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Simultaneous In-line Concentration for Spectrophotometric Determination of Cations and Anions

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Um sistema de análise em fluxo é proposto para a concentração simultânea de cátions e ânions. Um injetor-comutador com barra deslizante foi empregado para inserir colunas contendo resinas de troca catiônica e aniônica no fluxo de amostra para a retenção serial dos analitos. Na posição alternativa do injetor, soluções distintas fluíram através das colunas para a eluição em paralelo das espécies em percursos analíticos distintos. Válvulas solenóides de três vias permitiram a introdução intermitente dos reagentes nas zonas de amostra. Os sinais foram medidos com dois fotômetros construídos empregando LEDs como fontes de radiação e fotodiodos como detectores. Simultaneamente à pré-concentração, as zonas de amostra podem ser retidas em reatores helicoidais visando aumentar o tempo de residência e a taxa de conversão do analito. A potencialidade analítica foi demonstrada para a concentração em linha de amônio e fosfato seguida por detecção espectrofotométrica. Para um tempo de pré-concentração de 90 s, a freqüência de amostragem foi estimada em 40 determinações por hora, a qual é três vezes superior à obtida sem efetuar as tarefas simultaneamente. Fatores de enriquecimento de 8,0 e 18 foram estimados para fosfato e amônio, respectivamente, resultando em limites de detecção de 1 μg L^{-1} PO$_4^{3-}$ e 1 μg L^{-1} NH$_4^+$ (nível de confiança de 99,7%). O consumo de reagentes foi inferior a 2 mg por determinação. Resultados para amostras água foram concordantes com os obtidos pelos procedimentos de referência da APHA a nível de confiança de 95%.

A flow system is proposed for simultaneous in-line concentration of cations and anions. A sliding-bar commutator was employed to insert an anion and a cation exchange column into a flowing sample stream for serial retention of the analytes. In the injector alternative position, different solutions flowed through the columns for parallel elution of the species in different analytical paths. Three-way solenoid valves allowed the intermittent reagent introduction in the sample zones. Signals were measured by employing two flow-through LED-based detectors. The simultaneous retention of the sample zones in coiled reactors can be also performed to increase the residence time and the analyte conversion rate. The analytical potentiality was demonstrated by the in-line concentration of ammonium and phosphate followed by spectrophotometric detection. For a 90 s loading time, the sampling rate was estimated as 40 determinations per hour, which is three-fold higher than the obtained without performing the tasks simultaneously. Enrichment factors of 8.0 and 18 were estimated for phosphate and ammonium, respectively, yielding detection limits of 1 μg L^{-1} PO$_4^{3-}$ and 1 μg L^{-1} NH$_4^+$ (99.7% confidence level). The reagent consumption was lower than 2 mg per determination. Results for freshwater samples agreed with the obtained by reference APHA procedures at the 95% confidence level.

Keywords: flow-injection spectrophotometry, multicommutation, preconcentration, phosphate, ammonium

Introduction

Several alternatives are available to increase sensitivity in flow-based systems, such as in-line concentration by

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solid-liquid[1] or liquid-liquid[2] extraction, which have been successfully implemented by exploiting several manifold configurations and chemical strategies.[1-3] Moreover, there are a number of strategies to enhance reactions development with good precision and without increasing sample dispersion significantly.[4] By combining these strategies, improved detection limits can be attained with low cost instrumentation. However, the sampling rate is often hindered because these tasks are usually time-

consuming. An additional disadvantage is the waste of reagents when chemical reactions are involved.

The previously mentioned drawbacks can be circumvented by performing simultaneously the tasks involved in procedures for in-line concentration and increase of residence time. This can be suitably implemented by exploiting the multicommutation approach, which comprises the use of discrete commutators to setup the flow manifold.[5] As the commuting devices can be independently computer-controlled, the different steps involved in sample processing can also be independently implemented, aiming to perform the tasks simultaneously.[5] This approach was formerly exploited in a procedure for simultaneous trapping of multiple sample zones for increasing the residence time without hindering the sampling rate in the spectrophotometric determination of boron in plant digests.[6] A four-way solenoid valve directed the sample zones to three similar reaction coils allowing a mean residence time of 200 s with a sampling rate of 65 determinations per hour.

The simultaneous implementation of the steps involved in sample processing was also exploited in a flow system devoted to the inorganic speciation of nitrogen in natural waters.[7] After reagent addition, the sample zone for ammonium determination was trapped into a coiled reactor in order to enhance the reaction development. Simultaneously the sample zone was processed for determining nitrate and nitrite. In this way, 60 determinations per hour were performed with an 120 s residence time for ammonium, long enough for achieving a 95% conversion rate. The multi-task ability of multicommuted flow systems was also exploited when an improved procedure was proposed for the sequential determination of anions in waters.[8] In-line concentration into an anion exchange-resin was implemented simultaneously with the sample processing in order to further detect the analytes in lower concentration. A similar strategy had been previously exploited for the sequential determination of metallic ions and anions in waters by, Inducted Couple Plasma-Mass Spectroscopy, ICP-MS (Inductively Coupled Plasma Mass Spectrometry).[9] The sample flowed through an anion exchange resin before the direct measurement of Al, Cr, Mn, Ni, Cu, Zn, Cd, Ba and Pb and the retained anions were after eluted for sulfate and chloride determination.

The multicommutation approach was also exploited for improving the sampling rate in procedures involving in-line concentration.[10] A flow manifold constructed with solenoid valves was proposed for determination of cadmium, nickel and lead by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). In-line concentration and elution were simultaneously carried out by using three ion-exchange columns managed by a four-

way solenoid valve, allowing to achieve a sampling rate of 90 determinations per hour for a 120 s loading time.

In this work, it is proposed a flow system for simultaneous implementation of the tasks involved in procedures for increasing sensitivity, such as simultaneous in-line concentration of different species and retention of the sample zones in order to increase the residence time. The analytical features were demonstrated by the simultaneous in-line concentration of ammonium and phosphate from natural freshwater samples, followed by their determination with flow-through, LED (Light Emitting Diodes), LED-based photometers.

Experimental

Apparatus

The flow system was constructed with computer-controlled sliding-bar commutator and three-way solenoid valves (NResearch, 161T031), 0.7 mm i.d. polyethylene tubes and Perspex joint points. The resin columns were lab-made by using three Perspex blocs attached by screws. The central bloc was drilled to form two hollow cylinders (3 mm diameter, 15 mm long) in which the ion-exchange resins were deposited. An Ismatec IPC-8 peristaltic pump equipped with Tygon tubes was used for fluid propulsion. A 486 DX microcomputer equipped with an electronic interface (Advantech, PCL-711S) was employed to control the commuting devices and to perform data acquisition and processing. The control software was written in Microsoft Quick Basic 4.5.

Two flow-through detectors were built up by associating red LEDs (maximum emission at 660 nm) and silicon photodiodes (RS 308-067). Two "Z"-shaped glass flow cells (20-mm optical path) were inserted in Perspex pieces, into which the LEDs and the photodetectors were fixed and aligned. The electronic circuit of the photometer was similar to one previously employed.[7] The outputs of the detectors were connected to operational amplifiers (LM741) for offset adjustment. The output signals of the amplifiers were measured with the PCL-711S interface card.

Reagents and solutions

All solutions were prepared with deionized water further purified by a Mili-Q system and analytical-grade chemicals (except the hypochlorite solution, prepared from a commercial bleach solution containing about 2.5 % m/m active chlorine). Stock 1.00 g L^{-1} ammonium (5.54×10^{-2} mol L^{-1}) and phosphate (1.05×10^{-2} mol L^{-1}) solutions were prepared from $(NH_4)_2SO_4$ and KH_2PO_4, which were

dried at 110 ºC for two hours before weighting. Reference solutions containing 25.0-300 μg L^{-1} of both analytes were prepared by dilutions of the stocks.

Reagents for ammonium determination were 0.050 mol L^{-1} sodium salicylate, 0.025 mol L^{-1} sodium nitroprusside plus 0.060 mol L^{-1} Na$_2$EDTA (R$_1$). R$_2$ reagent was a solution containing 0.25 % (m/v) sodium hypochlorite. Solutions 4.0 mmol L^{-1} ammonium molybdate plus 1.0 mol L^{-1} HNO$_3$ (R$_3$) and 4.4 mmol L^{-1} SnCl$_2$ plus 0.30 mol L^{-1} HCl (R$_4$) were employed for phosphate determination. Solutions 0.50 mol L^{-1} NaOH and 0.50 mol L^{-1} HNO$_3$ were employed as eluent for ammonium and phosphate, respectively.

Ion-exchange resins (Bio-Rad, 100-200 mesh) AG 1-X8 (chloride form) and AG 50W-X8 (H$^+$ form) were employed for phosphate and ammonium in-line concentration, respectively. Before measurements, the eluent solutions flowed through the columns for 5 min in order to replace the counterions by nitrate and sodium.

Flow diagram and procedure

The flow manifold in the sampling position is showed in Figure 1. For triplicate measurements involving simultaneous in-line concentration of ammonium and phosphate, system is operated according to the valves switching course in Table 1, which allows also implementing the trapping of the sample zone for ammonium determination. In the first step, sample or reference solutions (S) flow at 3.0 mL min^{-1} through the columns C$_1$ and C$_2$ placed in series. After the loading time (*ca.* 90 s), the central bar of the computer-controlled commutator (I) is slid, the columns are placed in parallel and the eluent solutions flow through them. Simultaneously, the reagents are added to the sample zones in independent flow channels, by switching the solenoid valves V$_1$-V$_2$ and V$_3$-V$_4$ (step 2). After,

the sample zone for ammonium determination is trapped into the reaction coil B$_2$ by switching the valve V$_5$ on (step 3) and the sample zone for phosphate is directed towards the flow-through detector D$_2$ while the sliding-bar injector is moved back to its loading position (step 4). In this way, trapping of the sample zone, signal measurement for phosphate determination and sample concentration are simultaneously performed. After the trapping period (*ca.* 45 s) the retained sample zone is directed to the flow-through detector D$_1$ for ammonium determination (step 5). The time spent for signal measurement allows attaining the pre-established sample loading time and afterwards another measurement cycle starts.

Natural water samples were collected near Piracicaba in polyethylene bottles and analyzed in the same day by the proposed procedure and by employing batch procedures recommended by the American Public Association (APHA).[11] Before analysis, samples were filtered through 45-mm cellulose acetate membranes.
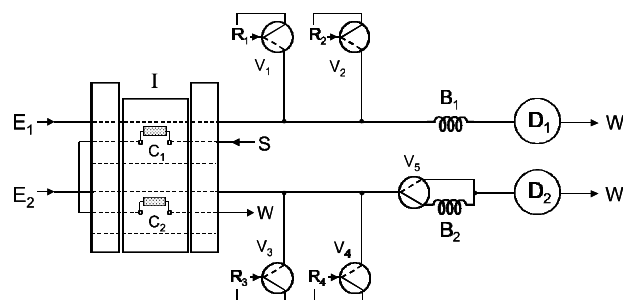

Figure 1. Flow diagram of the system for simultaneous in-line concentration: I – lab-made computer controlled sliding-bar commutator; V$_i$ – three-way solenoid valves; S – sample or reference solution (3.0 mL min^{-1}); R$_i$ – chromogenic reagents (1.0 mL min^{-1}); C$_1$ – cation exchange column (AG 50W-X8); C$_2$ – anion exchange column (AG 1-X8); E$_1$ – 0.50 mol L^{-1} NaOH (3.0 mL min^{-1}); E$_2$ – 0.50 mol L^{-1} HNO$_3$ (3.0 mL min^{-1}); B$_1$, B$_2$ – reaction coils (100 cm, 500 μL); D$_1$, D$_2$ – flow-through LED-based photometric detectors; W – waste vessels.

Table 1. Valves switching course for simultaneous in-line concentration of ammonium and phosphate (triplicate measurements). Numbers 1 and 0 indicate that the valves on Figure 1 are switched on or off respectively. L and E indicate sliding-bar commutator at loading and elution positions, respectively

Step	I	V$_1$	V$_2$	V$_3$	V$_4$	V$_5$	t(s)	Description
1	L	0	0	0	0	0	90	Sample loading
2	E	1	1	1	1	0	10	Elution / Reagent addition
3	E	0	0	0	0	1	0.1	Trapping of the sample zone (ammonium)
4	L	0	0	0	0	1	45	Sample loading / phosphate measurement
5	L	0	0	0	0	0	45	Sample loading / ammonium measurement
6	E	1	1	1	1	0	10	Elution / Reagent addition
7	E	0	0	0	0	1	0.1	Trapping of the sample zone (ammonium)
8	L	0	0	0	0	1	45	Sample loading / phosphate measurement
9	L	0	0	0	0	0	45	Sample loading / ammonium measurement
10	E	1	1	1	1	0	10	Elution / Reagent addition
11	E	0	0	0	0	1	0.1	Trapping of the sample zone (ammonium)
12	E	0	0	0	0	1	45	Sample loading / phosphate measurement
13	E	0	0	0	0	0	45	Sample loading / ammonium measurement

Results and Discussion

The determination of both phosphate and ammonium is often required in studies related to aquatic chemistry. These species are major nutrients needed to support the live in aquatic environments. However, they can cause eutrophication of water bodies when present in large amounts, resulting in a drastic depression of the concentration of dissolved oxygen and dead of fishes by asphyxia. The determination of these species is currently carried out by exploiting flow-based methods with spectrophotometric detection and the involved chemistries (Berthelot and indophenol blue reactions for ammonium and phosphate respectively) are well established. However, the application is frequently limited by lack of sensitivity, thus requiring tedious and time-consuming procedures for preconcentration. This results in lessening of the sampling rate and increasing of both sample/reagent consumption and production of wastes. These drawbacks can be minimized by improving the performance of the flow system by implementing simultaneously the tasks involved in sample processing.

The proposed flow system exploits the multi-commutation approach to combine in-line concentration, intermittent flows and zone trapping strategies to improve sensitivity without hindering the sampling rate or increasing the amount of wastes. As depicted in the diagram of Figure 1, during the sample-loading step the columns were placed in series in order to allow the sample stream to flow sequentially through both columns, thus avoiding wasting of time and sample. In this way, sample consumption was 30% lower than the required for determining ammonium and phosphate in previous procedures involving in-line concentration.[12,13] The sampling rate was estimated as 40 determinations per hour, which is three-fold higher than that obtained without performing the tasks simultaneously.

As the kinetic of the Berthelot reaction is slow, sample zone was trapped into the reaction coil B_2 to enhance the chemical reaction development. The trapping period was varied and it was observed that the steady state was achieved for time intervals longer than 120 s. A 45 s trapping interval was selected, which was enough to achieve a conversion rate of 75%. The retention of the sample zone was also implemented simultaneously to the sample loading step, avoiding hindering the sampling rate.

Eluent solutions were the same as previously employed: sodium hydroxide for ammonium[12] and nitric acid for phosphate.[13] The concentrations were varied and the best results by considering elution efficiency and reagent consumption were obtained for 0.50 mol L^{-1} solutions.

Besides the efficient elution, noted by narrow transient signals, these solutions provide suitable media for the reactions development. For ammonium, the elution is also favored by the conversion to ammonia in alkaline media. It was experimentally verified that a 10 s switching time of the valves V_1-V_4 was suitable to add the chromogenic reagents in the sample zone. Other operational parameters summarized in Table 1 were optimized using flow rates of sample and reagent solutions indicated in Figure 1.

Two LED-based photometers were used as detectors and the signals related to phosphate and ammonium were read by the microcomputer through the analog imput of the interface card. A set of signal recordings obtained by using the system parameters defined in Table 1 are shown in Figure 2. The record pattern and the repeatability of the signals is similar to those obtained with usual flow systems, therefore indicating that the combination of the different sample processing steps can be exploited to improve analytical performance. This can be exemplified by comparing the analytical parameters related to ammonium determination by exploiting zone trapping or in-line concentration (Table 2).

For sample loading times shorter than 270 s, the enrichment factors (E_f) are related to the sample loading time (t) by the equations: $E_f(NH_4^+) = 0.322 + 0.199\ t(s)$ and
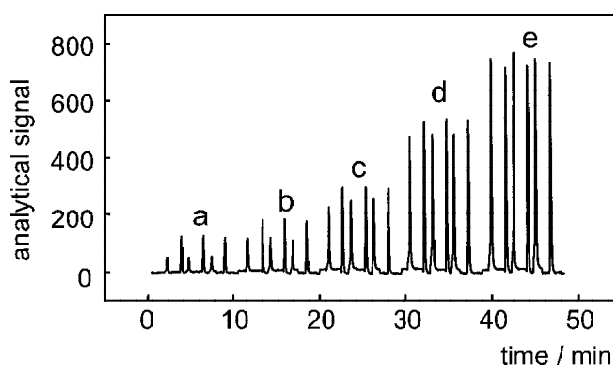

Figure 2. Transient signals obtained for simultaneous in-line concentration of ammonium and phosphate following the switching course in Table 1. Reference solutions containing: (a) 25, (b) 50, (c) 100, (d) 200 and (e) 300 µg L^{-1} phosphate and ammonium. For each group, the analytical signals refer to phosphate-ammonium determination in sequence performed in triplicate.

Table 2. Analytical parameters related to procedures for ammonium determination exploiting the Berthelot reaction

Analytical parameter	Proposed system	Zone trapping[14]	In-line concentration[12]
Trapping period (s)	45	48	—
Loading time (s)	90	—	60
Sampling rate (h^{-1})	40	58	40
Detection limit (µmol L^{-1})	0.05	0.4	0.2

Table 3. Mean values and uncertainties (n=3) for determination of ammonium and phosphate in freshwater samples

Water sample	Ammonium (μg L^{-1})		Phosphate (μg L^{-1})	
	Proposed system	Reference[11]	Proposed system	Reference[11]
1	48.5 ± 0.8	47.6 ± 0.8	256 ± 5	246 ± 11
2	31.2 ± 0.1	24.6 ± 1.7	171 ± 3	155 ± 7
3	40.4 ± 1.2	38.8 ± 1.1	274 ± 12	283 ± 25
4	104 ± 7	112 ± 4	162 ± 4	162 ± 7
5	47.1 ± 3.8	45.5 ± 3.4	190 ± 13	204 ± 19
6	166 ± 8	163 ± 10	272 ± 12	283 ± 25

$E_f (PO_4^{3-}) = 0.219 + 0.0871\ t(s)$, $r = 0.999$. For a 90 s loading time, enrichment factors of 8.0 and 18 were estimated for phosphate and ammonium, respectively. However, as stated by the previous equations, better sensitivities can be achieved by increasing the sample loading times. For the operational conditions established in Table 1, the detection limits were estimated as 1 μg L^{-1} PO$_4^{3-}$ and 1 μg L^{-1} NH$_4^+$ at the 99.7% confidence level. Coefficients of variation were 2.6% and 3.2% for phosphate and ammonium, respectively (n = 20). Linear responses (r = 0.999) were obtained within 5.00 and 300 μg L^{-1} of both analytes.

The proposed procedure is greener than the previously reported for the determination of these analytes involving in-line concentration and zone trapping, because the intermittent reagent addition allowed minimizing both the reagent consumption and the production of wastes. For ammonium determination, the reagent consumption was *ca.* 1.3 mg, which is up to ten-fold lower than the previously reported procedure.[14] Phosphate determination required 160 μg SnCl$_2$ and 800 μg ammonium molybdate, that is *ca.* 20% lower than the previously reported procedure.[13] Results for freshwater samples (Table 3) agreed with the obtained by reference APHA procedures at the 95% confidence level.

Conclusions

The proposed flow system allowed different tasks to be simultaneously implemented exploiting the multicommutation approach, thus improving system performance. Two species were determined in parallel after serial in-line concentration without impairing sampling rate or wasting of sample and reagents. Different cationic and anionic species can also be determined with the same flow setup by changing the chromogenic reagents. Moreover, the solid adsorvents can also be changed for determining other analytes, including organic substances after concentration in materials such as fullerene or C$_{18}$-bonded silica. Also, the flow system can be accomplished to other kind of detectors. In this sense, an interesting application would be the simultaneous determination of metallic ions in the effluent of the cation exchange resin by a multi-channel detector such as ICP-OES.

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