CONTINUOUS FLOW INJECTION REACTION/STOPPED FLOW DETECTION USING DERIVATIVE SPECTROPHOTOMETRY. DETERMINATION OF COBALT AND IRON IN MIXTURES

KEYWORDS: Flow injection, stopped flow, derivative spectrophotometry, simultaneous determination, cobalt, iron.

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Abstract

A continuous flow injection reaction-stopped flow detection method using derivative spectrophotometry was developed for the determination of cobalt and iron in mixtures. A conventional two-channel flow injection manifold was used to develop the analytical reactions between the analytes and ferrozine and drive the reaction products to the detector. After the signal (550 nm) reached the maximum the flow was stopped, the spectrum recorded between 400 and 700 nm and transformed into its first-derivative form using a $\Delta\lambda$ of 1.6 nm. Cobalt and iron were thus determined in the range 1.1-25 μg/ml and 0.15-6.0 μg/ml, respectively. The detection limits were found

to be 0.33 μg/ml of cobalt and 0.045 μg/ml of iron. The method was applied to the determination of the analytes in water samples.

INTRODUCTION

It is widely recognized that the reliability of a given analytical method can be enhanced considerably by adopting a flow injection processing of the solutions involved in the chemical system. Accuracy, precision, minimized human intervention and rapidity are the most important analytical features of an analytical assay. However selectivity, which is not directly dependent of the extent of automation, can also be increased usually by incorporating a continuous separation process of either the analyte or interferents[1]. Other efforts to improve selectivity are aimed to determine simultaneously two or more analytes in the same injection volume of sample. In this context, different continuous flow approches has been proposed such as: differential kinetics[2], zone penetration[3], several detectors[4], single detector with the aid of a special FIA configuration[5], sequential injection[6], multichannel photodiode array detector[7], and stopped flow techniques[8].

The simultaneous determination of analytes by flow injection analysis with conventional photometric detection is restricted to the selection of an appropriate wavelength to measure both species without mutual interference, which is considerably difficult on most occasions. However, when the spectrum obtained can be converted into its derivative counterpart, the information contained on it can be presented in a potentially more useful form making, consequently, the resolution of mixtures possible.

In this context, in order to record the absorption spectra of the reaction products generated in the FI manifold, the stopped flow technique must be implemented.

The combination of flow injection processing, stopped flow/derivative spectrophotometric detection is described in this work. The method involves the injection of the sample containing both analytes iron and cobalt in a conventional two-channel continuous flow manifold. the colour reaction with the reagent (ferrozine) which occurs in a reactor, and the subsequent detection is performed in the stopped flow mode. The absorption spectra provided by the detector were differentiated, in order to resolve the mixtures. A similar approach was proposed by Chen et al.[9] for the multi-determination of B_6 vitamines. However these authors used a flow-through sensing device and derivative synchronous fluorescence detection.

EXPERIMENTAL

Reagents

All reagents were analytical-reagent grade and the water used was distilled and deionized. The standard cobalt(II) solution was a 1000 μg/ml Titrisol Merck solution. A 1000 μg/ml iron (II) solution was prepared by dissolving 7.0220 g of ammonium iron (II) sulfate hexahydrate in 10 ml of 9M sulfuric acid and diluting to the volume in a 1000 ml standard flask. Different concentration solutions of the analytes were prepared by appropriate dilution. 3-(2-Pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine®, Aldrich, USA) solution 0.01 M was prepared in water. Sodium acetate-acetic acid buffer solution (pH 5) was prepared by adding

acetic acid to 0.02 M sodium acetate solution until pH 5 was reached. An aqueous hydroxylamine hydrochloride solution 0.1% was also prepared.

Instruments and apparatus.

A Shimadzu UV-160 spectrophotometer equipped with a Hellma 178.010-OS flow cell was used for measurements of the absorbance and derivative absorption spectra. An Orion Research Model 701 digital ion analyzer with glass and saturated calomel electrodes was used for pH determinations. A four-channel Ismatec fixed-speed peristaltic pump, a Rheodyne 5041 injection valve and one Omnifit 2407 mixing Y-piece were also used.

Manifold and procedure

The FIA manifold is depicted in Fig.1. Sample solutions were injected into a stream of buffer and reductor solution prepared daily (equal volumes of hydroxylamine and buffer) at a flow rate of 1.2 ml/min. The iron present in the sample was reduced in the mixing coil R_1 (50 cm x 0.5 mm i.d.) and then both analytes reacted in R_2 (100 cm x 0.5 mm i.d.) with ferrozine at a flow rate of 2.4 mL/min. The reaction products Fe(II)- and Co(II)-ferrozine complexes were transported to the detector and the signal was monitorized at 550 nm. When the signal reached the maximum height the flow was stopped. The absorption spectum was then scanned between 400 and 700 nm and transformed into its first-derivative form using a $\Delta\lambda$ of 1.6 nm. After this procedure the pump was switched to clean the cell and make it ready for the next sample.

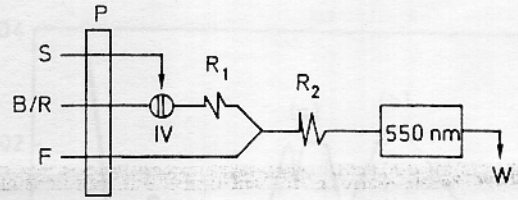

Fig.1 Manifold for implementation of the method. S denotes the sample, B/R buffer reductor mixture, F ferrozine, P, peristaltic pump, R reactor, IV injection valve and W waste.

RESULTS AND DISCUSSION

The batch determination of iron and cobalt with ferroin-type reagents, particularly the 5-phenyl-3-(4-phenyl-2-pyridinyl)-1,2,4-triazine (PPT) have been carried out previously by our group[10]. This method involves the extraction and reaction of the analytes with the reagent into 1,2-dichloroethane. This same well-behaved chemical system, but using a soluble version of the PPT reagent (ferrozine), to avoid the liquid-liquid extraction step, was implemented in the continuos FI system described in this work.

According to the procedure described above, different standard solutions of the analytes were injected into the manifold. When the FIA signal reached the maximum absorbance, the flow was stopped, remaining the signal in a constant value (fig.2) because the reactions were completed.

The overlapped spectrum of the mixture was then scanned (fig.3) and transformed into its first derivative-counterpart (fig.4). The baseline (zero-absorbance) was established at 550 nm for FIA monitorization, consequently the same zone of the

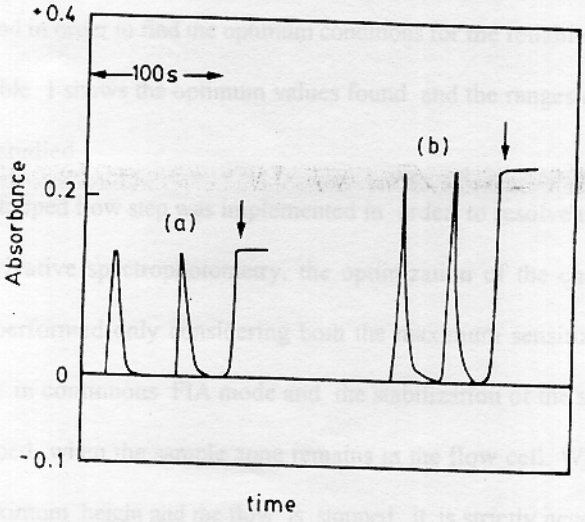

Fig. 2 Continuous signals obtained with the manifold shown in fig. 1. (a) 10 μg/ml cobalt and (b) 4 μg/ml iron. In both cases the third signal were obtained in stopped flow mode.

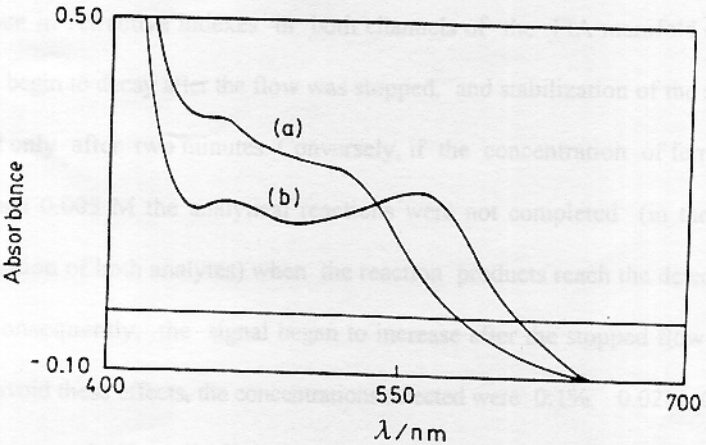

Fig. 3 Zero-order spectra of samples recorded after the stopped flow process. (a) 10 μg/ml cobalt and (b) 4 μg/ml iron.

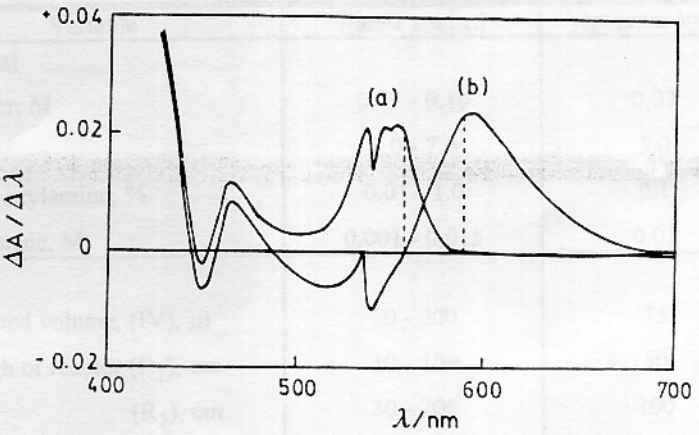

Fig.4 First derivative spectra recorded from the fig.2 spectra. $\Delta\lambda$, 1.6 nm. (a) Cobalt and (b) iron. Broken lines denote analytical wavelengths.

spectrum (fig.3) appears in negative absorbance. As can be seen in fig 4, the derivative spectra of the two complexes show differences which can be used for measurement. Iron was determinated by the graphical method at 580 nm and cobalt at 563 nm corresponding to the zero-crossing point of the derivative spectrum of the iron complex. The form of the first derivative spectra of the two complexes obtained under the present conditions are very similar to those observed in the batch method using PPT as reagent[10], however, the sensitivity is considerably lower because the present method does not involve a preconcentration step.

Study of variables

The variables affecting the performance of the method proposed here were divided into three groups: chemical, flow injection processing and those related with

the derivative spectrophotometric detection. The variables were optimized by the univariate method in order to find the optimum conditions for the reliable determination of mixtures. Table 1 shows the optimum values found and the ranges over which the variables were studied.

As the stopped flow step was implemented in order to resolve the mixtures of analytes by derivative spectrophotometry, the optimization of the chemical and FI variables was performed only considering both the maximum sensitization of the signal obtained in continuous FIA mode and the stabilization of the signal after the flow was stopped, when the sample zone remains in the flow cell. When the signal reaches the maximum height and the flow is stopped, it is strictly necessary that the signal remains constant in order to obtain reproducibility in the subsequent scans of the spectra. In this respect, if the concentrations of the buffer and hydroxylamine used in the analytical reactions were higher than 0.05 M and 0.2%, respectively, the difference in refractive indexes in both channels of the FIA manifold causes the signal to begin to decay after the flow was stopped, and stabilization of the signal was achieved only after two minutes. Conversely, if the concentration of ferrozine was lower than 0.005 M the analytical reactions were not completed (in the range of determination of both analytes) when the reaction products reach the detector.

Consequently, the signal began to increase after the stopped flow step. In order to avoid these effects, the concentrations selected were 0.1%, 0.02M, 0.01M for hydroxylamine, buffer and ferrozine, respectively. A pH value of 5 was chosen for the buffer system which is in the center of the optimum range.

Table 1. Study of variables

Variable	Range studied	Optimum Value
Chemical		
Buffer, M	0.01 - 0.10	0.02
pH	3.0 - 7.0	5.0
Hydroxylamine, %	0.01 - 1.0	0.1
Ferrozine, M	0.001 - 0.015	0.01
FIA		
Injected volume, (IV), μl	30 - 200	75
Lengh of reactor (R_1), cm	10 - 100	50
(R_2), cm	30 - 200	100
Flow rate, ml min^{-1}	1.0 - 4.3	2.4
Spectral		
Derivative order	0 - 4	1
$\Delta\lambda$, nm	0.8 - 8	1.6
Analytical wavelength, nm	400 - 700	580*, 563**

* Iron, ** Cobalt

In the chemical conditions selected the chemical reactions involved (reduction and complex formation) are fast and the hydrodynamic variables were not too critical in the performance of the method.

On the other hand, the spectral variables were the key to determine the optimum conditions for the resolution of the mixtures. The shape of the derivative spectra was strongly affected by the derivative order and the $\Delta\lambda$ value selected. The best signal-to-noise ratio was obtained using first derivative spectrophotometry and resolution is

achieved only for $\Delta\lambda$ less than 2.0 nm. Over this value the signal for cobalt begin to be dependent of the iron concentration, as it was observed previously[10].

Analytical Features

Calibration graphs were obtained for both analytes by plotting the derivative values at 563 nm and 580 nm versus the cobalt and iron concentrations, respectively. The determination ranges were 0.15-6.0 μg/ml for iron and 1.1-25 μg/ml for cobalt. The detection limits were found to be 0.045 μg/ml iron and 0.33 μg/ml cobalt. The precision of the method was tested with ten standard solutions containing 0.50 μg/ml iron + 3 μg/ml cobalt and the relative standard deviations were 3.2% and 3.0% for iron and cobalt, respectively. The sampling frequency determined for this approach was 18 h^{-1}. Evidently, the sampling frequency of the present method is considerably lower compared with that shown by the conventional FIA method for iron using ferrozine as reagent[11], because in this instance the method involves the stopped flow/derivative step. However with the conventional FIA method is not possible to determine both analytes simultaneously because cobalt interferes in the determination of iron. On the other hand, this method was considerably faster compared with its manual counterpart[10], though the sensitivity of the batch method involving PPT is approximately ten times higher. According to the aim of this work, we are interested here to resolve mixtures of analytes using derivative spectrophotometry coupled to FIA and not to increase sensitivity. Despite this, it is well known that the sensitivity of FIA methods can be easily improved by incorporating an on-line preconcentration step[12].

Table 2. Determination of cobalt and iron in synthetic samples

Added/μg ml^{-1}		Found */μg ml^{-1} (sd)		Recovery,%	
Cobalt	Iron	Cobalt	Iron	Cobalt	Iron
20.0	1.00	19.3 (0.90)	1.05 (0.04)	96.5	105
5.00	1.00	5.40 (0.19)	1.06 (0.04)	108	106
3.00	2.00	2.96 (0.12)	1.95 (0.07)	98.7	97.5
2.00	4.00	2.10 (0.09)	4.05 (0.16)	105	101.3
1.50	4.50	1.70 (0.12)	4.45 (0.12)	113	98.9

(*), Mean of five determinations. (sd), standard deviation.

The selectivity of the method was tested by injecting solutions containing 1.5 and 15 μg/ml of iron and cobalt, respectively containing known quantities of a desired foreign ion (alkaline, alkaline earth, and transition metal ions). The tolerance limits were equivalent to those reported in the manual method using PPT in dichloroethane and perchlorate as reagents, because the analytical reactions of complex formation are analogous. Interference from Hg^{2+}, Ni^{2+} and Cr^{3+} occurs when they are present over a 10:1 interferent to cobalt ratio. The common anions do not interfere but cyanide and EDTA inhibit the reaction at all levels.

The proposed method was applied to the analysis of different synthetic mixtures of cobalt and iron in different ratios. The results (table 2) were calculated from the calibration graphs and in all instances a good accuracy is achieved.

Determination of iron and cobalt in water samples.

Thermal and well water samples were assayed using the proposed method. Since cobalt was not detected in the samples, these were spiked with a known quantity of this analyte.

Table 3. Determination of cobalt and iron in water samples

Sample	Found/ppm		Added/ppm		Found/ppm		Recovery/%	
	Cobalt	Iron	Cobalt	Iron	Cobalt	Iron	Cobalt	Iron
1	---	3.96	5.0	1.0	4.7	5.21	94.0	105.0
2	---	4.03	5.0	1.0	4.8	5.10	96.0	101.4
3	---	3.89	5.0	1.0	4.8	5.05	96.0	103.3
4	---	2.32	5.0	1.0	4.9	3.20	98.0	96.4
5	---	2.01	5.0	1.0	5.3	3.19	106.0	106.0
6	---	1.78	5.0	1.0	4.7	2.68	94.0	96.4

Samples 1-3 (Morales baths), 4-6 (Well water)

Thermal water samples were collected at Morales baths (Metropolitan Region, Chile) on May 1995. Well water samples were collected at Algarrobo (V Region, Chile) on April 1995. Total iron was determined by treatment of 50 ml of sample with 0.25 ml of concentrated nitric acid and 25 ml of HCl 50%. The samples were boiled for 15 min and then diluted to 100 ml with acetate buffer pH=5. The determination of iron and cobalt was carried out using the proposed procedure (table 3).

Acknowledgements

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