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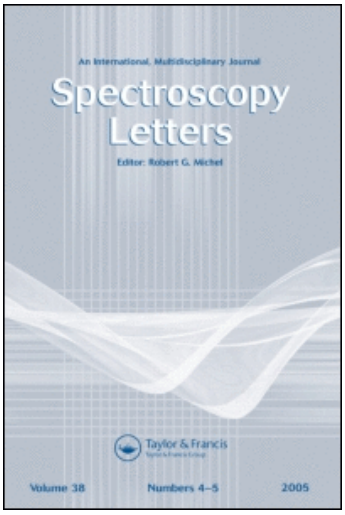

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Multi-commutation in Flow Analysis: A Versatile Tool for the Development of the Automatic Analytical Procedure Focused on the Reduction of Reagent Consumption

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Multi-commutation in Flow Analysis: A Versatile Tool for the Development of the Automatic Analytical Procedure Focused on the Reduction of Reagent Consumption

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Abstract: In this work, a general view of multi-commutation in flow analysis process is presented. The manuscript comprises a comprehensive description of the basic manifolds designed to demonstrate the multi-commutation concept including the operational modes and the inherent features. A multi-function flow system with the ability to carry out three-operation modes without any hardware modification is also described. Intending to demonstrate the versatility of the flow system based on multi-commutation process, a set of experiments was designed comprising an in-line dilution and stopped flow approach. Standard solutions of iron and of orthophosphate were selected as models to demonstrate the analytical potential of the proposed system. The results obtained are presented and discussed.

Keywords: Automation, flow injection analysis, multi-commutation, spectrophotometry

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INTRODUCTION

Multi-commutation in flow analysis process was introduced 12 years ago[1] and it can be considered to be a branch of the flow injection analysis process (FIA).[2,3] The main difference among them is related to the reagent solution management, which is accomplished employing an intermittent pattern, while in the usual flow injection system reagent solutions are pumped continuously.

The core of the flow system manifold comprises a set of solenoid valves assembled to work as an independent commutating unit,[1,4] which under computer control can allow the random access of the reagent solutions. Exploiting this facility, the working pattern of the flow system can be modified by software without any change in the manifold structure.

Analytical procedures based on the multi-commutation process have been developed for analytes determination in matrices such as plant digest,[5,6] pharmaceutical formulations,[7,8] surface water,[9,10] lubricant oil,[11] biologic fluid,[12,13] alcoholic beverage,[14,15] and silage extract.[16,17]

The procedures implemented based on the multi-commutation process have been employed as detection techniques: UV-Vis spectrophotometry,[18] potentiometry,[19,20] fluorimetry,[21,22] chemiluminescence,[23,24] and infrared spectrophotometry.[25,26]

Resources usually employed in the field of analytical chemistry such as liquid–liquid extraction using organic solvent,[27,28] solid-phase extraction with ion exchange resin,[29,30] immobilized enzyme on beads,[31,32] sample dilution,[33,34] hydride generation,[35,36] and flow-through sensors[37,38] have been employed in the analytical procedures implemented based on the multi-commutation process. In these cases, three main requirements such as versatility, low reagent consumption, and robustness, which could be expected when an automatic analytical procedure is proposed, were obtained without lessening any sacrificing of precision and accuracy.

The flow system is designed assembling a set of solenoid valves to work as independent commutation units controlled by a microcomputer, thus presenting ability to handle several reagent solutions in some cases using a single pumping channel. This facility was exploited to develop automatic procedures for sequential determination of two or three analytes by spectrophotometry sharing the same analytical path.[39,40] As an example, one can cite the procedures for the determination of ammonium (alkaline medium) and orthophosphate (acid medium),[5] which could be implemented designing a manifold simpler than those employed in the earlier works based on usual flow injection technique.[41,42]

Exploiting the multi-commutation ability to insert into the analytical path little volumes of titration solutions, true titration procedures could be implemented at the first time employing as detection techniques spectrophotometry[43,44] and potentiometry.[20,45] In both cases, the acid concentration was found without the use of the analytical curve as it was required in the earlier works based on flow injection analysis technique.[46,47]

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Nowadays, our conscience concerning environmental protection is focused on all activities that could cause any change in the ecosystem. In this sense, attention could be focused to find strategies to reduce the polluting charge of the analytical procedures. In this sense, effort should be directed to develop analytical procedures with ability to generate reduced volume of waste. This requirement could be accomplished by developing the analytical procedures based on the multi-commutation process.[28,35,36] In this case, reduction of waste volumes higher than 90% was achieved.

The detection had been performed using commercial equipment or homemade photometer prototype using LED as radiation source and photodiode as light detection.[48,49] Employing these facilities, the photometer and the flow system manifold were designed to comprise a compact instrument with little dimension, which could be housed in a metallic box with dimension of $30 \times 20 \times 15$ cm.[50,51]

Most works based on the multi-commutation process employed peristaltic pump for fluid propelling, however it was proved that gravity acceleration could be also employed.[52,53] The flow system presented an overall performance similar to that observed using peristaltic pump. Recently, the solenoid micropump was introduced as a new device for fluid propelling in flow analysis system.[54,55] The solenoid micropump is a compact commutating device, which has been employed to carry out solution propelling replacing the peristaltic pump.[56,57] The working pattern of the flow system is similar to that of the flow system based on multi-commutation, thus the micropump has been employed in multi-commuted flow systems replacing both peristaltic pump and solenoid valves,[58,59] thus allowing that versatile analytical procedures could be implemented with a significant reduction of the cost.

In this work, one intent was to demonstrate the overall features of the multi-commutation process, which was done by employing a basic flow system including comprehensive descriptions concerning the working pattern advantages and limitations.

Multi-commutation Solutions Inserting Model

The diagram shown in Fig. 1 is a pictorial view symbolizing the insertion of sample and reagent solution aliquots into a straight tube that is observed through the axial cutaway. In the first case (I), one aliquot of sample (S) and one of reagent solution (R) were inserted into the pipe, which were displaced through them by the carrier stream (Cs). Under this condition, mixing between solution aliquots could occur at the mutual liquid interface, thus permitting the development of the chemical reaction to produce the compound, which could be monitored to permit the analyte determination. In the second case (II), both aliquots of sample and reagent solutions were halved and the slugs were inserted into the pipe alternately. In the third case (III), the slugs were also halved, thus four slugs of each solution were

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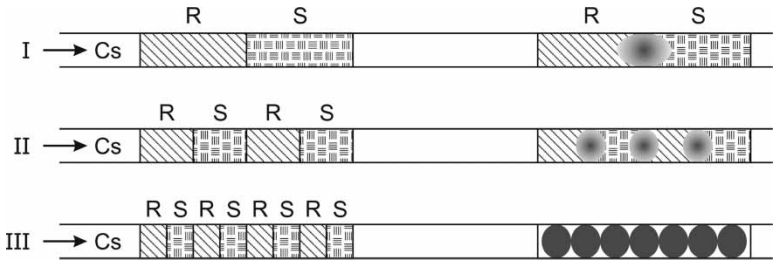

Figure 1. Multi-commutation model. I, II, and III = inserting stage; Cs = carrier solution; R = reagent solution; S = sample solution. The shadow symbolizes the sample and reagent solutions mixing interfaces.

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generated and inserted into the pipe thus maintaining the alternated pattern. Under this condition, a portion of the pipe was loaded with a string comprising slugs of sample solution in tandem with slugs of reagent solution. We could suppose that seven mutual liquid interfaces were formed, while in the first and second cases, the interfaces formed were one and three, respectively. In this sense, we could suppose that increasing the number solutions slugs pairs, an improvement of the mixing condition could be achieved. Maintaining the proposed solutions inserting pattern, we can deduce that the number of the solution slugs pair presents the following relationship: $Nsp = 2^{n-1}$ (n = number of inserting stage), while the number of liquid interfaces generated presents the following relationship: $Int = 2^n - 1$. Under this condition, the mixing between solution slugs could be improved without the enlarging of the sample bulk.

Requirements to Implement a Multi-commuted Flow System

Flow system based on the multi-commutation process requires some electronic and software facilities, which comprise the interface to generate control signals to drive solenoid valves and a computer to host an electronic interface card and to run the control software, which has been developed by the researchers according to the requirement of the analytical procedure. In this sense, the software to implement automated procedures has been developed using a very simple programming language such as Quick BASIC 4.5.[51,58]

The control signals are generated at TTL pattern (0–5 V), and solenoid valves required a potential difference of 12 V. In this sense, an external electronic interface is required in order to provide current intensity and potential difference necessary to switch on the solenoid valves. In the first works based on multi-commutation process, the interface comprised a set transistor, wired to work as electronic switches.[1,3,4] Actually, this interface has been easily mounted in the laboratory using the integrated circuit ULN2803.[51,58] To

generate the control signals, a commercial electronic interface card has been used,[7,8] nevertheless, the printer port available in the microcomputer has also been used.[58] This option is very interesting considering that some equipment available in the marketplace has data communication through a serial interface. In this case, an interface card for data acquisition becomes dispensable, thus contributing to reduction in the cost to implement an automatic analytical procedure.

When the detection setup does not incorporate data communication through a serial interface such as a homemade photometer, data acquisition has been performed by employment of a voltmeter with resolution of 0.1 mV and presenting a serial communication interface.[51] In this case, the control of the flow system manifold has been easily carried out through the printer port of the microcomputer. Some difficulty concerning the data format delivered by the voltmeter can be solved obtaining the communication protocol provided by the manufacturer.

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MATERIALS AND METHODS

Reagent Solutions

All chemicals were of analytical grade. Purified water presenting electric conductivity less than 0.1 $\mu S\ cm^{-1}$ was used throughout.

A 1000 mg L^{-1} Fe(III) stock solution was prepared by dissolving 2.42 g of $FeCl_3 \cdot 6H_2O$ (purity 99%) from Merck (Darmstadt, Germany) in 5 mL concentrated HNO_3 and diluting to 500 mL with water. Working solutions with concentration between 0.5 and 50.0 mg L^{-1} Fe(III) were prepared by dilution from the stock solution with water.

A 1000 mg L^{-1} PO_4^{3-} stock solution was prepared by dissolving 0.7163 g of KH_2PO_4 (purity 99%) from Merck in 500 mL of water and stored at 4°C. Working solutions with concentration between 0.5 and 4.0 mg L^{-1} PO_4^{3-} were prepared daily by dilution from the stock solution with water.

A 1.0% (w/v) ascorbic acid solution was prepared daily by dissolving 1.0 g of reagent (purity 99%) from Sigma-Aldrich (St. Louis, MO, USA) in 100 mL of water.

An 0.25% (w/v) 1-10-phenanthroline solution was prepared daily by dissolving 0.25 g of reagent (purity 99%) from Sigma-Aldrich in 100 mL of water.

An 0.5% (w/v) salicylic acid solution was prepared daily by dissolving 0.5 g of reagent (purity 99%) from Sigma-Aldrich in 100 mL of 0.001 mol L^{-1} HNO_3.

An 0.3% (w/v) ammonium molybdate solution was prepared daily by dissolving 0.3 g of reagent (purity 99%) from Merck in 100 mL of 0.05 mol L^{-1} HNO_3.

An 0.001 mol L^{-1} HNO_3 solution was prepared by dilution of concentrated HNO_3 with water. This solution was used as carrier fluid.

Apparatus

The equipment setup comprised an IPC-4 Ismatec peristaltic pump furnished with Tygon pumping tube; a microcomputer equipped with an electronic interface card (PCL711S, Advantech Corp, Cincinnati, OH, USA), a UV-Vis 460 Femto spectrophotometer equipped with a quartz flow cell, 80 μL inner volume, and 10 mm optical path; four three-way solenoid valves (161T031, Nresearch, New Jersey, NJ, USA); two solenoid pinch valves normally closed and one normally open (161P011 and 161P021, Nresearch, New Jersey, NJ, USA); a 12 V regulated power supply to feed the solenoid valves; a homemade driving electronic interface; reaction coil and flow lines of polyethylene tubing 0.8-mm inner diameter.

Implementation of the Multi-commutation Model

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In Fig. 2 is shown the diagram of the flow system, which computer controlled could generate the working pattern described in Fig. 1. The valve V_1 is a model normally open to permit the fluid stream through it while it was maintained switched OFF. Valves V_2 and V_3 are models normally closed, thus impeding the fluid stream through them while they were maintained switched OFF. Under this configuration, all valves are switched OFF, therefore only the carrier solution (Cs) flows suctioned by the peristaltic pump (Pp) through reaction coil (B) and detector (Det) toward waste (W).

The solenoid valves are coupled to the microcomputer through a digital interface,[51,58] thus when the control software was run the microcomputer sent the control signals through the interface to switch ON/OFF the solenoid valves. This step was done following the switching patter depicted in the valves timing course shown in Fig. 2. As we can see, in the first case (I), when the sampling cycle (Sc_0) was carried out, valve V_1 was maintained switched ON while valves V_2 and V_3 were switched ON sequentially one time during the time intervals Si_s and Ri_s, respectively. Under this condition, the stream of the carrier solution (Cs) was halted, while aliquots of sample and reagent solution flowed sequentially by suction through valves V_1 and V_2, respectively. Afterwards, all valves were maintained switched OFF and the carrier solution (Cs) flowed again, thus displacing the aliquots (slugs) of sample and reagent solutions through the reaction coil (B) towards the detector (Det). In this stage, one slugs pair of sample and reagent solution was inserted into the reaction coil (B), which was done by programming one sampling cycle (Sc_0) and settling equal time interval ($Si_s = Ri_s$) to switch ON valves V_2 and V_3. Under this condition, aliquots of sample and reagent solutions with equal volumes were inserted into the reaction coil (B). Because the flow rate was maintained the volumes of the solutions aliquots were $Vs = Vr = t_0\varphi$ (t_0 = time interval, φ = flow rate). In the second (II) and the third stages (III), the time intervals were settled halving

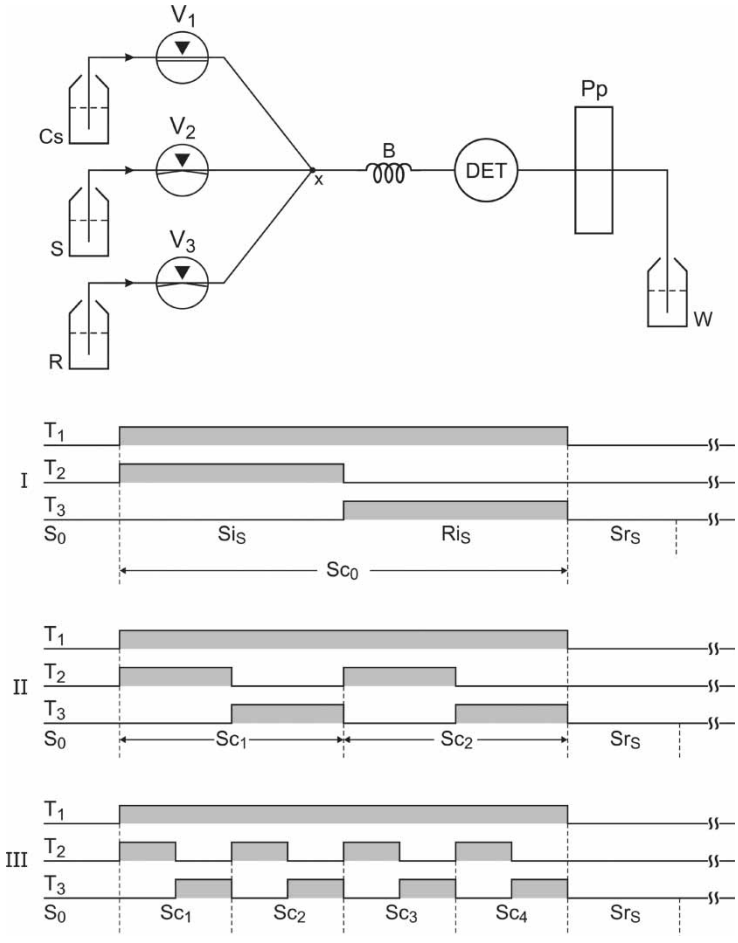

Figure 2. Diagram of the multi-commuted flow system. Cs = carrier solution, 0.001 mol L^{-1} HNO_3, flow rate at 30 μL s^{-1}; S = sample or standard solution; R = 0.5% (w/v) salicylic acid in 0.001 mol L^{-1} HNO_3 medium; V_1, V_2, and V_3 = pinch solenoid valves; x = joint device machined in acrylic; B = reaction coil, 30 cm long and 0.8 mm inner diameter; DET = spectrophotometer at 480 nm; Pp = peristaltic pump; W = waste. I, II, and III = inserting stage; T_1, T_2, and T_3 = timing course to switch valves V_1, V_2, and V_3, respectively; S_0 = standing by stage; Si_s and Ri_s = sample and reagent solutions inserting step, respectively; Sc_0, Sc_1, Sc_2, Sc_3, and Sc_4 = sampling cycles; Sr_s = signal reading step. The shadow surfaces beneath timing course lines indicate that the corresponding valve was switched ON.

the subsequent one as follow: $t_1 = t_0/2$; $t_2 = t_1/2$. In the first stage, valves V_1 and V_2 were switched ON one time, thus the inserting step comprised one sampling cycle (Sc_0). As it is depicted in the valves timing courses in the second and the third stages (II, III), the inserting steps comprised two

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(Sc_1, Sc_2) and four (Sc_1, Sc_2, Sc_3, Sc_4) sampling cycles, respectively. The numbers of sampling cycles were twofold and fourfold in order to insert equal volume of sample and reagent solutions.

A sampling cycle is defined as the event comprising the sequential insertion of one sample slug and one reagent solution slug. In this sense, we can deduce that maintaining the time interval halving pattern $t_1 = t_0/2$; $t_2 = t_1/2$; $t_3 = t_2/2$; ($t_{n+1} = t_n/2$), the number of sampling cycles presents the following relationship: $Nsc = 2^n - 1$ (n = number of inserting stage), which is equal the number of slugs pair deduced in the above section where the multi-commutation model was mentioned.

Intending to demonstrate the multi-commutation sampling model, assays were performed using a 20.0 mg L^{-1} Fe(III) standard solution and an 0.05% (w/v) salicylic acid solution as chromogenic reagent and detection by spectrophotometry at 480 nm. The initial time intervals (Si_s, Ri_s) settled to maintain valves V_2 and V_3 switched ON during the first stage (I) were halved ($t_{n+1} = t_n/2$) prior to carrying out the subsequent stage. The number of sampling cycles was increased to $Nsc = 2^{n-1}$ (n = number of inserting stage) in order to load reaction coil (B) with volumes of sample and reagent solutions equal to those inserted in the previous stage (n).

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Multi-function Flow System

The multi-commutation concept involves the ability to handle each commuting unit individually, thus different working conditions could be implemented by software without any change in the hardware of the flow system manifold. Aiming to demonstrate these facilities, the flow system depicted in the diagram of Fig. 3 was designed, which, under software control, could work as a usual multi-commuted flow system, presenting also abilities to perform programmed dilution or stopped flow.

In the configuration shown in Fig. 3, all valves are switched OFF, therefore only the carrier solution (Cs) is flowing suctioned by the peristaltic pump (Pp) through valves V_1, V_2, V_3, and V_4, reaction coils B_1 and B_2, and detector (Det) toward waste (W).

As it is indicated in the valves timing course (I), when the software was run to implement a usual multi-commuted procedure, valves V_1 and V_2 were switched ON at the same time, thus the stream of carrier solution (Cs) was halted and sample solution (S) flows suctioned by the peristaltic pump (Pp) through sampling loop (L) and valve V_2 through the bypassing line (BL) toward waste (W). After a preset time interval (Si_s) to fill the sample loop with sample solution, valves V_1 and V_2 were switched OFF and the carrier solution (Cs) flowed through the sampling loop (L), thus displacing the sample aliquot through the valve V_2 toward reaction coil B_1. As it is shown in the valves timing course, valve V_3 was switched ON/OFF sequentially three times. While this valve was maintained switched ON, a slug of the

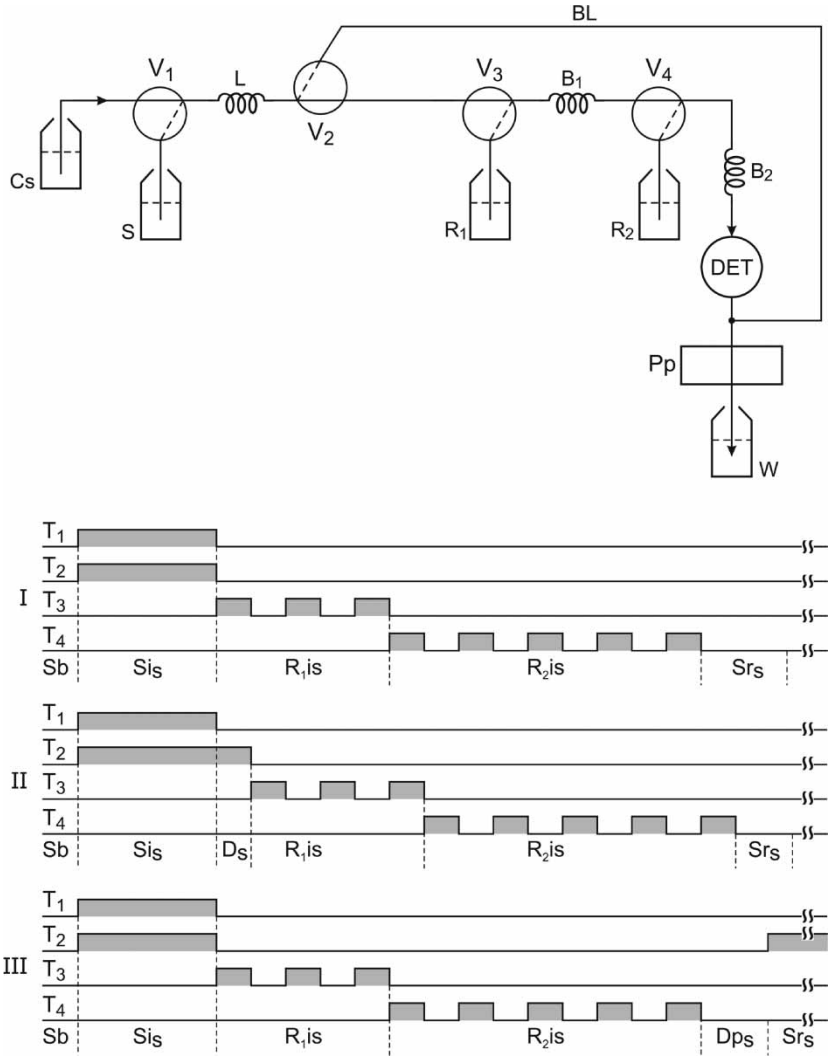

Figure 3. Diagram of the multi-function flow system. Cs = carrier solution, 0.001 mol L^{-1} HNO_3, flow rate at 30 μL s^{-1}; S = sample or standard solution; R_1 = 1% (w/v) ascorbic acid solution; R_2 = 0.25% (w/v) 1-10-phenanthroline or 0.3% (w/v) ammonium molybdate solution; V_1, V_2, V_3, and V_4 = three-way solenoid valves; L = sampling loop, 20 cm long and 0.8 mm inner diameter; BL = bypassing line, B_1 and B_2 = reaction coil, 30 and 50 cm long, respectively, and 0.8 mm inner diameter; DET = spectrophotometer at 540 nm; Pp = peristaltic pump; W = waste. I, II, and III = system operating modes; T_1, T_2, T_3, and T_4 = timing course to switch valves V_1, V_2, V_3, and V_4, respectively; S_b = standing by stage; Si_s, $R_1$is, and $R_2$is = sample and reagents solutions inserting step, respectively; D_s = dilution delay time; Dp_s = stop-flow delay time; Sr_s = signal reading step. The shadow surfaces beneath timing course lines indicate that the corresponding valve was switched ON.

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reagent solution (R_1) was inserted into the reaction (B_1), and while this valve was OFF, a slug of the sample solution was inserted into this reaction coil (B_1). Because it was programmed for three reagent sampling cycles ($R_1$is), the reaction coil (B_1) was loaded with a string comprising three slugs of sample in tandem with three slugs of reagent solution (R_1). As it is shown in the valves timing course, five sampling cycles ($R_2$is) were settled to insert reagent solution (R_2) into reaction coil (B_2), which was done by switching ON/OFF valve V_4 five times. Under this condition, the reaction coil (B_2) was loaded with a solution string comprising five slugs of reagent solution (R_2) in tandem with five slugs of the mix comprising sample and reagent solution (R_1). Reagent (R_1) could be a buffer solution, a masking reagent solution, a reagent solution that should be mixed with sample solution prior to adding the chromogenic reagent solution (R_2), and so forth. Considering that flow rate and reactor coil dimensions were maintained, the flow system variables are the time intervals to switch ON/OFF the solenoid valves and the number of sampling cycles settled to insert the reagent solutions R_1 and R_2.

Aiming to demonstrate the usefulness of the proposed system, a set of Fe(III) standard solution with concentration ranging from 0.5 up to 3.0 mg L^{-1} was processed employing ascorbic acid to reduce Fe(III) to Fe(II) and 1-10-phenantroline as chromogenic reagent.

Sample dilution is a common requirement in the analytical chemistry laboratory, and different strategies to accomplish this task have been proposed employing either flow injection analysis technique[60] or the multi-commutation process.[33,34] The flow system presented on the diagram of Fig. 3 could be programmed to implement this task, which could be done by settling a working pattern as depicted in the valves timing course (II). In the first step, valves V_1 and V_2 were switched ON at the same time to fill the sample loop (L) with sample solution. Afterwards, valve V_1 was switch OFF in order to stop the sample stream, thus the carrier solution flowed again through the valve V_1 to displace the sample aliquot from the sample loop (L) through the bypassing line (BL) toward waste (W). After a preset time interval (Ds), valve V_2 was switched OFF and the remaining portion of the sample aliquot into the sampling loop (L) was displaced toward the reaction coil (B_1), where reagent solutions R_1 and R_2 were inserted in the dispersed sample zone following valves V_3 and V_4 switching pattern depicted in the valves timing course (II).

While the sample aliquot was displaced through the bypassing line (BL) toward waste (W), analyte dispersion occurred into the sample loop, thus forming a descending concentration gradient. In this sense, controlling the time interval to maintain valve V_2 switched ON, we could select portions of dispersed sample with different analyte concentration. Intending to demonstrate that this operation mode was feasible, a standard solution 10.0 mg L^{-1} Fe(III) was processed varying from 1.0 up to 5.5 s the time interval (Ds) to turn OFF valve V_2.

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The stopped flow approach is a resource that had been employed in flow injection analysis technique to improve sensitivity when the analytical procedure was based on reaction of slow kinetic. The flow system depicted in Fig. 3 could afford this facility implementing the working pattern shown in the valves timing course (III).

Intending to demonstrate this ability of the proposed flow system, assays involving the procedure for spectrophotometric phosphate determination using ammonium molybdate as chromogenic reagent and ascorbic acid as reducing reagent were carried out. The steps to fill sample loop (L) and to insert ammonium molybdate and ascorbic acid solutions in the sample zone were performed similar to those previously described for iron(III). Afterwards, valve V_2 was switched ON in order to shunt the analytical path (B_1, B_2) and detector (Det). Under this condition, the carrier solution flowed through the bypassing line (BL) toward waste (W). The time interval for chemical reaction development could be controlled actuating on valve V_2. The assays were carried out settling time intervals of 80 and 160 s and using a set of standard solutions with concentration ranging from 0.5 up to 4.0 mg L^{-1} PO_4^{3-}.

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RESULTS AND DISCUSSION

The flow systems represented by the diagrams shown in the above sections were designed to implement the multi-commutation process, and a set of experiments were accomplished intending to demonstrate the usefulness of this approach as a tool for the development of the automatic analytical procedures. The results obtained running some demonstrative assays are presented and discussed in the following sections.

Assays Based on the Multi-commutation Model

The multi-commutation model depicted in Fig. 1 was implemented employing the flow system shown in Fig. 2. The results obtained using an Fe(III) standard solution and salicylic acid solution as chromogenic reagent are shown in Fig. 4. The first set of assays (records *a*) comprises three repetition of the first inserting stage. The time intervals to maintain valves V_2 and V_3 switched ON were settled at 8 s and one sampling cycle was carried out ($Nsc = 2^{n-1}$, $n = 1$), therefore the volume of each aliquot of sample and reagent solutions was 240 μL (flow rate = 30 μL s^{-1}).

According to the multi-commutation insertion model, prior to carrying out the second inserting stage, the time intervals to switch ON valves V_2 and V_3 were halved. Under this condition, the volume of solution slugs inserted when the corresponding valve was switched ON was 120 μL. In the second inserting stage, two sampling cycles ($Nsc = 2^{n-1}$, $n = 2$) were

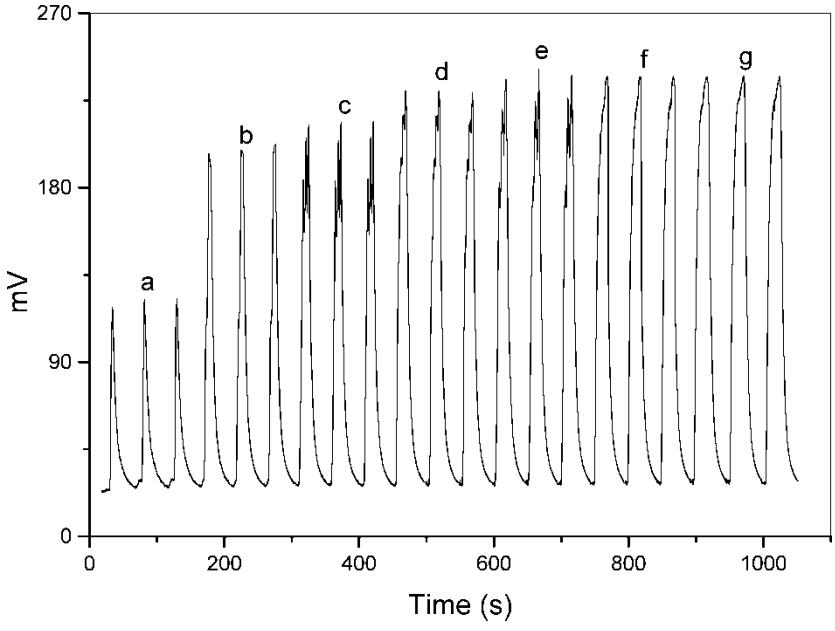

Figure 4. Effect of the mixing solutions interfaces. Records from *a* to *g* correspond with the mixing interface number 1, 3, 7, 15, 31, 61, and 127, respectively. Results obtained processing a 20 mg L^{-1} Fe(III) standard solution.

carried out, therefore the addition of the sample slugs comprised 240 μL. Because the time interval to switch ON valves V_2 and V_3 were equal, the volume of reagent solution inserted also was 240 μL. When these slugs were inserted into the reaction coil, three mixing interfaces ($Int = 2^n - 1$) were generated. The set of records *b* was obtained operating the system under this condition. The maximum peak height of the records *a* and *b* were 122 mV and 200 mV, respectively, therefore a signal increasing of 82% occurred. Because the volumes of sample and reagent solutions were equal, the gain in signal was caused by the mixing improvement. The numbers of the mixing interfaces ($Int = 2^n - 1$) associated to records *c*, *d*, *e*, *f*, and *g* were 7 ($n = 3$), 15 ($n = 4$), 31 ($n = 5$), 61 ($n = 6$), and 127 ($n = 7$), respectively. As we can see, the increasing of peak height occurred up to the cords *e*, which presented a maximum value of 240 mV that was maintained for the records *f* and *g*.

This set of records shows that appropriate mixing conditions were achieved when the mixing interfaces were equal to or higher than 31 ($n = 5$ or $n > 5$). In this case ($n = 5$), valves V_2 and V_3 were maintained switched ON during a time interval of 0.5 s, therefore the volume of each solution slug was 15.0 μL, which was inserted in tandem into the reaction coil (30 cm long, 150 μL inner volume), therefore the coil volume was 10-fold higher than the slugs volumes. In this sense, we could think that this relationship could be considered

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as a threshold condition to obtain an appropriate mix between sample and reagent solution. Nevertheless, other parameters such as viscosity of solutions, inner diameter of the reaction coil, hydrophilic feature of the reaction coil material, and so forth, can be considered.

The assays mentioned above were implemented varying the time intervals to switch ON valves V_2 and V_3 from 8.0 up to 0.125 s, then the solution slugs volume varied from 240 uL up to 3.8 uL. Analyzing the records of Fig. 4, we can observe that the repeatability of the measurements were very good, thus indicating that the precision was not affected, albeit the slug volume varied 61 times. In this sense, sensitivity could be the parameter elected in order to settle the slug volume.

Considering sensitivity as the goal parameter, a set of Fe(III) standard solutions were processed selecting the working condition employed to obtain the record *f*, where the slug volume of each solution was 7.5 uL. Operating the system under this condition, the following features were achieved: linear response raging from 1.0 up to 50.0 mg L^{-1} Fe(III) ($R = 0.999$); relative standard deviation of 1.0% ($n = 10$); reagent consumption 0.48 mg salicylic acid per determination; waste generation of 1.5 mL per determination; and throughput of 70 determination per hour.

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Assays Employing the Multi-function Flow System

As it was described before, the flow system shown in Fig. 3 could afford facilities to implement three different operation modes, which could be carried out by software without any change of the system hardware. Intending to demonstrate these possibilities, a set of assays were carried out and results are discussed in this section.

In the first case, the system was programmed to work as usual multicommuted flow system following the valves switching pattern shown in valve timing course (I) in Fig. 3. To ensure that sampling loop (L) was filled up, valves V_1 and V_2 were maintained switched ON during a time interval of 6 s. Afterwards, valves V_3 and V_4 were switched ON/OFF 8 and 12 times, respectively. In both cases, the time intervals to maintain the these valves ON or OFF were settled at 0.5 s. Processing a set of standard solutions with concentration ranging from 0.5 up to 3.0 mg L^{-1}Fe(III), a linear response ($R = 0.999$) was obtained. Other profitable features such as a relative standard deviation of 1.0% ($n = 9$); a low reagent consumption per determination 1.2 mg ascorbic acid, 0.45 mg 1-10-phenantroline; and a waste generation of 1.5 mL per determination were also achieved. An analytical throughput of 70 determinations per hour was estimated considering the time interval elapsed while the standard solutions were processed.

In the second case, the system programmed to work as depicted in the valves timing course (II) shown in Fig. 3. This operation mode was designed to perform in-line dilution exploiting the sample dispersion effect,

which occurred while sample solution was displaced to waste bypassing the reaction coils and detector. The microcomputer was instructed to operate the flow system following the valves switching pattern shown in the valves timing course (II). After the loop-filling step (Si_s), the dilution step (Ds) was carried out maintaining valve V_2 switched ON during time intervals, which were varied from 1.0 up to 5.5 s, and results yielded are shown in Fig. 5.

Analyzing these records, we can observe that when the time delay associated with dilution step (Ds) was 1.0, the peak height was around 325 mV, while for a time delay of 5.5 s, the maximum signal was around 50 mV. The signal decrease is associated with the remaining analyte concentration into the sampling loop (L) when valve V_2 was switched OFF. Intending to demonstrate that this effect could be exploited to accomplish in-line dilution, a set of Fe(III) standard solution was processed settling a time delay of 5.0 s to carry out the dilution step (Ds). Under this condition, a linear response ($R = 0.998$) in the concentration range of 2.5 up to 30 mg L^{-1} Fe(III) was obtained.

The records of Fig. 5 indicated that in the sampling loop was formed a descending gradient concentration while valve V_2 was maintained switched ON. Because this valve was commutated to the resting position without stopping the carrier stream, a little variation on the delay time (Ds) could affect unfavorably the precision of the measurements. Aiming to verify this assumption, one assay was carried out using a 15.0 mg L^{-1} Fe(III) standard solution and settling

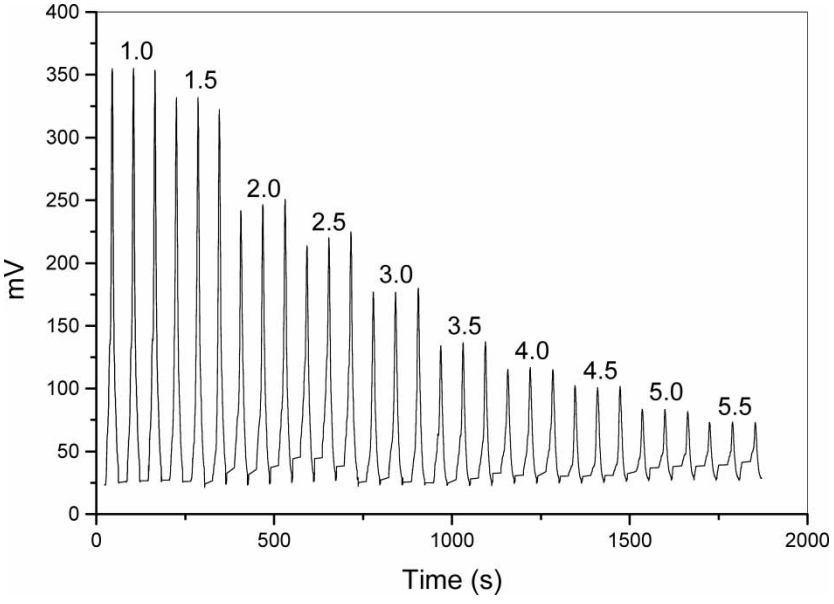

Figure 5. Dilution effect. Numbers on the records are the dilution delay times. Results obtained processing a 10 mg L^{-1} Fe(III) standard solution.

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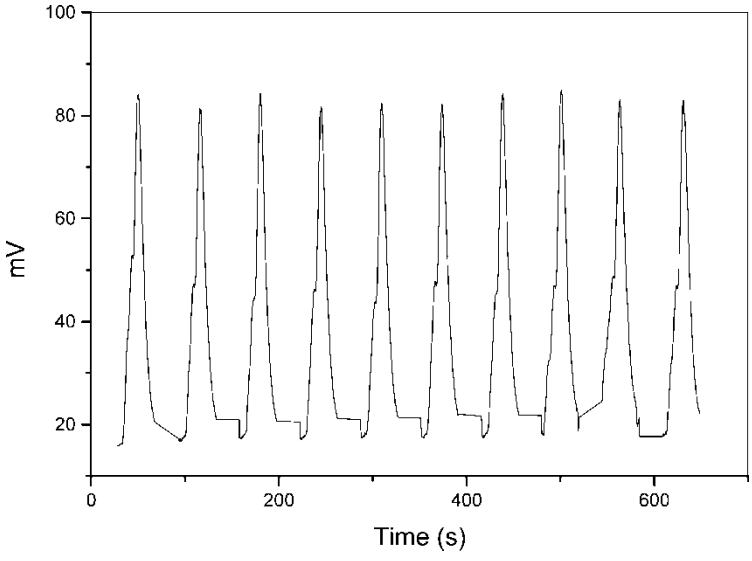

Figure 6. Dilution delay time effect on the precision of the measurement. Results obtained processing a 20 mg L^{-1} Fe(III) standard solution and programming a dilution delay time of 5.0 s.

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a delay time of 5.0 s yielding the results shown in Fig. 6. As we can see, the precision of the records is very good presenting a relative standard deviation of 1.4%, thus proving that this recourse could be employed with in-line dilution. In this sense, an adapting link could be incorporated in the software to accomplish the appropriate dilution when it was necessary.

The flow system shown in Fig. 3 was designed to perform the stopped flow approach following the valves switching pattern depicted in valves timing course (III). Intending to demonstrate its feasibility, the procedure for spectrophotometric determination of phosphate using ammonium molybdate as chromogenic reagent and ascorbic acid as reducing reagent was employed. The sets of assays were carried out using standard solutions with concentration ranging from 0.1 up to 4.0 mg L^{-1} PO_4^{3-} and employing two stop-flow delay times (Dps), which were settled at 80 and 160 s yielding the results shown in Fig. 7. As we can see, doubling the stop-flow delay time, the slope of the linear equation increased about 2.5 times, thus indicating the usefulness of the system to improve sensitivity.

The multi-commuted flow systems mentioned above comprised solutions displacing by suction employing a single pumping channel. This working operational condition was conveniently implemented placing the peristaltic pump behind the detector (see Fig. 2). Nevertheless, procedure based on solvent extraction or enzymatic reaction required other system configuration to allow the coupling to analytical path of an extraction chamber or a

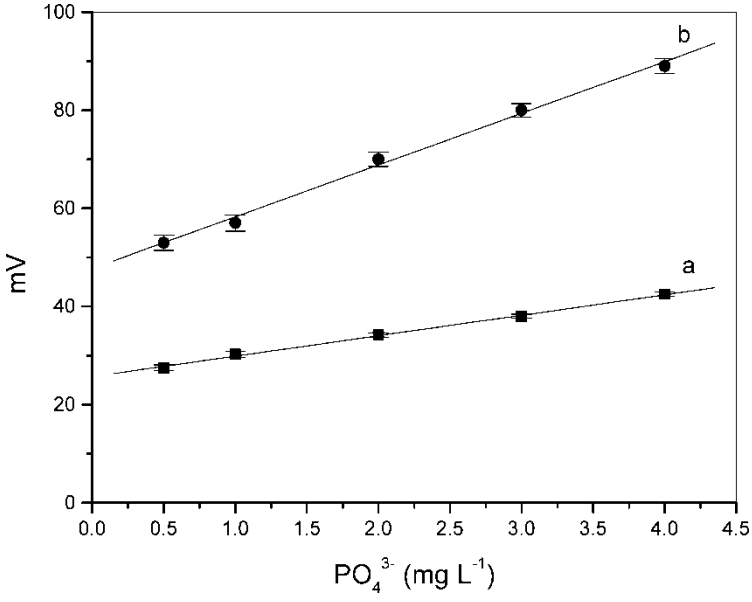

Figure 7. Effect of the stop-flow on the sensitivity. Curves *a* and *b* were obtained programming stop-flow delay times of 80 and 160 s, respectively. Linear equations (a) $y = (25.7 \pm 0.32) + (4.2 \pm 0.1)x$, $R = 0.999$; (b) $y = (48 \pm 1) + (10.5 \pm 0.4)x$, $R = 0.998$. y = mV and x = mg $L^{-1}PO_4^{3-}$.

column with immobilized enzyme. To accomplish these requirements, the multi-commuted flow system has been designed using one pumping channel for each solution.[13,15,23,27,31]

CONCLUSIONS

The flow systems presented in this manuscript allowed the demonstration of the multi-commutation process useful to accomplish different analytical assays, which could be implemented by instructing the control software without any change in the system manifold.

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