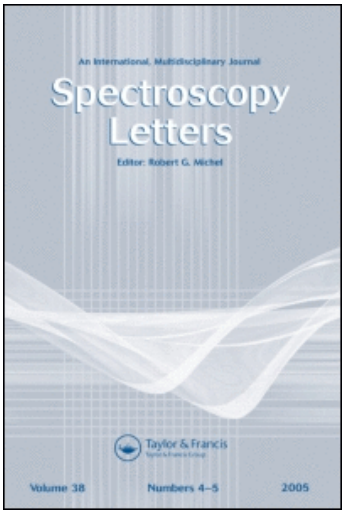

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A Multi-purpose Flow System Based on Multi-commutation

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A Multi-purpose Flow System Based on Multi-commutation

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Abstract: A multi-purpose flow system designed with solenoid valves and micropumps is proposed. The independent control of each active device was exploited to implement dilutions, calibration with a single solution, standard additions, titrations and strategies to increase sample residence time without changing the manifold hardware. Sample dispersion coefficients between 1 and 7800 were achieved by changing the sample volume and exploiting zone sampling, with coefficient of variation estimated as 6.5% for the highest dilution. Calibration curves obtained from a single standard showed slopes in agreement with those obtained by conventional batch dilutions with variations of lower than 2% between days. The possibility of implementing the standard addition method or the stopped-flow approach at different points of the manifold was demonstrated for creatinine determination in urine by the Jaffe reaction. Analytical curves for different sample residence times can be obtained for detecting matrix effects or for the analysis of colored samples. Titrations by the continuous variation method or binary search, as well as other possible applications, are discussed.

Keywords: Flow analysis, multi-commutation, multi-pumping, dilution, standard addition method, slow reactions, titrations

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INTRODUCTION

Flow systems have proved to be a useful tool for solutions management, allowing implementation of several steps of sample processing (e.g., controlled dilutions, in-line separation and concentration, reagent addition, mixing, and timing control). Applications can be found in several fields, in view of profitable characteristics such as the increase in sampling rate, precision improvement, and minimization of both reagent consumption and waste generation. Versatility is often indicated as an advantage of flow systems, because different procedures can be implemented by changing the hardware manifold. However, these changes are sometimes complex and can hinder the use of flow systems by nonexperts.

Some flow configurations and strategies have been proposed to implement different procedures without modifications in the hardware. In this sense, sequential injection analysis (SIA) has been proposed as a robust alternative for process monitoring and to implement different procedures without modifying the flow setup.[1] However, a literature survey revels that modifications in hardware are frequent. In addition, the analytical performance of such systems can be affected by the inefficient mixing by dispersion, which is characteristic of the SIA systems. The drawback is magnified when more than one solution needs to be mixed with the sample. Other proposals of multi-task flow systems can be mentioned.[2–5] A modular flow injection system coupling a sliding-bar injector and solenoid valves was proposed for implementing dilutions and the standard addition method for plant analysis by inductively coupled plasma optical emission spectrometry (ICP OES).[2] The SIA process was exploited to design a multi-task flow system devoted to operations involved in calibration, characterization, and applications of ion-selective electrodes[3] as well as for dilutions and calibration with a single standard.[4] A flow system designed with solenoid valves was proposed for implementing different flow approaches (sequential injection analysis, multi-commutation and binary sampling, sandwich techniques, and monosegmented flow analysis) for turbidimetric determination of sulfate.[5] The potential of SIA for developing multi-purpose flow systems, including those based on the lab-on-valve and bead injection processes, was emphasized and exemplified by the analysis of pharmaceuticals.[6]

In this work, a multi-purpose flow system based on multi-commutation is proposed. The flow system was designed with solenoid valves and micropumps in order to implement different procedures without changing the manifold setup. The proposal is demonstrated by implementing controlled dilutions, calibration with a single solution, the standard additions method, titrations and strategies to increase sample residence time.

MATERIALS AND METHODS

Apparatus

The flow system comprised four solenoid micropumps (Bio-Chem Valve, USA) with a nominal volume of 8 μL per pulse, two three-way solenoid valves (NResearch, USA), flow lines of 0.7 mm i.d. polyethylene tubing, and one Perspex joint point. A parallel port of a Pentium III (USA) microcomputer was used for controlling the active devices through a power drive based on an ULN2003 integrated circuit.[7] Spectrophotometric measurements were carried out with a multi-channel CCD spectrophotometer (Ocean Optics, USB2000, USA) with a tungsten–halogen light source (Ocean Optics, LS-1). Optical fibers (100 or 600 μm) were employed for the transport of the radiation. A quartz flow cell (1 cm optical path; 80 μL internal volume) was also employed.

The control software was developed in Visual Basic 6.0 (Microsoft), and the software furnished by the fabricant of the multi-channel spectrophotometer was employed for data acquisition.

Reagents and Solutions

All solutions were prepared with analytical grade chemicals and deionized water obtained from a Milli-Q system (Millipore, USA). Potassium permanganate solutions were employed to investigate sample dispersion and in the experiments with zone sampling and calibration with a single standard.

Reference solutions within 0.50 and 2.50 g L^{-1} creatinine were prepared by dilution in water of a stock solution containing 10 g L^{-1}. The buffered sodium picrate reagent (pH 13.0) was prepared by dissolving 0.80 g picric acid, 1.30 g NaOH, and 0.80 g $Na_2B_4O_7 \cdot 10H_2O$ in water and making the volume up to 100 mL. In all experiments, water was used as carrier.

For the iodometric titration, a solution was prepared by mixing 10 mL $KMnO_4$ 1.0 mmol L^{-1}, 100 μL H_2SO_4 (98%), 10 mL KI 0.10 mol L^{-1}, and 10 mL starch (1%). The volume was made up to 100 mL with water. For more accurate titrations, starch can be prepared separately or titration can be carried out by measuring directly the iodine consumption. Ascorbic acid solutions were prepared immediately before the experiment.

Flow Diagram and Procedures

The flow system (Fig. 1) was designed to implement different procedures involved in sample processing without changes in the hardware. As one micropump is employed for each solution handled, the flow system allows implementing procedures involving up to four solutions. During actuation,

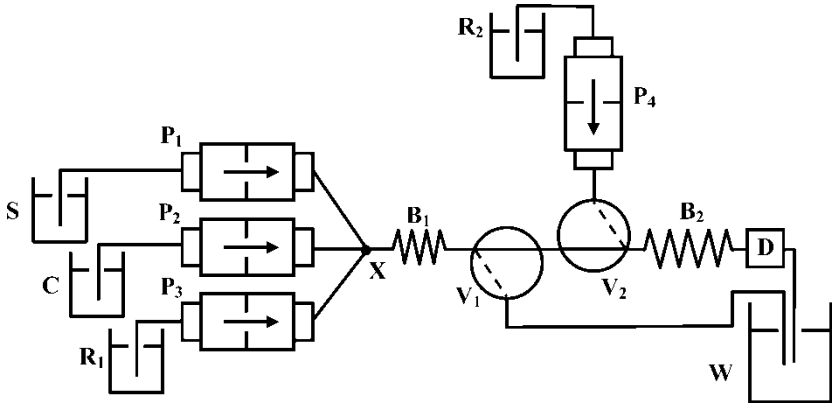

Figure 1. Flow diagram of the multi-purpose flow system. P_1–P_4, solenoid micropumps; V_1, V_2, solenoid valves; S, sample; C, carrier (H_2O); R_1, R_2, reagent, standard, or carrier; B_1, 50-cm-long coil; B_2, 100-cm-long coil; D, CCD spectrophotometer; W, waste. For details, see text.

the pumps were operated at 2 Hz (ca. 960 μL min^{-1}). Polyethylene tubes (0.7 mm i.d., 5 cm long) were employed to connect the micropumps to the valves and confluences.

In all procedures, the binary sampling approach[7] was adopted for solutions handling. The volume of each solution was defined by the programmed number of pulses of the corresponding micropump. Small aliquots of each solution were inserted in tandem in the flow system, generating a sampling profile that can be repeated until completing the programmed number of sampling cycles. One sampling cycle was employed except when mentioned. Valves V_1 and V_2 were employed for sample zone splitting and to avoid contamination when concentrated solutions were added by pump P_4, respectively. For sample replacement, valve V_1 is switched on while pump P_1 is actuated (10 pulses) to fill the connection tube with a new sample. Pump P_2 is then actuated (70 pulses), and the sample aliquot is removed by the carrier through valve V_1. Specific description of each procedure is presented next.

Controlled Dilution

Three strategies were implemented for controlled dilution with the flow system operated according to the switching course in Table 1. Experiments were carried out with $KMnO_4$ solutions, with spectrophotometric measurements at 525 nm. Formerly the sample volume was varied by changing the number of pulses of P_1 (1 to 160 pulses) to attain different sample dispersion (Table 1, A, step 1). Then, the pump P_2 was pulsed to carry the sample zone toward the flow cell (Table 1, B, step 2). Higher dilutions were implemented with the zone sampling

Table 1. Switching course of the active devices for online dilutions

Step	Description	P_1	P_2	P_3	P_4	V_1	V_2	Pulse
A. Online dilution								
1	Sample insertion	1/0	0	0	0	0	0	1–160[a]
2	Sample transport and signal measurement	0	1/0	0	0	0	0	200
B. Progressive dilution								
1	Sample insertion	1/0	0	0	0	0	0	1
2	Sampling zone transport and dispersion	0	1/0	0	0	0	0	35–100[a,b]
3	Sample zone (tail) carried toward waste	0	1/0	0	0	1	0	150
4	Sample transport and signal measurement	0	1/0	0	1/0	1	1	200
C. Zone sampling								
1	Sample insertion	1/0	0	0	0	0	0	1
2	Sample zone (front) carried toward waste	0	1/0	0	0	1	0	34–80[a]
3	Sample zone carried toward B_2	0	1/0	0	0	0	0	1
4	Sample zone (tail) carried toward waste	0	1/0	0	0	1	0	100
5	Sample transport and signal measurement	0	1/0	0	0	0	0	200

P_1, sample; P_2, P_4, H_2O; 0, valve off; 1, valve on; 1/0, electric pulse applied to the micro-pump. Other symbols are described in the text.

[a]Variable parameters.

[b]Correspond with 1–66 pulses of the dispersed sample in B_2.

approach. The first strategy, named "progressive dilutions" (Table 1, B), was implemented from one sample pulse that undergoes dispersion in B_1 (step 1). The number of pulses of P_2 (35–100) defines the portion of the dispersed zone (front) transported to coil B_2 (step 2), the tail being transported toward waste in step 3. Pumps P_2 and P_4 were then simultaneously actuated to carry the sample zone toward the flow cell (step 4). For the second strategy, the multi-purpose flow system was operated according to the switching course in Table 1, C. A sample aliquot (one pulse) is injected in coil B_1 (step 1) and

undergoes dispersion. The front and tail edges of the dispersed zone were transported toward waste through valve V_1 (steps 2 and 4, respectively) and an intermediary fraction (equivalent to one pulse) was transported to coil B_2. The portion of the sample zone to be resampled is defined by the number of pulses of P_2 in step 2. On the other hand, the amount resampled is defined by the number of pulses of the carrier in step 3 (in this work, one pulse was employed in all experiments). The fraction of the dispersed zone in coil B_2 was then sent to detector (step 5).

Calibration with a Single Standard

For obtaining an analytical curve from a single standard solution, the flow system was operated as described in Table 1, A, by varying the number of sample pulses between 1 and 20. Experiments were carried out with $KMnO_4$ solutions as sample.

Increase of the Sample Residence Time

For implementing the stopped-flow approach, the flow system was operated according to the switching course described in Table 2, A. Two sample pulses and eight reagent pulses were introduced into the analytical path by the micropumps P_1 and P_3, respectively (steps 1 and 2, Table 2, A). The sample zone was carried through coil B_2 by 100 or 178 pulses of the pump P_2, in order to stop the flow with the sample zone at B_2 or at the flow cell, respectively (step 3). After a predefined time interval (step 4), the sample zone was transported by actuating the pump P_4 (step 5). For the creatinine/picrate system, spectrophotometric measurements were carried out at 520 nm.

Sample processing is similar for measurements with two residence times (Table 2, B, steps 1 and 2), but pump P_2 is actuated to displace half of the sample zone to coil B_2 (step 3). It was observed in experiments with dye solutions that two equal signals were obtained with 55 pulses of the carrier in step 3. P_4 is then pulsed to carry the first sample zone aliquot to detection, while the second part remains trapped at B_1 (step 4). After measurement, P_2 is actuated (250 pulses) to transport the retained sample zone to the flow cell (step 5). Additional washing can be implemented in step 6.

The Standard Addition Method

For implementing the standard addition method, the flow system was operated as described in Table 3. Two pulses of the sample and variable amounts of a creatinine reference solution (0 to 10 pulses) were inserted in coil B_1 (steps 1 and 2). The sample zone was transported toward coil B_2 (step 3). Sample and standard solutions are mixed by dispersion and also by the pulsed flow characteristic of flow systems with solenoid micropumps before addition of

Table 2. Switching course of the active devices for implementing strategies to increase sample residence time

Step	Description	P_1	P_2	P_3	P_4	V_1	V_2	Pulse
A. Stopped flow								
1	Sample insertion	1/0	0	0	0	0	0	2
2	Chromogenic reagent insertion	0	0	1/0	0	0	0	8
3	Sampling zone transport	0	1/0	0	0	0	0	100[a] or 178[b]
4	Stopped flow	0	0	0	0	0	0	t[c]
5	Sample transport and signal measurement	0	0	0	1/0	0	1	200
B. Measurements with two residence times								
1	Sample insertion	1/0	0	0	0	0	0	2
2	Reagent insertion	0	0	1/0	0	0	0	8
3	Sample zone transported toward B_2	0	1/0	0	0	0	0	55
4	Sample transport and signal measurement 1	0	0	0	1/0	0	1	200
5	Sample transport and signal measurement 2	0	1/0	0	0	0	0	250
6	Additional washing	0	0	0	1/0	0	1	100

P_1, P_2, P_3, P_4: sample, H_2O, picrate reagent, H_2O. Other symbols are described in the text or in Table 1.

[a]Sample zone stopped at B_2.

[b]Sample zone stopped at the flow cell.

[c]Stopping time: $0 \leq t \leq 100$ s;

the picrate chromogenic reagent by binary sampling (steps 4 and 5) and detection (step 6).

Online Titrations

As described in Table 4, iodometric titration by the continuous variation method was performed by actuating the pumps P_1 and P_3 alternately (steps 1 and 2). In each step of the titration, the colored titrant volume is increased by maintaining constant the total pulse number (32 for the model titration). After each sampling step, the sample zone is carried toward the flow cell for the spectrophotometric measurement (step 3). Titration by binary search was analogously implemented,

Table 3. Switching course of the active devices for online standard additions

Step	Description	P_1	P_2	P_3	P_4	V_1	V_2	Pulse
1	Sample insertion	1/0	0	0	0	0	0	2
2	Standard insertion	0	0	1/0	0	0	0	0–10[a]
3	Sample zone transport	0	1/0	0	0	0	0	45
4	Reagent insertion	0	0	0	1/0	0	1	1[b]
5	Sampling zone transport	0	1/0	0	0	0	0	2[b]
6	Sample transport and signal measurement	0	1/0	0	0	0	0	200

P_1, P_2, P_3, P_4: sample, H_2O, 0.50 g L^{-1} creatinine, picrate reagent. Other symbols are described in the text or in Table 1.

[a]Variable parameter.

[b]Ten sampling cycles.

according to the strategy previously described.[8] Absorbance measurements were carried out at 580 nm. Suitable sample and titrant mixing was assured by the binary sampling strategy[7–9] and by the inherent pulsed flow.[10] Volumes were corrected considering the aliquots dispensed by each micropump (5.5 μL titrant and 8.5 μL sample).

Table 4. Switching course of the active devices for implementing online titrations

Step	Description	P_1	P_2	P_3	P_4	V_1	V_2	Pulse
1	Sample insertion	1/0	0	0	0	0	0	p[a]
2	Titrant insertion	0	0	1/0	0	0	0	q[a]
3	Sample transport and signal measurement	0	1/0	0	0	0	0	200

P_1, P_2, P_3: sample, H_2O, titrant. Other symbols are described in the text or in Table 1.

[a]$p + q = 32$ pulses.

RESULTS AND DISCUSSION

General Characteristics

The multi-purpose flow system presents profitable characteristics of the multi-commutation[7,9] and multi-pumping[10] processes. Versatility is increased

because each solenoid valve or micropump can be independently controlled, resulting in an active manifold that can be reconfigured by the control software in order to implement different tasks. The micropumps were actuated for sampling and propulsion, yielding a compact manifold to implement procedures characterized by low reagent consumption and reduced effluent generation. For example, as will be discussed next, the creatinine determination consumes 350 μg picric acid per determination, even with a reagent excess in the sample zone (reagent volumetric fraction = 0.7). The reagent consumption is ca. 1000 and 85-fold lower than that observed in batch[11] and continuous flow procedures,[12] respectively. Even a previously proposed flow system based on multi-commutation presents reagent consumption considerably higher.[13] Other important characteristic is the pulsed flow that contributes to improve the sample/reagents mixing. This goal is also achieved by the binary sampling strategy, because small sample and reagent aliquots alternately inserted into the analytical path are efficiently mixed. The active devices have been used for more than 500 hours without appreciable changes in performance.

Controlled Dilutions

Dilutions are often required in spectrophotometry in view of the narrow linear response range characteristic of the technique. In batch analysis, this task can be tedious and time-consuming when a lot of samples need to be analyzed or when analyte concentrations vary in a large interval. On the other hand, although controlled dilution can be suitably implemented in flow systems, changes in manifold setup are often necessary for implementing variable dilutions.

The multi-purpose flow system allows attaining different sample dilution degrees without changing the flow setup, as demonstrated by experiments with a colored solution ($KMnO_4$). This can be initially achieved by changing the number of sample pulses (Fig. 2). Sample dispersion coefficients (D)[14] within 1 and 33 were estimated for pulse numbers varying within 1 and 160. Limited dispersion is achieved for more than 14 pulses (>120 μL sample). On the other hand, a 33-fold sample dilution can be implemented from one sample pulse, with a coefficient of variation estimated as 3.0% (n = 10).

Higher dilutions can be implemented by the zone sampling approach, and two strategies were evaluated in this sense. In the former (Fig. 3A), the front of the sample zone dispersed in B_1 was carried toward B_2. Different dispersion degrees can be achieved by modifying the fraction of the sample zone directed to measurement. The highest dispersion ($D = 1368$) is achieved when the amount equivalent to one pulse of the disperse sample zone is processed. The lower dispersion ($D = 33$) corresponds with the processing of one sample pulse without zone sampling (Fig. 3A). This situation is achieved when more than 60 pulses of the carrier are employed in step 2B (Table 1). The second strategy was similar to that adopted in the original

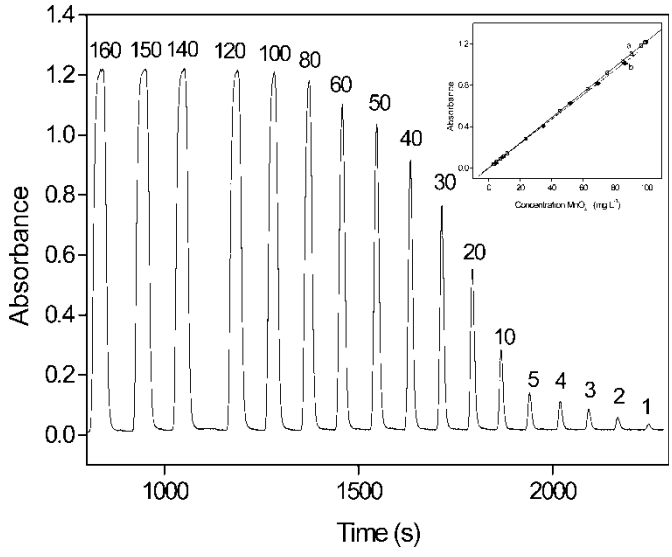

Figure 2. Online dilution and calibration with a single standard. Transient signals refer to a 100 mg L^{-1} $KMnO_4$ solution processed with different pulse numbers (upper numbers). The inset shows the linear response of the absorbance versus concentration of $KMnO_4$ for *a*, online dilution; *b*, conventional calibration.

proposal of zone sampling with a sliding-bar injector.[15] A fraction equivalent to one sample pulse was selected from different portions of the dispersed zone (Fig. 3B), resulting in dispersion coefficients within 430 and 7800. The precision is good (CV = 6.5%, n = 4) even for the highest dilution factor as a consequence of the reproductive volumes dispensed by the micropumps. Thus, the multi-purpose flow system is a powerful tool for controlled (and variable) dilutions. This characteristic is very interesting also in automatic procedures, in which sample processing conditions can be changed as function of a prior measurement to match the analyte concentration to the linear response range of the detector.[16]

When a chemical reaction is involved, it is necessary to ensure an excess of reagent in the sample zone. This can be implemented by selecting an appropriate reagent volume and concentration that can be distributed in the entire dispersed sample zone. This strategy is similar to that adopted in the well-established zone sampling approach.[13–16]

Calibration with a Single Standard

For the model system evaluated, a linear relation between the analytical signal (A) and the number of pulses (Np) was observed: $A = 0.00598 + 0.0274\ Np$, r = 0.999 ($1 < Np < 20$). The concentration of the reference solution can

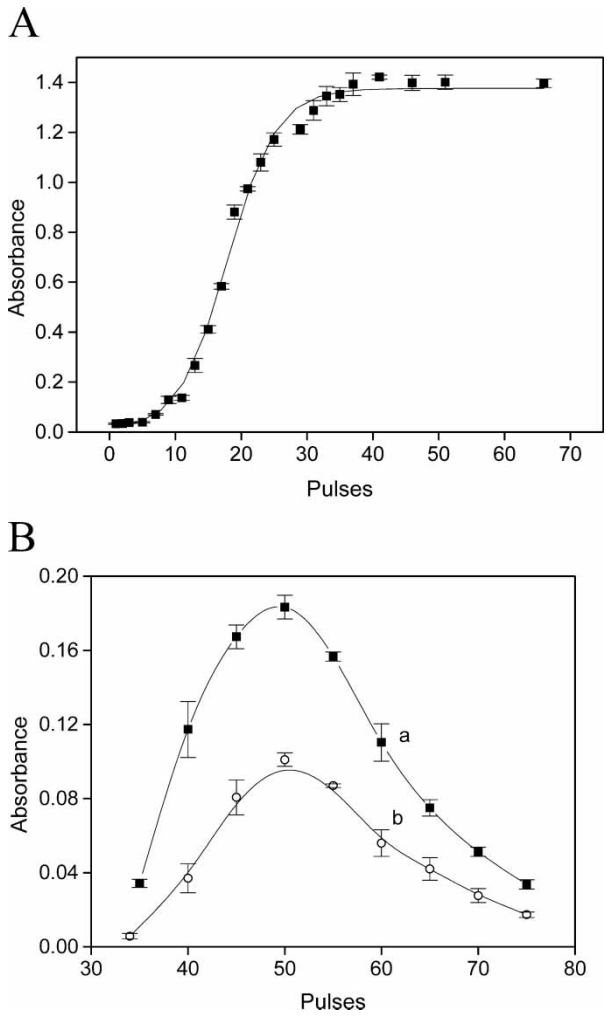

Figure 3. Zone sampling dilution by (A) increasing the volume of the dispersed zone transported toward detection and (B) selecting different fractions of the dispersed zone to be directed toward detection: *a*, 10,000 mg L^{-1}; *b*, 5,000 mg L^{-1} $KMnO_4$.

then be related to the number of pulses, and a calibration curve can be obtained by varying Np for a single standard. The insert in Fig. 2 shows calibration curves obtained with this strategy or with reference solutions with variable concentrations and a fixed sample volume. Even in different days, variations in slope of the curves were $<2\%$. This strategy can also be adopted for recalibration during the analysis of a lot of samples. About 10 min is required to obtain an analytical curve with five points and measurements in duplicate. This time is ca. 35% lower than the required for a conventional calibration.

As described in the previous section, a reagent excess needs to be assured in order to obtain a linear response.

Increase of the Sample Residence Time

Sample residence times (t_R) in flow injection systems are usually lower than 30 s, which are suitable for fast or moderately slow reactions. In conventional FIA systems, the increase in t_R usually results in increase of sample dispersion, decrease of the sampling rate, or both. Some strategies were proposed to increase sample residence time without affecting the sample dispersion, such as the zone trapping[17] and the stopped-flow[18] approaches. Stopping the flow at the measurement cell make it possible to monitor the reaction development, which is useful to obtain kinetic data or to improve selectivity. On the other hand, stopping the flow at the reactor coil is a preferred strategy when the reaction product is photosensitive. Both strategies were implemented for creatinine determination with the picrate reagent with the multi-purpose flow system (Fig. 4A). A linear relation between the analytical signal (A) and the stopping period (t) in B_2 was observed: $A = 0.144 + 0.00885\ t$, $r = 0.993$, in view of the increase of product formation without significant sample dispersion. Sensitivity was higher when the sample zone was stopped at the reactor coil B_2 in comparison with the stopped-flow at the flow cell. This can indicate that the product is photosensitive, but this effect needs to be further investigated.

Transient signals for two sample residence times were obtained when the flow system was operated according to the switching course described in Table 2, B (Fig. 4B). Sensitivity is ca. fourfold higher for the analytical curve referent to the second transient signal as a result of the higher residence time. This strategy can be explored for detecting matrix effects that affect the reaction kinetics and consequently the analytical signal in fixed-time methods, such as FIA.[19] Results determined by both analytical curves are in agreement only if such effects are absent. In the presence of significant matrix effects, the increase of the sample residence time as previously described can be an alternative. Other application of measurements in two sample residence times correspond with the compensation of absorption of radiation by colored species in the samples by employing the difference between the signals as the analytical parameter.[13]

The Standard Addition Method

Matrix effects are often observed in spectrophotometric procedures. For example, in the determination of creatinine in urine with the multi-purpose flow system, the slope of the analytical curve obtained in the sample medium was ca. 10% higher than that obtained for solutions prepared in water. The standard addition method is an alternative procedure for samples

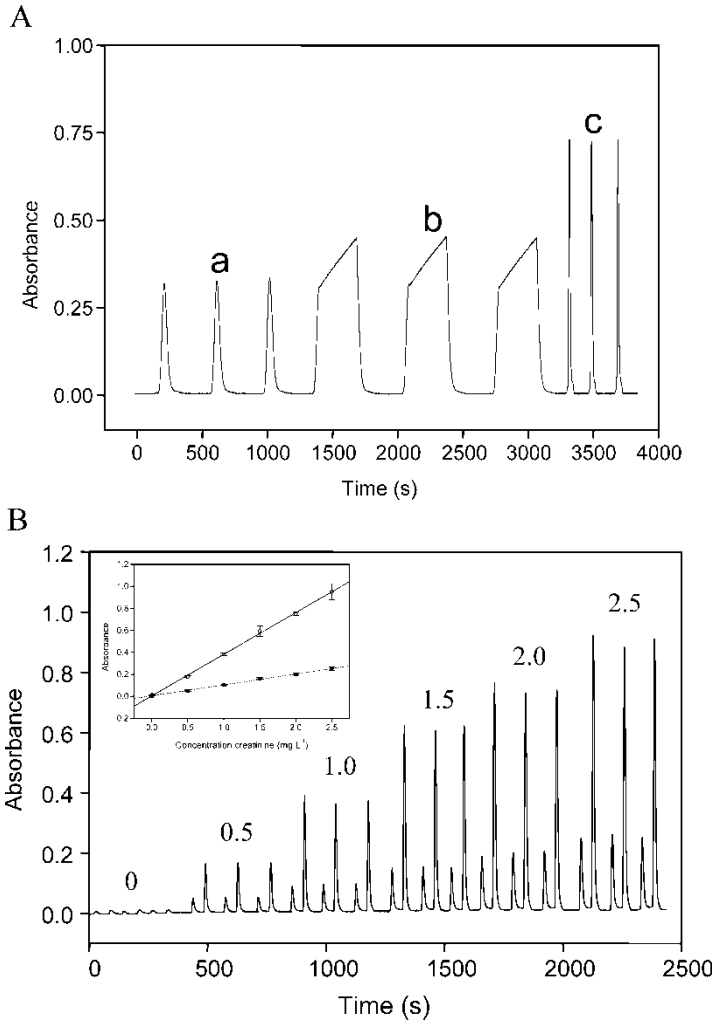

Figure 4. Strategies to increase sample residence time. (A) Measurements without stopping the flow (*a*), and stopping the flow at the flow cell (*b*) or in coil B_2 (*c*) for 60 s. (B) Measurements with two residence times; numbers indicate creatinine concentrations in g L^{-1}. The inset shows the linear response for reference solutions processed under two residence times.

presenting matrix effects. However, as a set of solutions needs to be prepared for each sample, the analysis can become very laborious. This strategy was implemented when the multi-purpose flow system was operated according to the routine described in Table 3. Analogous to the batch procedure, different volumes of the standard solution were added to a fixed volume of the sample by varying the number of pulses. The creatinine concentration

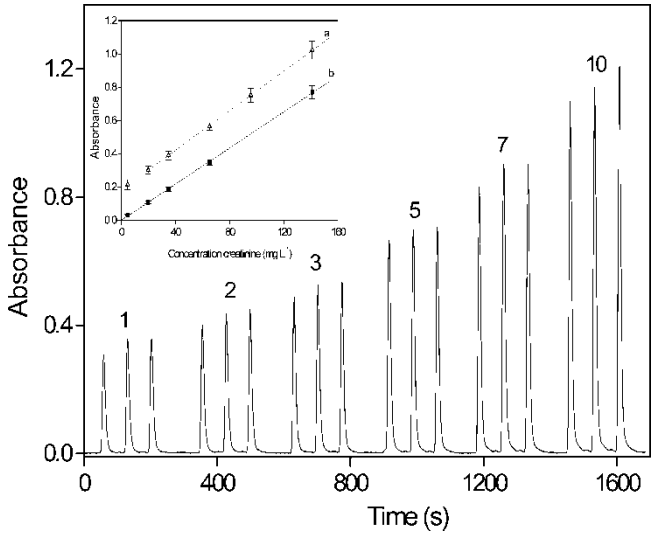

Figure 5. Online implementation of the standard addition method. Transient signals obtained for additions of 0.50 g L^{-1} creatinine to a urine sample. Numbers indicate the pulses of the reference solution. The inset shows the calibration curve for the online standard addition in *a*, urine sample; *b*, water.

added to the sample was estimated by considering the results for the model system presented in Fig. 2. Results presented in Fig. 5 show that a linear relation between the analytical signal and the creatinine concentration in the center of the sample zone was observed. The creatinine concentration in the urine sample determined with this strategy (0.558 ± 0.031 mg L^{-1}) was in agreement with that determined by the conventional procedure (0.588 ± 0.059 mg L^{-1}) at the 95% confidence level.

Online Titrations

Titrations have been performed with flow injection systems since the first articles,[20] but the proposed strategies generally require previous calibration, being more correctly named "pseudotitrations." Real titrations (without requiring a calibration curve) can be implemented with the multi-purpose flow system with different strategies. For the continuous variation method, for example, the number of pulses of sample and titrant were simultaneously varied by maintaining the total volume of the sample zone constant. As previously evaluated, mixing between sample and titrant aliquots is assured by the binary sampling strategy.[7,8] For the proposed system, the inherent pulsed flow also contributes for improving mixing.[10] The results for iodometric titration of ascorbic acid are presented in Fig. 6A. The inserted figure is very similar to a conventional spectrophotometric titration curve in which

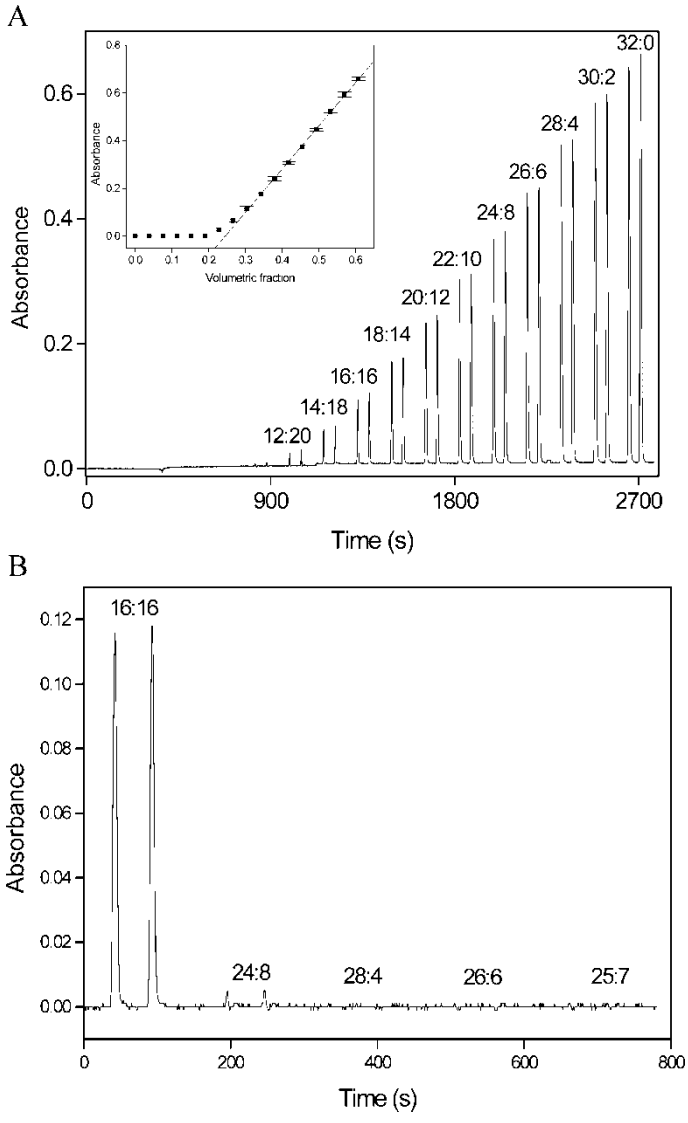

Figure 6. Iodometric titration of ascorbic acid solutions. (A) Continuous variation method. Numbers indicate the titrant/sample ratio in pulse numbers. The inset shows the titration curve as function of the titrant volumetric fraction. (B) Binary search strategy. For details, see text.

the end point can be determined by extrapolation (sample volumetric fraction = 0.25). This value is in agreement with that expected for titration of the 1 mmol L^{-1} ascorbic acid solution. This strategy can also be explored for determining stoichiometries of complexes and conditional equilibrium constants.

The end point of the titration can also be found by the binary search strategy[7] (Fig. 6B), in a time interval ca. 70% lower for the model system. As colored sample zones were generated in the first two steps (sample volumetric fraction = 0.5 and 0.75 for steps 1 and 2, respectively), the sample aliquot is increased for the third step (sample volumetric fraction = 0.875), resulting in a colorless sample zone. Sample is then processed in an intermediary condition in step 4 (sample volumetric fraction = 0.812) also resulting in a colorless sample zone. After step 5 (sample volumetric fraction = 0.781), it can be concluded that the end point of the titration is achieved for sample volumetric fractions within 0.75 and 0.78 (titrant volumetric fraction within 0.25 and 0.22). The end point can be found more accurately by increasing the total number of pulses for the titration.

Other Applications

The multi-purpose flow system can also be applied to implement procedures involving different chemistries with up to two reagent solutions. This makes feasible the sequential determination of different species by changing the solutions R_1 and R_2 as well as simultaneous determination exploiting kinetic discrimination or employing a modifier reagent. These strategies can also be adopted for speciation or to implement the accuracy assessment strategy.[21] As previously mentioned, the capability to determine conditional physical chemistry parameters is also inherent.

CONCLUSIONS

Different steps usually involved in sample processing were implemented without changing the manifold setup. The experiments involving dilutions showed that sample dispersion coefficients varying from 1 to 7800 can be implemented by changing the sample volume and exploiting the zone sampling approach for different portions of the dispersed zone. Even for the highest dispersion, the precision is not lessened, being much better than that achieved in a similar batch sequential dilution. As a reference solution can be submitted to different dilutions, online calibration with a single standard and implementation of the standard additions method can be performed, reducing time and sample/reagent consumption. Other laborious procedures, such as titrations, can be performed fast by continuous variation method or binary search strategy, minimizing time and sample/reagent consumption. Sample residence time can be changed in different ways in order to perform kinetic measurements or to implement fast or moderately slow reactions without hindering the sampling rate.

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REFERENCES

1. Ruzicka, J.; Marshall, G. D. Sequential injection: a new concept for chemical sensors, process analysis and laboratory assays. *Anal. Chim. Acta* **1990**, *237*, 329–343.
2. Reis, B. F.; Giné, M. F.; Krug, F. J.; Bergamin-Filho, H. Multipurpose flow injection system. Part 1. Programmable dilutions and standard additions for plant digests analysis by inductively coupled plasma atomic emission spectrometry. *J. Anal. At. Spectrom.* **1992**, *7*, 865–868.
3. Fernandes, R. N.; Sales, M. G. F.; Reis, B. F.; Zagatto, E. A. G.; Araújo, A. N.; Montenegro, M. C. B. S. M. Multi-task flow system for potentiometric analysis: its application to the determination of vitamin B6 in pharmaceuticals. *J. Pharm. Biomed. Anal.* **2001**, *25*, 713–720.
4. Baron, A.; Guzman, M.; Ruzicka, J.; Christian, G. Novel single standard calibration and dilution method performed by the sequential injection technique. *Analyst* **1992**, *117*, 1839–1844.
5. Vieira, J. A.; Raimundo Jr., I. M.; Reis, B. F. Turbidimetric determination of sulphate employing gravity flow-based systems. *Anal. Chim. Acta* **2001**, *438*, 75–81.
6. Solich, P.; Polásek, M.; Klimundová, J.; Ruzicka, J. Sequential injection technique applied to pharmaceutical analysis. *Trends Anal. Chem.* **2003**, *22*, 116–126.
7. Reis, B. F.; Giné, M. F.; Zagatto, E. A. G.; Lima, J. L. F. C.; Lapa, R. A. Multicommutation in flow analysis. Part 1. Binary sampling: concepts, instrumentation and spectrophotometric determination of iron in plant digests. *Anal. Chim. Acta* **1994**, *293*, 129–138.
8. Korn, M.; Gouveia, L. F. B. P.; de Oliveira, E.; Reis, B. F. Binary search in flow titration employing photometric end-point detection. *Anal. Chim. Acta* **1995**, *313*, 177–184.
9. Rocha, F. R. P.; Reis, B. F.; Zagatto, E. A. G.; Lima, J. L. F. C.; Lapa, R. A. S.; Santos, J. L. M. Muticommutation in flow analysis: concepts, applications and trends. *Anal. Chim. Acta* **2002**, *468*, 119–131.
10. Lapa, R. A.S.; Lima, J. L. F. C.; Reis, B. F.; Santos, J. L. M.; Zagatto, E. A. G. Multi-pumping in flow analysis: concepts, instrumentation, potentialities. *Anal. Chim. Acta* **2002**, *466*, 125–132.
11. Horwitz, W. *Official Methods of Analysis of AOAC International*, 17th edn; AOAC International, 2002.
12. Falcó, P. C.; Genaro, L. A. T.; Lloret, S. M.; Gomez, F. B.; Cabeza, A. S.; Legua, C. M. Creatinine determination in urine sample by batchwise kinetic procedure and flow injection analysis using Jaffe reaction: chemometric study. *Talanta* **2001**, *55*, 1079–1089.
13. Araújo, A. N.; Lima, J. L. F. C.; Reis, B. F.; Zagatto, E. A. G. Multicommutation in flow analysis. Part 3. Spectrophotometric kinetic determination of creatinine in

urine exploiting a novel zone sampling approach. *Anal. Chim. Acta* **1995**, *310*, 447–452.
14. Ruzicka, J.; Hansen, E. H. *Flow Injection Analysis*, 2nd edn; John Wiley Sons: New York, 1988.
15. Reis, B. F.; Jacintho, A. O.; Mortatti, J.; Krug, F. J.; Zagatto, E. A. G.; Bergamin-Filho, H.; Pessenda, L. C. R. Zone-sampling processes in flow-injection analysis. *Anal. Chim. Acta* **1981**, *123*, 221–228.
16. Rocha, F. R. P.; Martelli, P. B.; Frizzarin, R. M.; Reis, B. F. Automatic multicommutation flow system for wide range spectrophotometric calcium determination. *Anal. Chim. Acta* **1998**, *366*, 45–53.
17. Krug, F. J.; Reis, B. F.; Giné, M. F.; Zagatto, E. A. G.; Ferreira, J. R.; Jacintho, A. O. Zone trapping in flow-injection analysis. Spectrophotometric determination of low levels of ammonium ion in natural waters. *Anal. Chim. Acta* **1983**, *151*, 39–48.
18. Ruzicka, J.; Hansen, E. H.; Mosbaek, H. Stopped-flow and merging zones — a new approach to enzymatic assay by flow injection analysis. *Anal. Chim. Acta* **1979**, *106*, 207.
19. Zagatto, E. A. G.; Rocha, F. R.P.; Martelli, P. B.; Reis, B. F. Detecting and circumventing sources of inaccuracy in flow analysis. *Pure Appl. Chem.* **2001**, *73*, 45–54.
20. Ruzicka, J.; Hansen, E. H.; Mosbaek, H. Flow injection analysis. Part IX. A new approach to continuous flow titrations. *Anal. Chim. Acta* **1977**, *92*, 235.
21. Oliveira, C. C.; Sartini, R. P.; Zagatto, E. A. G.; Lima, J. L. F. C. Flow analysis with accuracy assessment. *Anal. Chim. Acta* **1997**, *350*, 31–36.