. For instance, dilution is currently performed for the determination of phosphate and other anions in chloride-rich samples such as sea water and wastewater before being injected into the IC system. High concentrations of certain metal cations also have an adverse effect on anion chromatography of phosphate. Ding and Mou [30] studied the influence of the concentration of alkaline, alkaline earth, and trivalent cations (Al^{3+} and Fe^{3+}) on phosphate determination, showing that the most significant effects were produced by high concentrations of trivalent metal ions. For instance, the presence of 10 mg L^{-1} Fe^{3+} produced a 33–91% decrease in the peak areas of phosphate at concentrations ranging from 1 to 10 mg L^{-1}. In contrast, phosphate peak areas increased slightly if Al^{3+} was present at the same concentration. In both cases, retention times of phosphate decreased. These authors also demonstrated that acidification of the sample to pH 2 reduces the influence of Fe, whilst precipitation of Al and Fe in basic solution was proposed in order to avoid interference.

It is commonly considered that chromatographic interference can occur between close eluting analytes. In the case of phosphate analysis by ion-exchange chromatography, this ion elutes at retention times higher than nitrate and before sulfate. Consequently, high concentrations of both anions can cause peak overlapping. In these cases elimination or reduction of the concentration of these interfering anions, generally using on-line coupling systems, is mandatory. Very high concentrations of chloride, as in the case of sea water samples, can also interfere with phosphate determination, even though it elutes at lower retention times [31,32]. Moreover, the response of phosphate in suppressed conductivity systems shows a significant decrease in the presence of high concentrations of chloride and other strong acid anions. This is probably due to the weak acid characteristics of phosphate in combination with the presence of high amounts of ions in the matrix. In the suppressor, all the influent cations are exchanged for H^+. When high concentrations of matrix anions elute, a large amount of exchanged H^+ cannot be neutralised by the eluent anions and they co-elute. Under these conditions, phosphate forms strong ion couples with hydronium ions, which results in a reduction of the charge of phosphate, thus decreasing its conductivity [31,32].

An interesting example of the use of the elimination of chloride ions is the work of Asada and Oikawa [33], who coupled a desalting cell and a dialysis cell on-line to remove high concentrations of chloride and filtrate in the sample prior to the determination of sulfate and phosphate in deep subsurface waters. The performance of this method was compared with the elimination of chloride using silver-form cation-exchange cartridges. A monovalent-anion-permselective membrane was used in the desalting cell to enable chloride removal from the samples, whilst multicharged anions, such as phosphate and sulfate, were sent to the dialysis cell. The dialysates were injected in a suppressed ion chromatography system. Recovery of sulfate and phosphate, evaluated at a concentration of 10 mg L^{-1}, was about 80–90% for concentrations of chloride ions lower than 10 mg L^{-1}, and approximately 100% for chloride concentrations higher than 20 mg L^{-1}. The limit of detection for phosphate was 500 μg L^{-1}, which was not low enough for use in diluted subsurface water.

A preconcentration method based on the formation of molybdophosphoric acid (MPA) has been published by Tikhomirova et al. [34]. In the first step, MPA was generated by addition of ammonium molybdate. Then, preconcentration of MPA as ion pairs with tetrabutylammonium (TBA) bromide was performed. The MPA–TBA ion pairs were passed in solution through a cartridge filled with glass

wool, from which they were desorbed with acetonitrile. The resulting ion pairs were analysed in a reversed-phase column with a mobile phase containing 0.2 mM TBA, and UV detected at 320 nm. The detection limit for phosphorus in the preconcentration from 100 mL of solution was 6.7 mg L^{-1} [34].

2.2.2. On-line procedures

2.2.2.1. Dialysis. The application of on-line dialysis systems for the removal of complex matrix components, such as proteins, surfactants, particulates, and high molecular mass organic compounds, has been reported by several authors. For instance, an automated on-line microdialysis-ion chromatography system has been developed for the determination of phosphate and other inorganic anions in olive oil mill wastewater [35]. In this approach, the sample is pumped into the dialysis cell in which the anions diffuse into an acceptor stream of water. The dialysate is then transferred to the injection loop and injected into the IC system. The authors evaluated the recoveries of the dialysis process by spiking wastewater samples and oil emulsions at different concentrations (25 and 50 μg L^{-1}), and found that phosphate recovery was 100% after 10 min at a sample flow-rate of 1.0 mL min^{-1}. Coupling of flow injection dialysis (FID) with ion chromatography has also been proposed for the simultaneous determination of phosphate and other common inorganic anions [36]. In this case, the sample is injected into a donor stream containing 22 mM Na_2CO_3 and 28 mM $NaHCO_3$, and flows into the dialysis cell. Then, the acceptor stream of water containing the sample anions flows to the injection valve and is partially injected into an IC system with suppressed conductivity detection. The scheme of the FID-IC system is shown in Fig. 1. An on-line dialysis-IC configuration in which the sample is continuously pumped into the dialysis cell was also used and compared with the previous system. The authors claim that the FID-IC method offers a number of advantages over the on-line dialysis-IC configuration, such as smaller sample volume (only microliter), and shorter total analysis time. Moreover, risk of clogging is minimised, as less sample passes through the dialysis cell. In addition, the authors indicate that a better detection limit was obtained for phosphate with FID-IC (0.37 mg L^{-1}) than with on-line dialysis-IC (0.97 mg L^{-1}), although this is still substantially poorer than that of conventional IC (0.08 mg L^{-1}).

2.2.2.2. Column-switching systems. Chromatographic configurations involving the use of two or more columns for on-line cleanup and/or preconcentration before the determinant chromatographic separation are commonly defined as coupled column systems. If, as frequently occurs, the columns are connected by manual or automatic switching valves that allow the modification of the eluent direction, these coupled systems are called column-switching systems.

Heart-cut column switching has been employed for the determination of phosphate and other trace anions in the presence of a high concentration chloride-matrix. This technology allows pre-separated chloride in the first column to be diverted to waste while the fraction containing the anions is transferred into the analytical separation column [31,37,38]. For instance, Dähllof et al. [37] applied this method to the determination of nitrate and phosphate in sea water. Two carbonate/hydrogencarbonate solutions at different concentrations were used as eluents. Chloride was separated from the rest of the anions in the precolumns with the weak eluent and eluted to the waste. Then, the weak eluent was replaced with the stronger one and the valve was switched to allow elution of nitrate and phosphate to the separation column. Linearity and limits of detection were calculated using standards in a sodium chloride solution to simulate marine water. The phosphate detection limit was in the order of 95 μg L^{-1}, and linearity to 2.8 mg L^{-1} was demonstrated. However, it should be noted that acceptable reproducibility of the results from heart-cut column switching methods is not easily obtained, and requires the accurate establishment of the column-switching time windows, taking into account the variation of the retention times with the concentration of the interfering anion in the matrix. Huang et al. [31] proposed a simplified technology that allows the easy determination of time windows by direct injection of a standard solution (10 mg L^{-1}) of the analyte. The authors developed a model for the retention behaviour of trace anions in the presence of high concentrations of co-eluent anions, and demonstrated that the effect of the matrix can be suppressed with the use of high concentration eluents. The system used by this author consisted of

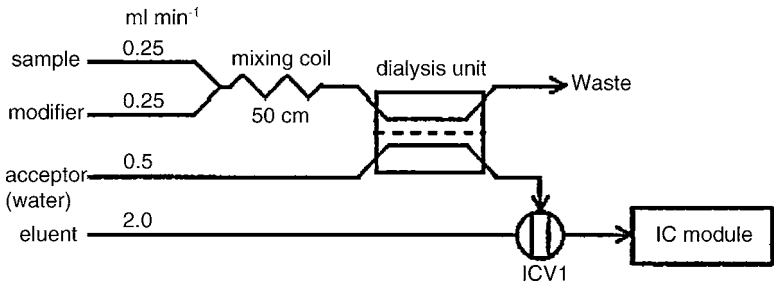

Fig. 1. Manifold for flow injection dialysis-ion chromatography (FID-IC). Donor/modifier (FID system) and eluent (IC system): 22 mM Na_2CO_3, 28 mM $NaHCO_3$; acceptor: water. IC system: anion separation column Lachat EPA 300A and suppressed conductivity detection with chemically regenerated suppressor column (250 mM sulfuric acid). Reproduced from Ref. [36] with permission from Elsevier.

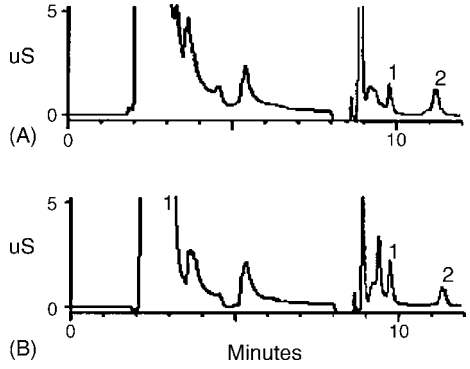

Fig. 2. Nitrate and phosphate determination on a heart-cut column-switching system. Separation system: AG11 precolumn and AS11 column. Concentration system: AG11-HC column. Eluent: 25 mM NaOH; flow rate: 1.0 mL min^{-1}; suppressed conductivity detection (ASRS-I suppressor in external water mode). Peak identification: 1 = nitrate, 2 = phosphate. (A) Mixed standard solution of 0.5 mg L^{-1} nitrate and 0.5 mg L^{-1} phosphate; matrix: 5000 mg L^{-1} chloride and 250 mg L^{-1} sulfate. (B) Sea water sample after 50 times dilution. Reproduced from Ref. [31] with permission from Elsevier.

a guard, a separation column, and a concentrator column. A 25 mM NaOH solution was used as eluent and conductivity suppression was achieved with an ASRS-I micromembrane suppressor in external water mode. By means of two valves, the elution fractions of the separation column corresponding to phosphate and nitrate time windows were collected onto the concentrator column. Once the majority of the matrix and strongly retained anions were eluted from the separation column to waste, the isolated nitrate and phosphate were transferred to the separation column. Chromatograms of a mixed standard solution containing 0.5 mg L^{-1} phosphate (matrix: 5000 μg mL^{-1} chloride and 250 μg mL^{-1} sulfate), and a diluted sea water sample acquired in the column-switching system are provided in Fig. 2. As can be seen in both chromatograms, using the column-switching technology, chloride and sulfate did not interfere at such high concentrations with the determination of phosphate and nitrate. Good correlation coefficients (>0.993) were obtained for phosphate in samples with chloride and sulfate at concentrations of 2000 and 100 mg L^{-1}, respectively. However, the response of phosphate varied with the concentration of the chloride and sulfate matrix. The increased concentration of these two anions in the matrix caused a decrease in the slope of the phosphate calibration curve, which indicates that a percentage of phosphate was lost from the concentration column at high concentrations. Excess exchanged protons, which are generated in the suppressor due to the high concentration of cations in the sample matrix, cannot be neutralised, and form proton-phosphate associations. The formation of these ion pairs results in the reduction of phosphate charge, and its consequent loss from the concentration column that collects the effluent fraction within the time windows in which phosphate elutes from the initial separation system. The developed system was applied to the determination of nitrate in sea water samples, but phosphate could not be determined due to interference by the contaminating phosphate present in the reagents, which was also concentrated in the concentration column.

Another example of the application of a column switching procedure is the chromatographic setup developed by Bruno et al. [38] for the determination of phosphate, nitrate, and nitrite in chloride-rich waters. The proposed arrangement contained a preseparation system composed of two in-line anion-exchange precolumns connected to an analytical column via a four way pneumatic valve. The use of two high capacity precolumns instead of only one allowed the separation of chloride and the remaining anions to be improved, and prevented overloading. Most chloride is separated in the precolumn system and eluted to waste. By switching the valve, residual chloride and the remaining anions are transferred to the analytical column. A phosphate detection limit of 1000 μg L^{-1} was reported using suppressed conductivity detection, a value that was not low enough to detect phosphate in sea water samples.

Recently, a coupled ion chromatography system was developed to determine phosphate, chloride, and sulfate as contaminants of concentrated nitric acid [39]. Two different separation and preconcentration columns were used along with two different eluents (NaOH and carbonate/hydrogencarbonate). Preseparation of the analytes from nitrate was carried out in a specially designed high-capacity anion exchange column with high retention for nitrate and low retention for the analytes. The stationary phase was prepared by functionalization of a PS/DVB copolymer with 5-bromo-1-pentene and *N*-methyldiethanolamine. The eluent containing the analytes was transferred to the second system, consisting of a preconcentration and a separation column, where they were separated using a carbonate/hydrogencarbonate eluent prior to quantification. The detection limit of phosphate was 5 μg L^{-1}.

2.2.2.3. Other systems. Solid-phase microextraction, a technique rarely used for inorganic anion analysis, has also been applied to phosphate extraction and preconcentration. Here, in-tube solid-phase microextraction was coupled to suppressed conductivity ion chromatography for the analysis of various common inorganic anions. The open tubular capillary was coated with polypyrrole, a conducting polymer that acted as an anion exchanger, providing high extraction efficiency. A 17-fold increase in the response was reported for phosphate when the method was applied to a 10 ppm standard solution. This preconcentration enabled the detection of phosphate in a tap water sample at mg L^{-1} level [40].

On-line sample preparation techniques for the removal of matrix interference, and neutralization of acidic and basic samples using electrochemically-regenerated ion suppressor (ERIS) and neutralizer cells have also been proposed [41]. Two systems have been developed based on the use of columns and cells with anion and cation resin: an ERIS suppressor and sample concentration and neutralization (SCAN) processors. Determination of phosphate and other inorganic anions in a rock fusion sample was performed by direct in-

jection of untreated sample into the SCAN sample processor. On-line neutralization enabled the artefact produced by hydroxide excess to be decreased, allowing determination of the sample anions in a suppressed carbonate/hydrogencarbonate selective system. This device can be also applied to the on-line preconcentration of trace level samples. The on-line sample concentration technique uses the detector effluent to carry the sample to the concentrator column without the need of a sample pump. The authors reported a calculated detection limit of 69 ng L^{-1} for phosphate using on-line sample concentration.

3. Chromatographic systems

Chromatographic analysis of phosphate, as well as common inorganic anions, has traditionally been performed using ion-exchange columns with alkaline eluents (carbonate/hydrogencarbonate solutions or alkaline hydroxides) and conductivity detection [9,17]. These systems have been established as the method of choice for routine applications, and a large number of regulatory and official methods involve the use of this chromatography configuration [13,14]. Extensive innovations in the field of ion chromatography technology have appeared in recent years, the development of new stationary phases and suppressor systems being the most significant of these [15–17,42–44]. Implementation of these new technologies in the analysis of phosphate and other inorganic anions provides more efficient separations and lower detection limits. Another focus of research in ion chromatography is the development of methods for the analysis of anions in modified reversed-phase columns. Depending on the modification, two different separation chromatographic modes are obtained: ion-interaction chromatography and electrostatic ion chromatography [45,46]. Applications of both modes to phosphate analysis are described in the following section.

Other stationary phases, such as non-modified porous graphitic carbon columns (Hypercarb S), have also been proposed for the separation of common inorganic anions [47]. In this case, retention is due to donor-acceptor electronic interactions between the lone pair electrons of the anions and the π-electrons of the PGC. Phosphate, as $H_2PO_4^-$, is the most poorly retained anion in this column, but separation between phosphate and chloride could not be accomplished. Moreover, LODs reported by the authors using an evaporative light scattering detector (ELSD) were higher than those obtained in conductivity detection, ranging from 10 to 50 μg mL^{-1}.

3.1. Ion-exchange chromatography

In ion-exchange chromatography, both the eluent and the stationary phase characteristics are highly dependent upon whether a suppressor device is used before the detection step. For this reason, ion chromatography systems are usually classified as suppressed or non-suppressed conductivity methods. Better detection limits and more robust conditions are obtained with suppressed modes. Therefore, the vast majority of the methods that have been developed for anion determination involve suppressed conductivity detection, those applied to phosphate being no exception. Recently described separation conditions (columns, eluents, and suppressors) for phosphate analysis in anion-exchange columns are covered in this section. Methods are described that have been developed specifically for this analysis, as well as those aimed at general inorganic anions that have also been applied to this objective. Experimental conditions and limits of detection are given in Table 1.

3.1.1. Suppressed conductivity detection

3.1.1.1. Columns. Ion-exchange chromatography of phosphate is usually carried out on polymer-based stationary phases, fewer applications having been developed on silica-based anion exchangers [9,17]. Most of the literature reviewed here describes the use of polymeric resins or latex-agglomerated anion-exchangers obtained from styrene/divinylbenzene copolymers or polymethacrylate supports. In comparison to resin exchangers, latex-agglomerated anion-exchangers are more efficient due to their pellicular structure. These latest stationary phases consist of a sulfonated polymeric substrate to the surface of which fully aminated porous polymer beads are attracted by electrostatic and van der Waals interactions. Selectivity of these columns can be altered by modification of the exchanger groups of the latex particles and the degree of cross-linking. During the last decade, the number of commercially available columns has increased considerably. Columns for universal anion separation, as well as those designed for specific applications, have been used in phosphate analysis. Bipolymeric resin-based columns, such as IonPac AS14 and AS15, and a large number of latex-agglomerate columns (IonPac AS4-SC, AS7, AS9, AS10, AS11, AS12, AS16, and AS17, among others) have been used. The characteristics of these modern stationary phases for ion chromatography have been comprehensively reviewed by Weiss and Jensen [17].

Depending on the composition of the ion-exchanger, and thus the selectivity of the column, separations are carried out using hydrogencarbonate/carbonate, or sodium or potassium hydroxide eluents. In principle, the use of hydroxides offers greater sensitivity than carbonate-based eluents, as the former are converted to water by the suppressor, which results in low background conductance, whilst carbonate eluent suppression produces carbonic acid. However, hydroxide is a monovalent anion and has poor selectivity for anion-exchange functional groups [43]. Therefore, high concentrations are required to elute highly retained anions, and as a consequence, separation selectivity can be better optimized in hydrogencarbonate/carbonate eluents. These buffered solutions contain both monovalent and divalent anions, showing high selectivity for anion-exchanger moieties. Thus, carbonate/hydrogencarbonate is a strong eluent, and lower concentrations than those for hydroxide are currently used. The

Table 1
Determination of orthophosphate by ion-exchange chromatography

Column	Mobile phase	Flow rate (mL min^{-1})	Detection	LOD[a]	Comments	Sample	Concentration	Reference
Carbonate/hydrogencarbonate systems								
IonPac AG14A/AS14A	8.0 mM Na_2CO_3 1.0 mM $NaHCO_3$	1 (4 mm) 0.5 (3 mm)	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode) AES (recycle mode) AMM (displacement chemical regeneration mode)	15.6 μg L^{-1} (ASRS) 10.8 μg L^{-1} (AES) 10.2 μg L^{-1} (AMMS)	Column and suppressor comparison	Standards, spiked environmental waters, spiked soil extract	10 mg L^{-1}	[48]
IonPac AG4A-SC/AS4A-SC	1.8 mM Na_2CO_3 1.7 mM $NaHCO_3$	2		17.8 μg L^{-1} (ASRS)				
IonPac AG14/AS14	3.5 mM Na_2CO_3 1.0 mM $NaHCO_3$	1.2		12.3 μg L^{-1} (ASRS)				
IonPac AG4A-SC/AS4A-SC IonPac AG17/AS17	1.8 mM Na_2CO_3 1.7 mM $NaHCO_3$ Electrolytically generated KOH gradient (1–40 mM)	2	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode)	17.8 μg L^{-1} 12.3 μg L^{-1}	Column comparison	Standard, spiked environmental waters, spiked soil extract	10 mg L^{-1}	[49]
IonPac AG4A-SC/AS4A	1.7 mM Na_2CO_3 1.8 mM $NaHCO_3$	1	Suppressed conductivity ASRS-I-4 mm			Sugar beet seed exudate	388 mg L^{-1}	[50]
IonPac AS4A-SC	1.8 mM Na_2CO_3 1.7 mM $NaHCO_3$	2	Suppressed conductivity AMMS-II ESI-MS			Standards	20 mg L^{-1}	[51]
IonPac AG9-HC/AS9-HC	28 mM Na_2CO_3	1.5–1.0	Suppressed conductivity AES	1.98 μg L^{-1} (AES)	System comparison	Standards	0.3–500 mg L^{-1}	[52]
	11.5 mM Na_2CO_3	1	ASRS-Ultra-4 mm (recycle mode)	0.77 μg L^{-1} (ASRS)				
IonPac AG9-SC/AS9-SC	1.8 mM Na_2CO_3 1.7 mM $NaHCO_3$	1	Suppressed conductivity AERS-II (external water mode)		Column comparison	Standards, drinking water	0.1–0.25 mg L^{-1}	[53]
IonPac AG9-HC/AS9-HC	9 mM Na_2CO_3	1						
IonPac AG12A/AS12A	2.7 mM Na_2CO_3 0.3 mM $NaHCO_3$	1	Suppressed conductivity ASRS-I-4 mm (recycle mode)			Standards, plant extracts	7.8–80.6 mg g^{-1} (dry plant tissue)	[54]

Table 1 (*Continued*)

Column	Mobile phase	Flow rate (mL min^{-1})	Detection	LOD[a]	Comments	Sample	Concentration	Reference
IonPac AG12/AS12	11 mM $(NH_4)_2CO_3$ pH 11.2	2	ICP-MS	36 μg L^{-1}		Standards	10–1000 μg L^{-1}	[55]
QS-A5G/QS-A5	10 mM Na_2CO_3 1 mM $NaHCO_3$	Not reported	Suppressed conductivity QE-A1 cartridge	0.006 mg L^{-1} HPO_4^{2-}–P	IC-FIA	Wastewater	0.41 mg L^{-1} HPO_4^{2-}–P	[57]
Shodex IC SI-904E	1 mM Na_2CO_3 2 mM $NaHCO_3$	1	Suppressed conductivity 753 Suppressor module	500 μg L^{-1}	Dialysis	Standards, spiked deep subsurface water	1 mg L^{-1}	[33]
Concentration col.: Metrosep A PCC 1 HC Separation col.: Star-Ion-A300	3.75 mM Na_2CO_3 3.6 mM $NaHCO_3$	0.5	Suppressed conductivity (Metrohm MSM)	0.14 μg L^{-1}	Coupled IC system	Ultrapure water		[58]
Metrosep Anion Dual 2	1.3 mM Na_2CO_3 2.0 mM $NaHCO_3$	0.8	Suppressed conductivity MSM suppressor	10 μg L^{-1}	On-line microdialysis-IC UV photolysis	Olive-oil mill wastewater, spiked standard oil emulsions	298–605 mg L^{-1}	[35]
IonPac AG9-HC	9 mM Na_2CO_3	1	Suppressed Conductivity DS-Plus or ASRS suppressors	1.4 μg L^{-1} (P)	System comparison	Standards	10 mg L^{-1}	[42]
IonPac AS4A-SC	[Na_2CO_3] + [$NaHCO_3$] = 5 mM [Na_2CO_3] = 44–100%	1.5						
Novosep A-1 Allsep A-2 IonPac AS4A-SC	Different carbonate gradient (0–20 mM Na_2CO_3)	1–1.5	Suppressed conductivity DS-Plus or ASRS suppressors		System comparison	Anions, wastewater, coffee, fermentation broth	3–10 mg L^{-1}	[43]
IonPac AG4-SC/AS4-SC	Initial: 2 mM $NaHCO_3$ Different KOH gradients (1–30 mM)	2	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode)		Carbonate gradient (KOH generated)	Anion standard	130 mg L^{-1}	[64]
IonPac AG4/AS4A	3 mM $NaCO_3$ 1.0–20.0 mM NaOH	1.5	Suppressed conductivity AMMS		Retention modelling	Standards	10 mg L^{-1}	[73]
IonPac AS4A-SC	35 Elution systems: [HCO_3^-] + [CO_3^{2-}]: 2–6 mM; [CO_3^{2-}]%: 10–90%	2	Suppressed conductivity ASRS-1		Retention modelling	Standards	2–200 mg L^{-1}	[74]

IonPac AS4A/AS4A-SC	2.97 mM Na_2CO_3 2.80 mM $NaHCO_3$	1.6	Suppressed conductivity AMMS-II	LOQ: 0.1 mg L^{-1} (P)	Column-switching, microwave digestion	Wastewater (urban, industrial, river)	0.21–0.82 mg L^{-1} (P)	[22]
IonPac AG4A/AS4A	1.8 mM Na_2CO_3 2.80 mM $NaHCO_3$	1	Suppressed conductivity AMMS-II	0.251 mg L^{-1} 0.012 % (w/w, sediment)	Microwave digestion	Standards: pyrophosphate, buffalo river sediment	9.78–31.8 mg L^{-1} (P) 0.0888 % (w/w, sediment)	[23]
IonPac AG12/AS12	2.7 mM Na_2CO_3 0.3 mM $NaHCO_3$	1.5	Suppressed conductivity ASRS	25 μg L^{-1}	UV photolysis	Plant material	1120–3720 mg kg^{-1} (P)	[25]
IonPac AG9/AS9	2.0 mM Na_2CO_3 0.75 mM $NaHCO_3$	1	Suppressed conductivity ASRS	35 μg kg^{-1} ($P–PO_4$)	UV photolysis	Edible vegetable oils and fats	<10–530,250 μg kg^{-1} (P)	[26]
IonPac AG14/AS14	3.5 mM Na_2CO_3 1.0 mM $NaHCO_3$	1.2	Suppressed conductivity ASRS	25 μg L^{-1}	UV photolysis	Milk	10,550 mg kg^{-1} (P)	[27]
IonPac AG14/AS14	3.5 mM Na_2CO_3 1.0 mM $NaHCO_3$	1.2	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode)		Effect of metal ions on anion response	Standards with variable pH and metal ions concentration	1–10 mg L^{-1}	[30]
Lachat anion guard column Lachat anion separation column EPA 300 A	2.8 mM Na_2CO_3 2.2 mM $NaHCO_3$	2	Suppressed conductivity	0.37 mg L^{-1} 0.97 mg L^{-1}	FIA-IC On-line dialysis-IC	Standards	1–50 mg L^{-1}	[36]
IonPac AS4A (2)/AS4A	Na_2CO_3–$NaHCO_3$ eluents: Eluent 1: pH 9.16; *I*: 1.4 mM Eluent 2: pH 9.5; *I*: 20 mM t_1: 0.4 min, t_2: 1.4 min	3	Suppressed conductivity AMMS	95 μg mL^{-1}	Column switching In-line precolumns (2)	Standards, sea water	95–950 μg mL^{-1}	[37]
IonPac AG9-HC (2)/AS9-HC	Pre-separation guard columns: 14 mM Na_2CO_3 3 mM $NaHCO_3$	1	Suppressed conductivity ASRS-Ultra-4 mm	1000 μg L^{-1}	Column switching In-line precolumns (2)	Standards, Cl-rich water (20,000 mg L^{-1} Cl^-), spiked sea water	852 μg L^{-1}	[38]

Table 1 (*Continued*)

Column	Mobile phase	Flow rate (mL min^{-1})	Detection	LOD[a]	Comments	Sample	Concentration	Reference
	Separation: 9 mM Na_2CO_3							
System 1: self-made PS/DVB functionalized DEMA	System 1: 100 mM NaOH	1	Suppressed conductivity (Metrohm 753 or 793 suppressor modules)	5 μg L^{-1} (system) 5 mg L^{-1} (nitric acid)	Coupled IC systems	Nitric acid	10–1000 μg L^{-1}	[39]
System 2: concentration col.: IonPac TAC-2 Separation col.: Metrohm ASupp 5	System 2: 2.5 mM Na_2CO_3 0.5 mM $NaHCO_3$	0.7						
IonPac AS14	3.5 mM Na_2CO_3 1 mM $NaHCO_3$	1	Suppressed conductivity ASRS-Ultra-4 mm (external water mode)		Solid phase microextraction (SPME)	Standards, tap water	10 ppm	[40]
Sarasep AN1 Allsep Anion	1.8 mM Na_2CO_3 1.7 mM $NaHCO_3$	1	Suppressed conductivity Alltech ERIS	69 ng L^{-1}	Sample concentration and neutralization: SCAN Sample Processor	Standards, rock fusion sample	5–15 ng mL^{-1} 723 mg L^{-1}	[41]
IonPac AS14	3.5 mM Na_2CO_3 1.0 mM $NaHCO_3$		Suppressed conductivity			Surface water ship channel	0.56–1.88 mg L^{-1}	[93]
IonPac AG9-HC/AS9-HC	9 mM Na_2CO_3	1	Suppressed conductivity ASRS-I			Standards, drinking water, sea water	25 μg L^{-1}	[94]
IonPac AG5/AS5	2.2 mM Na_2CO_3 2.8 mM $NaHCO_3$	2	Suppressed conductivity ASRS			Corrosion products in phosphoric acid treated lead pipes	10–75 mg g^{-1}	[95]
IonPac AG9-SC/AS-9 SC	2.0 mM Na_2CO_3 0.75 mM $NaHCO_3$	2	Suppressed conductivity AMMS-II		Coupled IC system	Degradation patinas on stones, mortars, historic buildings, monuments	<1–773 μg g^{-1}	[96]
IonPac AG4A-SC (2)/AS4A-SC	1.6 mM Na_2CO_3 1.5 mM $NaHCO_3$	1						
IonPac AG9/AS9	0.75 mM Na_2CO_3 5.5 mM $NaHCO_3$	1	Suppressed conductivity ASRS	1.3 mg L^{-1}		Lead-acid battery electrolyte	<1.30–4.95 mg L^{-1}	[99]

Hydroxide systems								
IonPac AS10	NaOH gradient (50–124 mM NaOH)	1	Suppressed conductivity: AMMS (4 mm)		Column comparison	High nitrate concentration samples (molten glass, diluted nitric acid)	Molten glass: 16.8 mg L^{-1} Spiked standards: 459 μg g^{-1}	[63]
IonPac AG10/AS10	NaOH gradient (10–200 mM NaOH)	0.25	AMMS (4 mm)					
IonPac AG15/AS15 (with Anion Trap Columns)	Electrolytically generated KOH gradient (48–100 mM)	0.2	ASRS (2 mm)	120 μg L^{-1}				
IonPac AG15/AS15	Electrolytically generated KOH gradient (7–40 mM)	0.5	Suppressed conductivity AES (recycle mode)	0.079 μg L^{-1}		Power plant water samples	0.78–1600 μg L^{-1}	[65]
IonPac AG11/AS11	NaOH gradients (0.5–40 mM NaOH)	0.25	Suppressed conductivity ASRS-Ultra-2 mm (external water mode)	0.8 μg L^{-1}	Column comparison	Standards, 30% hydrogen peroxide	30 μg L^{-1} standards	[66]
IonPac AG15/AS15		0.40		2.9 μg L^{-1}				
IonPac AG15/AS15	NaOH gradients. (10–40 mM NaOH)	0.4	Suppressed conductivity ASRS-Ultra-2 mm (external water mode)	1.0 μg L^{-1}	Matrix digestion	Standards, 30% hydrogen peroxide	30 μg L^{-1} standards	[28]
IonPac AS11	12 /32 mM NaOH 7.0 mM Na_2CO_3 1 mM $NaHCO_3$	0.3	Suppressed conductivity AMMS-II		Study of the effect of temperature (27–60 °C), system comparison	Standards	0.95 mg L^{-1}	[67]
IonPac AS14	3.5 mM Na_2CO_3 1 mM $NaHCO_3$ 32 mM NaOH	0.3						

Table 1 (*Continued*)

Column	Mobile phase	Flow rate (mL min^{-1})	Detection	LOD[a]	Comments	Sample	Concentration	Reference
Cryptand 2.2.2.	Capacity gradients (2.2.2.) 10 mM NaOH → 10 mM LiOH or H_2O	0.5	Suppressed conductivity ASRS-Ultra-2 mm (external water mode)		Anionic impurities removed by Anion Trap Column, (CR-ATC)	Standards, tap water	15 mg L^{-1}	[69]
Cryptand 2.2.1.	Capacity gradients (2.2.1.) 8 mM KOH → 10 mM LiOH or H_2O							
IonPac AS11	Electrolytically generated KOH gradients (3.720–27.84 mM)	1	Suppressed conductivity ASRS-II		Retention modelling, artificial neural network (ANN). Anionic impurities removed by Anion Trap Column, (ATC-1)	Standards	0.5–10 mg L^{-1}	[70] [71]
IonPac AG15/AS15	Electrolytically generated KOH gradient (25–60 mM)	1.0–1.95	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode)		Retention modelling, artificial neural network (ANN)	Standards	30 mg L^{-1}	[72]
Custom-made aminated PS-DVB high capacity resin	20 Ternary elution systems: $HClO_4$ (0–20 mM) NaOH (20–100 mM) $NaCO_3$ (0–20 mM)	1	Suppressed conductivity ASRS (external mode)		Experimental design for characterization of elution system	Standards	10 mg L^{-1}	[75]
Concentration column: IonPac AG11-HC Separation column: IonPac AG11/AS11	25 mM NaOH	1	Suppressed conductivity ASRS-I-4 mm (external water mode)		Column-switching	Standards	2.5–100 μg L^{-1}	[31]
IonPac AS11	NaOH gradients Eluent A: water Eluent B: 200 mM NaOH Eluent C: 0.75 mM NaOH Initial: A (50%)/C (50%) 7.0 min: A (85%)/B (15%) 7.1 min: A (50%)/C (50%)	2	Suppressed conductivity			Landfill leachate	149.15 mg L^{-1}	[90]

IonPac AG11/AS11	21 mM NaOH		Suppressed conductivity ASRS-I-4 mm	0.1 mg L^{-1} $H_2PO_4^-$		Groundwater	<0.1 mg L^{-1} $H_2PO_4^-$	[91]
IonPac AG16/AS16A	Electrolytically generated KOH gradient (1.5–55 mM)	1	Suppressed conductivity ASRS-Ultra (recycle and external water mode)			Standards, groundwater	μg L^{-1} levels	[92]
IonPac AG17/AS17	20 mM NaOH	1	Suppressed conductivity ASRS-Ultra-4 mm (recycle mode)	0.05 μg g^{-1}	Vapour phase digestion (VPD)	Purified quartz	0.73–7.72 μg g^{-1} (P)	[100]
IonPac AG17/AS17	15 mM NaOH	1	Suppressed conductivity ASRS	8 ng g^{-1}	Vapour phase matrix elimination (VPME)	Boric acid	92 ng g^{-1}	[101]
Non-suppressed								
Non-suppressed IC:								
Hamilton PRP-X100	4 mM *p*-hydroxybenzoic acid (pH 8.5), 2.5% MeOH	1	Non-suppressed conductivity	0.8 mg L^{-1}	System comparison	Standards, cane sugar liquors	50–89 mg L^{-1} (cane juice), 0.30% (molasse)	[59]
Suppressed IC:								
Metrosep Anion Dual 1	2.5 mM Na_2CO_3 2.4 mM $NaHCO_3$	0.5	Suppressed conductivity (Metrohm 761 IC)	0.05 mg L^{-1}				
Hamilton PRP-X110	0.6 mM hydrogen-phthalate (pH 4), 4% (v/v) ACN	1	Non-suppressed conductivity	4.1 mg L^{-1}		Coffee, tea	1.54–2.97 mg g^{-1} (dry sample)	[77]
Hamilton PRP-X100 Vydac 302 IC	Sodium phthalate (1–4 mM) pH (4.0–6.0)	Not reported	Non-suppressed conductivity		Retention modelling	Standards		[78]
Waters IC Pak A Anion Dual 1	1 mM *o*-phthalic acid-TRIS buffer, 2 % (v/v) ACN (pH 8)	0.8	Suppressed conductivity Indirect UV (273 nm)		System comparison	Spiked drinking water	4.9 mg L^{-1}	[79]

[a] As phosphate.

performance of different anion-exchangers with regard to the separation of phosphate and other inorganic anions has been discussed by some authors [48,49]. In general, a high number of carbonate selective columns (AS4A, AS4A-SC, AS4-SC, AS5, AS9, AS9-HC, AS9, AS12A, AS14, and AS14A) have been used for phosphate analysis. The A-columns are versions of the original column with smaller particle diameter (5 μm), HC standing for the high capacity and SC for the solvent compatible versions. The concentration of the eluent required depends on the capacity of the column, total carbonate concentrations ranging from 2.75 to 11 mM having been used, depending of the characteristics of the column and the specific separation. Among these columns, the latex-based anion-exchange column (IonPac AS4A) with a carbonate-hydrogencarbonate eluent and suppressed conductivity detection has been frequently used since its proposal in Method 300.0 of the US Enviromental Protection Agency (EPA) for the determination of inorganic anions, phosphate among them, in environmental waters and aqueous solid extracts [13]. Nevertheless, a significant number of columns with higher efficiencies have been developed for specific separations, and their use has generally been proposed to improve the method [48,49]. Separations in the AS4A column and its solvent compatible version, IonPac AS4A-SC, are performed with eluents at low concentration, in the order of 1.7 mM carbonate/1.8 mM hydrogencarbonate [50]. Both columns have the same capacity (20 μequiv col^{-1}), but differ in the degree of crosslinking of the particle cores. Solvent compatibility of the SC column is accomplished by a higher EVB-DVB crosslinking (55%), and high hydrophilicity is caused by the alkanol quaternary ammonium functionalized latex. These two columns are applied to common anion determination and phosphate elutes in approximately 8–10 min, between nitrate and sulfate [48]. The AS4A-SC was proposed by Corr and Anacleto [51] for the analysis of phosphate by IC with mass spectrometry detection (IC-MS). Detection was performed using a single-quadrupole mass analyser with prior on-line conductivity suppression using an anion micromembrane suppressor. Co-elution of orthophosphate with selenite was resolved due to the specificity of mass spectrometry, as orthophosphate was detected at *m*/*z* 97 as doubly protonated $H_2PO_4^-$, whilst selenite was monitored at *m*/*z* 129 as a singly protonated anion.

The performance of the latex-agglomerate IonPac AS4A-SC was compared with two polymeric resin columns, AS14 and AS14A [48]. Similar detection limits (17.8 μg L^{-1}) and linearity (in the range of 0.1–100 mg L^{-1}) were obtained in the three chromatographic systems, although higher concentrations of the carbonate-hydrogencarbonate eluents were required in the non-latex columns due to their higher capacity. Both polymeric columns (AS14 and AS14A) are also organic solvent compatible. They contain a polymethacrylate-based functional group grafted onto the surface of a macroporous resin consisting of EVB crosslinked with 55% DVB. The AS14A column is produced using a block-grafting technique that allows a high water content and has smaller particle diameters. Although the AS14 column offers advantages for the analysis of fluoride, the best performance in terms of efficiency and peak shape for phosphate is obtained with the AS14A, using an eluent at relatively high concentration (8.0 mM carbonate/1.0 mM hydrogencarbonate) [48]. Another latex-agglomerate column, AS9-HC, specially conceived for the separation of oxyhalides, in particular to resolve chlorate and nitrate, was tested for the analysis of phosphate [17,38,52,53]. This column consists of a medium hydrophobicity acrylate-based latex agglomerated on a macroporous EVB/DVB polymer. Due to its high capacity, a high carbonate concentration (9 mM) is required. Consequently, a highly efficient suppression procedure is needed to obtain good sensitivity. Highly hydrophilic latex-based columns, such as the AS12, designed to improve fluoride and oxyhalide analysis, have also been used to analyse phosphate. This column is composed of latex based on vinylbenzyl chloride functionalized with a hydrophilic quaternary moiety agglomerated on a macroporous EVB/DVB polymer. Intermediate carbonate concentration was required for the analysis [54]. An IC method coupled with ICP-MS has been proposed using this column for the determination of halogen and oxyhalogen anions, as well as phosphate and sulfate. The separation was performed with 11 mM ammonium carbonate as eluent in less than 4 min. Due to the specificity of the ICP-MS detection, complete resolution was not required, and under these conditions, phosphate co-eluted with selenite, as can be seen in Fig. 3, where chromatograms of the individual monitored elements are shown. Detection of phosphate was carried out at *m*/*z* 31, corresponding to the phosphorus ($^{31}P^+$) signal, and a detection limit of 36 μg L^{-1} was obtained [55]. However, despite the advantages of coupling IC to ICP-MS for ion analysis, such as low detection limits, wide linear range, and specificity, this approach has not been very popular for phosphate determination because of the presence of some inherent difficulties. Determination of phosphorus by ICP-MS is difficult, due to its high ionisation potential and its consequent low ionisation efficiency in an argon plasma.

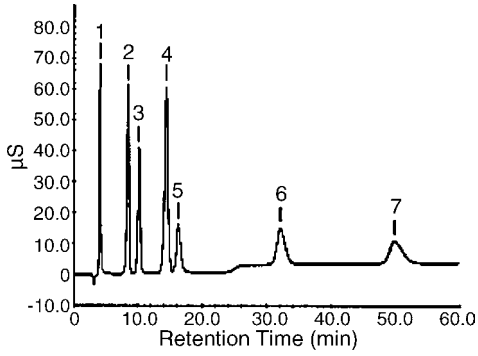

Fig. 3. Chromatogram of anion standard mixture. Peak identification (0.1 mM of each): (1) fluoride, (2) phosphate, (3) chloride, (4) sulfate, (5) nitrite, (6) bromide, (7) nitrate. Separation system: C18 column coated with Zwittergent 3-14/TTA (10 mM/10 mM) mixed micelles and 20 mM Na_2CO_3 as the eluent. Flow rate: 1.0 mL min^{-1}; detection: suppressed conductivity (ASRS). Reproduced from Ref. [80] with permission from Elsevier.

Moreover, interference of polyatomic ions, such as $^{15}N^{16}O^{+}$, $^{14}N^{16}O^{1}H^{+}$, and $^{12}C^{1}H_3{}^{16}O^{+}$ occurs at *m/z* 31, which results in higher background and detection limits than those corresponding to other ions [56].

Analysis of phosphate has also been performed in columns other than IonPac. For instance, Karmarkar [57] developed an IC-FIA system in which a QS-A5 column was used for separation in a 10 mM Na_2CO_3/1 mM $NaHCO_3$ eluent, and Asada and Oikawa [33] used a Shodex IC SI-904E for the separation of chloride, phosphate, and nitrate in a 1 mM eluent. Moreover, the separation of seven inorganic anions in 15 min using a Star-Ion-A300 (Phenomenex) column and an eluent consisting of 3.75 mM Na_2CO_3/3.6 mM $NaHCO_3$ has been reported by Kapinus et al. [58]. Polymeric columns, such as the polymethacrylate columns Metrosep Anion Dual 1 and 2, have also been used for phosphate analysis. These columns are composed of hydroxyethylmethacrylate (HEMA) and polymethacrylate, respectively, both funcionalized with quaternary ammonium groups. Suppressed and non-suppressed conductivity systems have been developed using carbonate/hydrogencarbonate and phthalate eluents, respectively [35,59].

Separations using carbonate/hydrogencarbonate are usually carried out under isocratic conditions, with a small range of eluent concentrations (Table 1). This explains the commercialisation of a wide variety of columns with slight modifications of the stationary phases developed to improve specific anion separations. Nevertheless, recent advances in suppressor technology have allowed the introduction of gradient elution with carbonate eluents, preventing baseline drifts produced by changes in carbonic acid concentration. For instance, Bose et al. [43] demonstrated the applicability of the DS-Plus suppressor, which removes the carbonic acid produced in the suppressor from the effluent. Several binary and ternary gradients, with carbonate, hydrogencarbonate, and water, and different columns have been used for the determination of common inorganic, organic, and polarizable anions. Thus, phosphate elution can be optimized such that it is separated from the rest of the anions present in the sample, as can be seen in Fig. 4, where the chromatogram of a refinery wastewater acquired using a carbonate/hydrogencarbonate elution gradient is shown. Initially, a mobile phase with low eluent strength (1 mM sodium hydrogencarbonate) was used up to 5 min in order to avoid the co-elution of early eluting anions (fluoride, propionate, bromate, and chloride). Then, the concentration was increased to 5 mM to elute more highly retained anions, such as phosphate and thiosulfate, in less than 20 min [43]. In contrast, gradient elution is frequently applied in hydroxide compatible systems. In fact, the commercialisation of hydroxide-selective columns that permit the use of dilute alkaline hydroxide as eluent has extended the number of applications of hydroxide anion-exchange chromatography systems to anion analysis. The introduction of new efficient suppressors and automated electrolytic on-line eluent generators [44] has facilitated both the preparation of high-purity hydroxide eluents and the application of elution

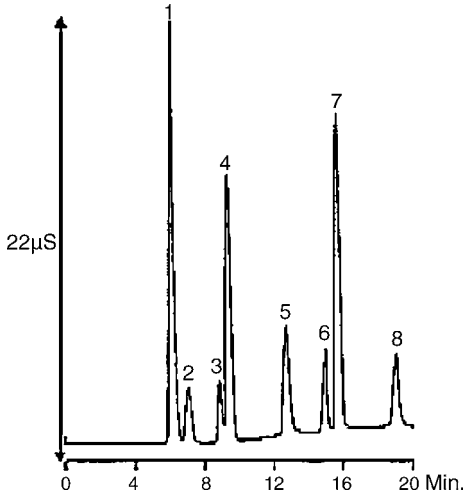

Fig. 4. Anions in refinery wastewater. Peak identification (10 mg L^{-1} of each): 1 = fluoride, 2 = propionate, 3 = bromate, 4 = chloride, 5 = nitrate, 6 = phosphate, 7 = sulfate, 8 = thiosulfate. Column: Novosep A-1, 150 × 4.6 mm; mobile phase: (A) 1.0 mM sodium bicarbonate; (B) 5.0 mM sodium carbonate. Gradient: 0% B for 5 min, to 100% B in 10 min, hold for 5 min. Flow rate: 1.5 mL min^{-1}; detection: suppressed conductivity (DS-Plus suppressor). Reproduced from Ref. [43] with permission from Elsevier.

gradients, avoiding the possible contamination of the eluent during preparation and the consequent negative effect on the gradient application. The high-pressure EG40 KOH eluent generator has been commonly used for the electrolytic generation of carbonate-free eluents, their KOH concentrations being directly related to the electrical current, and inversely proportional to the eluent flow-rate [60]. To ensure the removal of contamination due to traces of anions or cations in the column, a precolumn is often used before the injection port [56,61,62]. In particular, anion trap columns in the hydroxide form (ATC, ATC-1, and ATC-3) are frequently used to remove trace ionic impurities prior to reaching the analytical and guard columns, resulting in a chemically suppressed eluent reaching the column [16,63]. In addition, this device has also been used to generate gradients in carbonate/hydrogencarbonate systems by increasing the pH of the eluent [64].

Few columns have been specifically developed for hydroxide eluents, probably because selectivity can be easily modified by gradient elution [17]. Most applications have been carried out using IonPac AS11, AS15, and AS17columns. The IonPac AS11 column and its corresponding high capacity version are latex-agglomerated columns composed of highly cross-linked EVB/DBV that differ in their particle diameter (9 and 13 μm for the high capacity and AS11 columns, respectively) and latex size (also lower for the HC column). These columns are characterised by their gradient elution ability, enabling the simultaneous determination of low and highly retained anions in a single run. They have been used for both isocratic and gradient separations, and concentrations from 10–40 to 40–100 mM are commonly used. Moreover, with the HC columns, high amounts of sample

(up to 100 μL) can be injected without column overloading, and thus, detection limits of low $\mu g\,L^{-1}$ are obtained. The IonPac AS11 and AS15 columns have been proposed for the analysis of anion traces in highly concentrated matrices, such as nitric acid or hydrogen peroxide [28,63,65,66]. The AS15 (9 μm) column is composed of a highly crosslinked macroporous (EVB/DBV) core and an anion-exchange resin grafted onto the surface that was developed as an improvement on the AS11-HC. However, due to the higher hydrophobicity of AS15, adsorptive interactions can occur that lead to particular selectivity for late eluting species. The elution time of phosphate (25 min) under isocratic conditions is higher than that using an AS11-HC. The use of the shorter AS15A version, which contains smaller particles (5 μm), allows the analysis time to be reduced [17]. The performance of these two columns (AS11 and AS15) has been reported for the analysis of anion contamination in hydrogen peroxide [28,66]. Here, gradient elution with sodium hydroxide at up to 40 mM was used. Higher efficiencies and lower detection limits ($0.8\,\mu g\,L^{-1}$) were obtained for phosphate analysis using the AS11 column [66]. The AS15 column has also been used to determine phosphate contamination in a high-nitrate matrix [63]. Modification of ion chromatography selectivity can be accomplished by the variation of eluent concentration and column temperature. In order to optimize the separation between sulfate and phosphate the concentration of the eluent is modified, and the elution times of both anions reverse when the KOH eluent concentration increases from 33 to 48 mM. Different selectivity was observed using potassium hydroxide eluents at different concentration (33 or 48 mM) [44,63]. Selectivity can also be modified by changing column temperature. For instance, Hatsis et al. [67] studied the effect of column temperature (from 27 to 60 °C) on the separation of several anions on an AS11 column with NaOH as eluent, and an AS14 with carbonate/hydrogencarbonate as eluent, showing that retention of multiply charged anions such as phosphate, sulfate, oxalate, and thiosulfate increases with increasing temperature and elution order is reversed.

The performance of the hydroxide-selective AS17 column was studied by Jackson et al. [49]. This column is also a latex-agglomerated column that consists of microporous particles (10.5 μm) of the same sulfonated EVB-DB as the carbonate-selective AS4A-SC, but with a higher average number of hydroxyl groups in the latex. This accounts for its higher hydrophilicity and hydroxide compatibility, which allows higher efficiency. The combination of smaller particle size and gradient elution improves the peak efficiency for phosphate. Retention of most anions increases with the AS4A column, but maintains a similar overall analysis time. Different selectivity in the carbonate/hydrogencarbonate and hydroxide selective columns was observed. Whilst phosphate elution time is similar in both systems, occurring in less than 8 min, unlike in the carbonate/hydrogencarbonate system, phosphate elutes after sulfate in the hydroxide selective system [49]. Another high capacity hydroxide selective column, AS16, has been used for the separation of phosphate and other inorganic anions. This column, which contains highly hydrophilic ion-exchanger groups in its latex particles, was specifically designed for the separation of polarizable anions, such as iodide and arsenate. Nevertheless, its application to the analysis of polyphosphates up to P20 has been reported [68]. Using a hydroxide gradient of 1.5–55 mM, elution of phosphate occurs at approx 25 min [17].

Cryptand-based anion exchanger columns are a new class of anion resins produced by the covalent bonding of a cryptand to a cross-linked polymeric substrate [69]. The cryptand macrocycle complexes mobile phase cations (sodium, lithium, and potassium) of hydroxide solutions, providing anion exchange sites for the separation of sample anions. By changing the eluent composition during the separation run, both column capacity and eluent strength can be optimized for a particular application. Consequently, analysis of weakly retained anions, such as organic acids, and highly retained anions, such as polyphosphates, can be satisfactorily performed in a single run by the application of eluent gradients. A comparison of the performance of two cryptand based anion exchangers, 2.2.1. and 2.2.2., was published by Woodruff et al. [69]. One of the macrocycles, 2.2.1., contains one less oxygen and one less ethylene group in one of the bridges. In both cases the lowest exchange capacity is obtained when using lithium, whereas the highest capacity is obtained with sodium and potassium for the 2.2.1. and 2.2.2. columns, respectively. Similar selectivity was observed in both columns with standard capacity gradients, i.e. with sodium or potassium hydroxide as initial eluents and lithium hydroxide as the second eluent. Under these conditions, phosphate is baseline resolved from thiosulfate and iodide. The capacity of the 2.2.1. column is more significantly affected by a high concentration of cations in the samples than that of the 2.2.2. column, and thus, the authors proposed the use of the commercial equivalent of the latter column, Cryptand A1, for phosphate analysis.

In order to predict the retention factors of phosphate, as well as those of other inorganic anions, in suppressed IC, various retention models have been developed. For instance, Madden et al. [70] compared the ability of three mathematical models to describe anion behaviour in hydroxide selective systems using an AS11 column with isocratic elution. Whilst two models, empirical end points and three-point curve fitting, gave good prediction of the retention factors for all the 21 anions studied, the linear solvent strength model (LSSM) gave poor results when it was applied to phosphate, which could be due to its charge dependence on the concentration of hydroxide in the eluent. The same authors used artificial neural networks (ANNs) to predict retention times in this same column with several linear hydroxide gradients (0–40 mM KOH), obtaining a prediction error lower than 1% [71]. The use of artificial neural networks (ANNs) to model the retention of seven inorganic anions, including phosphate, in hydroxide selective systems was also reported by Srečnik et al. [72]. To build the model, an extensive study consisting of 128 experiments at different eluent concentrations (25–60 mM)

and flow rates (1.0–1.95 mL min^{-1}) was performed. Under these conditions, phosphate retention times varied from 9.08 to 70.60 min. The ANN model proved to be useful to predict the phosphate retention time, obtaining a correlation coefficient of 0.9965 for predicted and actual values [72]. The development of a non-linear function retention model for polyvalent weak acid anions, and its application to the prediction of phosphate behaviour in sodium hydroxide eluents has also been reported. A good correlation (0.992) between predicted and actual values was obtained [73]. Retention modelling in carbonate-selective IC systems has also been studied using six mathematical models [74]. As in the hydroxide systems, the empirical end-points model gave satisfactory modelling of anion behaviour. However, in the carbonate IC systems, the retention behaviour of phosphate was erratic, as indicated by the values of the normalised percentage differences between predicted and experimental data, which range from approximately −10 to 20% depending on the model. In another example, Nowak and Seubert [75] describe an experimental design for the characterization of a ternary elution system based on perchloric acid, sodium hydroxide, and sodium carbonate, using a high-capacity anion exchange column. A quadratic model that included interactions between the concentrations of the three reagents was proposed, providing a good correlation between predicted and actual retention values [75].

3.1.1.2. Suppressors. Recent developments in suppressor technology that simplify the suppression setup and allow detection limits to be improved were described in detail in a recent review [16]. In general, most current applications are performed using continuously regenerated high capacity micromembranes, and to a lesser extent, with packed column suppressors. For phosphate analysis, most recent applications have used electrolytic membrane-based suppressors that do not require external regenerants to achieve the suppression reaction (see Table 1). The design is similar to that of typical micromembrane suppressors, but containing two electrodes for the electrolysis of water to obtain the hydronium ions required for the suppression reaction. For this reason, these suppressors are termed self-regenerating suppressors (ASRS). These suppressors mostly operate in the recycle mode, using the effluent of the conductivity detector as the source of water for electrolysis, although better sensitivity can be obtained in the external water mode, in which deionised water is supplied to the suppressor in a similar way to the chemical regenerant of a micromembrane suppressor. A comparison of the performance of recycle and external water modes resulted in limits of phosphate detection of 44 and 18 μg L^{-1}, respectively, using the same chromatographic conditions [16,76]. Typical micromembrane suppressors with chemical regeneration, commonly known as AMMS, are still widely used, as they provide more stable baselines, and consequently, lower detection limits than other suppressor systems. They have high suppression capacity and are compatible with high buffer concentrations, high eluent flow rates, and in addition, facilitate the application of gradient elutions. A disadvantage of these suppressors is that they require the use of an additional pump and a regenerant solution. The eluent flows between two ion-exchange membranes while the regenerant, commonly 10–25 mM sulfuric acid, flows continuously in the opposite direction at high flow rates over their outer surfaces. Volumes of regenerant can be reduced in the displaced chemical regeneration mode, although higher concentrations of the regenerant are required.

Solid-phase regenerant cartridges can be used in the recycle line to remove waste products from the regenerant supply in both electrolytic and chemical micromembrane suppression systems. In addition, a solid-phase based suppressor with continuous electrolytic regeneration has recently been marketed under the name of DS-Plus suppressor [42,43]. The use of this suppressor is advantageous for carbonate/hydrogencarbonate systems, as in addition to suppressing the mobile phase it removes carbonic acid from the suppressor effluent prior to detection. An alternative approach consists of a new generation of anion electrolytic suppressors (AESs) with continuous electrolytic regeneration that provide the operational convenience of an electroanalytically regenerated device (ASRS), but give signal-to-noise ratios similar to AMMS [16,48]. The anion electrolytic suppressor is a packed-column suppressor based on the concept of 'ion reflux', an ion-exchange technique in which water is the source of the eluent as well as its means of suppression. This is accomplished by means of the circulation of water through an electrically polarized resin bed. This suppressor has been applied to the determination of phosphate and other common inorganic anions. An approximately two-fold improvement of phosphate detection limits is obtained with an AES suppressor compared to an ASRS suppressor. In the first case, a limit of detection of 7.3 μg L^{-1} has been reported for phosphate using a carbonate/hydrogencarbonate eluent, whilst with the ASRS the minimum detectable level is 4.8 μg L^{-1} [48].

3.1.2. Non-suppressed conductivity detection system

In recent years, the number of applications of non-suppressed conductivity systems has decreased considerably. In addition to the higher sensitivity of suppressed conductivity systems, the recent commercial availability of columns and suppressors has led to the widespread use of suppressed conductivity systems, especially for routine analysis. However, some applications have nevertheless been reported. For instance, the use of two anion-exchange resins, Hamilton PRP-X100 and PRP-X110, composed of spherical polystyrene/divinylbenzene particles functionalised with trimethyl amine, has been proposed for the analysis of phosphate and other standard anions [59,77]. Eluents consisting of mmolar solutions of *p*-hydroxybenzoic acid (pH 8.5) or hydrogenphthalate (pH 4), with percentages of MeOH or ACN lower than 4%, have been used in non-suppressed conductivity detection [59,79]. The limits of detection reported for phosphate were in the order of 0.8–4 mg L^{-1}. Mathematical retention models have also been used to optimize the separa-

tion of phosphate and 14 other anions in non-suppressed IC with phthalate eluents in three stationary phases (Waters IC Pak A, Hamilton PRP-X100 and Vydac 302 IC). The performance of the models to predict the retention factors improved as their complexity increased, and the best predictions were obtained using the empirical end points model [78]. Compared with suppressed IC, the retention behaviour in non-suppressed IC was found to be more difficult to model.

The use of aromatic acid solutions as eluents enables the alternative use of indirect UV detection, and this detection has also been used for the analysis of phosphate. For instance, Lazar et al. [79] proposed the use of an Anion Dual 1 column with a mobile phase consisting of 1 mM *o*-phthalic acid-TRIS buffer, 2% (v/v) ACN (pH 8), and indirect UV detection (273 nm) as a versatile and inexpensive approach to the analysis of phosphate and other inorganic anions, with detection limits lower than those obtained with conductivity detection.

3.2. Modified reversed-phase columns

Although it represents a less common approach for the analysis of inorganic ions, some authors have explored the possibilities of reversed-phase columns for the separation of phosphate and other anions [45,46,80,81]. This approach, which is sometimes regarded as ion interaction chromatography, consists of the modification of the reversed-phase by permanently or dynamically coating its surface with a charged cationic surfactant, such as cetyltrimethylammonium [82,83], tetradecylammonium [80], or didodecyldimethylammonium [84]. Although the mechanism is still not completely clear, this procedure seems to convert the reversed stationary phase into an anion exchanger. However, owing to its particular characteristics, the chromatographic behaviour of some anions differs from that observed in standard polymeric ion-exchange columns. Modification of the experimental conditions (columns, eluents, and surfactants) and control of the anion-exchange capacity of the column, which can be altered by changing the eluent, allow the chromatographic system to be optimized for particular separations. Applications for phosphate analysis are summarized in Table 2. Cetyltrimethylammonium (CTA) has been used as a coating agent for C8 [82] and PGC columns [83], and in both cases the coated columns were used for the separation of phosphate from other inorganic anions. Dynamic coating of the C8 column was performed by adding low concentrations (30 μM–3 mM) of the modifier to the hydrogen phthalate (1 mM) eluent [82]. Orthophosphate, as dihydrogen phosphate, elutes at the beginning of the chromatographic run, before chloride, and satisfactory reproducibility of retention times was reported after passing 120 mL of the eluent through the column. Nevertheless, performance of the system for quantification was not provided. Nagashima and Okamoto [83] reported the permanent coating of a PGC column using a solution of 0.5 mM CTA-Br in water:acetonitrile (75:25). The authors indicated that the resulting stationary phase was stable under the operating conditions (carbonate/bicarbonate eluent and suppressed conductivity detection) for at least two months. Addition of acetonitrile to the mobile phase slightly modified retention times, and in the case of divalent anionic forms (SO_4^{2-} and HPO_4^{2-}), retention increased with increasing acetonitrile. The detection limit for phosphate obtained with the PGC column was the highest of the seven anions tested, 3 ng mL^{-1}, but lower than that obtained by the same authors using tetrabutylammonium as ionic modifier in another PGC with a sodium carbonate eluent [85]. The latter modifier was also used by Fritz et al. [81] in a recently published study focused on different modification procedures for ion chromatographic separation in reversed-phase columns. Mobile phases containing tetrabutylammonium hydroxide (2.5–10 mM) and a substituted amino alkylsulfonic acid were tested for the separation of 10 common anions in a C18 column that was not previously coated. The best conditions were 2.5 mM TBA and 10 mM 3-morpholinopropanesulfonic acid (pH 7.5), but even working under these conditions, co-elution of nitrate and phosphate occurred. A possible improvement by the addition of organic solvent was suggested by the authors. The cationic surfactant tetradecyltrimethylammonium (TTA) has also been used as coating agent. Separation of phosphate among another six inorganic anions has been performed in a TTA-coated C18 column using a 25 mM sodium carbonate eluent. Elution order was coincident with that typical of anion-exchange systems. In addition, negative slopes of $\log K'$ versus $\log[E]$ plots (E denotes the eluting ion) were obtained, as in the case of fixed-site ion-exchangers. Both observations suggest that the chromatographic process on the TTA-coated column occurs through a conventional anion-exchange mechanism [80].

Recently, two fast chromatography methods based on the use of surfactant-coated columns have been published. Connolly and Paull [84] used a didodecyldimethylammonium (DDA)-coated C18 (4.6 μm, 3 cm ODS) column and a 5 mM phthalate eluent for the separation of nine inorganic anions with indirect UV detection. The elution time of phosphate was only 30 s, with a limit of quantitation in the order of 5 mg L^{-1}, which enabled the application of the method to phosphate-rich river waters. In the other method, a monolithic silica column (5 cm) was coated with a tetramethylammonium (TBA) solution. Phthalate was also used as eluent, but in contrast to the previous method, a low concentration of surfactant (0.5 mM) was added to the mobile phase, and detection was carried out by both non-suppressed conductivity and indirect UV detection. The use of high flow rates (up to 16 mL min^{-1}) enabled extremely fast separations. However, loss of efficiency was observed for phosphate, which is weakly retained at such flow rates, and thus, lower values (8 mL min^{-1}) were selected for the analysis of phosphate and other inorganic anions in industrial water [86].

A further development in anion chromatography with reversed-phase columns is based on the use of zwitterionic surfactants as coating agents. Here, a new stationary phase with both positively and negatively charged groups is gener-

Table 2
Determination of orthophosphate by modified reversed-phase LC

Column	Mobile phase	Flow rate (mL min^{-1})	Detection	LOD	Sample	Concentration	Reference
Waters NovaPak C_{18}	Eluent: 2.5 or 5 mM TBAOH 5 or 10 mM MOPS pH 7.18	1	Suppressed conductivity AMMS-Ultra-4 mm		Standards	20 μg mL^{-1}	[81]
	2.5 mM TBAOH 5 mM MES pH 7.2						
	2.5 mM TBAOH 5 mM Hepes pH 7.6						
Phenomenex Kingsorb	Eluent: 5 mM phthalate (pH 7.5) (T:45 °C) Coating solution: 10 mM DDAB	2 1	Indirect UV (279 nm)	0.5 mg L^{-1}	Standards, river and sea water	0.64 mg L^{-1}	[84]
Chromolith Speed ROD RP-18e	1.5 mM TBA-1.1 mM phthalate with 5% (v/v, ACN) pH 5.5	4–16	Non-suppressed conductivity	2 mg L^{-1}	Industrial water	<2 mg L^{-1}	[86]
		8	Indirect UV (255 nm)	1 mg L^{-1}			
Mightysil RP-18	ACN-water (60:40), 0.1 M acetate buffer (pH 3.9) 0.8 mM TBA	1	UV (310 nm)	1.56 μg mL^{-1} (P) Concentration (100 mL, 6.7×10^{-3} μg (P) mL^{-1})	Standards preconcentrated as molybdic heteropoly acid	0.02–0.15 μg mL^{-1} (P)	[34]
ODS column	Eluent: 25 mM Na_2CO_3 in TTA-coated column 20 mM Na_2CO_3 Coating solution: 10/10 mM TTA/Zwittergent 3-14	1	Suppressed conductivity ASRS	0.12 μM	Standards	up to 8.0 mM	[80]
ODS column	Eluent: 5 mM sodium tetraborate (1% coating solution) Coating solution: 20 mM Zwittergent 3-14/2.0 mM MTA	1 1	Suppressed conductivity ASRS	10.56 ng L^{-1} (sub-μM)	Standards, tap water	1 mM N.D.	[89]
Carbon IC BI-02	Eluent: 2 mM Na_2CO_3, 1 mM $NaHCO_3$ Coating solution (1 mL min^{-1}: 0.5 mM CTA-Br in H_2O/ACN (75:25)	1	Suppressed conductivity AMMS	3 ng mL^{-1}	Standards	5–50 μg mL^{-1}	[83]
Hypercarb S	300 mM HCOOH	1	Evaporative Light Scattering Detection (ELSD)		Standards	100–500 mg L^{-1}	[47]
	Mixtures: 10–30 mM HCOOH/10–20 mM pyridine						
Carbon IC BI-01	Eluent: 1 mM TBA, 2 mM Na_2CO_3, 5% ACN	0.8	Suppressed conductivity AMMS (chemical regeneration)		Standards	5–100 μg mL^{-1}	[85]
Silasorb C18 silica gel	1 mM hydrogen phthalate with: 3 mM CTAB, pH 6.0 or 30 μM CTAB, pH 4.05	2	Indirect UV detection 265 nm		Standards		[82]

ated. The presence of both charges simultaneously produces electrostatic attractions and repulsions between the analyte and mobile phase anions. Consequently, the separation mechanism is different to ion-exchange, and thus, the elution order is different in both systems. A wide variety of eluents, such as water, suppressible electrolytes, and strong acid or base salts, can be used [46]. This chromatographic mode, termed 'electrostatic ion chromatography' was reported by Hu at al. [87]. Since then, several papers addressing method optimization and applications to inorganic anion separation have been published both by this group and by other authors. The chromatographic behaviour of phosphate in EIC is different from that in ion-exchange columns. Phosphate, as well as sulfate, shows little retention on zwitterionic-coated stationary phases when water or electrolytes are used as eluents, in contrast to that observed in ion-exchange systems. Under these conditions, fluoride, phosphate, and sulfate elute near the void volume of the column, and their separation cannot be achieved [46,88]. The addition of a cationic surfactant to the coating solution allowed the separation of F^- and HPO_4^{2-}, and of HPO_4^{2-} and SO_4^{2-} in EIC [89]. The proposed method was based on the coating of an octadecylsilica column with mixed zwitterionic-cationic surfactant micelles. The best separation was obtained using 3-(*N*,*N*-dimethylmyristilammonium)-propanesulfonate (Zwittergent 3-14) and myristyltrimethylammonium (MTA) as the zwitterionic and the cationic surfactants in a 10:1 molar ratio, with a sodium tetraborate eluent (5 mM). A coating solution (50 mL of 20 mM Zwittergent 3-14/2.0 mM MTA) was passed through the column and afterwards the column was conditioned with the sodium tetraborate eluent prior to undertaking the analysis. Under these conditions, elution of fluoride, phosphate, and sulfate occurs in approximately 5 min, with satisfactory resolution. A detection limit of 10.5 ng L^{-1} was calculated for phosphate using suppressed conductivity detection. A similar detection limit (11.5 ng L^{-1}) was obtained using tetradecyltrimethylammonium instead of MTA as the cationic surfactant. Variation of the molar ratio of the zwitterionic and cationic surfactants changes the selectivity. Using a 10 mM/10 mM ratio of TTA/Zwittergent 3-14 solution to coat the column and 20 mM sodium carbonate as eluent, phosphate eluted before chloride, followed by sulfate, whilst under the same conditions, but with a 1 mM/10 mM ratio of TTA/Zwittergent 3-14 coating solution, sulfate immediately precedes the phosphate peak. The chromatogram of a mixture of anions acquired under the former conditions is given in Fig. 5. As can be seen, phosphate eluted in less than 10 min with high efficiency, in contrast to nitrate, which is highly retained in this system. The higher the mole fraction of the cationic surfactant, the more concentrated the eluent has to be, which results in longer elution times and higher detection limits [80]. Preconditioning of the sample by using a cation-exchange column for the conversion of the sample cations into a single cation form has proved to simplify the EIC chromatogram. Conversion to a divalent cation form is more effective than to a monovalent one, as higher retention is observed in the former case [9].

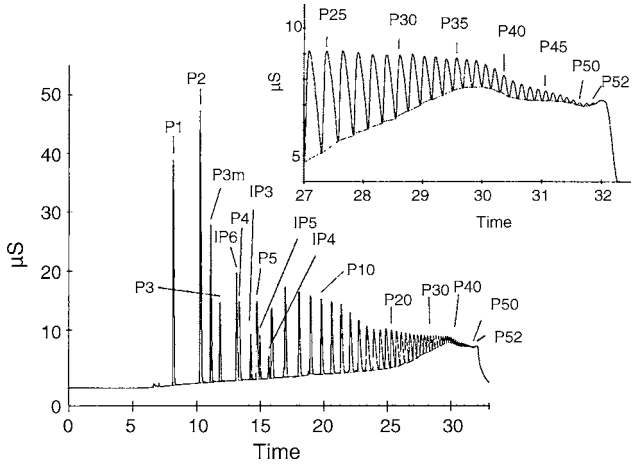

Fig. 5. Typical chromatogram of polyphosphates on IonPac AS11 column. IC conditions, eluent gradient 30–200 mM NaOH over 30 min; flow rate: 0.5 mL min^{-1}; suppressed conductivity detection (ASRS-Ultra). Sample: sodium phosphate glasses type 15 (Sigma) dissolved at 1 mL min^{-1} (Pn: polyphosphate, IP: inositol phosphate). Reproduced from Ref. [62] with permission from Elsevier.

4. Selected applications of IC to the determination of phosphate and other phosphorus species

The vast majority of IC applications for analysis of phosphorus involve the determination of orthophosphate, either as the specific analyte or as the final product of the sample treatment procedure in speciation studies. Moreover, IC methods have also been developed for the specific analysis of other phosphorus species. This section covers applications for the determination of both inorganic phosphorus species, such as phosphate, and condensed and reduced phosphorus species, and organic phosphorus species, such as phytic acid, inositol phosphates, alkyl substituted organophosphorus acids, and bisphosphonates. Applications of phosphate analysis are summarized in Tables 1 and 2, where they have been ordered according to the characteristics of the chromatographic systems used. The experimental conditions of the IC methods devoted to the analysis of other phosphorus species are given in Table 3. Applications in the environmental field are also reviewed along with relevant applications to phosphorus species in other samples.

4.1. Determination of phosphate and total phosphorus

Most recent papers concerning the determination of phosphate in the environment describe the application of IC methods to the general analysis of common inorganic anions in water [6,9]. The EPA regulatory method 300.0 published in 1993 remains widely accepted as the standard method [13]. This method specifies the experimental conditions for the

Table 3
Miscellaneous applications of IC to the analysis of different phosphorus species

Species	Column	Mobile phase	Flow rate mL min^{-1}	Detection	LOD	Sample	Concentration	Reference
Inorganic phosphorus species								
Hypophosphite Phosphite Orthophosphate	IonPac AG17 IonPac AS17	Electrolytically generated KOH gradient (0.5–35 mM)	1.5	Suppressed conductivity ASSR-Ultra (external water)	53 $\mu g\,L^{-1}$ (HPO_2^-) 31 $\mu g\,L^{-1}$ (HPO_3^{2-}) 34 $\mu g\,L^{-1}$ (HPO_4^{2-})	Geothermal water	1.25–20 μM	[103]
Phosphorous acid Phosphoric acid	BT S AG-P	1.5 mM Na_2CO_3	1.4	Suppressed conductivity BT × AN-S column (150 × 4 mm i.d.)	0.05 $\mu g\,mL^{-1}$ (HPO_3^{2-}) 0.1 $\mu g\,mL^{-1}$ (HPO_4^{2-})	Aerosols of phosphorus and phosphoric acids	0.1–100 $\mu g\,mL^{-1}$	[106]
Monofluorophosphate Orthophosphate	Guard col.: IonPac AG9-H4, CG2 Separation col.: IonPac AS9-HC	0.848 $g\,L^{-1}$ Na_2CO_3	1.2	Suppressed conductivity ASRS-Ultra-4 mm	5 $mg\,g^{-1}$ (MFP in HCP)	Acid extracts of hardened cement paste (HCP) containing monofluorophosphate (MFP)		[98]
Monofluorophosphate Orthophosphate	IonPac AG14 IonPac AS14	2.0 mM Na_2CO_3 2.5 mM $NaHCO_3$	2	Suppressed conductivity ASRS (recycle mode)		Aqueous and acid extracts of concrete treated with monofluorophosphate (MFP)	14.0–2639 $\mu g\,mL^{-1}$ (MFP)	[97]
Polyphosphates: (P1–P3)	IonPac AG11/AS11 (with Anion Trap Columns, ATC)	KOH gradient: (20–70 mM)		Suppressed conductivity ASRS (recycle mode)		Statistical quality control applied to IC calibrations (0.5–10 $\mu g\,mL^{-1}$)		[109]
Polyphosphates:(P2–P13)	Separon HEMA 1000-S Q-L	P2–P5: 2×10^{-3} M pyromellitic acid 8×10^{-5} M EDTA pH 9 P6–P10: 2×10^{-3} M pyromellitic acid 8×10^{-5} M EDTA pH 9	0.4	Indirect UV (296 nm)		Standard		[112]
P1–P3		2×10^{-3} M pyromellitic acid		Indirect UV (296 nm)		Phosphate washing agent		

Table 3 (*Continued*)

Species	Column	Mobile phase	Flow rate mL min^{-1}	Detection	LOD	Sample	Concentration	Reference
		8×10^{-5} M EDTA pH 9						
P2–P11		1×10^{-2} M pyromellitic acid 8×10^{-5} M EDTA pH 9		Indirect UV (296 nm)		Glassy phosphate mixture		
Polyphosphates: (P1–P7 and P3m)	Tosoh IC-Anion-PW or IonPac AS4A-SC	Eluent A: 48 mM dihydroxyphenylborane-32 mM NaOH	1	Suppressed conductivity (Chemical suppression)		Standards		[114]
		Eluent B: 32 mM mannitol Eluent C: 0.019 mM EDTA–16 mM NaOH		RI		Cheese extracts		
		Gradient: Isocr. (0–2 min) 50% A/50% B Linear (2–22 min) 50% A/0% B/50% C						
Polyphosphates: (P1–P4 and P3m)	IonPac AG11-HC/ASH11-HC	Electrolytically generated and off-line prepared KOH gradients (30–200 mM)	0.5	Suppressed conductivity ASRS-ULTRA (external water mode)		Standards Food products (ham, fish paste, cheese)	0.5–500 μM	[62]
Sodium trypolyphosphate	IonPac AG11-HC/ASH11-HC	Electrolytically generated KOH gradient (25–100 mM)	1	Suppressed conductivity ASRS (recycle mode)	5 mg kg^{-1} (fish)	Frozen cod and scallop adductor	0.0092–3.8 mg g^{-1}	[115]
Polyphosphates: (P1–P33)	IonPac AG11-HC/ASH11-HC	Electrolytically generated KOH gradient (30–200 mM)	1	Suppressed conductivity ASRS-Ultra-4 mm (autosuppression external water mode)		Standards Enzymatic digested fractions		[116]
Organic Phosphorus species								
Phytic acid, Inositol phosphates (IP2, IP3, IP4, IP)	CarboPac PA-100	Eluent A: 500 mM HCl Eluent B: H_2O Gradient: 0–92% B	1	UV Postcolumn (Fe^{3+}–HNO_3 reagent)		Standards, enzymatic hydrolysates		[118]
Phytic acid	OmniPac Pax-100	NaOH gradient: 69–129 mM NaOH	1	Suppressed conductivity	<0.0001 μM	Standards, food (cereal flour, oil, legume seeds)	0.01–0.16 mM	[121]
		1% (v/v) isopropanol		ASSR-I			0.29–1.28% (w/w)	

Dibutyl phosphate (DBP), monobutyl phosphate (MBP)	IonPac AG11/AS11	NaOH gradients: AS11 Eluent A: water Eluent B: 4 mM NaOH Eluent C: 100 mM NaOH Gradient: (multistep: 90% A/10% B; 100% B; 65% A/35% B)	1.5	Suppressed conductivity ASSR-Ultra (recycle mode)	150 μg L^{-1} (DBP) 25 μg L^{-1} (MBP)	Standards	Up to 900 μg L^{-1}	[124]
	IonPac AG5A/AS5A	AS5A Eluent A: 0.75 mM NaOH Eluent B: 200 mM NaOH Gradient: (multistep: 100% A; 85% A/15% B; 57% A/43% B)	1					
Methyl phosphonic acid Ethyl phosphonic acid	IonPac AS4A-SC	25 mM NH_4HCO_3 (pH 7.8)	1	Evaporative Light Scattering Detection	1 mg L^{-1} (200 ng)	Standards, spiked lake water	12.5–100 mg L^{-1}	[125]
Methyl phosphonic acid Isopropyl methyl phosphonic acid	Sarasep AN300 GC 2 Sarasep AN300	10 mM sodium tetraborate 3.75 mM NaOH	1.5	Suppressed conductivity Micromembrane suppressor	10–20 ng mL^{-1}	Standards, soil extracts	sub-ppm levels (μg g^{-1})	[126]
Glyphosate Aminomethylphosphonic acid (AMPA)	Metrohm Dual 2	Eluent A: pH 10.30 1.3 mM Na_2CO_3 2.0 mM $NaHCO_3$ Eluent B: pH 10.08 13 mM Na_2CO_3 20 mM $NaHCO_3$ Gradient: Isocr. (0–10 min) 95% A/5% B Linear (10–20 min) 50% A/50% B Isocr. (0–10 min) 50% A/50% B	0.5	ESI-MS Suppressed conductivity (Metrohm supp module 753)	1 μg L^{-1}	Spiked ground and surface water	7.1–8.0 μg L^{-1}	[127]
Methyl phosphate Dimethyl phosphate	IonPac AG11/AS11	Eluent A: 10 mM NaOH Eluent B: MeOH/H_2O (80:20) 70% A/30% B	1–1.5	Suppressed conductivity Alltech ERIS or ASRS-I ESI-MS		Standards, organophosphate insecticide	10 mg L^{-1}	[128]

Table 3 (*Continued*)

Species	Column	Mobile phase	Flow rate mL min^{-1}	Detection	LOD	Sample	Concentration	Reference
Methyl phosphate *S*-Methyl phosphoramidothioate *O*,*S*-Dimethyl phosphorothioate	IonPac AG11/AS11	NaOH gradient: 4–36 mM NaOH	1	Suppressed conductivity Alltech ERIS or ASRS-I ESI-MS		Organophosphate matrix		[129]
Bisphosphonates:	IonPac AG97/onPac AS7	1.5 mM HNO_3	1.4	ICP-MS	0.20 mg L^{-1}	Standards	0.6–20 mg L^{-1}	[56]
Alendronic acid (4-amino-1-hydroxybutane-1,1-bisphosphonic acid, AMDP) Etidronic acid (1-hydroxyethylidene-1,1-diphosphonic acid, HEDP) Orthophosphate	(Metal Trap Column MFC-1)	15 mM HNO_3	1		0.05 mg L^{-1}		0.16–16 mg L^{-1}	
Difluoromethylene bisphosphonate (F_2MDP), dichloromethylene bisphosphonate acid (Cl_2MDP), ethane-1-hydroxy-1,1′-bisphosphonic acid, 4-amino-1-hydroxybutane-1,1-bisphosphonic acid	Hamilton PRP-X100	Eluent: 30–40 mM $NaNO_3$ 4.1 mM NaOH Post-column reagent: 2 μM $Al(NO_3)$, 12 μM morin, 37 mM HOAc-NaOH buffer, ethanol-water (80:20) (1 mL min^{-1})	1	Post-column Indirect Fluorescence (Al^{3+}-morin reagent) λ_{exc}: 420 nm λ_{em}: 505 nm	4–6 ng	Standards, cell growth media, ambryonic rat bone	0.95 μg mL^{-1} (Cl_2MDP), 0.01 mM (F_2MDP)	[130]
2-Thioethane-1,1-bisphosphonic acid	Waters IC-Pak Anion HR	Eluent: HNO_3 isocratic: 5–50 mM HNO_3 gradient: Initial: 5 mM, final: 400 mM Post-column reagent: 10 mM sodium molibdate	0.8–1.2	Postcolumn UV (254 nm)	0.15 μg mL^{-1}	Standard	0.5–500 μg mL^{-1}	[131]

determination by ion chromatography of phosphate and six other anions (bromide, chloride, fluoride, nitrate, nitrite, and sulfate) in environmental and drinking waters by suppressed conductivity using an IonPac AS4A column or equivalent columns with a carbonate/hydrogencarbonate (1.8 mM/1.7 mM) eluent. Three years later, the EPA published Method 300.1, which includes some modifications that facilitate the quantitation of lower levels of bromate [14]. The new method uses a higher capacity column, AS9-HC, and a more concentrated eluent (9.0 mM sodium carbonate), and can be applied as an alternative to Method 300.0 for the determination of common anions. In addition to applications of the regulatory method, various modifications and their consequent applications have been published since the late 1990s.

4.1.1. Wastewater and industrial waters

Ion chromatography has been applied to the determination of nutrient anions, such as phosphate and sulfate, in industrial and urban effluents. As an example, Jackson et al. [48,49] compared the performance of new carbonate/hydrogencarbonate and hydroxide selective columns under the working conditions specified in EPA Methods 300.0 and 300.1 for the analysis of several water samples, including domestic and industrial wastewaters [13,14]. The results obtained show that the use of an IonPac AS17 column with on-line generated hydroxide elution gradient provides higher efficiency for phosphate peaks, although the elution time was slightly higher than under the EPA conditions. In addition, the performance of two carbonate/hydrogencarbonate selective columns, AS14 and AS14A, was has been compared with the AS4A-SC specified in the regulatory method, showing the suitability of these columns for the analysis of inorganic anions in low to moderate ionic strength water samples. Wastewater samples from a septic tank were analysed with these columns, obtaining concentrations in the order of 28.7 mg L^{-1}, clearly higher than the detection limits of these systems (12.3–17.8 μg L^{-1} as phosphate, 4.0–5.8 μg L^{-1} as P) [48]. Another interesting example of the application of IC to the analysis of phosphate was published by Karmarkar [57], who developed an IC-FIA method to analyse phosphate and two other nutrients, nitrate and ammonia, in sulfate-rich waters, in a single injection with a run time of 9 min. Complete resolution of phosphate and sulfate (910 mg L^{-1}) was accomplished even at a concentration ratio of 1:2200. Ion-exchange chromatography with suppressed conductivity detection in a carbonate/hydrogencarbonate selective system was used for the analysis of the anions. The determination of ammonia was carried out by FIA in the unsuppressed column effluent of the anion column, whilst off-line regeneration of the suppressor cartridge was carried out between injections. Phosphate concentration, as phosphorus, in the analysed wastewater samples was in the range of 0.21–5.0 mg L^{-1} (P-$H_2PO_4^-$). In some cases, an on-line pretreatment of the sample is required in order to simplify the matrix, to improve the separation, or to prevent contamination. For instance, total phosphorus, as phosphate, has been determined in persulfate-digested wastewaters using a column-switching method to remove excess sulfate [22]. Separation was performed using a carbonate/hydrogencarbonate eluent with IonPac AS4 and AS4A-SC columns. A quantification limit of 0.10 mg L^{-1} P was obtained, which was low enough to enable the determination of phosphorus in wastewaters, although the method is not sufficiently sensitive for the analysis of less polluted freshwaters. Recoveries higher than 96% were calculated from spiked samples (1–8 mg P L^{-1}), whilst the total P concentrations determined in six samples ranged from 0.47 to 3.16 mg P L^{-1}. Another example was published by Buldini et al. [35], who determined phosphate in olive oil mill wastewaters by on-line microdialysis-ion chromatography. In this case, microdialysis allows the organic load of the effluents to be removed in approximately 10 min, without affecting quantitation of the anions. Separation was performed in a Metrosep Anion Dual 2 column with carbonate/hydrogencarbonate isocratic elution and chemically suppressed conductivity detection. Recoveries higher than 96% were obtained, and phosphate concentrations of 298–605 mg L^{-1} were determined [35]. Carbonate/hydrogencarbonate has also been used for the analysis of phosphate and seven other anionic pollutants in refinery wastewater using a Novosep A-1 column, a DS-Plus suppressor, and gradient elution [43]. Another method using an on-line generated hydroxide gradient was developed by Manning and Bewsher [90] for the analysis of inorganic anions in landfill leachates. However, in this case an AS11 column was used for the separation, and sample dilution and pretreatment of the leachates with OnGuard-Ag cartridges were necessary in order to reduce the interference of the high level of chloride in the samples. Large-volume injection (1000 μL) and on-line electrolytically generated hydroxide gradient elution has been applied to the determination of phosphate in samples collected from a fossil fuel power plant. This study used an IonPac AS15 column and an electrolytic suppressor (AES) in the recycle mode. Phosphate was detected at different concentrations (0.78–1600 μg L^{-1}) depending on the additives used in the industrial process [65]. The applicability of an ultra-fast ion-interaction method has also been tested for the determination of inorganic anions in industrial wastewater samples, comparing the results with those of suppressed conductivity IC. The composition of the eluent in the ion-interaction method, consisting of 1.5 mM TBA, 1.1 mM phthalate, and 5% acetonitrile, allowed the use of conductivity and indirect UV detection. A phosphate concentration of 2.4 mg L^{-1} was determined by the standard suppressed conductivity method in an AS4A-SC column with a carbonate/hydrogencarbonate eluent. In contrast, the detection limits of the ion-interaction method were 2 and 1 mg L^{-1} for conductivity and indirect UV detection, respectively, preventing the analysis of phosphate using this method [86].

4.1.2. Natural and drinking waters

Phosphate concentration in natural water (sea, river, and groundwater), which is commonly in the range of 0.09–0.5 mg L^{-1} of P for non-eutrophic sites, as well as in

drinking water, is under the detection limits of most conventional ion chromatographic methods with conductivity detection. Consequently, application of the methods developed with anion standards to the analysis of natural waters is not easy, and requires validation of the methods for phosphate determination in such samples. The validation is commonly performed by recovery studies on synthetic and natural spiked samples [38,48,49]. Whilst the main difficulty in the determination of anions in drinking water is the low concentration of some analytes, in the case of natural water, especially in the analysis of sea water samples, the high salinity of the sample represents the main barrier. Dilution of the samples, especially for sea water, is usually performed before injection into the IC system in order to reduce the concentration of major anions, which improves the resolution and avoids saturation of the exchangers. Another approach to the removal of interfering chloride involves the use of a precolumn. For example, Dahllöff et al. [37] used AG4A precolumns and an AS4A-SC column with two carbonate/hydrogencarbonate eluents in combination with a diverting valve to determine phosphate in sea water samples at concentrations higher than 95 $\mu g\,mL^{-1}$ (1 $\mu mol\,L^{-1}$). In addition, Huang et al. [31] developed a 'heart-cut' column-switching method for the analysis of nitrate and phosphate in samples with high concentrations of chloride. However, due to phosphate contamination of the NaOH eluent, the method was not applicable to real sea water samples, and only nitrate could be determined. Another column-switching system was developed by Bruno et al. [38] for the analysis of phosphate and other inorganic anions in high salinity samples. In this case, separation was performed in a carbonate/hydrogencarbonate selective system with an IonPac AS9-HC column. Optimization was carried out with synthetic samples and the method was finally applied to the analysis of nutrients in sea water samples collected near a river outlet. Phosphate concentration was lower than the detection limit of the method (1 $mg\,L^{-1}$), and the recovery of the spiked sample at that level was 85% [38]. Another way of removing interfering ions in natural water is dialysis with a monovalent-anion-permselective membrane. This procedure has been used for the analysis of phosphate and sulfate in deep subsurface water, performing the separation on a carbonate/hydrogencarbonate IC system. Phosphate was not detected in the real water sample, but complete recovery was calculated from the spiked 50-fold diluted sample (1 $\mu g\,mL^{-1}$) [33]. On-line flow injection dialysis and ion chromatography has been applied to the determination of common inorganic anions in natural waters. The detection limit of phosphate in the FID-IC system using a carbonate/hydrogencarbonate selective column and chemical suppression was 0.37 $mg\,L^{-1}$, not low enough to detect this ion in the analysed samples [36].

Several papers have assessed the applicability of self-regenerator suppressors to the analysis of 'real world' waters. For instance, an extensive study of anion concentrations in Venezuelan groundwater was performed using a chemically suppressed IC method with 21 mM sodium hydroxide as eluent and an IonPac AS11 column with an ASRS suppressor. Elution of six common inorganic anions in a synthetic sample occurred in less than 4 min, phosphate being the most retained anion. Nevertheless, the minimum detectable concentration of phosphate was estimated as 0.1 $mg\,L^{-1}$, and the contents of the analysed samples were under that value, meaning that the method could not be proposed for the analysis of phosphate [91]. Also, groundwater samples collected in industrial and agricultural zones of Lombardia (Italy) have been analysed using an on-line electrolytically generated hydroxide eluent and a high capacity AS16 column with an ASRS module operating in the recycle and external water modes. The latter mode was used when serial conductivity and post column detection was performed. Separation conditions were optimized for the analysis of polarizable anions, and phosphate eluted between chromate and arsenate at retention times higher than 20 min. Although no quantitation of phosphate was reported, a peak corresponding to the elution time of the phosphate standard is observed in the chromatogram of a groundwater sample, indicating the possibility that this method could be used for its analysis [92]. Moreover, anionic pollutants were also determined in the Houston ship channel by IC using a carbonate/hydrogencarbonate (3.5 mM/1.0 mM) eluent in an AS14 column. A concentration range of 0.56–1.88 $mg\,L^{-1}$ was obtained for seasonally obtained sample from various sampling sites [93]. Another IC method has been developed for the simultaneous determination of common inorganic anions (chloride, nitrate, phosphate, etc.) and less concentrated anions (bromide, nitrite, bromate, iodate, etc.) in water [94]. In this case, separation was performed in the carbonate/hydrogencarbonate selective column AS9HC, which was specially designed for the analysis of oxyhalides under isocratic conditions (9 mM sodium carbonate). Its high capacity allows the injection of large sample volumes (200–500 μL). The method combines suppressed conductivity detection with an ASRS module for the major anions, phosphate being one of them, and subsequent postcolumn reaction and UV detection for minor anions. Although phosphate was not detected in the sea water sample, a detectable phosphate signal was present in the chromatogram of the analysed drinking water. The same conditions were used by other authors for the simultaneous analysis of oxyhalides, bromide, and common inorganic anions in simulated and real drinking water samples. For instance, Jackson et al. [53] found a concentration of 0.25 $mg\,L^{-1}$ in drinking water. Recently, an IC method using the same anion-exchange column was optimized for the determination of haloacetic acids and perchlorate in drinking water, although a more concentrated carbonate (28 mM) eluent was required. Under the optimized conditions, the performance of two suppression systems, ASRS and AES, were compared for these analytes, as well as for other common anions. In the case of phosphate, the detection limits using the two different suppressors were 0.77 and 1.98 $\mu g\,L^{-1}$, respectively. However, only haloacetic acids and perchlorate were quantified in the analysed drinking water samples [52]. Another example of the use of self-

regenerating suppressors for the analysis of common anions in tap water is the work of Wu et al. [40], who proposed the use of solid-phase microextration with a polypyrrole fibre in combination with suppressed IC. Automated in-tube SPME and the use of a separation system consisting of an IonPac AS14 column, a carbonate/hydrogencarbonate eluent, and an ASRS module allowed the preconcentration of phosphate and other anions. Although quantitation of the sample was not provided, a small peak corresponding to phosphate was observed in the chromatogram of the sample, indicating the applicability of the method [40]. Micromembrane suppressors with chemical regeneration have also been used. For instance, total phosphorus has been determined in a river sediment by microwave digestion and suppressed conductivity IC. Separation was performed in an IonPac AS4A column with isocratic carbonate/hydrogencarbonate. The method enabled determination of P levels higher than 0.012% (w/w), and the P concentration of the analysed sample was 0.0888% (w/w) [23].

As in the case of wastewater analysis, comparison of the performance of carbonate/hydrogencarbonate (AS14, AS14, and AS4A-SC) and hydroxide (AS17) selective columns for the analysis of surface, raw, and drinking water, as well as soil extracts, has been carried out [48,49]. Phosphate recoveries were satisfactory, and similar in all of the systems tested (>93.8%), and concentrations in tap water samples in the range of 0.1–0.48 mg L^{-1} were found. As in the previous case, higher efficiency for phosphate was obtained using the hydroxide gradient elution system with an AS17 column.

Non-suppressed IC with indirect UV detection has also been proposed for the analysis of groundwater and drinking water samples. Here, the mobile phase consisted of 1 mM *o*-phthalic acid-TRIS buffer, 2% (v/v) ACN (pH 8). Although phosphate concentration was under the limit of detection (not reported) in both samples, good recovery was obtained at a spiked concentration of 5 mg L^{-1} [79]. Indirect UV detection was also applied to the analysis of common inorganic anions in river and sea water samples by fast ion chromatography using a DDAB-coated short (3 cm) ODS column with a 5.0 mM phthalate eluent. Separation in less than 180 s was reported, with phosphate elution at 30 s. Phosphate was determined in a river water sample at a concentration of 0.64 mg L^{-1}, which is slightly higher than the limit of quantitation (0.5 mg L^{-1}) [84].

Electrostatic ion chromatography methods that allow very low detection limits to be achieved have also been applied to the analysis of drinking water samples. For instance, Hu et al. [89] used an ODS-coated column with mixed zwitterionic (Zwittergent 3-14) and cationic (MTA) surfactants as the stationary phase and a 5 mM sodium tetraborate eluent with suppressed conductivity (ASRS module) to analyse phosphate in tap water. Despite its low detection limit (10.56 ng L^{-1}), phosphate was not detected in this sample. Another method, employing a carbonate/hydrogencarbonate eluent in conjunction with a graphitized carbon column coated with CTA, has been applied to the anion analysis of tap and pharmacopoeial grade water with suppressed conductivity detection. As in the previous case, phosphate was not detected at concentrations higher than the limit of detection (3 ng L^{-1}) [83].

4.1.3. Miscellaneous applications

Phosphorus is present in most vegetable and animal foods in the form of inorganic and organic species. In addition to the presence of naturally occurring species, the use of inorganic phosphates and polyphosphates as fertilisers and additives increases the phosphorus content in these samples. Most applications in the field of food and plant analysis consist of the determination of total phosphorus, which requires convenient sample treatment to convert the different phosphorus species into phosphate [4–8]. Consequently, most IC methods are devoted to the determination of orthophosphate, which is usually performed using a carbonate/hydrogencarbonate eluent and suppressed conductivity detection [8,9,50,54], although non-suppressed conductivity methods have also been applied [77]. For instance, coffee and tea extracts have been analysed by non-suppressed ion chromatography with a PRP-X110 column using 0.6 mM potassium hydrogenphthalate with 4% (v/v) acetonitrile as eluent. Phosphate, in addition to chloride and organic acids, has been determined in various samples, obtaining concentrations in the order of 1.5–2.97 mg g^{-1} [77]. Organic and inorganic anions have also been simultaneously analysed in coffee using carbonate/hydrogencarbonate gradient elution in an Allsep A-2 column with the solid-phase conductivity suppressor DS-Plus, although phosphate quantitation was not reported [43]. Suppressed ion-chromatography using an ASRS suppression module operating in the recycle mode has been used for the analysis of aqueous extracts of edible plants with an IonPac AS12A column and carbonate/hydrogencarbonate as eluent. Phosphate contents, of 7.8–80.6 mg g^{-1} (dry plant tissue), have been determined in cotyledons, leaves, and root tissues [54]. This method, using an IonPac AS4A column with isocratic elution, has been proposed for the determination of phosphate and other inorganic anionic nutrients in sugar beet seed exudates [50]. Both non-suppressed and suppressed conductivity methods have been tested for anion determination in diluted sugar liquors and molasses. The non-suppressed conductivity analysis was performed in a Hamilton PRP-X100 column with a 4 mM *p*-hydroxybenzoic acid–2.5% methanol eluent, and a detection limit of 0.8 mg L^{-1} was obtained for phosphate. Good linearity was observed in the range of 0.5–100 mg L^{-1}. In contrast, in the suppressed system, the phosphate linear calibration range was limited to 5 mg L^{-1}, but a better detection limit (0.05 mg L^{-1}) was obtained. Suppressed conductivity analysis was carried out in a Metrohm Compact IC system with a Metrosep Anion Dual 1 column and isocratic carbonate/hydrogencarbonate elution [59]. Isocratic carbonate/hydrogencarbonate elution was also applied to the analysis of inorganic anions in milk after oxidative photodegradation of the matrix. Phosphate, corresponding to total phosphorus content, was determined at a concentration of 326 mg L^{-1}, whilst a detection limit of

25 μg L^{-1} was estimated using an ASRS suppressor module and an IonPac AS14 column [27]. A similar system, but using an IonPac AS9 column, was also used by the same group to analyse total phosphorus in edible vegetable oils and fats. After matrix removal, concentrations ranging from <10–530,250 μg kg^{-1} were determined in the samples and a detection limit of 35 μg kg^{-1} ($P–PO_4$) was obtained [26].

Ion chromatography has also been applied to the analysis of phosphate in degradation and corrosion products of different materials. For instance, dosage of orthophosphoric acid is used to reduce lead concentration in water from public lead pipes, which produces, in addition to a high concentration of phosphate in water (523 μg L^{-1}), the deposition of hydroxyapatite, as indicated by the characterization of the corrosion products. Peters et al. [95] used a carbonate/hydrogencarbonate IC system with an AS5 column and an ASRS module to analyse the samples after dissolving them with sodium carbonate at 900 °C. Another IC method using a carbonate/hydrogencarbonate selective system composed of two columns in series (AS4A-SC and AS9-SC) has been developed for the determination of anions in environment-exposed stones and mortars [96]. The method was applied to the analysis of aqueous solutions of degradation patinas on historic buildings and monuments. Resolution of 13 inorganic and organic anions was obtained in 25 min, with phosphate elution occurring at approximately 15 min. Concentrations of phosphate in the black crusts and surface layers of ancient monuments ranged from <1 to 283 μg g^{-1}. In addition, IC has also been employed for the determination of monofluorophosphate in concrete and hardened cement paste applied to avoid corrosion. Carbonate/hydrogencarbonate systems (AS14 and AS9HC) with ASRS suppression in recycle mode have been used to analyse aqueous and acidic extracts [97]. Whilst neither monofluorophosphate (MFP) nor its hydrolysis products were detected in aqueous extracts, monofluorophosphate hydrolysis products, phosphate and fluoride, were determined after acid extraction, reflecting the total amount of MFP [97,98].

The control of inorganic contaminants at very low levels in high purity reagents used for relevant applications, such as the semiconductor and electronics industries, is another example of the use of IC for phosphate analysis. Development of IC methods for this kind of application is focused on two objectives: the chromatographic separation of phosphate from large amounts of other matrix anions and the detection of very low concentrations of this analyte. Dilution of the samples, which is usually performed to accomplish the first objective, reduces sensitivity. Phosphate has been analysed in lead-acid battery electrolytes (usually 38% sulfuric acid) by IC with an IonPac AS9 column and carbonate/hydrogencarbonate eluent with an ASRS suppressor. Because of the relatively high detection limits (1.3 mg L^{-1}), phosphate was not detected in fresh solutions, but as levels increased up to 4.95 mg L^{-1} with aging of the electrolyte, it could be determined in used electrolytes [99]. The use of a microbore column (AS15) and an on-line KOH generator enhanced sensitivity, allowing a lower detection limit (120 μg L^{-1}). This method was used for the determination of anion contamination of high-purity nitric acid and reagent grade sodium nitrate solutions, showing that phosphate concentration was below the detection limit [63]. Recently, a coupled ion chromatography system was used for the determination of several contaminant anions (phosphate, chloride, and sulfate) in concentrated nitric acid (69%, w/w). Heart-cut transfer and a concentrator column were used to remove nitrate interference and to concentrate the analytes. The detection limit of phosphate in the concentrated acid was calculated as 5 mg L^{-1}, whilst the value corresponding to the 100-fold diluted injected solution was approximately 50 μg L^{-1} [39]. Semiconductor grade hydrogen peroxide (30%) has also been analysed for anion contamination [28,66]. Gradient elution of NaOH in microbore hydroxide-compatible columns (AS11 or AS15) and suppression with an ASRS module working in the external-water mode provided a detection limit as low as 1 μg L^{-1} for phosphate. Nevertheless, in order to eliminate the matrix, and consequently, protect the columns resins from peroxide attack, the use of a concentrator column for the anionic analytes and the application of a digestion procedure based on the platinum decomposition of hydrogen peroxide, allowing peroxide concentration to be reduced to 200 ppm, were proposed to reduce the amount of peroxide injected into the separation column. Recently, trace levels of phosphorus were determined in purified quartz by combining a vapour phase digestion (VPD) with a mixture of $HF–HNO_3–H_2O_2$, and the further determination of the phosphate generated by ion-exchange chromatography in an isocratic hydroxide selective system (AS17) with an ASRS module for suppressed conductivity detection [100]. The limit of detection of phosphate in quartz was estimated as 0.05 μg g^{-1}. The same authors applied a similar strategy to the determination of trace ions in boric acid. In this case, the borate matrix was eliminated as trimethyl borate ester using a mixture of glycerol–methanol for the vapour phase matrix elimination step. Phosphate concentration in two borate reagents was estimated as 0.092 and 0.213 μg g^{-1}, and the detection limit of the method was in the order of 8 ng g^{-1} [101]. Moreover, methodology for the determination of anion contamination in hydrofluoro ethers has recently been published. Extraction of trace anions with water, preconcentration with a concentrator column (TAC-LP1), and further separation in an AS11 column with a NaOH elution gradient using an ASRS module for suppressed conductivity detection were the basic steps. Good phosphate recovery (92%) was obtained at a spike level of 25 μg L^{-1} [102]. Another example of the applicability of IC to the analysis of pure reagents is the determination of trace levels of phosphate (LOD: 0.14 μg L^{-1}) in ultrapure water [58]. In this case, a preconcentration column was used instead of the sample loop, and the contaminant anions were transferred to the analytical column in a 500 μL fraction of eluent.

4.2. Other inorganic phosphorus species

In contrast to the extensive literature on applications of IC for phosphate determination, less work has been published reporting the separation of phosphorus oxyanions with lower oxidation states: phosphite(III) and hypophosphite(I) [4,19,103]. In fact, as it has traditionally been assumed that phosphorus occurs in the environment as P(V), standard speciation procedures, mainly based on the sequential determination of orthophosphate generated after conversion of the phosphorus species by different treatment procedures, would classify reduced phosphorus species mostly as organic phosphorus (persulfate-digested fraction) or condensed phosphorus species (acid-hydrolysed fraction) [19]. However, recent evidence indicates the presence and bioavailability of reduced phosphorus species in the inorganic reactive phosphorus fraction [19,103]. To separate phosphate, phosphite, and hypophosphite, non-suppressed ion chromatography with conductivity detection using 0.2 M succinic acid (pH)/5% ACN and an OmniPac PAX-500 column, or borate-gluconate with glycerol and a Waters IC-PAK A column has been proposed. In the former case, the elution order was hypophosphite, orthophosphate, phosphite, and the detection limits were estimated as 0.1 mg L^{-1} for hypophosphite and 0.5 mg L^{-1} for the other two anions [104]. The second system was applied to the detection of reduced oxyanions in combustion solutions of organophosphorus compounds [105]. An ion-exchange method with suppressed conductivity was applied to the determination of phosphorus (phosphite) and phosphoric (phosphate) acids in air. Aerosols collected on paper filters in poisoning chambers were treated with water prior to injection [106]. Moreover, a hydroxide selective suppressed IC system (AS17 column) with a multi-step gradient elution (0.5–35 mM) has recently been employed to analyse the same oxyanions in natural geothermal water. Suppression with an ASRS module with external water regeneration provided detection limits of 53, 31, and 34 μg L^{-1} for hypophosphite, phosphite, and phosphate, respectively. The chromatogram of an anion standard containing these species and other anions is given in Fig. 6. As can be seen, difficult separations of hypophosphite, fluoride, and phosphite from hydrogencarbonate were successfully accomplished with satisfactory resolution in a run time of less than 25 min [103]. The detection limits of this method are superior to those reported using indirect UV and a 4-amino-2-hydroxybenzoic acid eluent, which were 0.5, 1.2, and 1.4 μg mL^{-1} for hypophosphite, phosphite, and orthophosphate, respectively [107].

Condensed phosphates are phosphate oligomers that are commercially produced for a variety of applications, such as detergents, sequestering agents, and food additives (preservatives and acidulents). Thus, the determination of these compounds in water and food samples is of interest [4,8,108]. These compounds are classified according to their structure as polyphosphates (linear phosphates P2–Pn), metaphosphates (cyclic phosphates, Pm), or ultraphosphates (crosslinked phosphates). Chromatographic and electrophoretic methods

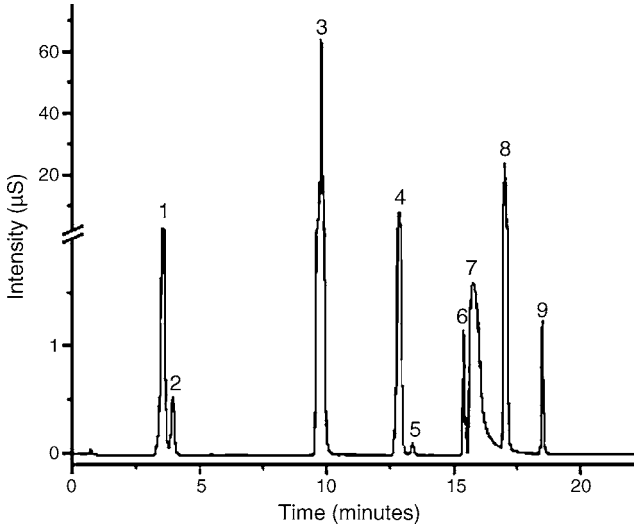

Fig. 6. Chromatogram of a 1:4 dilution of synthetic geothermal water spiked with hypophosphite, phosphite and orthophosphate at concentration of 20 μM each. Peak identification: (1) fluoride, (2) hypophosphite, (3) chloride, (4) bromide, (5) nitrate, (6) phosphite, (7) hydrogencarbonate, (8) sulfate, (9) phosphate. Separation system: IonPac AG17 precolumn and IonPac AS17 (4 mm i.d.) column. Mobile phase: electrochemically generated gradient KOH. Gradient: 0.5–35 mM. Flow rate: 1.5 mL min^{-1}; detection: suppressed conductivity (ASRS Ultra) in external water mode. Reproduced from Ref. [103] with permission from Elsevier.

for linear and cyclic polyphosphates have been reviewed by Rosset et al. [108]. In general, retention increases with increasing number of phosphorus atoms in the molecule. Ion chromatography methods using suppressed conductivity or indirect UV detection have been developed using hydroxide, isocratic, and gradient eluents in the first case [109,110], and naphtalenetrisulfonate [111] or pyromellitic solutions as non-suppressed eluents in the second [112]. These authors also demonstrated that addition of EDTA (80 μM) to the mobile phase resulted in improved peak symmetry and a reduction in peak tailing. This positive effect is due to the complexation of traces of heavy metal ions in the eluent, avoiding complexation and hydrolysis of polyphosphates. Linear polyphosphates (P2–P13) were separated using 2 or 20 mM pyromellitic acid/80 μM EDTA with indirect UV detection [112]. Post-column molybdate colorimetric detection has also been traditionally used in this analysis, although it requires the hydrolysis of condensed phosphate to orthophosphate after the separation step. Analysis of condensed linear phosphates in domestic wastewater using unsuppressed IC coupled with phosphate-specific post-column FIA UV-detection has been reported, obtaining low detection limits of 10–20 mg L^{-1} [113]. Capacity gradient elution based on complex formation between borate and diol compounds has been applied to the analysis of orthophosphate and condensed phosphates up to P7 in a synthetic mixture and in a cheese extract. The separation was performed in an IC-Anion-PW column using a dihydroxyphenylborane-mannitol eluent system and suppressed conductivity detection. In order to maintain a stable baseline, the borate concentration was kept constant during

the gradient of 16–0 mM mannitol. The decrease in mannitol concentration in a dihydroxyphenylborane-mannitol eluent accelerated the formation of the borate complex with diol groups on the resin, which causes a decrease in the capacity of the column and the consequent elution of the strongly retained polyphosphates [114].

Most new applications in this field involve the use of electrolytic on-line hydroxide generators for the production of gradient elution [62,68,115,116]. For instance, an ion chromatography method with gradient elution (30–100 mM KOH) using an on-line hydroxide eluent generator and an ASRS suppressor was used for the analysis of P1–P4, P3m and inositol phosphates. Injection of food extracts with trichloroacetic acid allowed the detection of phosphate and polyphosphates up to P7 in different matrices. Detection limits of 0.12–0.19 μM for the condensed phosphates and 0.5 μM for orthophosphate were obtained [62]. Similar conditions were applied to the determination of tripolyphosphate in fish, providing a detection limit of 5 mg kg^{-1} [114]. Moreover, as shown in Fig. 7, polyphosphate mixtures up to P50 can be separated in approximately 30 min using a hydroxide gradient of 30–200 mM and an AS11 column. This method was applied to the analysis of chain-length specificity of the enzyme polyphosphate kinase (PPK) [115]. In order to estimate the minimum calibration frequency required for the IC analysis of orthophosphate, pyrophosphate, and tripolyphosphate in control analysis, Stover and Brill [109] applied multivariate statistical tools. The chromatographic separations were performed in an AS11 column with a NaOH elution gradient (20–70 mM) and an ASRS in recycle mode. The response of orthophosphate and pyrophosphate has been demonstrated to be stable over 10 weeks, reducing the need for more frequent calibration. In contrast, tripolyphosphate response shows a decrease with eluent aging, suggesting the need for weekly calibrations.

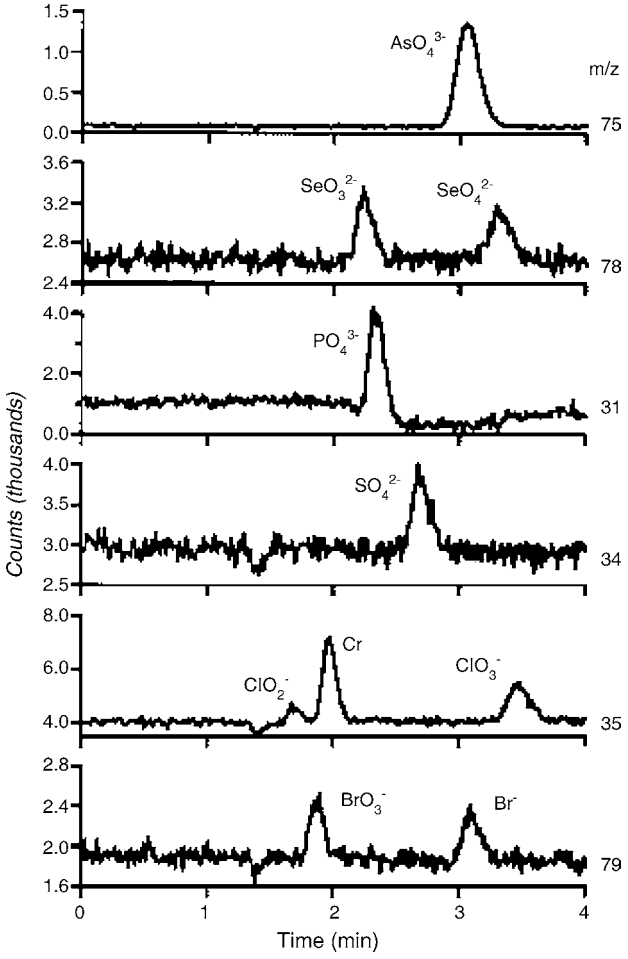

Fig. 7. IC-ICP-MS chromatograms of individual monitored elements for a standard solution of BrO_3^- and Br^- (each 1.0 μg L^{-1} Br), ClO_2^-, Cl^- and ClO_3^- (each 50 μg L^{-1} Cl), SO_4^{2-} (each 20 μg L^{-1} S), PO_4^{3-} (20 μg L^{-1} P), SeO_3^{2-} and SeO_4^{2-} (each 1.0 μg L^{-1} Se), and AsO_4^{3-} (1.0 μg L^{-1} As) at optimum operating conditions. Eluent: 11 mM ammonium carbonate; column: IonPac AS12A; flow rate: 2.0 mL min^{-1}; nebulizer, ultrasonic with membrane desolvator. Reproduced from Ref. [55] with permission from Elsevier.

4.3. Organic phosphorus species

Phosphorus occurs in several naturally occurring and industrially produced organic species. Natural species, such as sugar phosphates, display important biological functions. Moreover, anthropogenic organophosphorus species, such as alkyl substituted phosphonic acids, alkylphosphates, and bisphosphonates, have been used as herbicides, pesticides, detergents, and chemical warfare agents. Monitoring of their concentrations in the environment is required due to their toxicity and their negative effect on the euthrophication of environment systems. Ion chromatography methods have been developed to characterize organic phosphorus species, to study their degradation products, and to determine their concentration principally in environmental samples.

Among natural species, phytate and its degradation products, inositol phosphates, are the group of organic phosphorus compounds most frequently analysed by IC. Phytate, or phytic acid (inositol hexakis-phosphate, InsP6), is the most abundant form of phosphorus in plants. It has traditionally been considered an antinutrient since it binds minerals, thus decreasing their bioavailability. However, current interest in these compounds has been raised by studies that demonstrate their beneficial effects [117]. During food processing and digestion, phytate can be enzymatically and non-enzymatically degraded to inositol phosphates (InsP5-InsP1), and several isomers of inositol phosphate, as well as phytate, show important physiological effects in the treatment of cancer, diabetes, and heart disease, among others. The study of enzymatic degradation is another focus of research, as phytases also catalyze the hydrolysis of phosphate moieties. Moreover, supplementation of animal feed with phytase is thought to enhance the bioavailability of phosphorus, and as a consequence, can reduce phosphorus pollution in farm areas [118,119]. In this context, anion-exchange and reversed-phase chromatographic methods have been developed for the analysis of phytate and inositol phosphate. Gradient elution

allows the separation and quantitation of inositol hexakisto mono-phosphates (InsP6–InsP1) and their positional isomers. For instance, the performance of six different strong anion-exchange columns has been studied in two chromatographic systems. The first one uses acidic (HCl) gradient elution with postcolumn derivatization using a solution of iron nitrate and perchloric acid and UV detection, and was applied to InsP2-InsP6 isomer separation. For the separation of InsP3–InsP1 isomers, a sodium hydroxide gradient with chemically suppressed conductivity detection was used. The columns that showed better efficiency under acidic conditions were CarboPac PA-10 and PA-100, whilst OmniPac PAX-100 and AS11 where the best columns under alkaline conditions. The acidic system has been applied to the analysis of a cereal sample [120] and the postcolumn UV detection method has been used for the analysis of 35 inositol phosphates, allowing their separation into 27 peaks in 65 min. This method was applied to the study of the enzymatic hydrolysis mechanism of phytases [118]. An OmniPac PAX-100 column with suppressed conductivity detection with an ASRS module has also been used to determine Phytate (InsP6) content in oil, cereal, and legume seeds. In this case, a ternary gradient eluent containing sodium hydroxide, water, and isopropanol-water (1:1) was used. Comparison of the phytate contents obtained by absorptiometric and IC methods revealed that overestimation of phytate occurs in absorptiometry. Values ranging from 0.29 to 1.28% have been determined in sample seeds [121]. Recently, Harland et al. [122] analysed phytate in 82 commonly consumed foods derived from plant seeds to evaluate the risk of zinc deficiency. An ion-pair high-performance liquid chromatography method with refractive index detection has been developed on an analytical scale and as a preparative purification method. Phytate and its inositol phosphate degradation fractions (InsP3–InsP5) were separated in C18 columns using 51% MeOH containing 0.6–1% tetrabutylammonium hydroxide as ion-pair reagent and 0–0.025 M formic acid (pH 4.3) as eluent [123].

Tributyl phosphate (TBP) is a phosphoric alkyl ester that is usually applied in the nuclear industry as a uranium and plutonium extractant for recycling purposes. Degradation of TBP generates dibutyl phosphate (DBP) and monobutyl phosphate (MBP). Both analytes have been analysed by IC with suppressed conductivity detection. Comparison of the performance of two columns, AS5 and AS11, with two sodium hydroxide gradients has been published. Better resolutions between phosphate esters and inorganic anions were obtained in the AS5 column, with detection limits of 20 and 150 $\mu g\,L^{-1}$ for MBP and DBP, respectively [124].

Phosphonic acids (methyl phosphonic and ethyl phosphonic acids), which are hydrolysis products of alkyl substituted organophosphorus acids, have been determined by IC using evaporative light scattering detection [125]. A volatile mobile phase consisting of 25 mM ammonium hydrogencarbonate (pH 7.8) was used, and the separation was performed in an IonPac AS4A-SC column. This methodology was applied to the analysis of water with high levels of inorganic anions (1000 mg L^{-1} phosphate). Detection limits of 1 mg L^{-1} were estimated for both phosphonic acids. Methyl phosphonic and isopropyl methyl phosphonic acids have also been analysed in soil samples by chemically suppressed conductivity IC. An eluent composed of 3.75 mM sodium hydroxide/10 mM sodium tetraborate enabled the separation of both phosphonic acids and inorganic anions, such as chloride and phosphate, occurring in soil extracts. The compounds were determined at sub-ppm ($\mu g\,g^{-1}$) concentrations, and detection limits of 10 and 20 $\mu g\,L^{-1}$ were estimated for methyl phosphonic and isopropyl methyl phosphonic acids, respectively [126].

Compared with the analysis of inorganic species, ion chromatography coupled to mass spectrometry detection, with electrospray and thermospray ionisation sources, or by P-specific ICP-MS methods, have been more frequently used. For instance, glyphosate and its metabolite aminomethylphosphonic acid have been determined by ion chromatography-electrospray mass spectrometry (IC-ESI-MS) in the negative ion mode. The separation was performed in a Methohm Dual 2 column with carbonate/hydrogencarbonate gradient elution. Conductivity suppression using a suppression module composed of ion-exchanger columns before mass spectrometry provided a significant increase in sensitivity due to the reduction of both background signal and salt adduct formation. Analysis of both compounds in natural waters at a concentration level of 1 $\mu g\,L^{-1}$ has been reported [127]. Methyl and dimethyl phosphates in organophosphate insecticides have also been analysed by IC-MS using thermospray and electrospray interfaces. Separation was performed in a hydroxide selective column (AS11) with sodium hydroxide and methanol as eluents. Before entering the mass spectrometer, a high-resolution double focusing instrument with an EBEQQ geometry, sodium ions were removed from the eluent on-line with a solid-phase chemical suppressor. Dimethyl phosphate elutes before methyl phosphate, being detected at *m*/*z* 144 and 130, respectively, as M+18 ions in the thermospray chromatograms, whilst in the case of electrospray, $[M-H]^-$ ions were detected [128]. The same author reported the use of a similar instrument setup with a hydroxide eluent gradient for the elucidation of organophosphorus agricultural chemicals, methyl phosphate, and alkyl phosphoramidothioate, among others [129].

Recently, IC coupled to ICP-MS has been proposed for the analysis of bisphosphonates, analogs of pyrophosphate containing C–P instead of O–P linkages [56]. These compounds were originally synthesised as complexing agents for water treatment and detergent additives, although evidence in favour of their physiological effects on bone diseases, AIDS, and cancer have increased the interest in these products [56,130–131]. They are commonly determined by ion-exchange chromatography, although they exhibit high retention due to their ability to chelate trace metal contaminants retained by residual underivatized cation-exchange groups on the anion-exchange column. The IC-ICP-MS method developed for the analysis of two bisphosphonates

(alendronic and etidronic acids) and orthophosphate uses an AS17 column and dilute nitric acid (1.5 and 15 mM) as eluent and a metal trap column before the injector. Detection limits of 0.05–0.20 mg L^{-1} have been obtained at *m*/*z* 31 for phosphorus ions ($^{31}P^{+}$). Optimization of plasma conditions to reduce background signal due to interference of polyatomic ions at *m*/*z* 31 was required [56]. Post-column derivatization procedures have also been proposed for the detection of bisphosphonates after anion-exchange chromatography. For instance, post-column indirect fluorescence detection with a solution of Al^{3+}-morin complex was used to detect four bisphosphonates and pyrophosphate after their separation in a PRP-X100 column using a 40 mM sodium nitrate/4.1 mM sodium hydroxide mobile phase. Detection limits of 0.4–1.0 mg L^{-1} were reported [130]. Direct photometric detection after postcolumn derivatization with sodium molybdate has also been reported [131].

5. Concluding remarks

Nowadays, ion chromatography has become the method of choice for the routine determination of phosphorus species. The greatest number of applications has been developed for the analysis of phosphate, which is the most abundant form of phosphorus in the environment. In addition, determination of other phosphorus species is commonly performed by their conversion into phosphate and its further chromatographic analysis as this form. Ion-exchange chromatography with suppressed conductivity detection using either carbonate/hydrogencarbonate or hydroxide selective systems in combination with electrolytically self-regenerating membranes and solid-phase-based suppressors is the most common method used for phosphate determination. Well-established separation methods have been applied to the determination of phosphate in different samples, particularly in the fields of water and food analysis. In fact, experimental conditions for the determination of phosphate in water have been specified by some regulatory methods, and a significant number of the reviewed applications have been performed under these or similar conditions. In contrast, few applications of non-suppressed conductivity conditions have been reported, which is probably due to the poor sensitivity of these systems. Moreover, other different separation mechanisms, such as ion-interaction and electrostatic ion chromatography, have also been applied to the analysis of phosphate. Both chromatographic modes involve the use of reversed-phase columns modified with cationic and/or zwitterionic surfactants. In particular, a number of methods using electrostatic ion chromatography for the rapid determination of phosphate and other inorganic anions have recently been published.

Recent advances in ion chromatography technology, particularly on-line electrolytic hydroxide generator, new membrane and packed bed electrolytically self-regenerating suppressor devices, and high-capacity columns, have been implemented for the analysis of phosphate. Their application allows the use of elution gradients in both carbonate/hydrogencarbonate and hydroxide selective systems, improving sensitivity and reducing total analysis time when samples containing phosphate together with other inorganic anions are analysed. Another advantage of the use of these developments is the reduction of time and ease of operation for eluent preparation and sample treatment. However, despite the significant enhancement of sensitivity, further improvements are expected to extend the application of IC to the determination of ultra-trace levels in samples with very low phosphate concentration, such as drinking water.

Although to a lesser extent, ion chromatography has also been applied to the analysis of other inorganic (reduced and condensed) and organic (phytates, alkyl phosphate, and phosphonates) phosphorus species. As in the case of phosphate analysis, it is envisaged that the implementation of new technology will improve the performance of ion chromatography methods for the determination of these compounds.

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