Spectrophotometric flow system using vanadomolybdophosphate detection chemistry and a liquid waveguide capillary cell for the determination of phosphate with improved sensitivity in surface and ground water samples

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ABSTRACT

A flow injection procedure based on the vanadomolybdate method for the determination of dissolved reactive phosphorus in water samples is described. The system includes a liquid waveguide long pathlength cell connected to a charge coupled device (CCD) spectrophotometer. Calibration was linear up to $500 \mu g \, P \, L^{-1}$, with a detection limit of $17 \mu g \, P \, L^{-1}$ and a quantification limit of $56 \mu g \, P \, L^{-1}$. An injection throughput of 60 determinations h^{-1} and repeatability (R.S.D.) of 2.2% were achieved. Potential interference from silicate was effectively masked by addition of a tartaric acid stream. The accuracy of the proposed methodology was assessed through analysis of a certified reference material and recovery tests on water samples. The developed procedure allows the determination of phosphorus in water samples at trace levels with high sensitivity, reduced reagent consumption and low waste production.

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1. Introduction

Phosphorus is an essential element for the growth of plants and animals. It is the limiting nutrient in fresh water ecosystems, and an increase in its concentration usually leads to an increase in the aquatic vegetation. This process may result in eutrophic conditions and depletion of oxygen in water, due to the heavy oxygen demand of microorganisms as they decompose organic material. The disturbance of the normal functioning of the ecosystem will eventually cause degradation in water quality. European Union directive sets the limit of $100 \mu g \, L^{-1}$ P-PO$_4^{3-}$ as an indicator level for probable problematic algae growth [1].

In water samples, phosphorus can be found in the form of different inorganic and organic species and can be present in either the dissolved, colloidal or particulate form. The dominant and most stable inorganic species is dissolved in reactive phosphorus [2–4]. Reference procedures are usually based on phosphorus determination in this form, on aliquots of previously filtered samples. Most of them are based on the colorimetric assay of phosphomolybdate or vanadophosphomolybdate heteropoly acids.

Orthophosphate reacts with molybdate in acidic medium to yield 12-molybdophosphoric heteropolyacid; subsequently, detection is undertaken either on the molybdophosphate reduction product (molybdenum blue method) or on the yellow vanadomolybdate complex [2,5,6]. The molybdenum blue method became broadly used due to its higher sensitivity and lower susceptibility to interferences [4,7,8], than the vanadophosphomolybdate method. Nevertheless, the low concentration levels imposed by legislation are difficult to achieve. Therefore, the development of new methodologies capable of monitoring trace phosphate levels in environmental samples with adequate precision, has gained importance [9].

Flow injection analysis (FIA) is a widespread and well established approach to determine phosphorus. In fact, the first publication on FIA is dedicated to phosphate determination [10] and discusses both the yellow vanadomolybdate and the molybdenum blue method. Compared to the blue molybdenum method, the yellow vanadomolybdate analytical procedure can present some advantages that make it more suitable for flow systems: (i) the use of a single reagent mixture with extended stability makes the method suitable for field portable applications and for unattended operation in water monitoring [11,12] and (ii) the formation of a fine precipitate, accompanying the development of the blue colour, does not occur. However, maybe because of its limited sensitiv-

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ity, the yellow vanadomolybdate method has been rarely used in flow systems. This limitation might be overcome by resorting to instrumental advances in spectrophotometry. Recently, with the development of the liquid waveguide capillary cell (LWCC), the increase of the flow cell pathlength can be easily achieved without deteriorating other analytical characteristics of the method. The long pathlength spectrophotometric measurements can increase the method sensitivity by up to two orders of magnitude [4,13,14].

The objective of this work was to develop a flow system based on the vanadomolybdate method, capable of quantifying phosphate at trace concentrations in waters, using a LWCC coupled to a charge coupled device (CCD-array) detector. Different flow strategies were employed and the effect of silicate (the most commonly referred interfering compound) on the analytical measurements was assessed.

2. Experimental

2.1. Reagents and solutions

All chemicals presented an analytical reagent grade. Solutions and reagents were prepared with deionised water (with specific conductance lower than $0.1\,\mu S\,cm^{-1}$) and subsequent dissolution was done in appropriate solvents. Due to the low phosphorus level analysed in the LWCC flow system, all solutions were prepared with ultra-pure water (Barnstead-Easypure-LF) in volumetric material that was previously rinsed with this type of water.

Phosphorus stock solution ($100\,mg\,L^{-1}$) was prepared dissolving potassium dihydrogen phosphate (KH_2PO_4) previously dried overnight at $105\,^{\circ}C$. Silicate stock solution ($100\,mg\,L^{-1}$) was prepared by dissolving sodium metasilicate pentahydrate ($Na_2SiO_3{\cdot}5H_2O$). Working phosphorus and silicate standard solutions were prepared daily by suitable dilution of the stock solutions.

A 10 mM tartaric acid solution was prepared dissolving 0.37 g of L(+) tartaric acid ($C_4H_6O_6$) in 0.25 L of water.

The vanadomolybdate reagent was a solution of $25\,g\,L^{-1}$ ammonium heptamolybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}{\cdot}4H_2O]$ and $1.9\,g\,L^{-1}$ ammonium monovanadate (NH_4VO_3), in either 2 M or 3.25 M HCl.

All groundwater samples were filtered through a 0.45 μm Whatman cellulose acetate membrane. Mineral water samples had no previous treatment. Two surface waters certified reference materials were used: SPS-SW2 (Spectrapure, Oslo, Norway) and QC RW1 (VKI, Denmark).

2.2. Flow manifolds and instrumentation

The preliminary flow injection system used in this work is shown in Fig. 1(a). The initial conditions were based on the American Public Health Association (APHA) recommended batch procedure [5].

A peristaltic pump (Gilson, Minipuls 3) and Tygon PVC tubes were used to propel water (carrier, C), and reagent (R) solution into the system at flow rates of 3.2 and $0.8\,mL\,min^{-1}$, respectively.

The tubing (0.8 mm i.d.) connecting the different parts of the flow systems was made of poly(tetrafluoroethylene) with Gilson end fitting and connectors.

Sample and standard injections in the flow injection set-up were made using a Rheodyne type 5020 six-port rotary injection valve. Carrier and reagent streams then merged in a laboratory made acrylic Y-shaped connector used as confluence point and the resulting solution further mixed while passing a reaction coil (170 cm).

Another flow configuration (Fig. 1(b)) was used to reduce the effect of the silicate interference, with a stream of tartaric acid
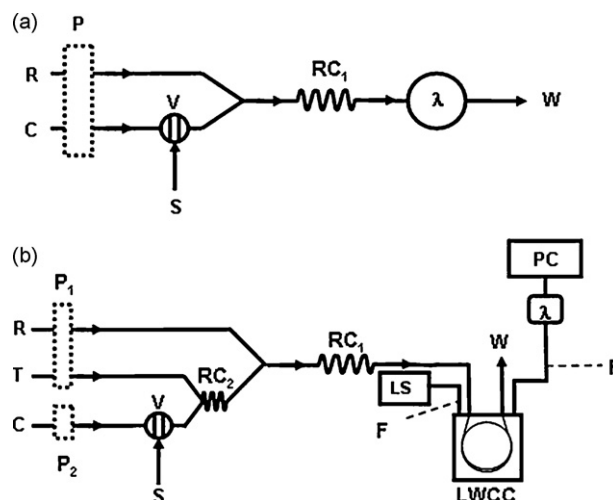

Fig. 1. Flow injection manifolds: (a) set-up used in preliminary studies and (b) manifold used for the determination of phosphorus in waters with long pathlength liquid core waveguide technology. R, vanadomolybdate reagent; C, carrier (deionised water); T, tartaric acid solution; P_i, peristaltic pump; S, sample or standard solution; V, injection valve; RC_1, reaction coil (170 cm); RC_2, reaction coil (50 cm); λ, detector (conventional or CCD array spectrometer); PC, computer; F, optical fibre; LS, light source; LWCC, liquid waveguide capillary cell; W, waste.

merging the carrier stream after the injection port. A reactor coil of 50 cm was used to enhance mixture between these solutions. Carrier (ultra-pure water), tartaric acid and reagent flow rates were reset to 2.7, 0.7 and $0.8\,mL\,min^{-1}$, respectively with the aid of a second peristaltic pump.

Different detection systems were used. For the manifold presented in Fig. 1(a), a Thermo Spectronic Helios Gamma UV–vis Spectrophotometer equipped with a Hellma model 178.712-QS flow cell (10 mm light path, inner optical volume 30 μL), connected to a Kipp & Zonen BD112 recorder was used. Subsequently this detector was replaced by an OceanOptics (Dunedin, FL, USA) USB2000-FLG Spectrometer (slit: 200 μm, grating: 600 lines blazed at 500 nm; bandwidth 380–1020 nm) connected via a 400 μm fibre optical cable (model P400-2-UV–vis) to the flow cell, placed in an Ocean Optics CUV cell support (Fig. 1(a)). A Mikropack (Ostfildern, Deutschland) Deuterium–halogen light source, model DH-2000-BAL was used with a 400 μm illumination optical fibre (model P400-2-UV–vis). Registration of the analytical signal was made using the OOIBase32 Spectrometer Operating Software. Dual-wavelength spectrophotometry was used to reduce the refractive index effect and the blank absorbance of the vanadomolybdate reagent. The analytical wavelength was 380 nm and the reference wavelength 600 nm; the absorbance signal at 600 nm was subtracted from the one at 380 nm for schlieren effect [15] correction. The collected absorbance data was treated and analysed in a lab-developed Microsoft Excel based software.

Afterwards, the conventional flow cell was replaced by a World Precision Instruments (Sarasota, FL, USA) liquid waveguide capillary cell, with a pathlength of 100 cm, model 2100. The absorbance change was monitored at 446 nm. During the optimisation process, it was observed that the LWCC gave the best day-to-day performance when sequentially flushed with 1 M NaOH, 1 M HCl and ultra-pure water (in counter current) at the end of each day.

2.3. Reference procedure

To assess the quality of the results obtained with the different analytical systems, results were compared with those given by the American Public Health Association recommended reference

method 4500-PC Vanadomolybdophosphoric Acid Colorimetric Method [5]. The methodology is based on the fact that dissolved reactive phosphorus reacts with ammonium molybdate under acid conditions to form a heteropoly acid–molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid $[(NH_4)_3PO_4NH_4VO_3\cdot16MoO_3]$ is then obtained being the intensity of the yellow colour proportional to phosphorus concentration. The determination range of phosphorus by this procedure is 1–20 mg L^{-1} with a minimum detectable concentration of 200 μg L^{-1} (in 1 cm spectrophotometer cells). A wavelength of 470 nm is usually used.

3. Results and discussion

3.1. Flow injection manifold with a conventional flow cell

The influence of several chemical and physical parameters – total flow rates, flow ratios, sample injection volume, reaction coil lengths, configuration and reagent concentrations – were then tested in order to maximise sensitivity and sample throughput, reduce reagent consumption and waste formation and minimise silicate interference. In this optimisation studies, the iterative univariate method was applied. Initial experimental conditions were set to: 1:1 flow ratio between reagent and sample streams, total flow rate of 1.5 mL min^{-1}, 280 cm reaction coil, 570 μL injection volume and vanadomolybdate reagent composition consisting of 25 g L^{-1} $(NH_4)_6Mo_7O_{24}\cdot4H_2O$, 1.25 g L^{-1} NH_4VO_3 and 4 M HCl.

Maintaining the total flow rate at 1.5 mL min^{-1}, different flow ratios between reagent and sample streams were assayed: 1:1, 1:2, 1:3, 1:4. A 22-fold increase in sensitivity (from 0.0015 to 0.033 L g^{-1}) was noticed with the decrease in the reagent to sample proportion. To maintain this lower sample dispersion but assure adequate reagent concentration and also enhance reaction sensitivity, vanadomolybdate reagent concentration was doubled—50 g L^{-1} $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ and 2.5 g L^{-1} NH_4VO_3 in 4 M HCl. Sensitivity increased to 0.044 L g^{-1}. A 1:5 flow ratio was then tested. A slight increase in sensitivity occurred (0.046 L g^{-1}) but repeatability decreased. Therefore, a 1:4 ratio was adopted throughout the rest of the work.

Under these conditions, total flow rate was re-evaluated – from 1.5 to 4.5 mL min^{-1}, with 0.5 mL min^{-1} increments – in order to further increase the determination rate. No significant change in sensitivity was noticed. A flow rate of 4.0 mL min^{-1} was chosen, as a compromise between determination rate, saving of reagent and waste formation.

The effect of sample volume was studied within the range of 73–930 μL. As expected, up to 570 μL, sensitivity increased with increasing sample volume (from 0.0067 to 0.045 L g^{-1}). This volume was set for the subsequent studies.

The effect of the reaction coil length was tested with 50, 100, 170 and 280 cm reactors. Although higher sensitivity was achieved with the 100 cm reactor, the 170 cm was chosen for the following experiments since it presented better repeatability due to a more efficient sample and reagent mixture. Regarding reactor configuration (knotted *versus* coiled) no difference was detected. Therefore, the initial coiled design was used.

Optimisation experiments continued with the study of the influence of the composition of the vanadomolybdate reagent components. Ammonium molybdate concentrations of 20, 30, 40 and 50 g L^{-1} were tested. Sensitivity (0.041, 0.042 and 0.042 L g^{-1}) was approximately the same for the three highest concentrations. A concentration of 40 g L^{-1} was chosen to guarantee an excess of reagent for high phosphate concentrations.

Four different concentrations of ammonium vanadate (0.6, 1.2, 1.9 and 2.5 g L^{-1}) were tested. Maximum sensitivity was achieved

Table 1
The results obtained in the study of Si interference with a bench-top spectrophotometer

[P] (mg L^{-1})	[Si] (mg L^{-1})	[P]$_{apparent}$ (mg L^{-1})[a]	RD (%)
5.00	5.00	5.09 ± 0.06	+1.8
5.00	8.00	5.36 ± 0.02	+7.2
5.00	10.00	5.41 ± 0.03	+8.2

[a] The results expressed as a mean of 3 determinations ± standard deviation.

with the 1.9 g L^{-1} solution; this concentration was used throughout the experimental work. The decrease in sensitivity that was noticed with the 2.5 g L^{-1} solution, can be attributed to an increase of baseline absorbance.

The acid concentration was the last parameter to be studied. It is well known that the vanadomolybdate mixture is only soluble at high acid concentrations [16]. The acid concentration also affects the blank absorbance signal. In this study, hydrochloric acid concentrations were varied between 1 and 4 M. No significant decrease in sensitivity was noticed with increased acid concentrations. A 2 M acidity was preferred, as lower acid concentrations can lead to precipitation in the tubing, and because silicate interference decreases if acidity increases. The silicate interference during phosphate analysis occurs because silicate also reacts with the vanadomolybdate reagent to form a molybdosilicate heteropoly acid. However, the vanadomolybdophosphate and molybdosilicate heteropoly acids complexes have different rates of formation—silicate reacts more slowly than phosphate and this reaction is impaired when pH is decreased [2,16–18]. Several 5.0 mg L^{-1} phosphorus solutions containing different silica concentrations – 5, 8 and 10 mg L^{-1} – were prepared, and their analytical signals evaluated. As expected, silicate interference increases with its concentration. However, results displayed in Table 1 show that even for the highest silica concentration (i.e. lower phosphorus/silica ratio) relative deviations lower then 8% were obtained.

3.1.1. Analytical characteristics of the method

The linear calibration graph (over the range 0.5–10 mg P L^{-1}) corresponds to the following equation: absorbance = 4.61×10^{-2} ($\pm2.4 \times 10^{-3}$) [P (mg L^{-1})] − 1.10×10^{-3} ($\pm2.40 \times 10^{-3}$) with a correlation coefficient (R^2) of 0.9997 ($\pm4 \times 10^{-4}$). This equation represents an average of the values obtained for seven independent calibration curves and between parentheses are the respective standard deviations. The limit of detection (LOD) was calculated as $3s_d/S$, and the quantification limit (LOQ) as $10s_d/S$, where s_d is the standard deviation estimated from the regression line established through 5 standard solutions each injected in triplicate, and S is the slope of the calibration graph [19]. The values obtained were 50 μg L^{-1} for the LOD and 150 μg L^{-1} for LOQ. A determination rate of 60 samples per hour was achieved. Repeatability, assessed from five consecutive injections of three samples with different phosphorus concentration, presented relative standard deviation (R.S.D.) values lower than 1.87%.

The accuracy of the proposed procedure was tested through the analysis of the dissolved reactive phosphorus content of four samples (ground waters), by the developed and reference methodologies. As samples presented analyte concentrations below the quantification limit of the reference APHA method, they were spiked with different phosphorus concentrations, prior to analysis. Relative deviations lower than 15% ($n = 3$) were obtained in all cases.

The applicability of the developed system was then tested with a surface water certified reference material, SPS-SW2, having certified phosphorus level of 0.500 ± 0.003 mg L^{-1}. The result obtained by the FIA methodology, for five consecutive assays of this sample, was 0.47 ± 0.02 mg P L^{-1}.

Table 2
Features of the developed FIA system and of the reference procedure (APHA)

Parameter	APHA procedure	Flow method
Concentration range	1 to 18 mg L^{-1}	At least, up to 50 mg L^{-1}
Minimum detectable concentration	0.200 mg L^{-1}	0.050 mg L^{-1}
Assay time	At least 10 min	1 min
Reagents consumption per assay		
Ammonium molybdate	250 mg	32 mg
Ammonium vanadate	12.5 mg	1.5 mg
Hydrochloric acid concentrated	3.3 mL	2.1 µL
Estimated amount of waste produced per assay	50 mL	4 mL

Table 3
Apparent phosphorus concentration for standard phosphorus solutions containing silicate

[P] (mg L^{-1})	[Si] (mg L^{-1})	[P]$_{apparent}$ (mg L^{-1})[a]	RD (%)
0.50	5.00	1.10 ± 0	120
5.00	2.00	5.18 ± 0.03	3.4
5.00	5.00	5.43 ± 0.03	8.4
5.00	10.00	5.82 ± 0.02	16

[a] Apparent phosphate concentrations are the mean of three determinations ± standard deviation.

Taking into account the accuracy of results obtained and the figures of merit of the methodology reported it can be concluded that this system presents advantages over the reference procedure in terms of detection limit, dynamic working range, determination rate, reagent consumption and produced waste (Table 2). However, the detection limit of the method makes it still not adequate for the determination of phosphate in non-contaminated natural water samples.

Subsequently, the conventional detector system was replaced by a CCD array spectrophotometer to improve the analytical features and portability of the overall apparatus. Because sensitivity was slightly higher at 380 nm than at the recommended (>400 nm), this wavelength was used. A reference wavelength of 600 nm was used for background and sample matrix correction.

In these conditions, linear calibration curves were obtained over the range 0.5–10 mg P L^{-1}. The following equation represents an average of six independent analytical curves (and the values between parentheses are the respective standard deviations): absorbance = 8.31×10^{-2} ($\pm 2.9 \times 10^{-3}$) [P (mg L^{-1})] $- 1.63 \times 10^{-3}$ ($\pm 8.15 \times 10^{-3}$); $R^2 = 0.9999$ ($\pm 2 \times 10^{-4}$). With the increase in sensitivity, a concomitant a decrease in the limit of detection (30 µg L^{-1}) was obtained.

The repeatability of the method was assessed by the determination of the relative standard deviation (R.S.D.) values (expressed as percentage) of 10 consecutive injections of three samples with 0.57,

Table 4
Determination of dissolved reactive phosphorus (mg P L^{-1}) in ground waters by a FIA system with a CCD spectrometer connected to a conventional flow cell and by the reference method and corresponding relative deviations (RD)

Sample	FIA (mg P L^{-1})[a]	Reference method (mg P L^{-1})[b]	RD (%)
1	4.60 ± 0.02	4.19 ± 0.01	9.80
2	1.00 ± 0.05	0.99 ± 0.01	0.80
3	2.01 ± 0.05	2.04 ± 0.01	−1.75
4	3.78 ± 0.02	4.03 ± 0.02	−6.25
5	1.21 ± 0.03	1.05 ± 0.01	14.6
6	2.22 ± 0.02	2.09 ± 0.03	6.30
7	4.09 ± 0.06	4.08 ± 0.01	0.23

[a] The results are expressed as the mean of five determinations ± standard deviation.

[b] The results are expressed as the mean of three determinations ± standard deviation.

Table 5
Apparent (blank corrected) phosphorus concentration in phosphorus standards containing silicate

[P] (µg L^{-1})	[Si] (µg L^{-1})	[Tartaric acid] (mM)	[P]$_{apparent}$ (µg L^{-1})[a]
100	50	–	107 ± 3
100	100	–	110 ± 2
100	200	–	113 ± 2
100	50	2	102 ± 4
100	100	2	103 ± 3
100	200	2	105 ± 2

[a] The results are expressed as the mean of three determinations ± standard deviation.

1.9 and 3.5 mg P L^{-1}. Values of 3.80, 1.28 and 1.59%, respectively, were obtained.

Due to the change in the monitoring wavelength and increase of sensitivity, the interference of silicate was re-assessed (Table 3).

The results confirm that silicate interference is more significant when the shorter wavelength is used, being this difference greater for the solutions having lower phosphorus to silica ratio.

Four groundwater samples were analysed for dissolved reactive phosphorus content by the developed and reference methods. Again, samples presented analyte concentrations below the quantification limit of the reference method and therefore were spiked with different phosphorus concentrations. Paired results and relative deviations, are presented in Table 4.

With these results, the following linear correlation was obtained: $C_{FIA} = 0.998$ (± 0.163) $C_{REF} + 0.067$ (± 0.480) with a correlation coefficient of 0.9784. Confidence limits for the slope and intercept, at 95% significance level for 6 degrees of freedom (values shown above between parentheses after the respective values), point out that no evidence for systematic differences between procedures exists.

The surface water certified reference material, SPS-SW2, was also analysed. A concentration of 0.49 ± 0.05 mg P L^{-1} (five determinations) was found.

3.2. Flow injection systems with long pathlength absorbance spectrophotometry

Although the detection limit was 30 µg L^{-1}, the quantification limit (185 µg L^{-1}) was still too high. With the objective of decreas-

Table 6
Recovery tests for determination of phosphorus in surface and ground waters using the modified FIA-LWCC method

Sample type	Added concentration (µg L^{-1})	Found concentration (µg L^{-1})[a]	Recovery (%)
Ground	0	96.3 ± 1.8	–
	50	136 ± 2	78.7 ± 3.4
	100	186 ± 2	90.1 ± 2.2
	200	274 ± 2	88.7 ± 0.7
Surface	0	44.7 ± 1.7	–
	50	93.3 ± 0.7	97.2 ± 1.5
	100	124 ± 1	79.5 ± 1.1
	200	214 ± 2	84.7 ± 1.2
Surface	0	226 ± 3	–
	50	278 ± 2	104 ± 4
	100	326 ± 4	101 ± 4
	200	413 ± 3	93.5 ± 1.4
Surface	0	88.0 ± 2.1	–
	50	143 ± 1	110 ± 1
	100	182 ± 2	94.0 ± 1.7
	200	261 ± 4	86.5 ± 1.8

[a] The results are expressed as the mean of three determinations ± standard deviation.

Table 7
Comparison of some analytical characteristics of the developed flow systems for phosphate determination

Parameter	Conventional flow cell		LWCC-CCD detector	
	Conventional spectrophotometer	CCD detector	Without tartaric acid addition	With in-line tartaric acid addition
Monitoring wavelength (nm)	470	380	446	446
Linear calibration range	0.5–$10\,\mathrm{mg}\,\mathrm{P}\,\mathrm{L}^{-1}$	0.5–$10\,\mathrm{mg}\,\mathrm{P}\,\mathrm{L}^{-1}$.	20–$500\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$	20–$500\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$
Sensitivity ($\mathrm{L}\,\mathrm{mg}^{-1}$)	4.61×10^{-2} ($\pm 2.4 \times 10^{-3}$)	8.31×10^{-2} ($\pm 2.9 \times 10^{-3}$)	8.80×10^{-1} ($\pm 1.1 \times 10^{-3}$)	1.36 ($\pm 3.4 \times 10^{-2}$)
Detection limit ($\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$)	50	30	8	17
Quantification limit ($\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$)	150	185	28	56
Repeatability (R.S.D.)[a]	<1.9%	<3.8%	<4.0%	<2.2%

[a] Assessed from at least five consecutive injections.

ing the limit of determination and increasing the sensitivity of the method, the next approach was to replace the conventional flow-cell by an increased optical pathlength cell. A LWCC was incorporated and the sample volume was changed to 1500 μL. However, the increase of the pathlength led to a concomitant increase in the blank absorption signal. In practice, this resulted in the impossibility of using the same light source and this lower (380 nm) monitoring wavelength, due to the insufficient light intensity at the detector. Therefore, the data acquisition was carried out at a higher wavelength (446 nm).

Linear calibration curves were performed over the range 20–$500\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$. The following equation represents an average of three independent analytical curves and the values between parentheses are the respective standard deviations: absorbance $= 8.80 \times 10^{-1}$ ($\pm 1.1 \times 10^{-3}$) [P ($\mathrm{mg}\,\mathrm{L}^{-1}$)] $+ 2.10 \times 10^{-3}$ ($\pm 2.86 \times 10^{-3}$); $R^2 = 0.9998$ ($\pm 2 \times 10^{-4}$). If all analytical experimental conditions were maintained, an 100-fold increase in the slope of the calibration curve would be expected (due to change in the optical path from 1 to 100 cm). However, as mentioned earlier, the analytical wavelength had to be altered from 380 to 446 nm. In these conditions, a 10-fold increase in the method sensitivity and improvement in detection and quantification limits were noticed. Detection and quantification limits of 8 and $28\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$ were obtained making the determination of phosphorus in natural waters possible according to the limits imposed by European Directives.

Repeatability, assessed by 15 consecutive injections of a sample containing $100\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$, was 4.0%.

To ascertain method accuracy, a surface water certified reference material, QC RW1 ($0.0987 \pm 1.15\,\mathrm{mg}\,\mathrm{P}\,\mathrm{L}^{-1}$) was analysed. The mean phosphorus concentration for the 15 determinations performed was $0.0981 \pm 0.0043\,\mathrm{mg}\,\mathrm{P}\,\mathrm{L}^{-1}$.

3.2.1. Interference studies

A recent study [17] pointed out that silicate interference could be reduced using tartaric acid as masking agent, provided that it was added prior to molybdophosphate or molybdosilicate formation. Therefore, two sets of $100\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ phosphorus standard solutions containing either 50, 100 or $200\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ of silica (i.e. phosphorus and silica ratios between 2 and 0.5) were prepared. One of these groups also contained tartaric acid at a 2 mM concentration. The results obtained are given in Table 5.

These assays demonstrated that an increase in silicate concentration leads to an increased interference. However, within both sets of solutions, smaller differences were found for the solutions containing tartaric acid. In fact, the change between apparent phosphorus concentration for the $200\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ of silica solutions and the equivalent blank solutions was significantly reduced in the set of standards containing the masking agent (from 12.8 to 4.6%).

From the data in Table 6, it can be concluded that tartaric acid should be added in-line so that its concentration could be maintained at the same level for both standards and samples. Therefore,

the system was reconfigured to accommodate the in-line addition of tartaric acid stream (Fig. 1(b)).

Silicate interference was then assessed using a $100\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ of phosphorus solution with $2\,\mathrm{mg}\,\mathrm{L}^{-1}$ of silica. This silica level was chosen because, levels as high as $2\,\mathrm{mg}\,\mathrm{L}^{-1}$ of silicate can be found in water and because the improvement of the sensitivity of phosphate analysis made possible to quantify the silicate interference signals relative to the low phosphate concentrations typical of most surface water samples [17].

Using this new system configuration, experiments using vanadomolybdate hydrochloric acid concentrations of 2 or 3.25 M and a tartaric acid stream with concentrations between 7 and 20 mM (1.5–4.5 mM at the confluence point, respectively) were conducted. The best conditions for minimizing the interference of silicate corresponded to using a 10 mM tartaric acid solution (2 mM at the confluence point) and a 3.25 M hydrochloric acid concentration. The apparent phosphorus concentration in these experimental conditions was $108 \pm 4\,\mu\mathrm{g}\,\mathrm{L}^{-1}$. These concentrations were used throughout the work.

3.2.2. Analytical characteristics of the proposed method

Linear calibration curves over the range 20–$500\,\mu\mathrm{g}\,\mathrm{P}\,\mathrm{L}^{-1}$ were traced. The following regression equation represents an average of the values obtained for five independent calibration curves: absorbance $= 1.36$ ($\pm 3.4 \times 10^{-2}$) [P ($\mathrm{mg}\,\mathrm{L}^{-1}$)] $- 1.68 \times 10^{-2}$ ($\pm 4.99 \times 10^{-3}$); $R^2 = 0.9990$ ($\pm 5 \times 10^{-4}$), between parentheses are the respective standard deviations. Improved limits of detection and quantification of 17 and $56\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ were obtained, respectively. Repeatability, assessed through 10 consecutive injections of two water samples, was better then 2.2%.

To test the applicability of the developed system, recovery tests were made using several water samples (surface and ground waters). Caution was taken so that the added volumes of phosphorus standard solution did not affect the overall characteristics of the sample matrixes. The results obtained were in the 80–110% range and are shown in Table 6.

A surface water certified reference material, QC RW1 ($98.7 \pm 1.15\,\mu\mathrm{g}\,\mathrm{L}^{-1}$), was also analysed. Mean concentration phosphorus of $98.7 \pm 2.8\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ (corresponding to 11 consecutive injections) was found.

The results show that the developed method presents high sensitivity for the determination of phosphorus at low levels as demanded by European Union regulatory directives, and that silicate interference was quenched up to a range of $2\,\mathrm{mg}\,\mathrm{L}^{-1}$.

4. Conclusions

This paper describes a flow methodology based on the vanadomolybdate reaction applicable to the determination of trace levels of phosphate in natural waters (European Union regulatory directives set the limit of $100\,\mu\mathrm{g}\,\mathrm{L}^{-1}$ P-$\mathrm{PO}_4{}^{3-}$). This is a significant breakthrough as this colorimetric reagent is more stable than those

based on the alternative molybdenum blue detection chemistry. Additionally, problems associated with tube clogging and baseline instability are avoided. This was achieved by replacing a conventional flow cell by liquid waveguide capillary cell, increasing the sensitivity and setting the LOD to 17 μg L^{-1}. Some of the figures of merit of the flow systems studied are summarized in Table 7.

When compared to some previous works, using the same reaction [7,16,20], the proposed system presents some significantly better analytical features. The potential interference of silicate was successfully masked by the in-line addition of tartaric acid.

Acknowledgements

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