Analytical Letters

An Automated Multi-Pumping Pulsed Flow System with Spectrophotometric Detection for the Determination of Phosphate in Natural Waters

Marta F. T. Ribeiro [a b] , Cristina M. C. M. Couto [a b] , Pedro M. M. Conceição [b] & João L. M. Santos [a]

[a] REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy , University of Porto , Porto , Portugal

[b] CICS, Research Centre on Health Science, Superior Institute of Health Sciences-North (ISCS-N), CESPU , Gandra , Paredes , Portugal
Accepted author version posted online: 27 Jun 2012.Published online: 11 Jul 2013.

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Flow Injection Analysis

AN AUTOMATED MULTI-PUMPING PULSED FLOW SYSTEM WITH SPECTROPHOTOMETRIC DETECTION FOR THE DETERMINATION OF PHOSPHATE IN NATURAL WATERS

Marta F. T. Ribeiro,[1,2] Cristina M. C. M. Couto,[1,2] Pedro M. M. Conceição,[2] and João L. M. Santos[1]

[1]*REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal*
[2]*CICS, Research Centre on Health Science, Superior Institute of Health Sciences–North (ISCS-N), CESPU, Gandra, Paredes, Portugal*

In this work, an automated multipumping pulsed flow system was implemented for the determination of phosphate in natural waters. The developed flow approach was based on the spectrophotometric determination of phosphate by using the vanadomolybdate reaction. The exploitation of a very simple manifold configuration relying on the utilization of just two active components, in this case two solenoid actuated micropumps that were accountable for sample and reagent insertion and commutation, reaction zone formation, and solutions propelling, provided a great operational and optimization simplicity, low reagent consumption, and waste minimization.

Linear calibration plots for phosphate concentrations of up to 20 mg L^{-1} (R^2 = 0.999, n = 6) were obtained, with a detection limit of 0.2 mg L^{-1}. The sampling rate was about 60 samples per hour. The system was applied to the monitoring of phosphate in local streams at specific sampling stations.

INTRODUCTION

Nutrients—especially phosphorus—are key water quality parameters in estuaries. Depending on both their chemical form and species, phosphorus can have

significant direct or indirect impacts on plant growth, oxygen concentrations, water clarity, and sedimentation rates (Cunningham, Cunningham, and Saigo 2005). Phosphate, the form of phosphorous that is readily available for use by aquatic plants, is of particular interest in relation to rivers and lakes as it is typically the limiting nutrient in these freshwater ecosystems. Excessive loadings of phosphate by agriculture runoff (fertilizers and animal wastes), treated and untreated municipal wastewater discharges or other industrial sources often lead to eutrophication (Carpenter et al. 1998; Jarvie, Withers, and Neal 2002).

Due to the widely acknowledged need to protect water resources and to ensure compliance with increasingly stringent environmental legislation, there is a growing demand for improved monitoring of environmental water quality (Coquery et al. 2005). In view of the growing demand for such analysis, continuous flow techniques have advantageous features as they enable the development of simple, compact, versatile, fast, and low-cost analytical methods. In this sense, several methods based on Flow Injection Analysis (FIA) (Estela and Cerdà 2005; Motomizu and Li 2005), Sequential Injection Analysis (SIA) (Mesquita et al. 2011), and also Multisyringe (Mirabó, Forteza, and Cerdà 2009) have been proposed for the determination of phosphate. These typically involve the reaction between phosphate and molybdate in acidic conditions in order to form a heteropolyacid. Detection is generally undertaken either on molybdophosphate reduction product (molybdenum blue method) (Ruzicka and Hansen 1975; Mirabó et al. 2009; Gentle et al. 2010; Mesquita et al. 2011) or on the yellow vanadomolybdate complex (vanadomolybdate method) (Munoz et al. 1997; Silva et al. 1998; Bowden et al. 2002; Neves et al. 2008). Other alternatives employing mostly SIA and based on the formation of ionic pairs of either molybdophosphate or vanadomolybdophosphate with basic dye compounds such as Rhodamine 6G (Frank et al. 2006a, 2006b), Malachite Green (Munoz et al. 1997), Cristal Violet (Burns, Chimpalee, and Ittipornkul 1991) or Methylene Blue (Matsuo, Shida, and Kurihara 1977) have also been proposed.

Although less sensitive than the molybdenum blue method, the vanadomolybdate method presents several advantages. This method is quite tolerant to interfering ions, and no precipitates are formed during the reaction or in storage. In addition, the long reagent lifetime makes it more suitable for on line monitoring purposes (Bowden et al. 2002; Motomizu and Li 2005). On the other hand, the reactional scheme is simpler, facilitating implementation and being more cost-effective, and less prone to errors. In situations involving the analysis of a large amount of samples and when the control of phosphate levels is essentially to guarantee that they remain below the maximum allowable values to prevent the occurrence of environmentally harmful effects, the vanadomolybdate method, for its stability, simplicity, and ruggedness presents noteworthy advantages.

Multipumping flow systems (MPFS) represent one of the most recently approaches to flow techniques (Lapa et al. 2002). MPFS presents several advantages compared to the previous flow systems, such as excellent cost-effectiveness, precision, accuracy, greatly reduced reagent consumption or waste, and robustness and miniaturization features that imply that MPFS could be advantageously used for developing of portable fieldwork instruments for in situ environmental analysis. MPFS make use of multiple solenoid actuated micropumps as the only active components that act simultaneously as sample-insertion, reagent-introduction,

commuting, and solution-propelling units. This way, and in addition to the high simplicity of flow manifold configuration, operation and optimization, it is possible to attain a noteworthy reagents saving and liquid waste minimization as the reaction zone is clearly and promptly established by proper timing and synchronization of sample and reagents mixing. Moreover, the pulsed flow inherent to the micropumps actuation ensures good mixing conditions, thus improving the reaction zone homogenization, even under limiting dispersion conditions that occurred when using reagent solutions with considerable viscosity (Lima et al. 2004).

The objective of this work was to develop an automated multipumping pulsed flow system for the determination of phosphate in natural waters. Further on, to test the applicability of the developed flow system and offer new chemical monitoring data, surface water samples taken from the main streams of the Porto city in Portugal were analyzed. The study was carried out during April to October 2011, and involved six fixed stations where phosphate input to Douro estuary was predicted (Vieira 2009). Two of these stations (Abade and Gramido streams) crossed rural areas, while the remaining four (Tinto river 1 and 2, and Granja stream 1 and 2), involved mainly industrial and urban areas.

EXPERIMENTAL

Reagents and Solutions

The solutions used in this work were prepared with deionized water (specific conductivity $< 0.1\,\mu S\,cm^{-1}$) and reagents of analytical grade quality.

Working standard solutions of phosphate were prepared by dilution of a $100.0\,mg\,L^{-1}$ phosphate stock solution prepared from potassium dihydrogen phosphate (KH_2PO_4, Sigma), in water.

The vanadomolybdate reagent consisted of a $0.010\,mol\,L^{-1}$ ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, Sigma-Aldrich) and $0.015\,mol\,L^{-1}$ ammonium vanadate (NH_4VO_3, Sigma-Aldrich) in $0.5\,mol\,L^{-1}$ hydrochloric acid (Sigma-Aldrich).

Sampling and Storage

Water samples were collected from the main streams of Porto city, from late April to October 2011, at different distances from river Douro mouth: stations 1 to 4 were located upstream, while stations 5 and 6 were close to the ocean. For this reason, samples analyzed exhibited assorted salinity. Water samples were collected into 1 L amber glass bottles, which were previously rinsed with ultrapure water and rinsed on site, with the water sample. The water samples were collected from 30-cm below the water surface. After sampling, all water was filtered through a 0.45-μm membrane filter (GF/C Whatman Germany) prior to analysis.

Equipment

The MPFS flow set-up consisted of two solenoid micropumps (120SP1210, Bio-Chem Valve Inc., 10 μL per stroke) (Lima et al. 2004), coupled by a homemade confluence point to a Jenway 6300 spectrophotometer equipped with a flow cell with

18 µL internal volume and 10 mm optical path. Measurements were performed at 420 nm. Reaction coils and transmission lines were made of polytetrafluoroethylene (PTFE) tubing (0.8 mm internal diameter).

The automated MPFS was controlled by computer with specifically developed software. This was equipped with a PC-LAB card model PCL-711 B (Advantech) interface card and was used both for system control and for data acquisition and processing. A homemade power drive based on the ULN2003 chip or a Cool Drive (NResearch Inc.) was used to operate the solenoid micropumps (Lima et al. 2004). The reference procedure (Greenberg, Clesceri, and Eaton 1998) was carried out by using a Unicam UV/Vis spectrometer.

Flow Manifold

In the developed MPFS (Figure 1), the micropumps P_1 and P_2, were used for sample and vanadomolybdate insertion, respectively, as well as for propelling the reaction zone toward detection. As micropump P_1 allowed simultaneous propelling and insertion of sample there was no need for a specific sample insertion valve. Thus, the MPFS comprised a reduced number of active components, therefore contributing to minimizing errors and ease of system operation. The sample solution was used as carrier solution not only because it provided enhanced analytical simplicity, arising from the suppression of an extra inert carrier propelling/inserting micropump, but also because there was no evident shortage of sample, due to the unlimited available amount, its utilization as carrier reduced the dispersion of the reaction zone improving sensitivity and facilitated sample replacement. On the other, this manifold configuration reduced reagent consumption and improved reproducibility.

The analytical cycle started by inserting the sample solution into the MPFS, by actuating P_1, at a fixed pulse frequency, in order to establish the baseline.

Then, the vanadomolybdate reagent was inserted through the confluence point X, by actuating P_2. The reagent volume was defined by the number of pulses of actuation of P_2 and was always a multiple of 10 µL, the pulse volume. Micropump P_1 was also active during the insertion of the reagent, contributing to the establishment of the reaction zone due to the merging (at confluence point X) of reagent and sample. Subsequently, by operating P_1 and deactivating P_2, the sample solution was introduced in the analytical system and the sample zone was carried out toward detection where it produced an analytical signal whose magnitude was proportional to the phosphate concentration.
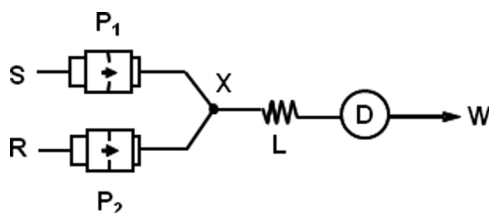

Figure 1. Flow manifold of the proposed MPFS system: S, sample; R, vanadomolybdate reagent; P1 and P2: solenoid micro-pumps; X, confluence point; L, reaction coil (50-cm); D, detector (420 nm); W, waste.

RESULTS AND DISCUSSION

Optimization of Experimental Parameters

Different factors affecting the performance of the MPFS system were optimized, such as reagent volume, sampling strategy, coil length, flow rate, and reagents concentration. Optimized conditions enabled a compromise between sensitivity, sampling rate, and low reagent consumption.

Reagent volume was optimized by inserting in the analytical path an increasing number of pulses (5 to 30 pulses) of the vanadomolybdate reagent. For this purpose phosphate standard solutions within the range of 5.0 to 20.0 mg L^{-1} were tested as well as different sampling strategies in the flow manifold. The three strategies tested involved i) either the insertion of the reagent as a unique volume, creating only two reaction interfaces; ii) the intercalation of multiple reagent and sample aliquots, creating multiple reaction interfaces (binary sampling); or iii) the simultaneous insertion of reagent and sample aliquots (merging zones). The individual and independent control of the solenoid micro-pumps, allowing different activation combinations without physical reconfiguration of the flow path, made these different strategies possible and feasible.

The results obtained for the three strategies evaluated were similar, although binary sampling and merging zones assured improved reproducibility due to the intrinsic improved mixing conditions (Dias et al. 2003). It was also observed that the sensitivity increased up to a volume of 25 pulses (250 μL, Figure 2) remaining almost constant above this value. This reagent volume was then selected for further experiments. Reagent was also inserted via the merging zones strategy as it allowed a higher flow rate and consequently a higher sampling rate.

Different reactor lengths (25–150 cm) were also tested in order to evaluate the influence of the residence time on the development of the reaction. The results showed that the sensitivity increased up to a 50-cm reactor length and then approached
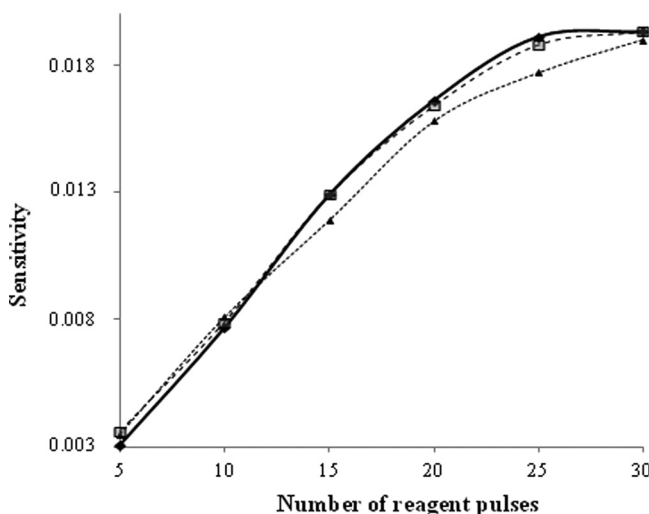

Figure 2. Influence of the number of reagent pulses in the analytical signal: (◇) unique volume; (△) binary sampling; and (□) merging sampling strategy.

stabilization. A 50-cm reactor was then chosen for subsequent experiments, as a compromise between analytical sensitivity and sampling rate.

Flow rate was also evaluated since it affects residence time as well as reaction development. In a MPFS, the flow rate is defined by both the pulse volume and the pulse frequency of the respective micropump. Pulse frequency is defined in terms of pulse time, corresponding to the time interval between two consecutive pulses. Pulse times within the range of 0.3 and 1.0 s (corresponding to pulse frequencies of about 200 to 60 pulses min^{-1}) were evaluated for the insertion the sample solution, used as carrier stream. The sensitivity increased up to a pulse time of 0.6 s, probably as a consequence of the increase in the residence time affecting the available time for reaction development and then approached stabilization. A pulse time of 0.6 s, corresponding to a flow rate of 1.0 mL min^{-1}, was then selected for subsequent experiments as a result of the higher sensitivity.

The combined reagent concentration consisting of ammonium molybdate and ammonium vanadate was optimized in the range of 0.01 to 0.04 mol L^{-1} and 2.5×10^{-4} to 0.025 mol L^{-1}, respectively. The obtained results showed that sensitivity increased up to a concentration of 0.015 mol L^{-1} and 0.01 mol L^{-1} for ammonium molybdate and ammonium vanadate, respectively, approaching stabilization above these values. In view of the higher sensitivity, those concentrations were then selected for the following experiments.

Another chemical parameter affecting analytical signal magnitude was the hydrochloric acid concentration used in the preparation of the combined reagent. Considering that the ammonium molybdate is slightly soluble in lower concentrations of hydrochloric acid and that the silicate interference in the reaction development is also minimized with the acidity increase (Mas-Torres et al. 1997) HCl concentration was evaluated between 0.5 and 4.0 mol L^{-1}. It was observed that the sensitivity markedly decreased as the hydrochloric acid concentration increased (Figure 3). Taking into account these results, the silicate interference was further evaluated by using a 0.5 mol L^{-1} hydrochloric acid concentration in the combined reagent and by testing
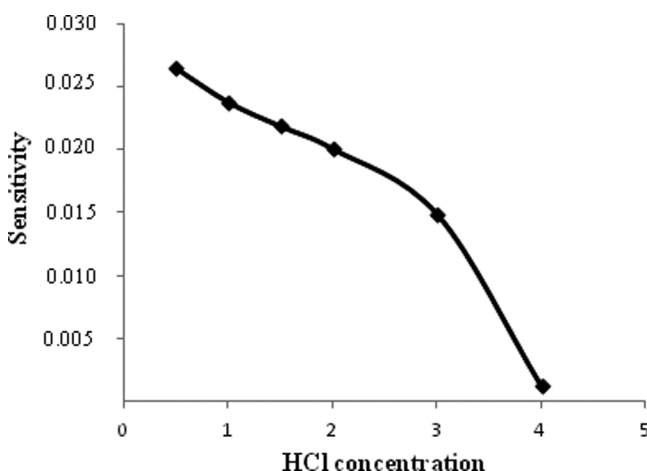

Figure 3. Influence of the hydrochloric acid concentration (mol L^{-1}) in the analytical signal.

standard solutions of phosphate with a concentration of $5\,mg\,L^{-1}$ added with different amounts of silicate in the range of 5.0 to $10.0\,mg\,L^{-1}$. The added silicate concentrations were selected taking into account the phosphate/silicate ratios described for natural waters. It was observed that for the different solutions evaluated (different phosphate/silicate ratios) the relative deviations were always lower than 10%, and therefore a $0.5\,mol\,L^{-1}$ hydrochloric acid concentration was chosen for subsequent experiments as a result of its higher sensitivity together with the minimal possibility of reagent precipitation and no silicate interference.

The effect of some potential inorganic interfering species, such as chloride, nitrate, sulfate, carbonate, sodium, and potassium was also investigated. The presence of these ions at concentrations as high as $500\,mg\,L^{-1}$ had no significant effect on the phosphate determination.

Figures of Merit

The linear calibration graph was described by the equation $A = 0.023\ (\pm0.001)$ $[P\ (mg\,L^{-1})] + 0.001\ (\pm0.004)$, where A is the absorbance and [P] the phosphate concentration in $mg\,L^{-1}$ ($r = 0.999$, $n = 6$), being linear up to $20\,mg\,L^{-1}$.

The detection limit calculated by using the regression equation (with $YB = a$ and $SB = Sy/x$) (Miller and Miller 2000) was $0.2\,mg\,L^{-1}$ and the value of the relative standard deviation was 0.5% for a $15\,mg\,L^{-1}$ phosphate standard solution ($n = 10$). The sampling rate was about 60 samples h^{-1}.

Confirmation of accuracy was performed by running some samples previously analyzed by the reference procedure (Greenberg et al. 1998). There were no significant differences between the proposed and the reference procedure, with relative deviations always lower than 7.7% (Table 1). No significant statistical differences were also confirmed by a paired t-student test, for a 95% confidence level (t calculated $= 0.622$; t tabulated $= 2.57$).

Application of the Present Method to Field Samples

To evaluate the applicability of the validated method, water field samples were collected from the main streams of Porto city (S1 to S6), from late April to October 2011, covering three annual seasons.

Table 1. Comparative results. Phosphate concentrations ($mg\,L^{-1}$) as determined by the developed flow system and the APHA reference procedure

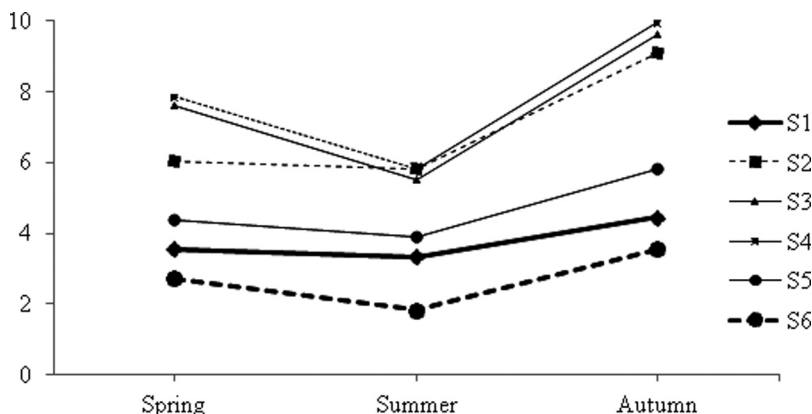| Site	Proposed method	Reference method	Relative error (%)
S1	3.50 ± 0.21	3.42 ± 0.02	2.34
S2	6.03 ± 0.01	6.39 ± 0.02	-5.63
S3	7.59 ± 0.36	7.58 ± 0.15	0.13
S4	7.70 ± 0.43	7.58 ± 0.14	1.58
S5	4.38 ± 0.11	4.27 ± 0.20	2.58
S6	2.51 ± 0.15	2.33 ± 0.05	7.72

Figure 4. Spatial distribution of phosphate concentration ($mg\,L^{-1}$) in stations S1 to S6 during April to October 2011.

The sampling stations were chosen according to three main criteria: i) accessibility to streams (some of other existent streams are almost completely underground); ii) representative coverage of all extension of Douro estuary, using geographical considerations and taking into account the activities developed along the stream (predominantly rural activities for Abade and Gramido streams (S1 and S2), and industrial and urban discharges for Tinto and Granja streams (S3 and S5); and iii) evaluation of the "auto-depuration" ability of the Douro estuary, by selection of collecting points at the confluence of the streams with Douro river (S4 and S6).

The phosphate content ranged from a minimum value of $1.8\,mg\,L^{-1}$ at S6 in summer and a maximum of $9.9\,mg\,L^{-1}$ at S4 in autumn (Figure 4). Similar results were recorded by other research during January and July 2007 (Vieira 2009). The high values of phosphate found at stations S3 and S4 may be attributed to agriculture runoff and sewage wastes disposal (Vieira 2009). Statistically significant differences in the phosphate content were also observed during the sampling period. Phosphate content decreased during the summer season and then increased. This may be due to the much higher rainfall during autumn and the winter period.

With respect to the "auto-depuration" ability of the Douro estuary, the differences obtained between S3 and S4 stations were negligible. This may be explained by the fact that in S4 the water was collected too close to the mouth of the stream (due to geographical limitations) and therefore no dilution effect from estuarine water was observed. On the other hand, significantly differences between S5 and S6 stations were observed and, therefore, estuarine water was clearly diluting the stream water. In this station an auto-depuration effect was clearly observed.

CONCLUSIONS

The developed multipumping pulsed flow system could be a valuable strategy for the determination of phosphate in superficial and wastewater samples. It can be an advantageous alternative to other available procedures, because it exhibits a

high degree of automation, it is simple, fast, precise, accurate, requires low reagent consumption, and minor operator intervention.

The developed multipumping pulsed flow system has also made possible the recognition of a seasonal variation in the phosphate content in the main stream waters of the Porto city. The majority of the streams were heavily contaminated, probably by agriculture activities, sewage discharges, or industrial effluents. In order to fully assess the pollution in these streams and to get further information, further monitoring studies are in progress.

REFERENCES

Bowden, M., M. Sequiera, J. P. Krog, P. Gravesen, and D. Diamond. 2002. Analysis of river water samples utilizing a prototype industrial sensing system for phosphorus based on micro-system technology. *J. Environ Monit.* 4: 767–771.

Burns, D. T., D. Chimpalee, and S. Ittipornkul. 1991. Flow-injection spectrophotometric determination of phosphate using Crystal Violet. *Anal. Chim. Acta* 254: 197–200.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8: 559–568.

Coquery, M., A. Morin, A. Bécue, and B. Lepot. 2005. Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality. *Tr. Anal. Chem.* 24: 117–127.

Cunningham, W. P., M. A. Cunningham, and B. W. Saigo. 2005. *Environmental Science: A Global Concern*. New York, NY: McGraw-Hill Science/Engineering/Math.

Dias, A. C. B., J. L. M. Santos, L. F. C. Lima, and E. A. G. Zagatto. 2003. Multi-pumping flow system for spectrophotometric determination of bromhexine. *Anal. Chim. Acta* 499: 107–113.

Estela, J. M., and V. Cerdà. 2005. Flow analysis techniques for phosphorus: An overview. *Talanta* 66: 307–331.

Frank, C., F. Schroeder, R. Ebinghaus, and W. Ruck. 2006a. A fast sequential injection analysis system for the simultaneous determination of ammonia and phosphate. *Microchim. Acta* 154: 31–38.

Frank, C., F. Schroeder, R. Ebinghaus, and W. Ruck. 2006b. Using sequential injection analysis for fast determination of phosphate in coastal waters. *Talanta* 70: 513–517.

Gentle, B. S., P. S. Ellis, P. A. Faber, M. R. Grace, and I. D. Mckelvie. 2010. A compact portable flow analysis system for the rapid determination of total phosphorus in estuarine and marine waters. *Anal. Chim. Acta* 674: 117–122.

Greenberg, A. E., L. S. Clesceri, and A. D. Eaton. 1998. *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association (APHA), American Water Works Association, and Water Environment Federation.

Jarvie, H. P., P. J. A. Withers, and C. Neal. 2002. Review of robust measurement of phosphorus in river water: sampling, storage, fractionation and sensitivity. *Hydrol. Earth Syst. Sci.* 6: 113–132.

Lapa, R. A. S., J. L. F. C. Lima, B. F. Reis, J. L. M. Santos, and E. A. G. Zagatto. 2002. Multi-pumping in flow analysis: concepts, instrumentation, potentialities. *Anal. Chim. Acta* 466: 125–132.

Lima, J. L. F. C., J. L. M. Santos, A. D. B. Dias, M. F. T. Ribeiro, and E. A. G. Zagatto. 2004. Multi-pumping flow systems: An automation tool. *Talanta* 64: 1091–1098.

Mas-Torres, F., A. Munoz, J. M. Estela, and V. Cerdà. 1997. Simultaneous determination of phosphate and silicate in waste water by sequential injection analysis. *Analyst* 122: 1033–1038.

Matsuo, T., J. Shida, and W. Kurihara. 1977. Extraction—spectrophotometric determination of phosphate as the methylene blue. *Anal. Chim. Acta* 91: 386–387.

Mesquita, R. B. R., M. T. S. O. B. Ferreira, I. V. Tóth, A. A. Bordalo, I. D. Mckelvie, and A. O. S. S. Rangel. 2011. Development of a flow method for the determination of phosphate in estuarine and freshwaters – Comparison of flow cells in spectrophotometric sequential injection analysis. *Anal. Chim. Acta.* 701: 15–22.

Miller, J. N., and J. C. Miller. 2000. *Statistics and chemometrics for analytical chemistry*. England: Pearson Education.

Mirabó, F. M. B., R. Forteza, and V. Cerdà. 2009. A multisyringe sequential injection method for monitoring water in the energy cogeneration system of a municipal waste incinerator. *Talanta* 79: 1011–1020.

Motomizu, S., and Z. Li. 2005. Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques. *Talanta* 66: 332–340.

Munoz, A., F. Mas-Torres, J. M. Estela, and V. Cerda. 1997. Evaluation of spectrophotometric methods for determination of orthophosphates by sequential injection analysis. *Anal. Chim. Acta* 350: 21–29.

Neves, M. S. A. C., M. R. S. Souto, I. V. Tóth, S. M. A. Victal, M. C. Drumond, and A. O. S. S. Rangel. 2008. 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. *Talanta* 77: 527–532.

Ruzicka, J., and E. H. Hansen. 1975. Flow injection analyses: Part I. A new concept of fast continuous flow analysis. *Anal. Chim. Acta* 78: 145–157.

Silva, F. V., A. R. A. Nogueira, G. B. Souza, and E. A. G. Zagatto. 1998. A polyvalent flow injection system for multielemental spectrophotometric analysis of plant materials. *Anal. Chim. Acta* 370: 39–46.

Vieira, N. M. S. M. 2009. Chemical and Bacteriological Quality of Water of the streams of Porto. Dissertation submitted to the University of Aveiro for obtaining a Master Degree in Geochemistry. Department of Geosciences: 1–134.