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Flow analysis techniques for phosphorus: an overview

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Abstract

A bibliographical review on the implementation and the results obtained in the use of different flow analytical techniques for the determination of phosphorus is carried out. The sources, occurrence and importance of phosphorus together with several aspects regarding the analysis and terminology used in the determination of this element are briefly described. A classification as well as a brief description of the basis, advantages and disadvantages of the different existing flow techniques, namely; segmented flow analysis (SFA), flow injection analysis (FIA), sequential injection analysis (SIA), all injection analysis (AIA), batch injection analysis (BIA), multicommutated FIA (MCFIA), multisyringe FIA (MSFIA) and multipumped FIA (MPFIA) is also carried out. The most relevant manuscripts regarding the analysis of phosphorus by means of flow techniques are herein classified according to the detection instrumental technique used with the aim to facilitate their study and obtain an overall scope. Finally, the analytical characteristics of numerous flow-methods reported in the literature are provided in the form of a table and their applicability to samples with different matrixes, namely water samples (marine, river, estuarine, waste, industrial, drinking, etc.), soils leachates, plant leaves, toothpaste, detergents, foodstuffs (wine, orange juice, milk), biological samples, sugars, fertilizer, hydroponic solutions, soils extracts and cyanobacterial biofilms are tabulated.

Keywords: Flow techniques of analysis; Phosphorus analysis

1. Sources, occurrence and importance of phosphorus

Phosphorus is one of the key elements necessary for the growth of plants and animals. Phosphorus in the elemental form is very toxic and is subject to bioaccumulation. Phosphates, which derive from this element, are present in three forms: orthophosphate, polyphosphate and organically bound phosphate. Ortho forms are produced by natural processes and are found in sediments, natural waters and sewage. Poly forms are used for treating boiler waters and in detergents. In water, they change into the ortho form. Organic phosphates play an important role in nature, and their occurrence may result from the breakdown of organic pesticides containing phosphates. They may exist in solution, as particles or in the bodies of aquatic organisms. Phosphorus in aquatic systems may originate from natural sources such as the mineralization of algae and the dissolution of phosphate minerals, from anthropogenic point source discharges of sewage and industrial effluents and from diffuse inputs from grazing and agricultural land. Phosphate stimulates the

Abbreviations: 12PM, 12-phosphomolybdate; AAS, atomic absorption spectrometry; AIA, all injection analysis; AP, apatite phosphorus; BAP, bioavailable phosphorus; BIA, batch injection analysis; CFA, continuous flow analysis; CL, chemiluminiscence; FAHP, filterable acid-hydrolysable phosphorus; FCP, Filterable condensed phosphates; FIA, flow injection analysis; FIP, flow injection potentiometry; FOP, filterable organic phosphorus fraction (FOP = TFP − (FAHP + FRP)); FRP, filterable reactive phosphorus; HGAAS, hydride generation AAS; ICP-AES, inductively couple plasma atomic emission spectrometry; IP, inorganic phosphorus; ISE, ion-selective electrodes; MCFIA, multicommuted flow injection analysis; MG, malachite green; MRP, molybdate reactive phosphorus; MSFIA, multisyringe flow injection analysis; MPS, multipump system; μTAS, micro-total analysis system; OP, organic phosphorus; PAP, particulate acid-hydrolysable phosphorus (PAP = TAHP − FAHP); PMB, phosphomolybdenum blue; POP, particulate organic phosphorus (POP = TOP − FOP); PRP, particulate reactive phosphorus (PRP = TRF − FRP); PVM, vanadomolybdophosphate complex; QCM, quartz-crystal microbalance; r-FIA, reversed flow analysis systems; r-SIA, reversed sequential injection analysis; SFA, segmented flow analysis; SIA, sequential injection analysis; TAHP, total acid-hydrolysable phosphorus; TFP, total filterable phosphorus; TOP, total organic phosphorus; TP, total phosphorus; TPP, total particulate phosphorus (TPP = TP − TFP); TRP, total reactive phosphorus; NAIP, non-apatite inorganic phosphorus

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growth of plankton and aquatic plants which provide food for fish. However, if an excess of phosphate enters in the aquatic system, algae and aquatic plants will grow wildly, choke up the aquatic system and use up large amounts of oxygen. This condition is known as eutrophication or overfertilization of receiving waters. The rapid growth of aquatic vegetation can cause the death and decay of vegetation and aquatic life due to the decrease in dissolved oxygen levels. Phosphorus is an essential mineral required by every cell in the human body for normal function [1]. The majority of the phosphorus in the body is found as phosphate (PO_4^{3-}). Approximately 85% of the body's phosphorus is found in bone [2]. Phosphorus is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. Phospholipids (e.g., phosphatidylcholine) are major structural components of cell membranes. All energy production and storage are dependent on phosphorylated compounds, such as adenosine triphosphate (ATP) and creatine phosphate. Nucleic acids (DNA and RNA), responsible for the storage and transmission of genetic information, are long chains of phosphate-containing molecules. A number of enzymes, hormones and cell signalling molecules depend on phosphorylation for their activation. Phosphorus also helps to maintain normal acid–base balance (pH) in its role as one of the body's most important buffers. The phosphorus-containing molecule 2,3-diphosphoglycerate (2,3-DPG) binds to hemoglobin in red blood cells and affects oxygen delivery to the tissues of the body [1]. Phosphorus is found in most foods since it can be considered a critical component of all living organisms. Dairy products, meat, and fish are particularly rich sources of phosphorus. Phosphorus is also a component of many polyphosphate food additives and is present in most soft drinks as phosphoric acid. The phosphorus in all plant seeds (beans, peas, cereals, and nuts) is present in a storage form of phosphate called phytic acid or phytate. Only about 50% of the phosphorus from phytate is available to humans owing to the lack of enzymes (phytases) which liberate it from phytate [3]. Yeasts possess phytases and, therefore, whole grains incorporated into leavened breads have more bioavailable phosphorus than whole grains incorporated into breakfast cereals or flat breads [2]. Some investigators are concerned about the increasing amounts of phosphates in the diet which can be attributed to phosphoric acid in soft drinks and phosphate additives in a number of commercially prepared foods [4,5]. Since phosphorus is not as tightly regulated by the body as calcium, serum phosphate levels can rise slightly with a high phosphorus diet, especially after meals. High blood phosphate levels reduce the formation of the active form of vitamin D (calcitriol) in the kidneys, reduce blood calcium and lead to increases in the parathyroid hormone (PTH) released by the parathyroid glands. However, high serum phosphorus levels also lead to decrease urinary calcium excretion [2]. If sustained, elevated PTH levels could have an adverse effect on bone mineral content, however, this effect has only been observed in humans on diets which are high in phosphorus and low in calcium. The most serious adverse effect of abnormally elevated blood levels of phosphate (hyperphosphatemia) is the calcification of non-skeletal tissues, most commonly, the kidneys. Such calcium phosphate deposition can lead to organ damage, especially kidney damage [1].

2. Analysis of phosphorus and terminology

Phosphorus determination is of great importance from the environmental, nutritional and clinical point of view. Most of the analytical methods for the determination of phosphorus require the samples being in solution or being put in solution. The analysis of water samples (natural, waste, etc.) are especially complex owing to the fact that phosphorus can be found in the form of different inorganic and organic species [6] which in turn can be present in either the dissolved, colloidal or particulate form. However, the dominant species is always orthophosphate. Usually, in the analysis of water samples the analysis of the phosphorus content is carried out on aliquots of the whole sample and on aliquots of the sample previously filtered through membrane filters of 0.45 and 0.2 μm nominal pore size [7] or glass fiber filters (GF/F 0.7 and 1.2 μm) [8]. The aim of this procedure is to obtain the data required for the calculation of the parameters which allow the evaluation of aspects such as the content of phosphorus in several organic and inorganic species, the eutrophication of aquatic systems or the amount of bioavailable phosphorus (BAP). Parameters determined on the filtered fraction contain the word filterable, namely: filterable reactive phosphorus (FRP), total filterable phosphorus (TFP) and filterable acid-hydrolysable phosphorus (FAHP), however, in the literature it is indistinctively used together with the words dissolved or soluble. On the other hand, the term reactive refers to the phosphorus species which react with molybdate to form 12-phosphomolybdate (12PM) or phosphomolybdenum blue (PMB), the latter if a reducing agent is present in the reaction medium. Filterable condensed phosphates (FCP) are comprised of inorganic polyphosphates, metaphosphates and branched ring structures. The term acid-hydrolysable phosphorus refers to the required acidic hydrolysis for the conversion of condensed phosphates to orthophosphate. Therefore, FCP = FAHP and if the formation reaction of 12PM or PMB is used for the corresponding determination, thus, FRP + FAHP is obtained. The filterable organic phosphorus fraction (FOP = TFP − (FAHP + FRP)) consists of nucleic acids, phospholipids, inositol phosphates, phosphoamides, phosphoproteins, sugar phosphates, aminophosphonic acids, phosphorus-containing pesticides as well as organic condensed phosphates [8–11]. The parameters obtained on the aliquots of the whole sample (without filtration processes) contain the word total, namely: total reactive phosphorus (TRP), total acid-hydrolysable phosphorus (TAHP), total phosphorus (TP) and total organic phosphorus (TOP) and are equivalent to those previously mentioned, however, they also consider the particulate fraction. Determination of FOP, TFP, TP or TOP requires a previous digestion of the sample for

the conversion of the organic phosphates into the orthophosphate reactive specie. This digestion can be performed in different ways, which can be classified in three groups, namely: thermal methods (wet chemical [12–16], high temperature combustion and fusion [17–19] and microwave [20–23]), ultraviolet photo-oxidation methods [24–28] and combined thermal hydrolysis and photo-oxidation methods [29]. In this context, the methodology proposed by Solórzano and Strickland [28] should be mentioned. The authors have observed that UV photo-oxidation alone is insufficient to convert condensed phosphates into orthophosphates and suggest that the use of this procedure provides a basis for the discrimination between the organic and condensed phosphorus fraction. The numerical treatment of the parameters determined on the filtrated and the whole fractions of the sample allow the evaluation of other parameters related to the contents of phosphorus in the particulate phase, namely: total particulate phosphorus (TPP = TP − TFP), particulate reactive phosphorus (PRP = TRF − FRP), particulate acid-hydrolysable phosphorus (PAP = TAHP − FAHP) and particulate organic phosphorus (POP = TOP − FOP). All determined, as previously, based on the transformation into orthophosphate and the reaction of 12PM or PMB. Thus, both TP and FRP are the most measured parameters. TP provides a measurement of the maximum potential bioavailable phosphorus, whereas FRP, comprising mostly orthophosphate, provides an indication of the amount of most readily bioavailable phosphorus. Nevertheless, several researches [30,31] have found discrepancies between the bioavailable phosphorus (BAP) estimated by using the previous parameters (quick and easy to determine) and that classically used, i.e., the algal bioassay [32] (slow, uncomfortable and with poor sensitive). The use of iron oxide adsorption methods in their variants of iron-impregnated strips [33,34] and the diffusive gradients in thin films (DGT) [35] constitute convenient alternatives for the estimation of BAP [36]. The enzymatic methods are an alternative way of determining bioavailable phosphorus, thus, alkaline phosphatase has been used in both soluble [37,38] and immobilized form to hydrolyse FOP in natural waters, and the resultant orthophosphate has been detected along with the FRP [39]. The alkaline phosphatase hydrolysable phosphorus (APHP) has been determined in a range of natural waters and wastewaters [40]. The immobilization of the enzyme is required since in this way its inhibition by substances present in the samples is avoided, which, on the other hand, takes place when the enzyme is used in the dissolved form. In the case of solid samples, such as soils, sediments, etc., a fractionation of the sample is carried out in order to obtain its distribution among the different phases. For this purpose, chemical fractionation methods using extracting agents have been widely applied [41–43]. These methods attempt to differentiate the sediment phosphorus pool in the following fractions: labile, associated to Al, Fe and Mn oxides and hydroxides, associated to Ca, organic and residual [44]. Several compounds are included in the organic fraction: sugar phosphate, nucleotides, humic and fulvic susbstances, phosphate esters, phosphonates [45,46]. Although some authors have proposed methods for the fractionation of organic phosphorus in sediments, most of them consider the organic phosphorus in one single fraction, due to the difficulty involved in the separation and identification of these compounds. The SMT (Standards Measurements and Testing) protocol, based on the Williams method [47] and modified by Burrus et al. [48], lead to the obtaining of five phosphorus fractions: non-apatite inorganic phosphorus (NAIP), bound to Al, Fe and Mn oxyhydrates; apatite phosphorus (AP), bound to Ca; inorganic phosphorus (IP); organic phosphorus (OP) and total phosphorus (TP). Phosphate determination is carried out by UV–vis spectrophotometry, using the molybdenum blue method. Recently, a method consisting of performing two of the five extractions established by the SMT protocol (AP and NAIP) and determining the extracted phosphorus by both UV–vis spectrophotometry and inductively coupled plasma-atomic emission spectrometry (ICP-AES) has been published [49]. The three remaining fractions (TP, IP and OP) were then estimated from combining the results obtained in the AP and NAIP steps using the following relationships: $TP^S = AP_{ICP} + NAIP_{ICP}$, $IP^S = AP_{UV-vis} + NAIP_{UV-vis}$ and $OP^S = TP^S - IP^S$. The application of this methodology to sediment reference materials gave a good approximation to the results obtained when the SMT protocol was applied.

Determination of phosphorus can be carried out by classical analysis methods, namely: gravimetric methods (phosphate was directly precipitated as magnesium pyrophosphate, magnesium ammonium phosphate hexahydrate, ammonium phosphomolybdate, or nitratopentammine cobalt phophomolybdate) [50] and volumetric methods (by titration of ammonium phosphomolybdate with sodium hydroxide). The gravimetric method proposed by Iitaka et al. [51] based on a quartz-crystal microbalance (QCM) coated with insoluble orthophosphate salts, such as $CePO_4$, $CrPO_4$ and $BiPO_4$, which allows the determination of orthophosphate within the range of nanograms should be mentioned. The mass change caused by the selective adsorption of the ion on solid/aqueous interfaces of the immobilized insoluble orthophosphate salts was detected by the QCM. The observed frequencies of the QMC ion-sensors were found to decrease with increase in the orthophosphate ion concentrations in adjacent sample solutions ranging from10^{-6} to10^{-2} M at pH 7.0. These responses were mainly due to single-ion adsorption of orthophosphate ions.

Due to the poor sensitivity of classical methods most samples, after dissolution, are analysed by means of instrumental methods. The basis and classification of the instrumental methods applied to the determination of phosphorus, among other aspects related to the analysis of phosphorus in water samples, have been carried out by McKelvie [32]. In this bibliographical study it is observed that optical methods based on molecular spectroscopy techniques (visible photometry, thermal lens spectroscopy, chemiluminiscence and fluorescence) are usually used for molybdate reactive phosphorus (MRP), atomic spectroscopic techniques (atomic absorption spectrometry, inductively coupled plasma-atomic emission

spectrometry) are the usual method for MRP or TP and electrochemical techniques (potentiometry, amperometry and voltametry) for orthophosphate or MRP. Chromatographic methods such as high-performance liquid/ion chromatography, gel filtration/exclusion chromatography and capillary electrophoresis together with the use of several detection systems have enabled carrying out speciation.

3. Flow techniques

Flow analysis chemical methods were introduced in the field of chemical analysis in the middle of the last century [52]. At first, they aimed to mechanize the step of collecting the fractions eluted in chromatographic separations and the step of sampling in monitoring processes of physicochemical parameters in industrial plants. At the end of the fifties this objective was enlarged and the automation, in a simple and practical way, of all the steps of an analytical methodology, by means of sample handling within a fluid segment was attempted in a certain flow system and using a flow detector. Together with mechanization, determinations based on flow analytical measurements revealed, in relation to manual procedures, a better precision, a higher analysis throughput and a reduction in sample contamination. However, the results of the determinations in flow systems did not only depend on the applied chemistry but were also a function of the dynamic processes which took place and when the measurement was carried out. These factors in addition to those derived from the possibilities of carrying out preconcentration and/or separations of the analyte, from the sample matrix, favourably affected the selectivity of the methods based on flow measurements. The first practical application of flow measurements was carried out by a fluid stream segmentation technique with air segments which was named segmented flow analysis (SFA) [53]. Such approach was quickly accepted by chemical laboratories dedicated to clinical analysis to perform routine analyses and subsequently by laboratories dedicated towards environmental, agricultural and industrial analyses. In the middle seventies a new flow analysis technique named flow injection analysis (FIA) [54], which used no kind of gas segmentation, thus, providing great simplicity and robustness and which has proved to be a useful and efficient instrumental method for carrying out analytical determinations, is nowadays a well-established technique in the field of chemical analysis. The easy implementation together with the analysis throughput are its main advantages, whereas the vulnerability of the elastic tubes of the liquid drivers usually employed (peristaltic pumps) and the high reagent and sample consumption (due to the continuous flow) are its two main drawbacks. Several methodologies such as the FIA variant named merging zones [55], the construction of μFIA systems or micro-total analysis system (μTAS) [56,57] or techniques of reagents/enzymes immobilization on adequate supports [52,58,59] have been established to solve this aspect. In each case, although these variants solved several problems, however, they caused the appearance of new inconveniences or restrains such as, for example, the loss of enzyme activity due to the chemical processes required for the immobilization, the complication of the designs with the consequent difficulty involved in the implementation together with the loss of availability for researchers and analysts. In the decade of the nineties new approaches to the flow analysis techniques appeared such as the so-called sequential injection analysis (SIA) [60] and batch injection analysis (BIA) [61], although certain authors consider the latter modality as a 'non-flow injection-based technique' owing to the fact that no transportation of either samples or reagents takes place through the tubing. Leaving these discrepancies aside, there is no doubt that, nowadays, of the two new approaches the SIA modality has become more successful. In the SIA technique the substitution of the injection valve, usually employed in FIA, for a selection valve is accomplished, thus, sample and reagents segments are sequentially introduced in a tube, named the holding coil, such that in a characteristic movement which changes the flow direction, are subsequently impelled through a tube, named reactor coil towards a flow detector by a piston pump. A great robustness and flexibility of the SIA designs together with an important saving on the consumption of reagents and samples constitute its main advantages, whereas a low analysis rate, difficulties involved in the use of rapid chemical reactions and in the implementation (the use of computers or microprocessors as well as a certain knowledge in computers are essential in order to perform the SIA methodologies) are its main drawbacks. Nowadays, it can be stated that SIA methodologies have become popular and are well established in the field of chemical analysis. Subsequently, new approaches to flow analysis techniques have appeared such as the so-called multicommuted flow injection analysis (MCFIA) [62], the all injection analysis (AIA) [63], the multisyringe flow injection analysis (MSFIA) [64] or that which could be named as multipumped system (MPS) [65]. In Fig. 1 schematic illustrations of manifolds characteristic of the different cited flow techniques are depicted. In some cases, as in the AIA, there are scarcely any publications revealing its possibilities, contrarily to the remaining techniques where their potential is clearly shown. In the AIA all reagent solutions are injected into a reaction coil and all solutions are circulated for a definite time. By this circulating process, the amount of consumption of the reagents is extremely reduced, even in intermittent measurements. According to the inventors of this technique the system can be used for various analytical reaction systems without rearranging the construction of the FIA assembly. The MCFIA refers to flow systems designed with discrete computer-controlled commutators resulting in flow networks in which all the steps involved in sample processing can be independently implemented. The flow systems can be re-configured by the control software, thus, presenting increased versatility, potential for automation and for minimization of both reagent consumption and waste generation. Multicommutation is usually accomplished by taking

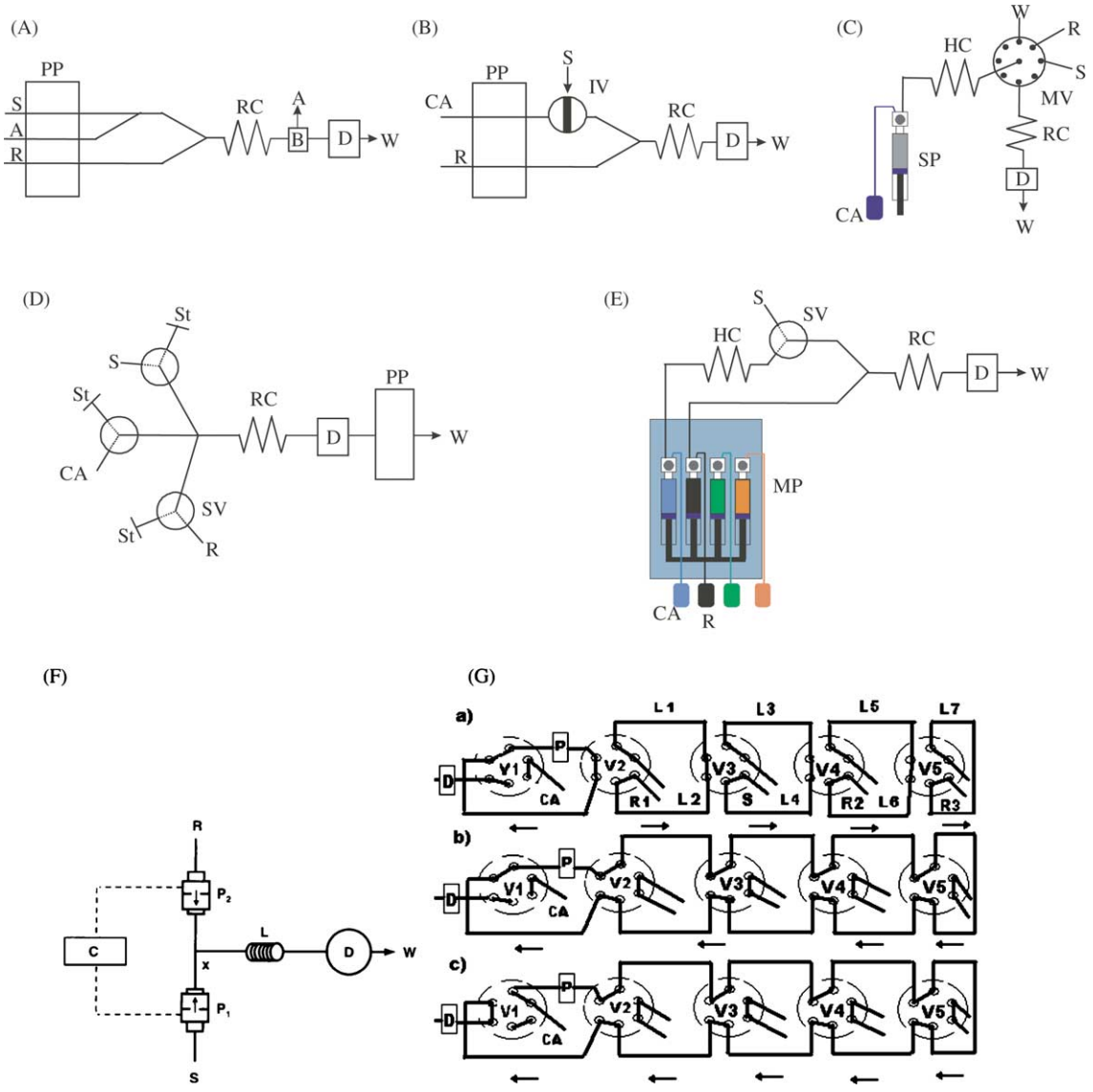

Fig. 1. Schematic illustration of flowing stream manifolds assembled for determination of chemical parameters: (A) segmented flow analysis (SFA), (B) flow injection analysis (FIA), (C) sequential injection analysis (SIA), (D) multicommutation flow analysis (MCFIA), (E) multisyringe flow analysis (MSFIA), (F) multipumped flow analysis (MPFA), and (G) all injection analysis (AIA): (a) sample and reagent loading; (b) mixing by circulation; (c) to detector and washing of line. S, sample; R, reagent; CA, carrier; A, air; D, detector; IV, injection valve; HC, holding coil; RC, reaction coil; B, debubbler; MV, multiposition valve; SV, solenoid valve; SP, syringe pump; MP, multisyringe pump; PP, peristaltic pump; St, stopper; W, waste; P1 and P2, micropums; C, control interface; x, confluence point; V1–V6, six way valves; L1–L7, Teflon tubes; R1–R3, reagents.

advantage of valves, timing devices and other artefacts for improving system performance. These devices can be operated in a passive or active manner and external timing is often exploited for versatility enhancement. The MSFIA was conceived with the aim of coupling the overall advantages of the parent FIA, SIA and MCFIA. The system comprises a multisyringe burette constructed by adaptation of an automated syringe pump analogous to that employed in SIA manifolds in order to simultaneously move four syringes which are connected in block to the same step-by-step motor. Each syringe presents at the head a three-way solenoid commutation valve which allows its connection with the flow system or with the reagent reservoir independently of the piston displacement. As one of the syringes of the module cannot be used as a sample reservoir due to carryover between consecutive samples, an additional injection system, such as an injection, multiposition or solenoid valve, needs to be connected to the multisyringe piston pump to perform analytical applications. Robustness, versatility, economy of the consumption of samples and reagents, possibility of carrying out different sample injection modalities such as time-based injections using the configurations in a multicommuted way are the most remarkable characteristics. The analysis throughput is intermediate between that obtained in the FIA and SIA systems and in several designs it can be similar or even higher [66] to that obtained in the FIA systems. However, the use of comput-

ers and certain knowledge in computers are required in this new approach, as well as in all the approaches recently proposed. The MPS is a novel strategy for the implementation of flow-based analytical procedures using several micropumps. The pumps are switched individually or in combination, in order to create a pulsed flowing stream through the analytical path and are the only active devices acting simultaneously as liquid propelling units, sample insertions ports and commuting elements. The micropumps produce distinct flow rates at distinct pulse frequencies with high reproducibility ensuring the attainment of very stable flow rates which enables the utilization of different approaches for sample management including step-wise variable sample volume, binary sampling and merging zones without reconfiguration of the system hardware. This technique is characterized by a pulsed flow ensuring a fast sample/reagent mixing which contributes to improve the reaction development, thus, sensitivity, even in situations of limited dispersion.

4. Flow techniques and phosphorus analysis

Liquid, aqueous and water samples are specially well adapted to be analysed by flow techniques. The relevance of flowing stream methods for water quality is demonstrated by extensive reviews [67–73] and several publications issued to date in this field comprising multiparametric [74,75] or phosphate determinations [32]. Although originally flow techniques were performed for off-site measurements, their outstanding feature is the capability for both in situ and real-time monitoring of chemical parameters in waters [68,75–78], some of the flow analysers being included in EPA directions. It can be stated that generally speaking the analysis of phosphorus, as occurring in the determination of other elements and compounds, has benefited from the different flow analytical techniques which have appeared throughout the years. Facility, reproducibility, selectivity, high analysis throughput and possibility of automation are the main features the methodologies based on these techniques possess. Automation has allowed carrying out more easily several steps (preconcentration and/or elimination of interferents, on-line digestion, etc.) involved in the determinations of the different fractions of phosphorus. Next, a detailed account will be outlined on the incidence of the different flow techniques in the analysis of phosphorus according to the detection technique used, which will act as a connection link between these techniques and help in their study. A classification of the detection techniques has been previously carried out in Section 2.

4.1. Optical techniques

4.1.1. Visible spectrophotometry

The chemical basis of spectrophotometric flow methods for determination of phosphorus in a wide range of samples such as water (ground, marine, residual, river, lake, etc.), soil and sediments extracts, beverages (soft drinks, beer, etc.), urine, nutrients solutions, digested plant material etc. lies in the reaction between orthophosphate ions with molybdate in acidic medium to form 12-molybdophosphoric heteropolyacid. In most of the flow analysis applications, detection is undertaken either on the molybdophosphate reduction product (molybdenum blue method, PMB), by ascorbic acid in the presence of antimony tartrate serving as a catalyst [52,79] or on the yellow vanadomolybdophosphate complex (PVM). The former became widely accepted as a routine methodology due its to high sensitivity and on which the EPA certified methods for phosphorus analysis in water are based. In addition to ascorbic acid [80] several other reducing agents have been reported and reviewed in the literature for molybdenum blue such as hydrazine [81] and tin(II) chloride [82]. In spite of the relatively unstable nature of tin(II) chloride, this reductant usually gives the best overall results [83]. Silicate, arsenate and germanate are the main interferences of these methods since these ions also form heteropolyacids, which on reduction yield molybdate blue species with similar absorption maxima. Other alternatives for the determination of phosphate are the methods based on the formation of ionic pairs of either molybdophosphate or vanadomolybophosphate with basic dye compounds such as Malachite Green, Rhodamine B, Crystal Violet, Methylene Blue, etc.

Continuous flow techniques have been widely used for automated phosphorus analysis of water since the introduction of the segmented-flow analysis systems in the 1950s. SF-manifolds described include those which are suitable for the determination of phosphorus in water in the presence of high silica [84] for highly sensitive detection of FRP in the presence of mercuric chloride preservative [85] and for the determination of total filterable phosphorus [86]. Nowadays, few flow analytical systems not involving the use of the molybdenum blue method have been proposed, and even fewer are based on a segmented flow (SF) for the analysis of phosphorus. In this context, the SF system proposed by Zhang et al. [87] should be cited, which carries out the automated continuous flow analysis (CFA) of phosphate using the molybdenum blue method which is essentially similar to the Murphy and Riley method [88], however, the specific reaction conditions are optimized with regard to minimizing coating and silicate interference while maintaining high sensitivity. Another system to be taken into account is that reported by Teixeira et al. [89] which uses the monosegmented flow system (MSF) with simultaneous multiple injection for the determination of low contents of phosphate in natural water based on the reaction of association between molybdophosphate and malachite green. This proposed system presents a better sensitivity compared with other methods described in the literature based on the same chemical reaction (Fig. 2a). Muñoz et al. [90] have carried out a comparative study, in the same SIA design, using the PMB employing tin(II) chloride as a reductant, PVM and vanadomolybdophosphate-malachite green (PVM-MG) methods and have compared the results obtained between them, with the corresponding FIA designs described in the literature and with those obtained by the corresponding batch

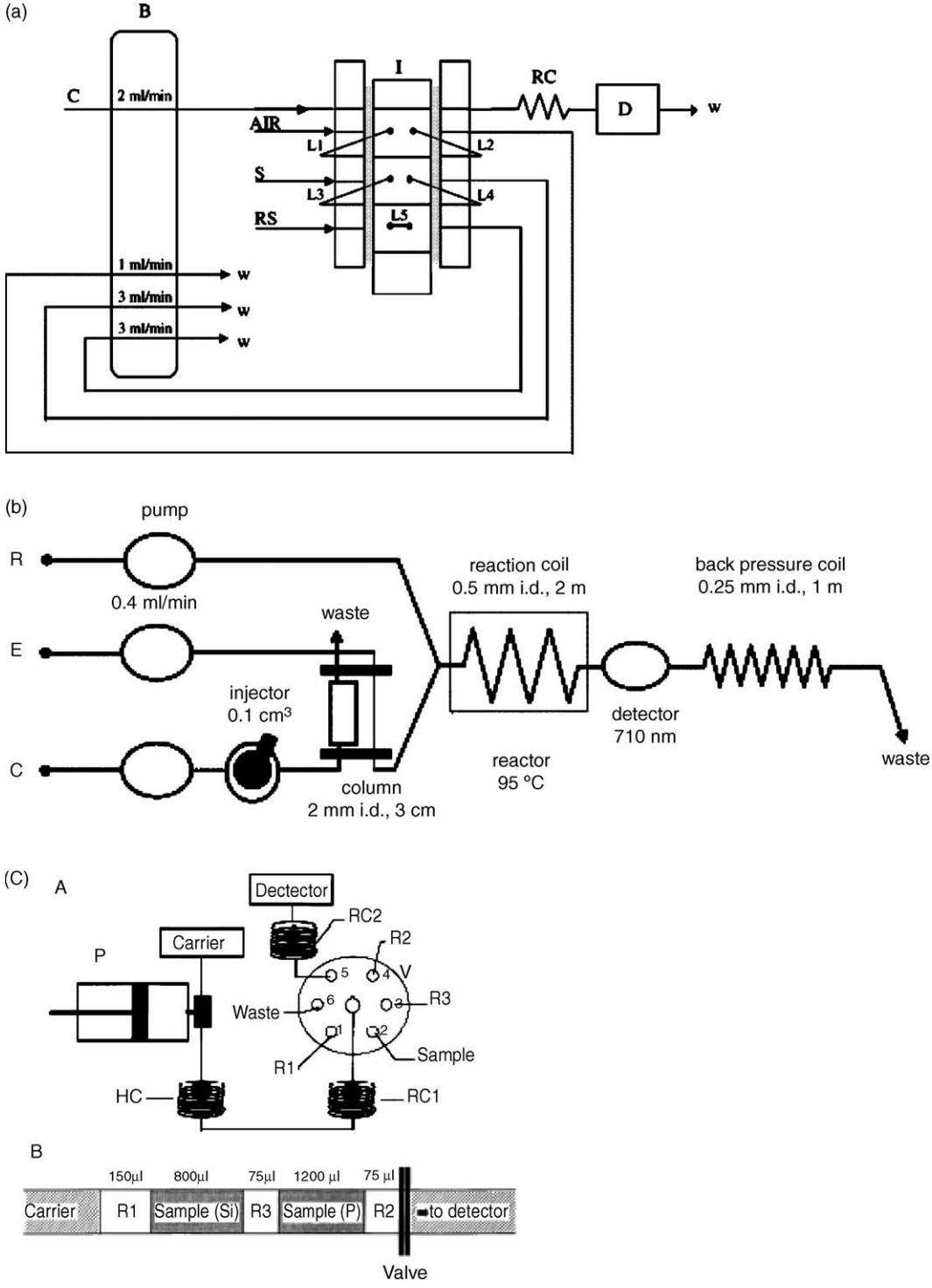

Fig. 2. (a) Schematic diagram of the monosegmented flow system (MSFA) with simultaneous multiple injection for determination of phosphate in natural water [89]. B: peristaltic pump; I: injector; RC: reaction coil; S: sample or reference solution; C: carrier (water); RS: reagent solution; w: waste; D: detector; L1 and L2: air loops; L3 and L4: sample loops; L5: reagent loop. (b) Flow system (FI) for preconcentration of phosphate at sub-ng ml^{-1} level with chelating polymer-gel loaded with zirconium(IV) [92]. C: carrier stream (0.02 mol dm^{-3} HNO_3 solution); E: elution stream (0.1 mol dm^{-3} KOH solution); R: reagent stream (a mixed molybdenum solution for color-development). (c) (A) Schematic diagram of the SIA system used for the simultaneous determination of phosphate and silicate [107]. P, titration burette; V, six-port valve; HC, holding coil; RC1, reaction coil1; RC2, reaction coil2; R1–R3, reagents; S, sample; C, carrier and D detector. (B) Illustrated zone sequence aspiration of sample and reagents.

methods when analysing samples from different types of waters. Summing up, these authors state that the FIA and SIA methods based on PMB and PVM possess very similar analytical features and that only in the case of using the ionic association (PVM-MG) the FIA method presents a higher sensitivity. Nevertheless, all the SIA methods present the advantage of the considerable saving of reagents together with the easy set up of the manifold, their main drawback the low being sampling frequency (30 samples per hour). The PVM SIA-method presents a good reproducibility together with the stability of the reagents, within the range suitable for wastewater analysis. The linear range of the PVM-MG SIA-method is very narrow, the sensitivity is not sufficient for some natural waters and dilution of wastewater samples prior to analysis is necessary. The PMB SIA-method shows a good reproducibility, wider linear range than that of the PVM-MG SIA-method for the same sensitivity. It would be possible to achieve the range required for waste samples by diluting the sample in situ, varying the volume ratios or by means of dialysis. The main disadvantage regarding the use of this method for monitoring purposes is the instability of the tin(II) chloride solution which must be prepared daily.

There has been an attempt to increase and improve the sensitivity and/or selectivity of the flow analysis methods based on the previous chemical systems by means of different strategies, namely; (a) using anion-exchanger packed reactors [91], chelating polymer-gel loaded with zirconium(IV) [92] (Fig. 2b), reversed phase polymers, styrene-divinylbenzene, methacrylate, dextran, etc. [93] or organotin extractant-containing extraction chromatographic minicolumns [94] for separation and solid-phase pre-concentration. It should be outlined that the simultaneous determination of phosphate, silicate and arsenate [95,96] is also feasible using these systems; (b) using Nafion or Accurel membrane tubing for reagent introduction and the combination of a semiconductor laser with a thin long flow cell [97]; (c) detection limits of approximately 0.1 ng ml^{-1} phosphorus are reported, previous ion-pair preconcentration, using either solvent extraction or filtration-dissolution procedures [98]; (d) using reversed flow analysis systems r-FIA [80,99,100] and r-SIA [101] which are usually more sensitive than the non-reversed systems. In the case of r-SIA the additional use of a new standard addition methodology should be mentioned; (e) using the multicommutation principle combined with binary sampling and sample zone trapping in order to increase the spectrophotometric analytical range, improve the sensitivity [102] and allow the simultaneous determination of orthophosphate and ammonium [103]; (f) using kinetic factors which enhance both aspects. Thus, for example, FIA-methods based on the different rates of formation of the heteromolybdic acids of phosphate and silicate anions have been described [104]. The measurements are based on the colour of the ion-pairs formed between the former molybdic acids and Rhodamine B, or stopped-FIA systems using molybdenum blue [105,106] which allow the simultaneous determination of phosphate and silicate or arsenate. In the case of the method proposed by Petterson and Karlberg [106] the application of multiway partial least squares (PLS) models based on the combined effect of spectral and kinetic differences is required. Analogously, SIA-methods can also be found based on the formation of yellow vanadomolybdophosphate and molybdosilicate in addition to the use of large sample volumes [107] (Fig. 2c) and based on the different reaction rates of the heteropolymolybdate formation reaction of phosphate and silicate ions which uses a stopped-flow technique [108] which also allow the simultaneous determination of both species; (g) by the use of chemical masking agents. The simultaneous determination of silicate and phosphate [109] in boiler water at power plants based on a series of flow cells has been published. The principle of the method is that the total concentration of silicate plus phosphate is determined when an injected sample plug is passing through the first flow cell and then the concentration of silicate is serially determined at a second flow cell of the same detector after continuously masking the yellow molybdophosphate in the sample zone. The concentration of phosphate is obtained by difference.

These chemical systems have been also used for the determination of phosphorus with spectrophotometric field monitors for water quality parameters using either FIA or SIA designs. A FIA system based on reagent injection for determination of phosphate in natural waters employing a double-bean photometric detector incorporating light-emitting diodes (LED) and photodiodes has been described [100]. In this context, the use of a compact flow injection analysis system for surface mapping of phosphate in marine waters has been proposed [110]. This portable system employs gas pressure for reagent propulsion and computer-controlled miniature solenoid valves for precise injection of multiple reagents into a flowing stream of filtered sample. A multi-reflection flow cell with a solid state LED photometer is used to detect filterable reactive phosphate (0.2 μm) as phosphomolybdenum blue. Based on a SIA configuration a multiparametric monitor has been constructed [78] which permits the determination of orthophosphate and total phosphorus among other parameters such as total organic carbon (TOC), chemical oxygen demand (COD) biochemical oxygen demand (BOD), total suspended solids (TSS), nitrate, nitrite, ammonium and total nitrogen in wastewaters samples.

The Schilieren effect as well as the need of the manifold reconfiguration for each determination in the FIA systems have been aspects taken into consideration by the researchers. Conventional flow injection manifolds with sample injection for the determination of phosphorus in certain types of water samples are limited by the Schlieren or refractive index (RI) effect which can cause major errors in quantification. A simple flow injection manifold which obviates this effect on reactive phosphorus measurements in estuarine waters has been reported by McKelvie et al. [111]. It involves the injection of acidic molybdophosphate reagent into a carrier stream of sodium chloride solution of similar refractive index, which is then sequentially merged with a sample and a reductant.

On the other hand, a polyvalent flow injection system for multielemental spectrophotometric analysis of plant materials has been proposed by Silva et al. [112]. This system consisting of a single manifold suitable to perform different determinations after only minor adaptations was conceived. Its use is particularly attractive for laboratories processing a large number of samples including several analytes. The system was applied to the spectrophotometric determination of iron, copper, manganese and zinc (micronutrients) as well as calcium, magnesium and phosphorus (macronutrients).

The unstableness of the chemical reductants used in the phosphomolybdenum blue method has the researchers to attempt to substitute them for the electrochemical reduction. Scholz et al. [113] propose a flow system for the electrochemical reduction of molybdophosphoric acid at a bubble-electrode at a potential of −0.3 V (versus Ag/AgCl/satd. KCl electrode) followed by the spectrophotometric detection of the species reduced at 805 nm. Recently, an automatic SIA method has been described [114] for the determination of orthophosphate in beverages, wastewaters and biologic samples by electrogeneration of molybdenum blue using tubular flow-through electrodes, a stainless steel tube as the working electrode and a graphite tube as a counter electrode, with spectrophotometric detection at 760 nm (Fig. 3a). The proposed methodology enables the determination of orthophosphate in samples with high concentrations using a dilution chamber as well as flow-reversal techniques.

The determination of dissolved organic phosphorus through the molybdenum blue method is also possible following mild in-line photo-oxidative decomposition with low pressure mercury lamps [181] or UV tubes [115] using peroxydisulfate in alkaline or acidic conditions. Speciation of low and high-molecular weight (i.e. dissolved inorganic and organic) phosphorus species was achieved through a flow injection gel filtration strategy [116]. Determination of total dissolved phosphorus using the above chemistry requires previous acidic hydrolysis of condensed phosphates (viz. pyro-, metha-, polyphosphates) into orthophosphate. Since these species are not susceptible to UV photo-decomposition, thermal [117] and microwave-induced [22] digestion which provided recoveries higher than 85%, were successfully implemented into flow injection systems or hyphenated techniques. As a consequence of the different reaction conditions needed for the successful conversion of simple organic phosphates and condensed phosphates to orthophosphate, a two-stage process involving UV photooxidation/thermal digestion using a combined oxidizing/hydrolysing reagent was described [29] (Fig. 4a). An outstanding feature of the latter approach is the capability to quantify particulate- and colloidal-associated phosphates and, thus, measurement of total phosphorus is feasible. With regard to the thermal decomposition, the mineralization step can be speeded up by in-line implementation of a capillary digestor containing a platinum wire acting as a catalyst for the oxidation of organic species. The selective determination of orthophosphate and total inorganic phosphate in detergents [118] is based on the performance of a previous acidic hydrolysis. The orthophosphate was directly determined in the presence of other phosphates by utilizing the kinetic discrimination of flow injection analysis and total inorganic phosphate was analysed after on-line hydrolysis of polyphosphates in 2.5 mol l^{-1} sulphuric acid for 50 s at 145 °C. Sodium dodecylsulphate (SDS) was added to mask the interference of non-ionic surfactants. A SIA procedure for in-line sample preparation for the spectrophotometric determination of total phosphorus in foodstuffs based on the molybdenum blue method was proposed by Oliveira et al. [119] (Fig. 4b). A natural suspension slurry is transported together with nitric acid towards a home-made digestion bomb placed inside a microwave oven for subsequent digestion. The sample zone is stopped inside the oven and, after digestion, directed in reverse flow towards a holding coil and then towards detection. The proposed system is very robust and yields reproducible measurements for 20–400 mg l^{-1} P and the results are in agreement with a conventional spectrophotometric procedure involving manual sample digestion. FI-determination of specific fractions of phosphorus through enzymatic reactions involving immobilized alkaline phosphatase [39,120] or 3-phytase [121] coupled to the molybdenum blue method has potential use as an indicator of the pool of biological available and unavailable dissolved organic phosphorus, respectively. The alkaline phosphatase immobilized on a cellulose nitrate membrane has also been used for the quick spectrophotometric determination of monofluorophosphate [122] (Fig. 3b) and the simultaneous spectrophotometric determination of orthophosphate and monofluorophosphate [123] in toothpastes after on-line hydrolysis in a flow-injection system.

Photographic and wet etching techniques were used to fabricate a micro-FIA system [124] (Fig. 5a) based on electroosmotic flow for the determination of phosphate as orthophosphate based on the molybdenum blue reaction. The manifold channel dimensions were 200 μm wide and 50 μm deep and the μFIA methodology is comparable in performance to conventional FIA for the detection of orthophosphate. Analogously, two micro-SIA systems can be found in the literature [125,126] for spectrophotometric determination of phosphorus with accommodate EPA-approved methods for the spectrophotometric determination of phosphates in the ppb concentration range. These use a lab-on-valve manifold whose technical improvement, apart from micro miniaturization, is the use of the stopped-flow technique, which resulted in improved detection limits and reduced reagent consumption (Fig. 5b).

4.1.2. *Fluorescence spectrophotometry*

Since fluorometry is inherently more sensitive and selective than UV–vis spectrophotometry, flow analysis methods with fluorometric detection are, therefore, expected to be so in relation to those based on UV–vis spectrophotometric detection. Although this statement is basically true, certain discrepancies may be found in the literature which may be attributed to the previous use of preconcentration and/or sep-

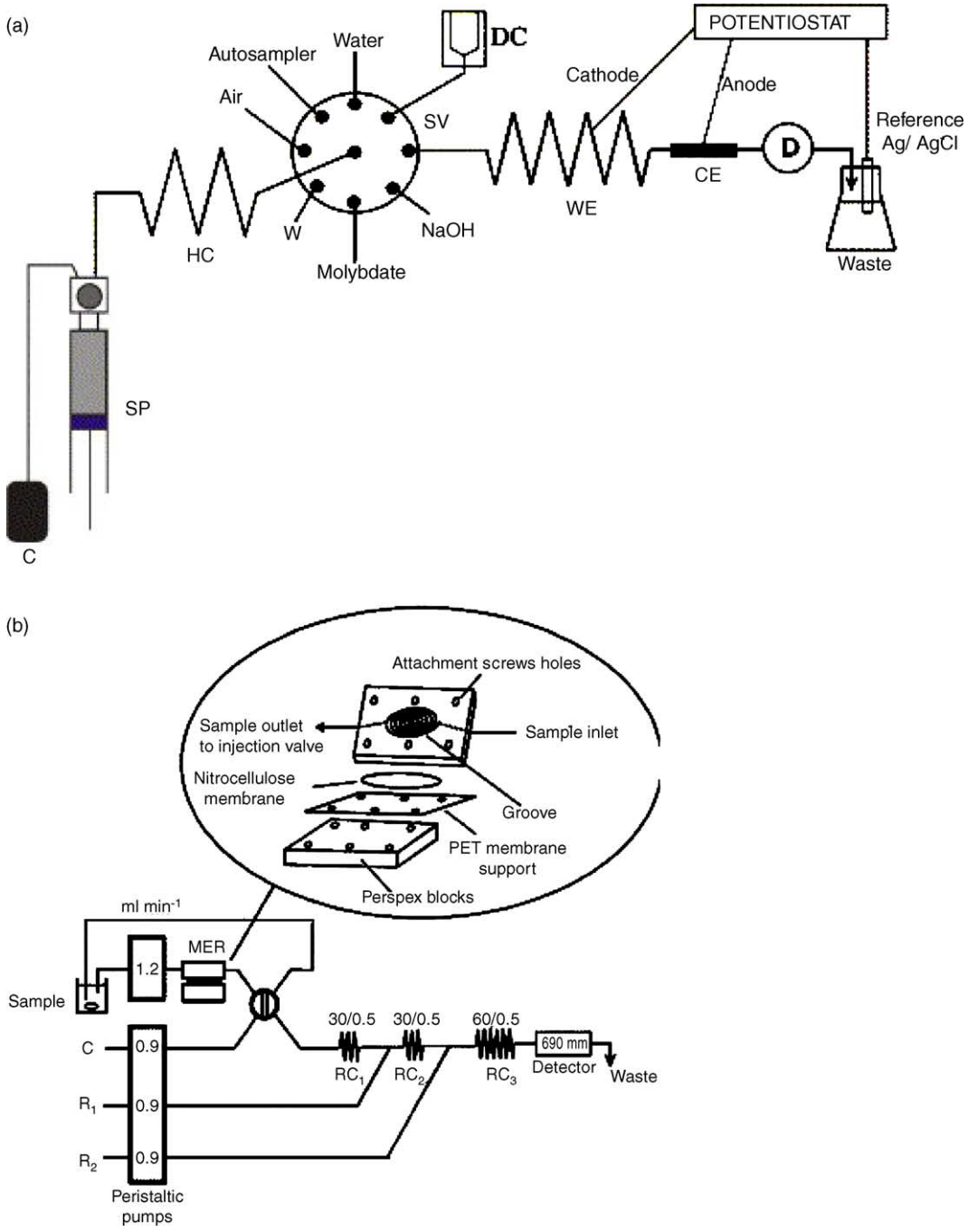

Fig. 3. (a) Schematic illustration of the sequential injection (SIA) set-up devised for the spectrophotometric determination of orthophosphate based on the electrochemical generation of molybdenum blue [114]: C, carrier; SP, syringe pump; HC, holding coil; SV, selection valve; DC, dilution chamber; WE, working electrode; CE, counter electrode; D, detector; W, waste. (b) FI setup for the determination monofluorophosphate ion [122]: MER, membrane enzymatic reactor; C, carrier streams (water); R_1, molybdate stream; R_2, tin(II) stream; MC, mixing coil; RC_1, RC_2 y RC_3, reaction coils; numbers above coils denote coil length (cm)/inner diameter (mm).

aration systems, to the way the reaction and flow conditions are optimized, to the way the sensitivities are calculated or to the features of the particular fluorometric reactions used.

The detection limits of these methods range between 0.3 and 100 ppb of phosphorus, whereas in the literature detection limits of the order of 0.06 ppb of phosphorus can be found using the spectrophotometric FIA-method based on malachite green [98] or 0.1 ppb by means of the μSIA-method based on molybdenum blue [126] reactions. In the literature several FIA methods are described involving the indirect fluorimetric determination of orthophosphate over a wide dynamic range and relied on the reduction of the vanadomolybdophosphate ion, previously formed, by non-fluorescent thiamine to generate highly fluorescent thiochrome. The calibration

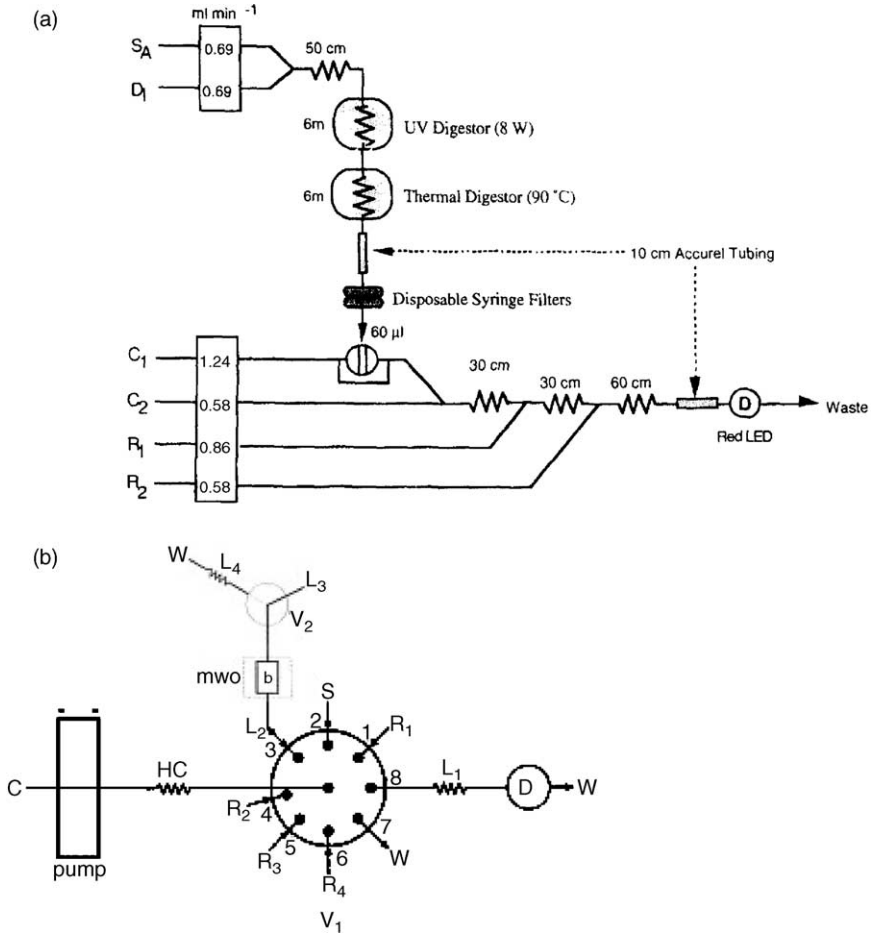

Fig. 4. (a) FI manifold for the determination of TP with UV/thermal digestion [29]: S_A, sample; D_1, digestion reagent; R_1, acid molybdate reagent; R_2, tin(II) chloride/hydrazinium sulphate reagent. C_1 and C_2 are Milli-Q water carrier streams. (b) Sequential injection (SIA) system for sample preparation and sprectrophotometric determination of TP in food samples [119]: C, carrier stream; pump, peristaltic pump; HC, holding coil; V_1, stream directing rotary valve (clockwise rotation); D, detector; S, sample; R_1, concentric nitric acid; R_2, ascorbic acid; R_3, ammonium heptamolybdate; R_4,sodium hydroxide; L_1 and L_2, transmission lines; MWO, microwave oven; b, digestion bomb; V_2, six-port valve; L_3 and L_4, outlet coils; W, waste; W′, waste with sodium hydroxide. Numbers 1–8, valve ports.

curve of the method established by Khisida and Aoki [127] was linear within the concentration range of 5.0×10^{-7} to 2.0×10^{-5} M. The addition of vanadate enabled the removal of interference from silica and increased more than 50 times the sensitivity for the determination of phosphate. In this context, Perez-Ruiz et al. [128] proposed FI-system based on the same reaction, without using vanadate and the reaction medium being nitric acid instead of sulphuric acid. This system was used for the determination of inorganic and organic phosphorus in water samples (spring, mineral and well) and included a simple ultraviolet photoreactor for the on-line photodegradation of organophosphorus compounds in the presence of peroxydisulphate and an external decomposition unit for hydrolysing condensed phosphates with hydrochloric acid. The FIA configuration designed allowed two injections to be performed for each sample; the first, without irradiation, corresponded to inorganic phosphorus and the second, with irradiation, to total phosphorus. Calibration graphs were linear up to 350 ng P ml^{-1} and the detection limit was 0.3 ng P ml^{-1}. The heteropolyacids of silicate and arsenate can also oxidize thiamine to thiocrome, resulting in enhanced fluorescence intensity. However, in the conditions selected in the method the rate of formation of these heteropolyacids was very slow and ratios as high as 70 for Si/P and 50 for As/P were tolerated. Also based on the same reaction a rapid, automated and sensitive FIA-method for the determination of phosphate in natural water samples has been proposed [129]. A linear response was obtained over the range of 0.02–20 mg l^{-1}, with a detection limit of 0.01 mg l^{-1}. Other flow injection methods for the indirect fluorometric determination of phosphate are based on the quenching of the fluorescence of Rhodamine B derivatives [130] or Rhodamine 6G [131,132] through the formation of ion associations with phosphate. In the method proposed by Motomizu et al. [130] the calibration graph was linear over the range 10^{-8} to 3×10^{-6} M phosphate and the method was applied to the determination of phosphate in seawater and river water samples. Taniai et al. [132] developed an

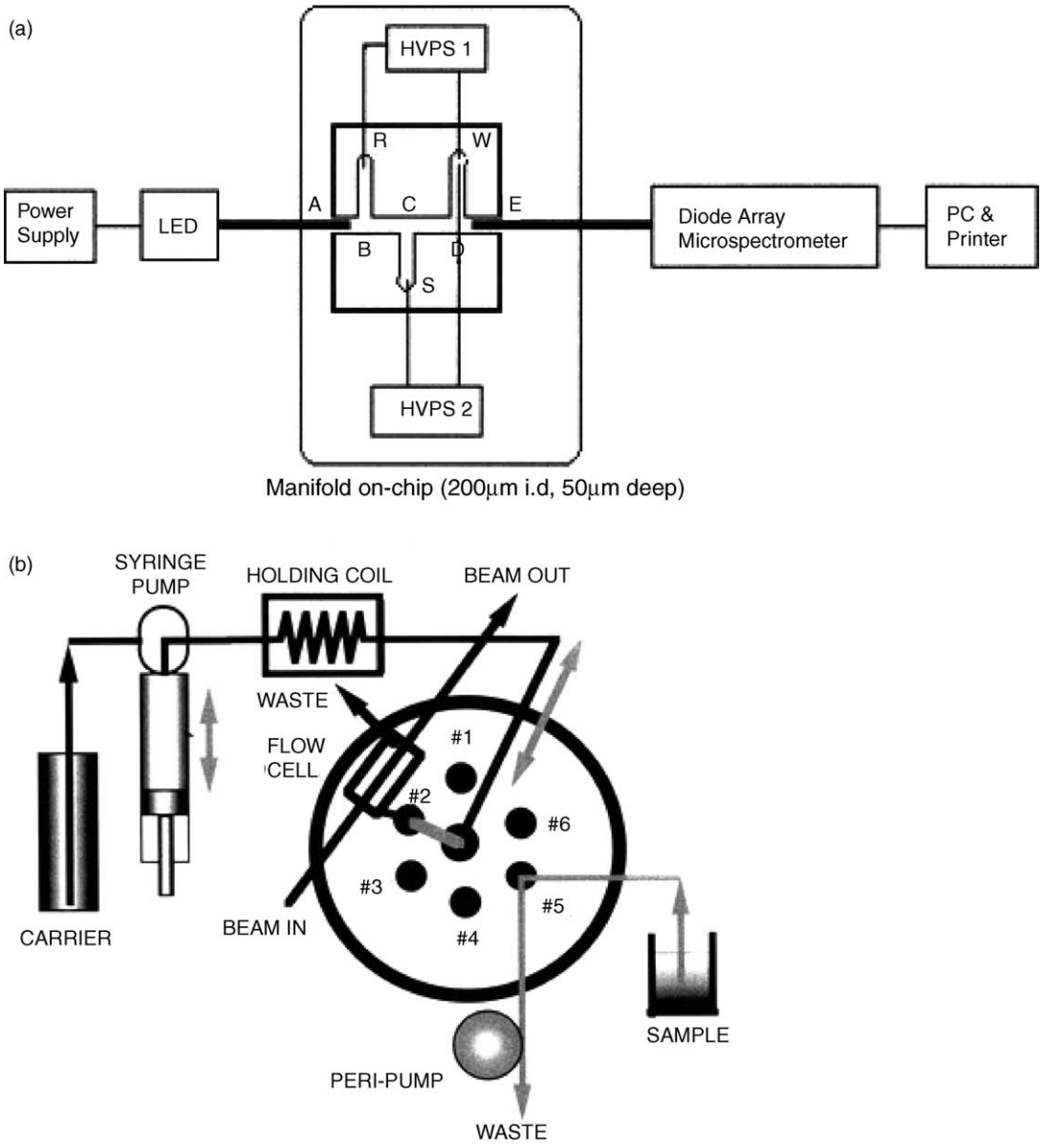

Fig. 5. (a) μFIA manifold based on electroosmotic flow and detector setup for phosphate analysis as orthophosphate [124]: AB = BC = DE = 3 mm; CD = 4 mm, BD = 7 mm; and RB = SC = WD = 6 mm. (b) Micro SI system [125] comprising a high precision syringe pump (500 μl volume) and an auxiliary peristaltic pump, shown serving the flow through sampling port (#5). The central sample processing unit, integrated with a flow cell (FC), is mounted atop a six-position valve. The syringe pump operates in reversed-stop-forward flow sequences, for metering the sample (#5) and the reagents (#3, 4, 5) into the holding coil. Flow reversal is used to direct the reaction mixture into the flow cell through port #2.

automated FIA-system for measuring the concentration of phosphate in natural water samples with a preconcentration column, which was packed with an ion-exchange resin in the molybdate form to collect and preconcentrate the phosphate. Rhodamine 6G was chosen because the reaction with phosphomolybdate was fast and did not require heat. The linear range of the calibration graph for phosphate was from 1.6 to 0.2 μg P, the sensitivity was the same as with the molybdenum blue method. The inhibitory effect of orthophosphate on the photo-oxidation of acridine catalysed by iron(III) [133] has been also used for the determination of phosphate. The analysis procedure is automated and the development of the photochemical reaction was monitored by making use of the fluorescence of acridine. A linear calibration graph was obtained over the range 0.95–9.5 μg ml^{-1} phosphate. The method was applied to the determination of fluoride and phosphate in waters.

4.1.3. Chemiluminiscence photometry

The use of chemiluminescence (CL) detection usually provides highly sensitive methods of analysis for the determination of a great variety of analytes both organic and inorganic and in different types of samples. The difficulty involved in its performance and the lack of selectivity are

the main drawbacks when attempting to carry out its implementation in the analysis of samples with real matrixes. The analytical application of chemiluminescence detection has received a strong impetus by the advent of flow systems, mainly flow injection analysis, as exemplified in several monographs and exhaustive fundamental reviews [134–137]. The on-line sample treatment on the basis of the use of reagents in the solid phase in order to concentrate the analytes and/or remove the sample matrix and the use of more selective chemiluminescence reactions have considerably improved its selectivity. The reproducible mixing of streams and precise timing control inherent to flow injection techniques are crucial conditions for reliable CL measurements. Flow injection should be highlighted as a unique strategy to accommodate fast CL reactions, very often involving the catalysed-luminol (3-aminophthaloylhydrazine) oxidation in alkaline medium, wherein light emission occurs instantaneously upon merging sample and chemiluminogenic reagent with half-life times of only a few seconds. It should be stressed that CL assays in the analytical field usually lie in indirect determinations, so that one of the essential ingredients for the CL reaction is on-line generated in a preceding reaction. In the chemiluminescence analysis of phosphorus the construction of highly sensitive FIA-chemiluminescence-based biosensors involving enzymatic packed bed reactors is usual. Such sensors have the generation of hydrogen peroxide as a final product in common which acts as the oxidant species of the chemiluminiscent reagents. The purine nucleoside phosphorylase-xanthine oxidase [138] systems were devised for FIA analysis of orthophosphate in different environmental waters. In this system the enzyme catalyses the phosphorolysis of inosine to hypoxantine, which is oxidized by xanthine oxidase to generate hydrogen peroxide. Hydrogen peroxide was detected by chemiluminescence in reaction with bis[2-(3,6,9-trioxadecanyloxycarbonyl)4-nitrophenyl]oxalate. Concentrations of phosphate below the level of 0.1 μM were determined. There are several FIA-biosensors which use pyruvate oxidase to produce the chemiluminescence reaction and which by the measurement of the intensity of the produced light will allow phosphate detection. In the literature the first FIA-sensor can be found [139] which used pyruvate oxidase (PyrOx) from Pediococcus sp immobilized on controlled pore glass beads packed into a column for the analysis of river water. The chemiluminiscent detection is based on the reaction of hydrogen peroxide previously generated by reaction of pyruvate with phosphate. This occurs in a medium containing $MgCl_2$, thiamine pyrophosphate chloride (TPP) and flavin adenine dinucleotide (FAD), in the reactor with pyruvate oxidase immobilized with luminol in the presence of *p*-iodophenol as an enhancer of the chemiluminescence and immobilized peroxidase (POD) from Horse Radish which acts as a catalyser. In order to improve the sensitivity, the detection unit was placed directly in front of the immobilized peroxidase. A linear response was observed from 0.37 to 7.4 μM phosphate, whereas the detection limit was 74 nM. Subsequently, a second sensor has been reported for the determination of phosphates. This is an automated FIA system [140] combining piruvate oxidase G (PyrOxG) from Aerococcus viridians immobilized on *N*-hydroxysuccinicacidimido beads without a cross-linker and CL detection. The hydrogen peroxide generated reacts in a homogeneous phase with luminol in an alkaline medium containing the dissolved peroxidase. The sensor presents a detection limit of 96 nM phosphate ion and a range between 96 nM and 32 μM. Treatment with activated carbon could improve the response of the sensor when inhibited by dissolved substances in river water, except for the manganese ion and uric acid. Good correlation between the results obtained with this sensor and the modified molybdenum method was obtained in analysis of river water samples. The enzymatic system maltose phosphorylase-mutarotase-glucose oxidase (MP-MUT-GOD) combined with an Artromyces ramosus peroxidase-luminol reaction system has been used by Nakamura et al. [141] for the construction of a FIA-biosensor for the detection of phosphate-ion. This system is coenzymeless and consists of a column packed with MP-MUT-GOD inmobilized on *N*-hydroxysuccinimide beads, a mixing joint for the chemiluminescence reaction and a photomultiplier. The response provided by this system was linear, with a wide range between 10 nM and 30 μM phosphate ion. The sensor was able to detect 1 μM phosphate ion for at least 2 weeks. In a subsequent work [142] these authors carried out a study of the interferences that this sensor presents and of the systems for their removal. They compare the effects of the removal of dissolved interferent by a chelating reagent, ion exchange resin, or combination of ion exchange resin and UV irradiation on the sensor response. They proposed as a pre-treatment method the use of a cation-exchange resin (sulphonated), which improved most effectively the sensor response. The constructed sensor and the established methodology have been used for the determination of phosphate in river and pond water with results in agreement with those obtained with the molybdenum-blue method.

In the literature, other chemiluminescence methods can be found for the determination of phosphates which are not based on the use of biosensors. Thus, Yaqoob et al. [143] have proposed a FIA-method for determination of phosphate (as molybdate reactive P) in freshwaters based on luminol chemiluminiscence detection (Fig. 6a). The molybdophosphoric heteropolyacid formed by phosphate and ammonium molybdate in acid conditions generated chemiluminiscence emission via the oxidation of luminol. The detection limit was 0.03 μg P l^{-1}, with a sample throughput of 180 h^{-1}. The calibration graph was linear over the range 0.032–3.26 μg P l^{-1}. Interfering cations were removed by passing the sample through an in-line iminodiacetate chelating column. Silicate interference was effectively masked by addition of tartaric acid. The only chemiluminiscence system for the analysis of phosphorus based on a configuration different from FIA has been recently reported [144]. It consists in a flow-through solid-phase based chemiluminescence optical sensor for the trace determination of orthophosphate in waters (mineral,

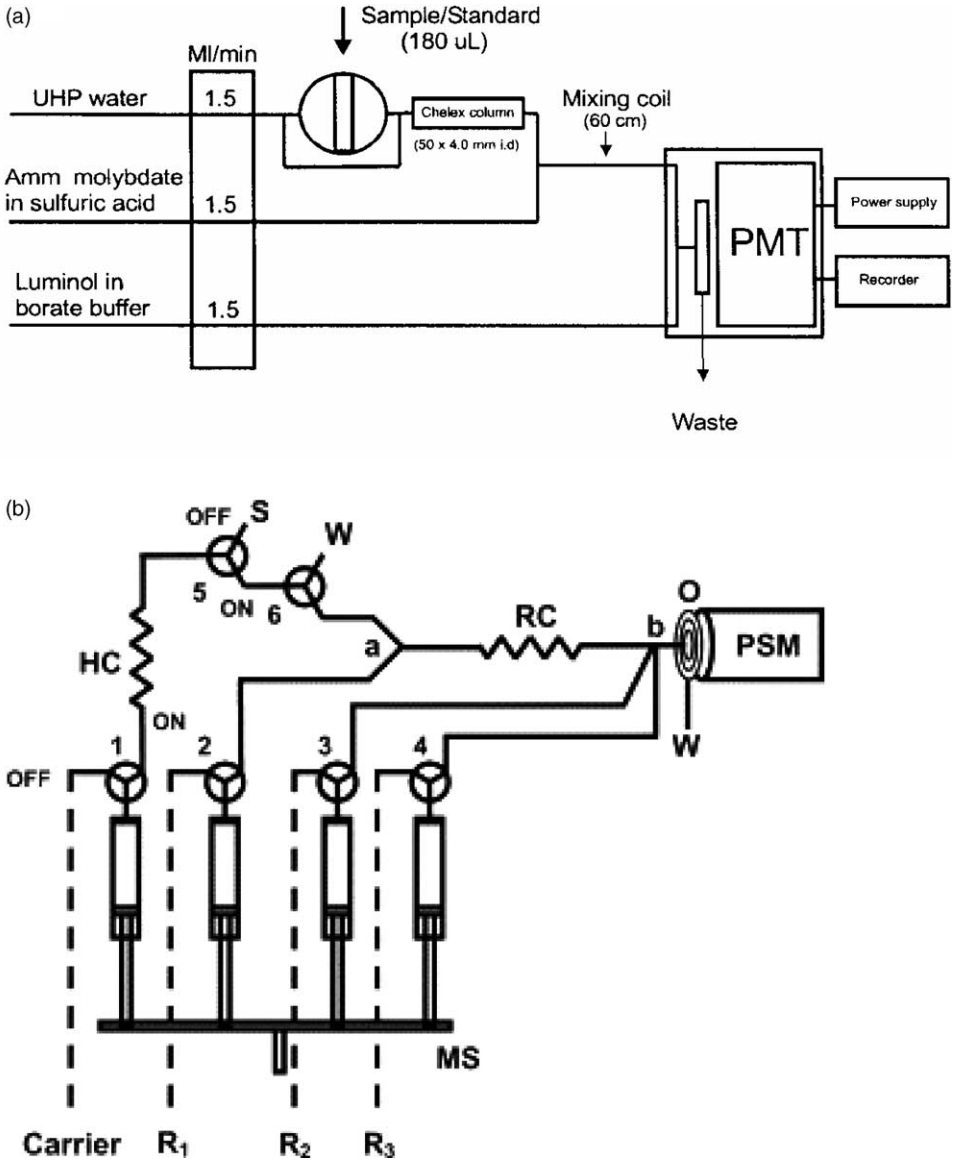

Fig. 6. (a) Flow injection-chemiluminescence (FI-CL) manifold for the determination of phosphate in freshwaters [143]. (b) Manifold for the determination of trace levels of orthophosphate in waters using a flow-through MSFIA-CL optical sensor [144]. MS, multisyringe; PSM, photosensor module; O, flow-through solid-phase optical sensor; HC, holding coil (230 cm); RC, reaction coil (160 cm); a and b, confluences; W, waste; S, sample or standard; Carrier, distilled water; $R_1 = 1.5 \times 10^{-3}$ M ammonium molybdate and 1.5×10^{-3} M ammonium vanadate in 0.2 M H_2SO_4; $R_2 = 5 \times 10^{-3}$ M luminol in 0.035 M NaOH; $R_3 = 80\%$ (v/v) MeOH.

ground, tap, and pond water and water-steam cycle of an incineration plant) and exploits the MSFIA concept with multicommutation (Fig. 6b). The proposed time-based injection system relies upon the in-line derivatization of the analyte with ammonium molybdate in the presence of vanadate, and the transient immobilization of the resulting heteropolyacid in an *N*-vinylpyrrolidone/divinylbenzene copolymer packed spiral shape flow-through cell located in front of the window of a photomultiplier tube. Simultaneous injection of well-defined slugs of luminol in alkaline medium and methanol solution towards the packed reactor produces the light emission from the luminol oxidation by oxidant species retained on the sorbent material which is readily detected. At the same time, the generated molybdenum-blue compound is eluted by the minute amount of injected methanol, rendering the system prepared for a new measuring cycle. The noteworthy features of the developed CL-MSFIA system are the feasibility to accommodate reactions with different pH requirements, the ability to determine trace levels of orthophosphate in high silicate content samples (Si/P ratios up to 500) and the absence of the typical drawbacks of the molybdenum-blue based spectrophotometric procedures such as the presence of excess molybdate anion, which causes high background signals due to its self-reduction. It can be concluded that chemiluminescence detection applied to phosphorus analysis is essentially based on FIA designs which provide sensi-

tive methods which involve relatively simple and economical instrumentation.

4.1.4. Atomic spectroscopic techniques

Atomic absorption spectrometry (AAS), hydride generation AAS (HGAAS) and inductively couple plasma atomic emission spectrometry (ICP-AES) are the most relevant techniques. Although several methodologies based on non-flow techniques involving the use of AAS for the indirect determination of phosphorus may be found in the literature, molybdenum is determined after the solvent extraction of phosphomolybdate or malachite green-phosphomolybdate ion pair, there are few references regarding the use of this detection technique combined with flow analysis techniques. This fact may be attributed to the poor sensitivity of the detection of phosphorus by AAS. The detection of this element is more sensitive by ICP-AES and, therefore, a greater number of analytical methods which use this detection technique can be found, and particularly based on flow injection techniques. The combination of flow-injection techniques with atomic spectrometry (AAS, HGAAS and ICP-AES) in agricultural and environmental analysis has been reviewed by Fang et al. [145]. These authors indicate that the flow injection systems are valuable for sample introduction; appropriate dispersion control allows the analysis of solutions containing as much as 40% (w/v) phosphate in fertilizers. The flow injection techniques have been fundamentally used to carry out pretreatments of the samples before being introduced into the detection system which is a feature of the methods based on atomic spectroscopy techniques. McLeod et al. [146] determine a phosphorus in steels by a flow injection analysis system incorporating a microcolumn of activated Al_2O_3 devised for performing rapid analyte enrichment/matrix removal in ICP-AES. Linear calibration was established over the range 0–50 $\mu g\,P\,ml^{-1}$. The rapid differential determination of orthophosphate and total phosphate in wastewater by FIA-ICP-AES has been proposed by Miyazaki and Bansho [147]. Orthophosphate was determined using the molybdovanadophosphoric acid method, and the solution after passing through the flow cell was introduced into the ICP to determine total P. A 213.618 nm wavelength for the ICP determination of P was selected because the elevation of the background intensity by Mo and V in the reagents for phosphate determination was smaller than at 214.912 nm which is the optimum wavelength for P determination alone. A cyclone spray chamber was better than a Scott-type spray chamber in order to obtain a signal with higher resolution. This method was further improved [148] by using a vacuum ultraviolet emission line of 177.499 nm. Although the intensity of phosphorus emission is not as high as it is at 213.618 nm it has very low background emission and suffers no interference from molybdenum or vanadium. The detection limit of the FIA-ICP-AES method was measured to be 0.5 $\mu g\,ml^{-1}$ when 200 μl of standard solution was injected into system. The detection limit was 0.07 $\mu g\,ml^{-1}$ when standard solutions containing the same matrix as used for the FIA method were nebulized directly and continuously into the plasma. The FIA detection limit was inferior compared with direct nebulization but was better than that reported by Miyazaki and Bansho [147].

4.1.5. Other optical techniques

In the analysis of phosphorus other optical detection techniques have been used, although much less frequently than those previously described, and mainly in FIA designs. In the literature a FIA-method with Fourier transform infrared (FTIR) detection proposed as a versatile technique for the determination of phosphate in aqueous solutions [149] can be found. Calibration graphs of standard solutions were obtained in the concentration range 100–1000 $mg\,l^{-1}$ phosphate. The high selectivity of the method was demonstrated by the analysis of six sugar and non-nutritive sweetened soft drink samples containing 350–600 $mg\,l^{-1}$ phosphate. The FIA determination of phosphate as aggregates of ion associates by light-scattering detection has been proposed [150]. Molybdophosphate with a chloro derivate of Malachite Green (Cl-MG) and Rhodamine B (RB) form aggregates in acidic solution, to stabilize the aggregates and prevent their precipitation in aqueous medium poly(vinyl alcohol) solution was added after the formation of the ion association. Traces of phosphate were determined by detecting the light-scattering intensity with a spectrofluorometer at the same wavelength of emission as that of excitation, 460 nm for Cl-MG and 460 and 580 nm respectively for RB. The calibration graph was rectilinear from 2×10^{-7} to $10^{-6}\,mol\,dm^{-3}$ phosphate and the detection limit was $6 \times 10^{-8}\,mol\,dm^{-3}$. Interferences from bulky and hydrophobic anions were slight. A rapid turbidimetric flow-injection method for the determination of phosphate in water has been used [151]. The method is based on the reaction of phosphate with ammonium molybdate in the presence of Triton X-100. Rapid mixing in a geometrically deformed Teflon capillary (a knitted capillary) prevents a parabolic concentration profile, thus decreasing the band width of the peaks. A 250 cm capillary permitted 0.2 $mg\,l^{-1}$ phosphate to be determined at reaction time of 4 s. A radioluminescent light source has been used in a detector for the flow injection determination of phosphorus in aqueous solutions by means of the vanadomolybdophosphoric (VMP) acid spectrophotometric method [152]. A comparison with previous studies in which lamp-based instrumentation was utilized proves that radioluminescence (RL) is a viable alternative source. RL enables the design of compact, inexpensive light sources for spectroscopic studies. Through the selection of a radioisotope and scintillation medium, the spectral and temporal characteristics of the source can be chosen independently. In this study, a broadband radioluminescent source provided detection limits of 0.4 $mg\,P\,l^{-1}$ which are similar to those of conventional lamp-based detectors but a dynamic range (two orders of magnitude) that is superior to conventional sources (1.3 orders of magnitude).

4.2. Electrochemical techniques

The most relevant electrochemical detection techniques for phosphates in flow analysis are potentiometry, voltammetry and amperometry.

4.2.1. Potentiometry

Potentiometric methods for the detection of phosphate include both direct and indirect methods using ion-selective electrodes (ISE). These methods, which have detection limits in the range of 30–300 $\mu g\ P\ l^{-1}$ are generally too insensitive for wastewater analysis [32].

4.2.1.1. Direct methods. Ion-selective electrodes (ISE) and flow injection potentiometry (FIP) has been used in a variety of fields, in particular, in the direct determination of phosphate. In spite of the lack of a reliable phosphate ISE, many ISEs have been developed for the detection of this ion; however, only a few of them have been used for the direct determination of phosphate in FIP. An excellent potentiometric response for a tri(thiourea)copper(I) monohydrogen phosphate $(Cutu_3)_2HPO_4$ and a silver(I) sulfide (Ag_2S) [153] solid-state membrane system was reported initially, but it loses its sensitivity with continued use, due to the oxidation of Ag_2S to $AgSO_4$ resulting in chemical transformation of the response characteristics of the electrode. A cyclic polyamine ionophore/PVC-based sensor displays a high sensitivity and selectivity towards dibasic phosphate over other commonly found anions in water samples [154]. However, this ISE possesses high detection limits, an impractically short lifetime, and suffers from significant interferences in the presence of other common anions (e.g. Cl^-, Br^-, I^-, S^{2-}, CH_3–COO^-, etc.) [155]. Notably, the most promising results were obtained using bis-(4-chlorobenzyl)tin dichloride electrodes with respect to sensitivity, selectivity against Cl^- and detection limit (viz. a detection limit of 33 μM). A phosphate ISE coated wire field effect transistor (CWFET) electrode which responds directly to hydrogenphosphate has also been reported [156]. Despite the CWFET's linear response over a wide phosphate range, its applicability is severely hampered by its relatively short lifetime and excessive cost. Alternatively, an ESL-51-07 glass ISE has been employed for PO_4^{3-} in natural water samples by direct potentiometry [157], yielding a good detection limit of 0.22 ppm. A cobalt wire phosphate ISE has been developed for use in direct FIP in aqueous media [158]. Significantly, the cobalt wire FIP system is a potentially useful ISE field technique because it is robust, highly selective, inexpensive, simple to operate, highly stable in FIP and provides a rapid response to phosphate. Cheng et al. [158] reported a linear response towards phosphate over the concentration range 5×10^{-5} to $5 \times 10^{-3}\ mol\ l^{-1}$.The mechanism and the applicability of this FIP method to real samples, wastewaters and fertilizers [159], soils extracts [160] and hydroponic nutrients [161] have been established. Mechanistic studies [159] using X-ray photoelectron spectroscopy (XPS) and electrochemical impedance spectroscopy (EIS) have demonstrated unambiguously that the response of the cobalt wire electrode involves a phosphate and hydrogen ion dependent charge-transfer process. The dihydrogenphosphate species dissolves the cobalt oxide overlayer of the electrode, facilitating the corrosion process which controls the response of the metallic electrode in aqueous media. Anion corrections for interferences by Cl^-, NO_3^- and SO_4^{2-} were applied to samples of hydroponics nutrients using the selectivity coefficients determined independently using a fixed interference method.

4.2.1.2. Indirect methods. They are alternative methods to the FIP methods with direct detection. The potentiometric response of a metallic copper wire as an indicator electrode has been measured in a FIA system [162] when inorganic anions are injected into a buffered carrier stream without the addition of excess Cu^{2+}. The response to pyrophosphate and tripolyphosphate and other anions has been shown to give a slope and sensitivity dependent on the complex stoichiometry and stability at the electrode surface. The calibration graph for pyrophosphate has been established between 10^{-4} and 10^{-2} M and between 10^{-4} and 10^{-1} M for tripolyphosphate ion. A flow injection analysis system was developed for the determination of various analytes with the lead(II) ion [163]. The system was optimized for the determination of the sulphate ion but the same flow system, with an aqueous rather than an ethanolic reagent, was used for the determination of orthophosphate and tripolyphosphate ions. While calibration curves tended to be non-linear for these ions, reproducibility was adequate for many analytical purposes. The continuous-flow determination of phosphate is described with emphasis on theoretical calculations to explain the experimental calibration data [164]. In the determination system, phosphate standards are introduced into a reagent stream containing Pb^{2+}, resulting in the formation of $Pb_3(PO_4)_2$. The associated decrease in free lead ion concentration is measured by a lead ion-selective electrode. The detection limit is 10^{-6} M phosphate. The Ca ion-selective electrode has been applied to the FIP determination of ligands which complex Ca ions. The response of the electrode is measured when ligand solutions are injected into a buffered carrier stream containing Ca. Injections of EDTA, citrate, tripolyphosphate, and pyrophosphate provide peaks with heights dependent on both the ligand and Ca concentrations. Ligand concentrations $\geq 2 \times 10^{-5}$ M can be detected. Tripolyphosphate is easily determined in detergents [165]. The flow injection determination of phosphate with the cadmium ion-selective electrode has also been proposed [166]. Phosphate samples are introduced into a reagent stream containing Cd^{2+}, resulting in the formation of $Cd_3(PO_4)_2$. The associated reduction in free metal concentration is sensed by a cadmium-selective electrode. With the exception of major interference from iodide and moderate interference from bromide and thiocyanate, the system exhibits excellent response to phosphate and selectivity over several common anions in solutions buffered at pH 8.4. The calibration graph has been established between

10^{-5} and 10^{-1} M. The immobilization of an enzyme on the surface of ion-selective membranes has also been used to obtain potentiometric biosensensors such as the biosensor based on inhibition of the hydrolysis of glucose 6-phosphate by potato acid phosphatase [167] proposed by Katsu and Kayamoto [168] for determination of inorganic phosphate using a salicylate-sensitive membrane electrode and an alkaline phosphatase enzyme. No application has been made of these biosensors to water analysis or FIP analysis because of the relatively poor sensitivity.

4.2.2. Voltammetry

Electrochemical reduction of the heteropolyacid to molybdenum blue at a glass carbon electrode in a wall-jet configuration flow-through cell without silicate interference was reported by several researchers [169–173]. While the approach is convenient and rapid, it is less sensitive than photometric detection of PMB and is similarly non-selective for orthophosphate.

Phosphate [169] and phosphate plus arsenate, silicate and germanate [170] were determined voltammetrically in aqueous samples at a glassy carbon electrode by flow-injection of their preformed heteropolyacids by direct injection of phosphate into the molybdate reagent. The detection limit was 0.5 ng of phosphate when the sample volume/reagent volume ratio is 9:1. The calibration graph was linear at 10^{-6} to 10^{-4} M. The phosphate determination in human blood serum by a flow-injection voltammetric method has been proposed by Abdalla et al. [171] also using direct injection into aqueous acidic molybdate reagent. A flow-injection voltammetric system for the determination of phosphate and nitrite by injection of reagents into a sample stream without mutual interference and with good precision has been used by Fogg and Bsebu [172]. The water sample is made the carrier in the FIA system (r-FIA) and the reagents for the determination of individual components are injected into the sample stream sequentially. A sample solution containing phosphate and nitrite ions was analysed in this way by using acidic molybdate and bromide reagents for determining these analytes voltammetrically at a glassy carbon electrode.

4.2.3. Amperometry

For the determination of total phosphorus in waters by flow-injection analysis, continuous microwave oven decomposition with subsequent amperometric detection of orthophosphate is proposed [21]. The percentage digestion was examined for two different decomposition reagents and by varying the pH of the carrier and the length and diameter of the digestion coil. With potassium peroxydisulphate decomposition the recoveries of phosphorus vary from 91 to 100% for organic phosphorus compounds, and with perchloric acid decomposition the recoveries vary from 60 to 70% for inorganic polyphosphates. Calibration graphs are linear up to 30 mg P l^{-1} and the determination limit is 0.1 mg P l^{-1}. Batch injection analysis (BIA) for the determination of phosphate using amperometric detection was recently investigated [174]. The phosphomolybdate complex, formed by addition of nitric acid, ammonium molybdate and phosphate, was reduced at a carbon paste electrode polarized at +0.3 V (versus Ag/AgCl). The major characteristics observed were simplicity of the equipment, a limited consumption of reagents and a low detection limit (0.3 μmol l^{-1}) with a linear range between 1 and 20 μmol l^{-1}. The interference of silicate was completely eliminated using an appropriate concentration of nitric acid and ammonium molybdate. The amperometric detection of orthophosphate in seawater was reported. Moreover, a carbon paste microelectrode was constructed. Its use allows the analysis of small volumes of samples with little dilution in supporting electrolyte. This method was applied to the determination of orthophosphate in cyanobacterial biofilms collected from Roman catacombs.

FIA systems has been developed with integrated amperometric biosensors with a wide variety of different possibilities of enzyme immobilization. These immobilization methods obey entrapment within the electrode material, physical attachment or chemical binding to the electrode surface or immobilization on the membrane placed at the electrode surface. Most of these enzyme sensors are based either on the inhibitory effect of orthophosphate on the alkaline phosphatase reaction or the use of purine nucleoside phosphorylase, maltose phosphorylase and pyruvate oxidase, which require orthophosphate as a cosubstrate. Biosensors based on a trienzyme system composed of maltose phosphorylase, mutarotase and glucose oxidase entrapped in an inorganic laponite clay and involving amplification by adding acid phosphatase have been proposed. A flow injection analysis biosensor system for the determination of phosphate was constructed using immobilized nucleoside phosphorylase and xanthine oxidase and an amperometric electrode (platinum versus silver/silver chloride, polarized at 0.7 V) for the determination of phosphate in various food products and plasma [175]. When a phosphate-containing sample was injected into the detection cell, phosphate reacted with inosine in the carrier buffer to produce hypoxantine and ribose-1-phosphate in the presence of nucleoside phosphorylase. Hypoxantine was then oxidized by xanthine oxidase to uric acid and hydrogen peroxide, which were detected by the amperometric electrode.

Several unique designs are worth mentioning. The concept of amplification of the response of the enzymatic detection system by substrate recycling was employed to enhance the sensitivity of the FIA enzymatic determination of phosphate. The principle of this approach is enzymatic reproduction of the substrate in the measuring system in the course of detection, which results in an increase of the measured signal. Bio-amperometric flow-injection systems are proposed [176] for the highly selective and sensitive determination of phosphate. One system studied is based on the use of a co-immobilized purine nucleoside phosphorylase-xanthine oxidase reactor, which responds to phosphate with high selectivity, and a detection limit of 3×10^{-7} M for a 20 μl injection. Another system with a co-immobilized purine nucle-

oside phosphorylase-xanthine oxidase-alkaline phosphatase reactor gives responses amplified by substrate recycling during passage through the enzyme reactor. Phosphate can be determined with 12 times the sensitivity in the latter system compared with the former, but the latter system responds to nucleotides and pyrophosphate in addition to orthophosphate.

A bioamperometric FIA system allows the simultaneous determination of phosphate and pyrophosphate [177]. The sample is split so that part passes through an immobilized inorganic pyrophosphatase reactor before passing through a coimmobilized nucleoside phosphorylase-xanthine reactor. The other portion passes only through the latter reactor. Since each channel has a different residence time, two peaks are obtained. The first peak corresponds to phosphate and the second peak to the total phosphate (phosphate plus pyrophosphate). Orthophosphate and total phosphates (inorganic phosphates plus purine nucleotides) can be determined simultaneously in a novel flow-injection system made up using a 16-way switching valve with two sample loops [178], an acid phosphatase (AcP) immobilized reactor and a delay coil needed to separate two peaks corresponding to two sample portions injected simultaneously. An orthophosphate enzyme electrode with a hybrid membrane of trienzyme film and poly(1,2-diaminobenzene) film was used to selectively detect both the endogenous orthophosphate and orthophosphate generated enzymatically in the AcP immobilized reactor. Since two sample portions passed through the flow line with different residence times, two peaks were obtained. The first peak corresponded selectively to orthophosphate and the second peak to the total of inorganic phosphates and purine nucleotides. The maximum currents of both peaks were linearly related to the concentration of orthophosphate and total phosphates (as orthophosphate) in the range 5×10^{-7} to 8×10^{-4} M, respectively.

5. Analytical characteristics, interferences and some other aspects of the application of flow techniques to phosphorus analysis in samples with real matrixes

In Table 1 are compiled the main analytical characteristics, including the type of sample analysed, of numerous flow analysis methods published up to date for the determination of phosphorus in its different forms. Firstly, the large number of publications that use FIA and UV–vis spectrosphotometric detection to evaluate this element in different types of samples should be highlighted. This can be explained in terms of the ease of implementation of FIA compared to SFA, as well as the fact that the remaining flow techniques are more recent. Also, UV–vis spectrophotometric detection is easy to implement, inexpensive and does not require specialized personnel. The PMB method is definitively the most widely used and, although not being specific for the determination of P in the orthophosphate form, it can be considered as an almost universal procedure for the determination of P, independently of the forms in which the element may be present and of the sample matrix. The ease of implementation of the on-line digestion, preconcentration, separation systems or enzymatic reactors in FIA systems have allowed the spread of the use of the PMB method to all types of samples, in an easy and comfortable way and with a high degree of automation, although water samples and aqueous solutions prevail for which flow techniques are well adapted. The PMB method is so well established in the field of the analysis of phosphorus that is constitutes the reference method for new ones, bearing in mind that the EPA methods are based on the PMB method. Besides, many flow methods based on other detection techniques use the formation of the heteropolyacid in some of their analysis steps (electroactive species, reagent or detected reaction product, for the preconcentration or speciation, etc.) The SIA and SFA methodologies have also mainly used spectrophotometric detection and the PMB method for the analysis of P. In spite of SFA being long established more research work in relation to the SIA methods for the determination of P can be found in the literature. This is because, SFA systems are more difficult to implement and, thus, are essentially commercial designs used by routine analysis laboratories to process a large number of samples, even determining several analytes or in certain research with very specific objectives. Discrepancies between analytical parameters for assemblies involving the same chemistry are attributed to the dissimilarity of the chemical and physical variables selected, which in turn greatly influence the sensitivity of the methodology as well as the tolerance to heteropolyacid forming oxoanions such as silicate.

The analytical techniques used to determine P in even eutrophic waters must be quite sensitive. In pristine waters, very low concentrations are observed, e.g. 0.001 mg P l^{-1} or less of FRP and it is generally only in polluted waters and wastewaters that concentrations in the mg l^{-1} range are found [32]. In the data shown in Table 1 it can be observed that the FIA methods for the determination of P with spectrophotometric detection and based on different chemistries range in P concentrations between 0.001 and 200 mg l^{-1} (detection limit (DL): 0.00006–0.8 mg P l^{-1}, sampling rate (SR): 6–144 h^{-1}); the SFA methods between 0.02 and 0.102 (D.L.: 0.0007–0.019, SR: 9–72); the SIA methods between 0.05 and 400 (DL: 0.01–0.15, SR: 16–30) and the MCFIA methods between 0.01 and 1.00 (DL: 0.005, SR: 56–180). In many cases, r-FIA (or r-SIA) systems have been used. These involve the concept of reverse or reagent injection FIA, which are shown to be inherently more sensitive than the conventional sample injection flow injection approach. The r-FIA systems reported in the literature range in concentrations of P between 0.002 and 0.10 mg l^{-1} (DL: 0.002, SR: 30) and the r-SIA methods between 0.1 and 1.0 (DL: 0.02, SR: 22). On the other hand, the μFIA and μSIA systems present ranges within 1–10 mg l^{-1} (DL: 0.1, SR: 60) and 0.001–0.030 (DL: 0.0001, SR: 60), respectively. Thus, except for water samples with very low concentrations, flow methods possess adequate detection limits and concentration ranges for their use in the analysis of the P content in these types of samples. Among the flow analysis

Table 1
Analytical performance of relevant flowing stream methods applied to determination of phosphorus in different samples

Flow technique	Detection technique	Reagents	Linear or determination range (mg P l^{-1})	RSD% (mg P l^{-1})	Detection limit (mg P l^{-1})	Sample	Sampling rate (h^{-1})	Reference
SFA	Spec	Mo-Sb/Asc	0.002–0.062	–	0.002	Synthetic water	9	[87]
MSFA	Spec	Mo-MG	0.005–0.075	2 (0.020)	0.0007	Lakes waters	72	[89]
CFA	Spec	Mo-MG	0.028–0.102	ResSD: 1.2 (0.028–0.102)	0.019[a]	Sea water	–	[180]
FIA	Spec	Mo-V	Up to 200	2 (10)	0.8	Waste water	8	[148]
FIA	Spec	Mo/Sn-Hy	0–25	0.4 (8.75)	0.05	Waste water	20	[82]
FIA	Spec	Mo/Sn	0.005–0.05	1 (0.03)	0.002	Natural water	80	[91]
FIA	Spec	Mo/Sn-Hy	0.005–0.10	<4 (0.01–0.032)	0.003	River water	12	[94]
FIA	Spec	Mo-Sb/Asc	0.001–0.05	1.0 (0.020)	0.0006	Waste water	12	[97]
FIA	Spec	Mo/Sn-Hy	0.025–0.250	1.3 (0.025–0.25)	0.020	River water	38	[183]
FIA	Spec	DR: Perox + H_2SO_4, Mo-Sb/Asc-NaDS	0–0.10–1.0	2.25–0.13 (0.024–3.03)	0.001	River and waste water	20	[181]
FIA	Spec	DR: Perox + H_2SO_4, Mo/Sn-Hy	0–1.5	<1 (0.50)	0.007	Soils leachates and runoff waters	40	[115]
FIA	Spec	MWD in HNO_3 medium, Mo/Asc	Up to 6.53	<5.0 (0.033–6.53)	0.033	Waste water	30	[22]
FIA	Spec	DR: Perox + $HClO_4$, Mo/Sn-Hy	0–18	≤2.0 (10.2)	0.15	Waste water	32	[29]
FIA	Spec	Mo-V	Up to 30.0	3.5 (–)	0.680	Plant leaves	83	[112]
FIA	Spec	Mo-MG	–	–	0.0005	Natural water	–	[179]
FIA	Spec	Mo-MG	Up to 0.018	0.57 (0.006)	0.00006	Sea water	30	[98]
FIA	Spec	3-Phytase, Mo/Sn-Hy	0.025–0.5	DRP: ≤2.5 PHP: ≤2.5 (0.025–0.5)	DRP: 0.003 PHP: 0.004	Estuary, lake and river water	40	[120]
FIA	Spec	Mo/Sn-Hy, NaCl 60 g/l carrier	0.025–0.3	–	0.006	Estuarine waters	85	[111]
FIA	Spec	CNM-$Phos_{im}$, Mo/Sn	MFP: 1.24–18.6	0.7 (3.1)	0.124	Toothpaste	72	[123]
FIA	Spec	PBR-$Phos_{im}$, Mo/Sn-Hy	0.005–5.0	–	–	Aqueous solutions	20	[39]
FIA	Spec	CNM-$Phos_{im}$, Mo/Sn	Or and MFP: 0.031–6.2	Or: 0.5 (3.2) MFP: 1.7 (3.2)	Or and MFP 0.016	Toothpaste	–	[122]
FIA	Spec- PBLED	Mo/Sn-Hy	0.025–0.10	1.95 (–)	0.005	Marine waters	225	[187]
FIA	Spec	PC-DVB/Mo/Sn/NaOH	Up to 0.1	4.0 (0.0005)	0.0002	Tap water	6	[93]
FIA	Spec-SFC	Mo/OxA	0.03–7.83	1.3 (0.05)	–	Boiler water at power plants	60–120	[109]
FIA	Spec-PBLED	Mo/Asc	0.5–3	–	–	Pond, irrigation, reservoir waters	–	[105]
FIA	Spec-RL	Mo-V	Up to 105	1 (6–35)	0.40	Aqueous solutions	144	[152]

Table 1 (*Continued*)

Flow technique	Detection technique	Reagents	Linear or determination range (mg P l^{-1})	RSD% (mg P l^{-1})	Detection limit (mg P l^{-1})	Sample	Sampling rate (h^{-1})	Reference
FIA	Spec	Or: Mo-Sb/ASc TIP:H_2SO_4/Mo/Asc/SDS	Or: up to 8.73 TIP: up to 52.39	Or: 1.2 (2.18) TIP: 2.0 (21.83)	Or: 0.44 TIP: 1.09	Detergents	Or:80 TIP:40	[118]
r-FIA	Spec	Mo/Asc	0.002–0.10	2.8 (0.048)	0.002	Estuarine water	<30	[80]
GF-FIA	Spec	Mo/Sn-Hy	0–0.180	<5 (0.001–0.180)	0.001	Natural and waste water	15	[116]
IC-FIA	Spec	TD in H_2SO_4 medium, Mo/Sn-Hy	Or: 0.010–1.00 Pyr and Tri: 0.020–2.00	≤3.0 (1.00)	Or:<0.01 Pyr and Tri: 0.020	Waste water	5	[117]
μFIA	Spec-EOF	Mo/Asc	1–10	5 (1)	0.1	Aqueous solutions	60	[124]
SIA	Spec	Mo-V	Up to 18.00	2.1 (5.00)	0.15	Drinking, ground and waste water	30	[90]
SIA	Spec	Mo-V-MG	0.05–0.40	18 (0.10)	0.01	Drinking, ground and waste water	30	[90]
SIA	Spec	Mo/Sn	0.05–4.00	1.7 (2.50)	0.01	Drinking, ground and waste water	30	[90]
SIA	Spec	MWD in HNO_3 medium, Mo/Asc	20.0–400.0	<3 (–)	–	Foodstuffs (wine, orange juice, vegetable, milk)	16	[119]
SIA	Spec	Mo/SSTFTE	0.3–20	1.8 (10)	0.1	Beverages, waste water and urine	18	[186]
SIA	Spec	Mo-V	Up to 12	1.4 (9)	0.2	Waste water	23	[107]
SIA	Spec	Mo-V	0.8–15	2.1 (5.0)	0.23	Waste water	30	[108]
r-SIA	Spec	Mo/Sn	0.1–1.0	2.1 (0.75)	0.02	Tap and ground water	22	[101]
μSIA	Spec-LOV	Mo-Sb/Asc	0.001–0.030	6 (0.001–0.003)	0.0001	Tap and lake waters	60	[126]
MCFIA	Spec	Mo/Asc	2.5–12.5	1.8 (8.3)	–	Digest of plant material	80	[184]
MCFIA	Spec	Mo-Sb/Asc	Up to 3	1.4 (2.47)	–	Water of sewerage treatment plant	180	[185]
MCFIA	Spec-PBLED	Mo/Asc	Up to 1.00	0.7 (0.5)	0.005	River water	56	[103]
FIA	Spec + ICP-AES	Spec: Mo-V	Up to 200 Or and TP	– ICP: 2.01 (10)	Spec: 0.8 ICP: 0.5	Waste water	80	[148]
FIA	Spec + ICP-AES	Spec: Mo-V	–	–	Or: 5 TP: 0.6	Waste water	40	[189]
FIA	ICP-AES	PC-Al_2O_3 activated	0–50	1.6 (20)	0.6	Steels	–	[146]
FIA	Flu	DR: Perox + $NaBH_4$, Mo/Thia	0.0016–0.35	<1 (0.20)	0.0003	Spring, mineral and well water	–	[128]
FIA	Flu	Acr-Fe(III)	0.78–7.80	1.2 (1.24)	0.1	Synthetic water	45	[133]
FIA	Flu	Mo-RhB	0.0003–0.093	1.2 (0.025)	0.0003	Sea and river water	15	[130]
FIA	Flu	Mo-Rh6G, PC-IE	0.01–0.4	1.5 (0.2)	0.01	Natural water	–	[132]
FIA	LS	Mo-MG or RhB/ PVA	0.006–0.031	1.1(0.025)	0.0019	Aqueous solutions	15	[150]
FIA	FTIR	HCl or NaOH	32.6–326	0.5 (163)	–	Sugars and soft drink	60	[188]
FIA	CL	PyrOxG/Lu	0.003–1.0	2.3 (0.003–1.0)	0.003	Marsh and river water	30	[182]
FIA	CL	GOD_{im}/Lu	0.0003–0.93	4.3 (0.0003–0.93)	0.0003	River water	20	[141]
FIA	CL	MP-MUT-GOD_{im}/Lu	0.0003–0.930	4.3 (0.0003–0.930)	0.0003	River water	20	[141]
FIA	CL	$PyrOxG_{im}$/Lu-ARP	0.003–1	2.3 (0.003–1)	0.003	River and marsh water	–	[140]
FIA	CL	$PyrOxG_{im}$/Lu -4iph-POD_{im}	0.012–0.229	5 (0.012–0.229)	0.002	River water and aqueous solutions	20	[139]

FIA	CL	Pur-Xan/Triox	Down 0.003	–	–	Synthetic water	20	[138]
FIA	CL	Mo/Lu	0.00003–0.003	1.2–4.7 (0.00003–0.003)	0.00003	Freshwater	180	[143]
MSFIA	CL	Mo-V/NVDC-PSSFTC/Lu	0.005–0.05	3.0 (0.011)	0.004	Natural water and water-steam cycle of an incineration plant	11	[144]
FIA	Pot	Pht-CoW	3.1–310	4.0 (31)	0.093	Fertilizer and waste water	–	[159]
FIA	Pot	Pht-CoW	15.5–310	2–5 (15.5)	0.093	Hydroponic nutrient	12	[161]
FIA	Pot	Pht-CoW	0.31–155	3.8 (31)	0.031	Soils extracts	30	[160]
FIA	Pot	Cu wire electrode	Py: 6.2–620 Tri: 9.3–9300	–	–	Aqueous solutions	72	[162]
FIA	Pot	Pb-ISE, Pb^{2+}	3.1–310	0.6 (–)	–	Aqueous solutions	20	[163]
FIA	Pot	Cd-ISE, Cd^{2+}	0.31–3100	0.23 (3100)	–	Aqueous solutions	160	[166]
FIA	Vol	GCE/Mo	0.031–15.5	–	0.062	Aqueous solutions	–	[169]
r-FIA	Vol	GCE/Mo	0.155–15.5	–	0.155	Aqueous solution	–	[172]
FIA	Amp	Trines and P12Dia membrane electrode-AcP	0.16–24.8 Or and TP	< 2.5 (0.31)	0.008	Aqueous solutions	30	[178]
FIA	Amp	MWD/DR: Perox or $HCLO_4$	Up 30	3 (5.0)	0.10	Domestic waste water	21	[21]
FIA	Amp Amp-ASR	ER: Pur-Xan ER-ASR: Pur-Xan-Phos	0.031–15.5 ASR: Up to 1.55	5 (0.062) –	0.009; 0.0006	Aqueous solutions	20	[176]
BIA	Amp	Mo in nitric acid, CPE	0.031–0.62	6 (0.031–0.62)	0.0093	Cyanobacterial biofilms and sea water	–	[174]

Acr, acridine; Acp_{im}, acid phosphatase immobilized; AES, atomic emission spectrophotometry; Amp, amperometric; ARP, arthromyces ramosus peroxidase; Asc, ascorbic acid; ASR, amplification substrate recycling; BIA, batch injection analysis; CFA, continuous flow analysis; CL, chemiluminescence; CNM, cellulose nitrate membrane; CoWE, cobalt wire electrode; CPE, carbon paste electrode; Cu, cupper metal; DR, digestion reagent; DRP, dissolved reactive phosphorus; DVB, reversed phase polymers styrene-divinylbenzene; EOF, electroosmotic flow; ER, enzyme reactor; Fe, iron(III); FIA, flow injection analysis; Flu, spectrofluorimetric; FTIR, Fourier transform infrared spectrometry; GCE, glassy carbon electrode; GF, gel filtration; GOD_{im}, glucose oxidase immobilized on *N*-hydroxysuccinimide beads; Hy, hydrazine; IC, ion chromatography; IE, ion exchange resin; ICP, inductively coupled plasma; ISE, ion selective electrode; LOV, lab on valve; LS, light scattering; Lu, luminol; MCFIA, multicommutation FIA; MFP, monofluorophosphate; MG, malaquita green; Mo, potasium ammonium molybdate; MP, maltose phosphorylase; MSFIA, multisyringe FIA; MSFA, monosegmented flow analysis; MUT, mutarotase; MWD, microwave digestion; NaCl, sodium chloride; NaDS, sodium dodecylsulfate; NVDC, *N*-vinylpyrrolidone/divinylbenzene copolymer; Or, orthophosphate; Oxa, oxalic acid; Pb, lead; PBLED, photometer-based light emission diode; PBR, packed-bed reactor; PC, preconcentration column; Perox, sodium peroxydisulfate; $Phos_{im}$, immobilized alkaline phosphatase; PHP, phytase hydrolysable phosphorus; Pht, phtalate buffer; POD_{im}, peroxidase immobilized; Pot, potentiometric; PSSFTC, packed spiral shape flow-trough cell; Pur, purine nucleoside phosphorylase; PVA, poly(vinyl alcohol); Pyr, pyrophosphate; PyrOxG, pyruvate oxidase G; $PyrOxG_{im}$, pyruvate oxidase G immobilized on *N*-hydroxysuccinicacidimido beads; P12Dia, poly(1,2-diaminobenzene) film; ResSD, residual standard deviation; r-FIA, reverse FIA; RhB, rhodamine B; Rh6G, rhodamine 6G; RL, radioluminicent light source; R.S.D., relative standard deviation; r-SIA, reverse SIA; Sb, antimony tartrate; SDS, sodium dodecyl sulfate; SFA, segmented flow analysis; SFC, series flow cells; SIA, sequential injection analysis; Sn, tin(II); Spec, spectrophotometric; SSTFTE, stainless steel tubular flow-through electrode; TD, thermal digestion; Thia, thiamine; TIP, total inorganic phosphorus; TP, total phosphorus; Tri, triphosphate; Trienz, trienzyme film; Triox, bis[2-(3,6,9-trioxadecanyloxycarbonyl)4-nitrophenyl]oxalate; V, ammonium vanadate; Vol, voltametric; Xan, xanthine oxidase; 4iph, 4-iodophenol.

[a] Lowest *P* concentration analysed using robust regression methods.

methods with spectrophotometric detection described in the literature those based on the FIA, SFA and MCFIA techniques present lower detection limits and higher analysis rates. However, the μSIA system [126] is worth mentioning because of these aspects as well as other relevant analytical characteristics such as the small volumes of reagents and samples consumed together with the small amount of residues generated. The interferences caused by species present in the samples which also form heteropolyacids, especially silicate, which yield products presenting similar absorption spectra in the spectrophotometric method with PMB are eliminated by the addition of oxalic or tartaric acid to the molybdate reagent, hindering the formation of silicomolybdic acid. If the heteropolyacids are already formed, the addition of these organic acids destroys the phosphomolybdic acid, thus, allowing the determination of silicate in the presence of phosphates. Alternatively, there are several FIA and SIA methods which allow the simultaneous or sequential determination of phosphates and silicates based on kinetic factors in the formation of the corresponding heteropolyacids or other strategies. In several samples the interferences due to hydrolysis of labile phosphorus species (dissolved organic and condensed phosphates) produced by the conditions of acidity and the presence of molybdate may give rise to results which overestimate the orthophosphate concentration present in the sample, unless the correct measures are taken. Kinetic-extraction methods or complexation of the phosphomolybdate formed by means of citrate–arsenite reagent have been used in non-flow-based methods. Analogously, the interferences produced by samples with high contents of Fe, Al, Ca and chloride should be considered. Cations interfere due to competitive complexation of the phosphate, whereas interference by chloride is probably due to inhibition of the phosphomolybdate reduction if tin(II) is used as a reducing agent. The interference of chloride in the PMB method is particularly problematic, especially for the determination of phosphate in marine and estuarine waters, and for this reason ascorbic acid is often favoured. Another aspect to be taken into account is the case of determining the TP in samples with high chloride content or water samples with high salinity, since chlorine is formed during the digestion step with peroxydisulphate. This is not problematic if the sample is digested in an open vessel, where the chlorine is boiled off. If digestion is performed in a closed system the chlorine is trapped and subsequently interferes in the detection process involving the ascorbic acid reduction step. This problem is avoided by introducing sodium sulphite into the reactor.

The FIA-methods based on ICP-AES detection present a lower sensitivity than the UV–vis spectrophotometric methods and the range of determinable concentrations corresponds to 0.6–200 mg P l^{-1} (DL: 0.5, SR: 40–80). However, these methods are useful for certain types of samples (agricultural, steels, etc.) and to carry out the determination and calculation of important fractions of P in waters and sediments. It is usual in this type of determination to employ a combination with the spectrophotometric determination using PMB with this aim. The methods based on fluorometric and chemiluminiscence detection also provide appropriate flow methods of analysis for the determination of P in waters. The reported fluorometric methods, all based on FIA configurations, show concentration ranges between 0.0003 and 7.8 mg P l^{-1} (DL: 0.0003–0.1, SR: 15–45), whereas the CL-FIA methods between 0.00003 and 1 (DL: 0.00003, SR: 20–180). The presence of metal traces constitutes the most relevant interference, especially with chemiluminiscence detection, however, it can be conveniently avoided on-line by the pretreatment of the sample with masking agents or by making the sample initially pass through a minicolumn of chelating resins or ion exchangers. The economy and simplicity of the instrumentation required in the implementation of the CL-FI methods should be emphasized.

Among the flow methods using electrochemical detection, the potentiometric and amperometric methods in FIA designs have been the most widely used for the determination of P species (orthophosphate, triphosphate and pyrophosphate) and TP in samples with real matrixes. Among the FPI methods, the direct methods based on the use of ESI have been used for the determination of P in fertilizer, waste water, hydroponic nutrient and soils extracts. The determined concentration ranges are comprised between 0.31 and 3100 mg P l^{-1} (DL: 0.031, SR: 20–160). These are only a few ISE methods for the analysis of P that provide the conditions of robustness, selectivity, sensitivity, economy, stability and speed of response required for their application to real samples in flow systems. Among these it is noteworthy mentioning the cobalt wire phosphate electrode [159–161]. The FIA-methods which use amperometric detection are equipped with immobilized enzymes reactors or sensitive phosphate-selective enzyme electrodes which provide a viable and selective alternative to PMB spectrophotometry, especially if the concept of amplification substrate recycling (ASR) is used [176]. These FIA methods provide determination ranges between 0.031 and 24.8 mg P l^{-1} (DL: 0.009; ASR-DL: 0.0006, SR: 20) and have been applied to the determination of orthophosphate and TP in waters.

6. Future outlook

As stated in this review most of the implementations of the flow techniques to the analysis of P have been carried out through SFA, FIA and SIA designs. In the future the development of the alternatives flow techniques, namely MSFIA, MCFIA and MPS is expected together with relevant contributions in the field of chemical analysis, particularly in the analysis of P. Moreover, these new techniques, based on technological advances, are favourable to achieve an almost complete automation of the steps involved in the analysis of P in samples with real matrixes, especially in the case of waters, although the automation is also slowly spreading to the field of solid samples. It seems that the spectrophotometric determination of P based on the PMB method will continue

to be widely applied, however, the advances to be achieved in ICP, both in improvements in the sensitivity and the combination with other techniques (hyphenated techniques) involving more advantages, such as ICP–MS, will constitute viable alternatives for the determination of P. The chemiluminiscence methods also constitute very interesting alternatives due to their low cost, instrumental simplicity and high sensitivity. The selectivity of these methods is the major drawback, however this aspect is likely to be overcome satisfactorily by the use of new and more selective chemiluminiscence reactions and/or better devices for the pretreatment of samples. The electrochemical detection techniques are far less used than the optical techniques, however, it is likely that in the future the FPI methods based on ESI, containing immobilized ionophores [190], will be developed especially because of their low cost and simplicity. These techniques present sufficient selectivity, sensitivity, robustness and other chemical and physical characteristics which will probably allow determination of P with the same ease, selectivity and sensitivity already attained for other ions.

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