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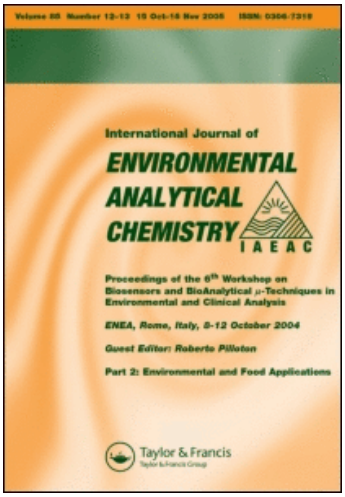

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SIMULTANEOUS DETERMINATION OF IONIC SPECIES BY SEQUENTIAL INJECTION ANALYSIS USING A SANDWICH TECHNIQUE WITH LARGE SAMPLE VOLUMES

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In this work we investigated the feasibility of using large sample volumes in sequential injection analysis with a view of determining various analytical parameters in a unique sample injection.

The use of two reagents separated by a large sample volume allows the determination of two different parameters in the sample. The accuracy with which the two resulting peaks (one for each reaction) can be resolved and the linear working ranges are dependent on both the injected sample volumes and reagent, together with their concentrations. Under given conditions, the proposed methodology can be applied over a certain analyte concentration range.

For validation purposes the proposed methodology was applied to the simultaneous determination of Fe(II) and nitrite ions for their well-known *o*-phenanthroline and Griess reactions, respectively.

KEY WORDS: Sequential injection analysis, nitrite, Fe(II).

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INTRODUCTION

The development of sequential injection analysis (SIA) in 1990 by Ruzicka and Marshall[1] gave rise to a substantial operational simplification of injection techniques. In fact, the SIA technique has been the subject of many studies regarding development and assessment over the past few years. The widespread use of SIA in routine analyses has been mainly constrained by the high degree of automatization required by this technique together with the existence of few liquid drivers such as those originally employed by its proponents (sinusoidal flow pumps). In order to popularize the former technique, Ivaska and Ruzicka[2] proposed the use of a more affordable type of liquid driver, *viz.* the peristaltic pump. Cladera *et al.*[3] found titration autoburettes to provide acceptable results and to allow the construction of SIA assemblies from affordable parts usually available in laboratories. Based on the former system, Gómez *et al.*[4] used multicomponent SIA for the simultaneous determination of Ca and Mg in waters by using a chromogenic reagent.

Alonso *et al.*[5] reported on the use of an 8-port injection valve to insert fairly large sample volumes between two different reagents in FIA systems (sandwich techniques). This technique has been applied to various multicomponent determinations;[6–8] besides,

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Montesinos *et al.*[9] developed a mathematical model to describe the shape of the peaks obtained under different experimental conditions.

The ease with which samples and reagents can be introduced into a sequential injection system prompted us to employ the sandwich technique by using a large sample volume in order to physically separate the two reagents involved thus subjecting the sample to two different reactions. This should enable the sequential determination of two analytes in a unique sample injection. This paper demonstrates the feasibility of the proposed application.

EXPERIMENTAL

Apparatus and software

The experimental set-up used was an automatic SIA system previously developed by the authors[3]. The system involves the following elements:

An IBM PC or compatible computer to control all other elements in addition to acquiring and processing data in real time.

A Crison 738 autoburette (Barcelona), with programable speed, governed by the computer via a serial RS-232C interface and acting as the propelling pump.

One laboratory-made electromechanical valve controller that was interfaced to the computer via a PC-8225 board from Flytech Technology (Taiwan). The controller was fitted with two independent valves (*viz.* a Rheodyne 5020 injection valve and a Rheodyne 5011 six-way valve).

A Hewlett-Packard HP-8452A diode array spectrophotometer connected to the computer via its HP-IB interface and furnished with a flow-through cell of 1-cm light path and 18 μL inner volume.

A Gilson Sample Changer-222 (France) automatic sampler governed by the computer via a RS-232C interface.

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Both instrumental control and data acquisition and processing were performed with the aid of the program DARRAY*, v. 2.0, developed by the authors' group.

Connectors were built from pieces of PTFE tubing of the following dimensions: 300 cm × 1.5 mm ID (connection between the burette and the selection valve) and 54 cm × 0.8 mm ID (connection between the selection valve and the detector). All other tubing was 0.8 mm ID. The autoburette was fitted with a 5-mL syringe which dispensed the fluid at a flow-rate of 2.1 mL/min (the lowest available).

Reagents

The hydrodynamic variables of the system were characterized by using a solution containing 0.0016% (w/v) Methylene Blue, the absorbance of which at the observation wavelength (612 nm) was 1.76 A.U.

* The software used in this work can be obtained on request from SCIWARE, Banco de Programas, Departament de Química, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain.

The following solutions were used in the experiments involving a chemical reaction:

(a) 10 mg L^{-1} Bromocresol Green in 2 10^{-4} M HCl.
(b) 0.01 and 0.001 M NaOH, prepared from pro-analysis sodium hydroxide.
(c) A 2.52 × 10^{-3} M Fe(II) standard in 0.5% (w/v) hydroxylamine, prepared from ammonium ferrous sulphate (pro-analysis) and hydroxylamine hydrochloride (pro-analysis).
(d) 2.8 × 10^{-4} M *o*-phenanthroline in acetic acid/acetate buffer at pH 4, made from 99% pure reagent.
(e) 0.011 M sodium nitrite, prepared from pro-analysis $NaNO_2$.
(f) The Griess reaction mixture, which consisted of 2% (w/v) sulphanilamide and 0.05% (w/v) *n*-(1-naphthyl)-ethylenediamine.

Procedures

The operating sequence was started by flushing the tubing and filling the reagent channels with their respective solutions. For this purpose, an appropriate volume of reagent was aspirated and driven to tube T_1 (loop situated between the burette and the selection valve), which was subsequently flushed with water. The sample channel, which received the sample from the autosampler, was similarly flushed after each sample was processed. The detector cell was filled with the carrier liquid (water) before the instrumental zero was performed.

The flow-rate was kept at 2.11 mL/min throughout reagent aspiration and data acquisition.

Dye injection

The system was hydrodynamically characterized by performing injections of variable volumes of 0.0016% (w/v) Methylene Blue at different positions. In order to change the position in the T_1 tube (*viz.* the distance travelled by the injected plug), an appropriate volume of water was aspirated after each dye injection. Changes were monitored via absorbance readings at 612 nm, which were corrected at 750 nm in order to minimize the effects of the changes in the refractive index.

Experiments with Bromocresol Green

The tests carried out with this indicator involved the successive injection of variable volumes of 0.01 M NaOH (R_1), 10 mg L^{-1} Bromocresol Green and 0.001 M NaOH (R_2). The peaks corresponding to the basic form of the indicator were monitored at 616 nm and the corresponding acid form at 444 nm, both readings being corrected at 750 nm.

Experiments with Fe(II)

Tests for the determination of Fe(II) with *o*-phenanthroline at both ends of the sample plug were carried out by aspirating according to the following sequence: 150 µL (0.5 $V_{1/2}$) of *o*-phenanthroline (R_1), 2000 µL (6.7 $V_{1/2}$) of sample containing Fe(II), and 75 µL (0.25 $V_{1/2}$) of *o*-phenanthroline (R_2). The *o*-phenanthroline concentrations of the solutions located before and after the sample were different and changed throughout the experiments. Peaks were recorded at 505 nm and corrected at 750 nm.

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Determination of Fe(II) and nitrite

The aspiration sequence for the simultaneous determination of ferrous ion and nitrite was as follows: 150 μL (0.5 $V_{1/2}$) of 1.4×10^{-3} M *o*-phenanthroline (R_1), 2000 μL (6.7 $V_{1/2}$) of sample containing Fe^{2+} and NO_2^-, and 75 μL (0.25 $V_{1/2}$) of Griess reagent (R_2). The process was monitored at 505 nm (with corrections at 750 nm), which corresponds to the absorption maximum for the product of the Fe(II)-*o*-phenanthroline reaction. The spectrum obtained for the Griess reaction was extensively overlapped with that of the Fe(II) reaction, and besides produced a signal—however, not the maximum possible—at the monitoring wavelength. The diode array spectrophotometer used would have allowed both products to be monitored simultaneously at the optimum wavelength respectively, however, a unique wavelength was used since the sensitivity shown for both products was adequate.

As an example, Table 1 describes the operational sequence used in the determination of both Fe(II) and nitrite.

RESULTS AND DISCUSSION

Characterization of hydrodynamic parameters

The SIA system used was hydrodynamically characterized by determining $V_{1/2}$ (*viz.* the injected volume that resulted in an extent of dispersion such that the product concentration at the maximum of the obtained peak was one-half of its original concentration). This parameter can be used as a measure of dispersion and hence facilitates extrapolation of the volumes used in other assemblies.

$V_{1/2}$ was determined by injecting increasing volumes of a dye (Methylene Blue) into a colourless carrier. Based on previous experience,[3] a carrier volume required to ensure that the centre of the dye plug remained at the same position (250 μL from the selection valve) was aspirated in all experiments after each dye aspiration. In this way, effects of changes regarding the distance travelled by each injected volume were avoided. The results thus obtained are shown in Figure 1; the curve was fitted to the following linear equation:[10]

$$-\log\left(1-\frac{A_{max}}{A_o}\right)=\frac{0.693\,V_s}{2.303\,V_{1/2}}$$

where A_{max} is the absorbance of the dye at the peak maximum, and A_0 corresponds to that prior injection, and V_s is the injected volume. From the slope of the curve, $V_{1/2}$ was calculated to be *ca.* 300 μL.

The recommended injected both sample and reagents volumes for SIA experiments are 0.5 $V_{1/2}$ and $V_{1/2}$, respectively[1], which are seemingly the best compromises between low sample dispersion and adequate interpenetration of sample and reagents. Being the aim of this work the simultaneous reaction of the sample with two reagents by using a sufficiently large sample volume, the volumes to be used were obviously rather different from those routinely employed for other purposes.

The dispersion of the first injected reagent increases with the sample volume due to the larger distance to be travelled by the reagent inside the system. Figure 2 shows the profiles obtained by injecting the same dye volume at different positions in relation to

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Table 1 Operational sequence used for the determination of Fe(II) and nitrite.

Step	*Switching valve*	*Burette*	*Sampler*	*Acquisition*	*Notes*
1		Initialize (initial piston pisition 0 ml)	Initialize (resting position)	No	
2	4 (Waste)	Dispense 2.225 mL		No	Place burette piston for subsequent operations
3			Next sample	Mark: M	Signals sample changeover on data record
4	6 (Sample)	Aspirate 1 mL (intermediate speed)		No	Flush and load sample channel
5	4 (Waste)	Dispense 1 mL(intermediate speed)		No	
6	3 (R_1)	Aspirate 0.150 mL (low speed)		No	Introduce sample and reagents into the reaction channel (T_1)
7	6 (Sample)	Aspirate 2 mL (low speed)		No	
8	2 (R_2)	Aspirate 0.075 mL (low speed)		No	
9				Mark: I	Signals each injection
10	1 (Cell)	Dispense 5 mL (low speed)		Yes	Acquire data
11		Load(*) 2 mL (high speed)		No	Flush detector cell
12	1 (Cell)	Dispense 2 mL (high speed)		No	
13		Load(*) 2.775 mL (high speed)		No	Adjust piston position for next cycle
14	Repeat from step 6, number of injections				
15	Repeat from step 3, number of samples				
16		Reset	Reset	No	

(*) The burette is loaded via its own two-stop valve, and therefore none of the lines of the switching valve need be used.

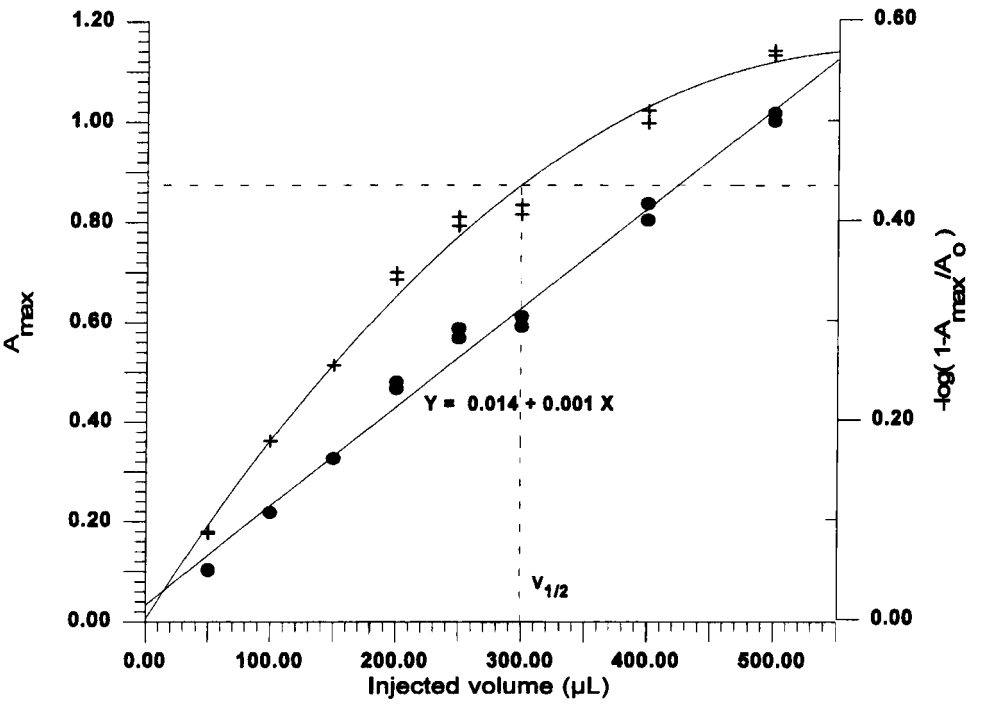

Figure 1 Determination of $V_{1/2}$. Variation of peak height with the injected volume (+) and fitting to equation 1 (•).

the selection valve. It should be noted that, while small changes in the injection position resulted in a considerably decrease in the peak height, the effect diminished as the separation was increased. It could also be observed that, using equal concentrations for the two reagents, with a separation between them of 3 $V_{1/2}$ the overlap between the signals corresponding to the two reactions is sufficiently small.

The experiments described so far only consider dispersion phenomena, however, in the presence of a reaction, kinetic and stoichiometric factors are bound to affect the profiles of the reaction products obtained. Therefore, several chemical reactions were tested in subsequent experiments in order to analyze their effects.

Influence of the injected volumes on peak resolution

Due to the large sample volume used, the first injected reagent (R_1) is bound to be more extensively dispersed than the second reagent (R_2). Therefore, it seemed logical to use higher concentrations and injected volumes of R_1 in relation to R_2.

In order to investigate the influence of the sample volume on spatial resolution in the presence of a kinetically fast reaction, a series of experiments were carried out by using an acid-base indicator (Bromocresol Green) as the sample and NaOH solutions at concentrations of 10^{-2} M and 10^{-3} M reagents R_1 and R_2, respectively. The advantage of the selected indicator is that both acid and basic form spectra are well resolved, which allowed the profiles for the reaction product or unreacted sample to be recorded provided an appropriate monitoring wavelength was used.

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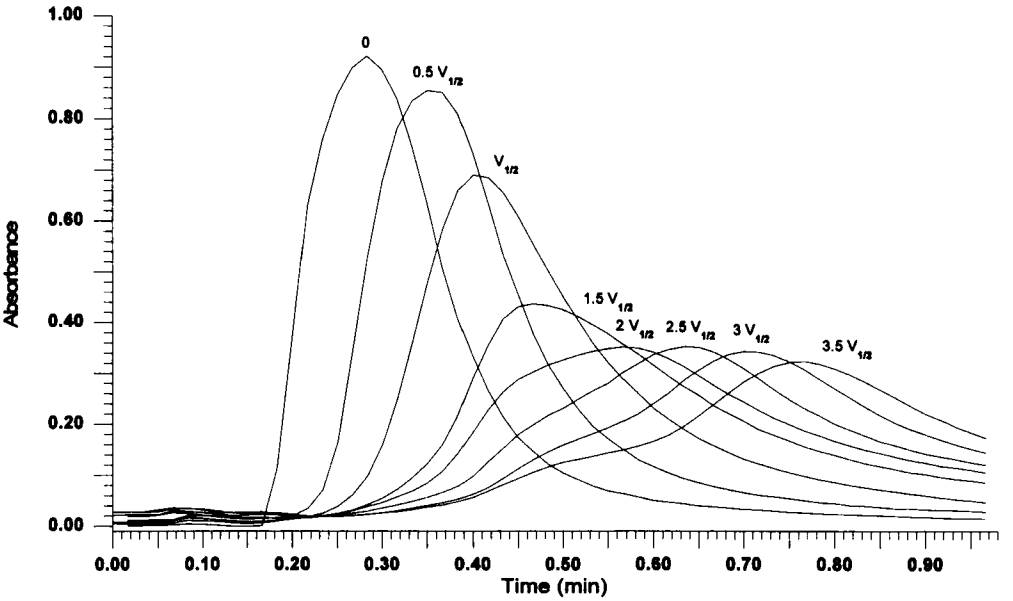

Figure 2 Dispersion profiles obtained by injecting a volume $V_{1/2}$ of dye followed by a variable volume of water.

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In Figure 3 the obtained peaks for different sample volumes are represented. These peaks were monitored through both the reaction products and unreacted sample. In this figure it can be observed -as expected- that peak separation is improved with the increase of the sample volume. A sample volume greater than 1500 μL (5 $V_{1/2}$) is required with the selected volumes and reagent concentrations in order to avoid the mutual influence of the two reactions. This fact is demonstrated by the presence of a peak corresponding to the unreacted sample.

Influence of the concentrations on resolution and linearity

The above results suggest that using R_1 and R_2 volumes of 0.5 $V_{1/2}$ and 0.25 $V_{1/2}$, respectively and a sample volume over 5 $V_{1/2}$ allows a correct spatial resolution of the peaks at given concentrations. In order to investigate the dependence of the reagent concentrations on both the spatial resolution and the linear determination range, the Fe(II)-*o*-phenanthroline reaction was used. Several experiments were performed in which the injected volumes remained constant (V_{R1} = 0.5 $V_{1/2}$, V_{R2} = 0.25 $V_{1/2}$ y V_s = 6.7 $V_{1/2}$) and the relation between the concentration of *o*-phenanthroline in both R_1 and R_2 reagents was varied. The tested concentrations were the following: $C_{R1} = 28 \times 10^{-3}$ M and $C_{R2} = 2.8 \times 10^{-3}$ M (C_{R1}/C_{R2} = 10); $C_{R1} = 14 \times 10^{-3}$ M and $C_{R2} = 2.8 \times 10^{-3}$ M (C_{R1}/C_{R2} = 5); $C_{R1} = 14 \times 10^{-3}$ M and $C_{R2} = 5.6 \times 10^{-3}$ M (C_{R1}/C_{R2} = 2.5). For each pair of concentrations the corresponding signals regarding several concentrations of Fe(II) within the range (0.7–11.3 mg/L) were obtained.

The result obtained showed that, on the one hand, the spatial resolution was quite acceptable, as expected from previous results for Bromocresol Green. On the other hand,

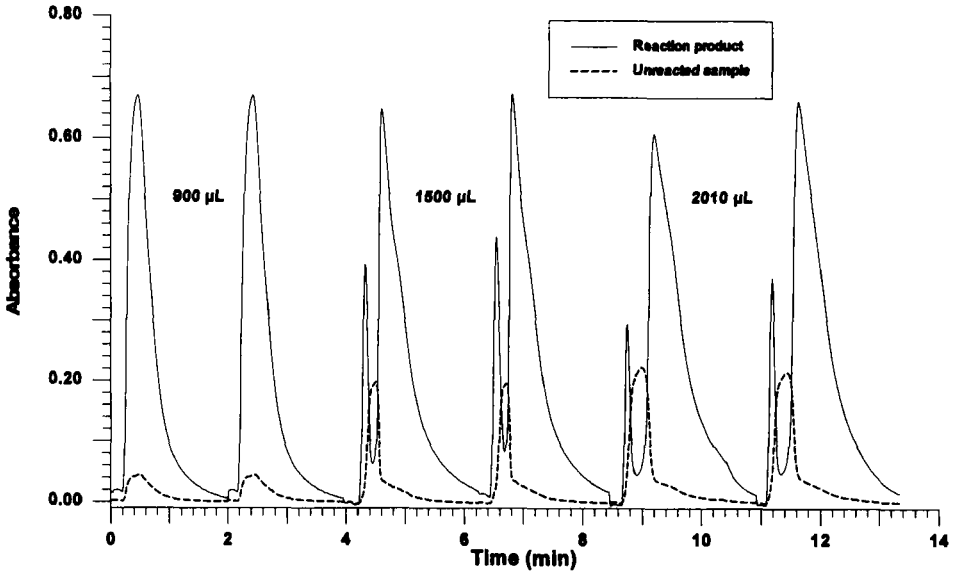

Figure 3 Peaks obtained by duplicated injections of variable volumes of Bromocresol Green by reaction with NaOH and reading at 616 nm (Reaction product) and 414 nm (Unreacted sample). $V_{R1} = 0.5\ V_{1/2}$, $[NaOH]_{R1} = 10^{-2}$ M, $V_{R2} = 0.25\ V_{1/2}$, $[NaOH]_{R2} = 10^{-3}$ M.

with regard to linearity, R_1 behaved quite acceptably in all experiments, however R_2 required a higher concentration in order to obtain a sufficiently wide linear range. By using the highest concentration of R_2 a linear range response for this reagent of 0.7–7 mg/L of Fe(II) was obtained, whereas for R_1 the linear range was in all cases the range of concentrations corresponding to the Fe(II) studied.

It should be noted that lower concentrations of R_1 did not affect the width of the linear range, but improved the resolution at very low sample concentrations. Therefore, an appropriate choice of the reagent concentrations should ensure sufficiently wide determination ranges for both peaks.

Determination of two analytes

In order to examplify the determination of two different analytes in a unique sample injection was quantified Fe(II) with *o*-phenanthroline and nitrite by the Griess reaction. The former reaction was previously studied in depth and the results extrapolated to the present application. The Griess reaction was previously optimized by our group for implementation in FIA and SIA systems. We used the Griess reagent as R_2 and *o*-phenanthroline as R_1.

In order to study both the spatial resolution and the application of the linear ranges regarding the proposed methodology two experiences were performed. In the first experience the concentration of nitrite (3.06 mg/L) remained constant, whereas the concentration of Fe(II) varied within the range 0.84—11.26 mg/L. In the second experience, concentration of Fe(II) (2.81 mg/L) remained constant, whereas concentration of nitrite varied between 1.02 and 7.15 mg/L.

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In all cases, both analytes were correctly resolved in space; besides, linearity was quite acceptable and each reaction contributed insignificantly to the other. The linear ranges obtained agreed with those of the concentration ranges studied. The relative standard deviation of the peaks for the analyte whose concentration was kept constant was 5.3% for Fe(II) and 2.5% for nitrite. The sample rate was approximately 2.5 min/sample.

Table 2 summarizes the analytical figures of interest for the analysis of a batch of synthetic samples containing both nitrite and Fe(II) concentrations within the linear range. The peaks obtained are shown in Figure 4. As it can be observed, the results are acceptable in all cases.

CONCLUSIONS

The results obtained in this work suggest that using large sample volumes in sequential injection analysis systems allows the determination of two different chemical species by using a different reagent at each end of the sample plug.

The proposed methodology obviously requires further research into its foundation and scope of application in both theoretical and practical terms. However, this is the first step in the development of a variety of applications of a potentially high practical interest, applications that, on the other hand can be most readily and conveniently implemented with the assistance of SIA systems. Some anticipated applications of this methodology include, but are not limited to, the following:

(a) The validation of the results obtained for a given analyte in the same injection by using two chemically disparate reagents.
(b) The determination of two analytes, or the same analyte over two different linear ranges, in a unique sample injection by using two different reagents, similarly as in reported FIA applications.[6-8]

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Table 2 Results obtained in the determination of Fe(II) and nitrite in various mixtures.

Added (mg/L)		*Found (mg/L)*		*Error (%)*	
NO_2^-	*Fe*	NO_2^-	*Fe*	NO_2^-	*Fe*
3.06	2.81	2.97	2.51	-3.23	-10.95
3.06	2.81	2.96	2.70	-3.41	-4.10
2.04	2.81	2.12	2.67	3.56	-5.05
2.04	2.81	2.08	2.71	1.63	-3.87
1.02	5.63	1.21	5.72	18.95	1.66
1.02	5.63	1.16	5.68	13.44	0.95
5.11	1.41	5.03	1.38	-1.60	-1.70
5.11	1.41	4.88	1.39	-4.47	-1.23
2.04	4.22	2.06	4.16	0.80	-1.44
2.04	4.22	2.06	4.25	1.08	0.61
3.06	0.00	3.19	0.00	4.12	
3.06	0.00	3.08	0.00	0.63	
0.00	2.81	0.00	2.76		-1.98
0.00	2.81	0.00	2.86		1.56

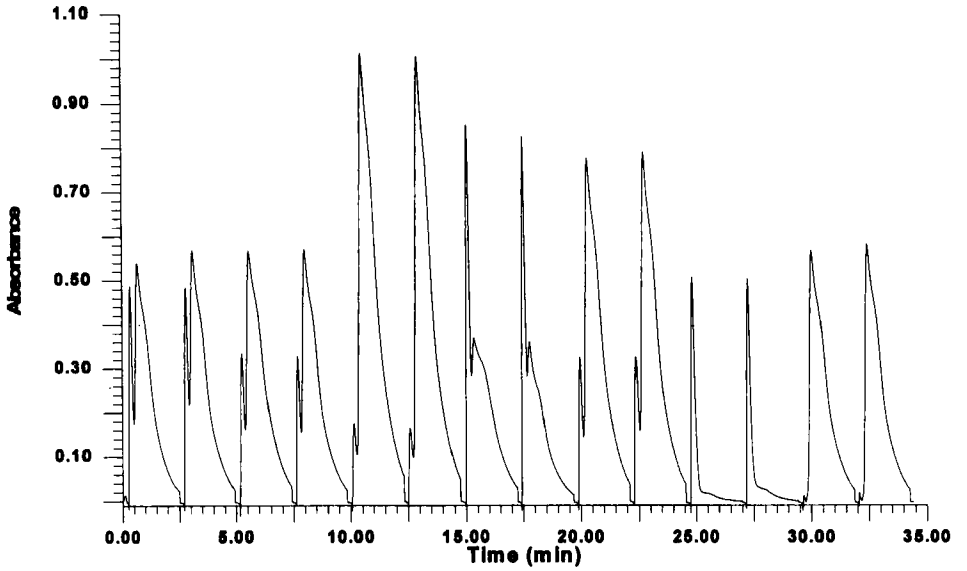

Figure 4 Peaks obtained in the analysis of the Fe(II) and nitrite mixtures listed in Table II. Each mixture was injected in duplicate.

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(c) The determination of several analytes at each end of the sample plug by using multicomponent techniques involving different reagents. The physical separation of the two reactions involved should allow the determination of species with mutually interfered signals and may be of special use with incompatible reagents. With compatible reagents, the two analytes could be determined by using classical multicomponent methods provided the signals produced by both are resolvable. In the latter case, the two signals may be overlapped to some extent and hence result in decreased sensitivity for either or both analytes. The proposed methodology enables physical discrimination, and therefore no sensitivity need be lost.

The ability to determine several parameters from a unique injection can be highly useful for monitoring samples exhibiting temporal variability.

The optimization of such a system is, in general, slightly more complex than a standard SIA system, since it is necessary to optimize not only the sensitivity conditions for each of the involved reactions, but also the injected volumes in order to avoid the mutual interference of both reactions. It is convenient to underline that volumes and concentrations are correlated, for when the reagent concentrations are modified the peaks distance changes and, therefore, the sample volume has to be readjusted.

On the other hand, the methodology developed in the present work presents with regard to its homologue in FIA systems the same advantages as those of the SIA conventional systems in relation to FIA systems. Among the former can be mentioned the considerable saving on reagents, a great versatility in the use of the same manifold for different reactions, the possibility of modifying the injected volumes without requiring instrumental modification, and finally, an accurate control of the incubation times for kinetically slow reactions.

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