

Flow Injection with an Integrated Retention/Photometric Detection Unit for the Determination of Iron in Water

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An integrated retention/photometric detection method for the determination of microamounts of iron was developed. The method is based on the retention of the reaction product of the analyte with ferrozine in a flow cell packed with an anion exchange support, and on the subsequent elution of the retained complex with a solution of 1.5% sodium peroxide, which allows the reuse of the system. The method is highly selective and has a detection limit (3σ) of 4.3 ng ml^{-1} . Iron was thus determined in the range $10\text{--}200 \text{ ng ml}^{-1}$ with a relative standard deviation of 1–3% ($n = 11$). The method was applied to the determination of iron in tap and river water and the results were consistent (2%) with those provided by AAS. © 1996 Academic Press, Inc.

INTRODUCTION

The increasing evolution of trace analysis of metal ions has demanded the development of suitable methods for separating and preconcentrating trace constituents from different matrices, together with techniques for handling them.

The use of chemical sensors in continuous flow systems, in which a retention process and/or the analytical reaction are integrated with detection, has resulted in a powerful means of improved analytical properties in trace determinations (1–8). Selectivity and sensitivity can be increased dramatically through the *in situ* concentration and separation of the analyte (or reaction product) on a support packed in the flow cell of a flow-through chemical sensor. In this respect, flow-through sensor approaches offer salient advantages compared with both manual and flow injection methods using the same analytical reaction.

It is well known that a large number of manual-photometric methods of determining iron at trace level have been developed. Most of these procedures have some drawbacks regarding sensitivity, selectivity, or simplicity. On the other hand, automated methods based on air-segmented continuous flow systems (9) and more recently on flow injection analysis (FIA) (10–15) have been also applied to the determination of this analyte using different manifolds and detection systems. Despite the high potentiality of the direct FIA methods (without a preconcentration step), their sensitivity and selectivity are not usually large enough for application at trace levels.

Recently, Lazaro *et al.* (8) proposed a flow-through sensor device to determine iron (III), which is based on the formation and retention of the iron (III)–thiocyanate complex in an anion exchange resin placed in the flow-cell. This method shows a high level of precision and sensitivity; however, copper interferes seriously.

In this paper a highly selective, sensitive, and reliable integrated retention/photometric

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detection flow injection method for the determination of iron is described. The method is based on the temporary immobilization of the reaction product between the analyte, which is previously reduced to Fe(II) species, and 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) on an anion exchange resin packed in the flow cell of a photometric detector. Fast elution with sodium peroxide allows reuse of the system.

MATERIALS AND METHOD

Reagents

All reagents were of analytical reagent grade and the solutions were prepared with high purity water.

Standard iron (II) solution. A $1000 \mu\text{g ml}^{-1}$ solution was prepared by dissolving 7.0220 g of ammonium iron(II) sulfate hexahydrate in 10 ml of 9 M sulfuric acid and diluting to the volume in a 1 liter standard flask. More diluted solutions were prepared from it as required.

3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (Ferrozine). A $5 \times 10^{-3} M$ solution was prepared by dissolving 2.3525 g of the compound in 1 liter of water.

Hydroxylamine hydrochloride solution. This solution was prepared by dissolving 2.0 g of the salt in 1 liter of water.

Sodium acetate solution. A solution of 0.4 M sodium acetate was prepared by dissolving the calculated amount in 1 liter of water.

Foreign ion solutions. Solutions of diverse ions were prepared for the interference studies by dissolving the calculated amount of each compound in order to give 10–1000 $\mu\text{g ml}^{-1}$ solutions of each species.

Sodium peroxide solution (eluent). A 1.5% eluent solution was prepared daily.

Apparatus and Instruments

A Hellma 178.010-OS flow cell (10-mm path length; 3-mm diameter) was packed with Dowex 1-X1 strongly basic anion exchange resin (50–100 mesh). The flow cell was filled with resin only for 3 mm of the light-path portion and was blocked with nylon fiber wool packing, as shown in Fig. 1. The anion exchange material was previously rinsed with deionized water and conditioned by triplicate treatment with 3 M HCl, 2 M NaOH, and deionized water, which convert the resin into its chloride form (16).

A Shimadzu UV-160 spectrophotometer equipped with the above described flow cell was used.

Two four-channel Ismatec fixed-speed peristaltic pumps, two Rheodyne 5041 injection valves (one of them acting as a selecting valve), and two Omnifit 2407 mixing Y-pieces were also used.

Manifold Design and Procedure

The FIA manifold and the schematic diagram of the flow-through cell used are depicted in Fig. 1. The sample (2 ml) was inserted into a deionized water stream at a flow rate q_1 of 0.6 ml min^{-1} . This stream was merged with a stream of buffer and reductant mixture (equal volumes of hydroxylamine and acetate solutions, prepared daily) at a flow rate of 0.6 ml min^{-1} (q_2). The analyte was reduced in mixing coil L_1 (50 cm \times 0.5 mm i.d.) and then reacted in L_2 (200 cm \times 0.5 mm i.d.) with ferrozine at a flow rate q_3 of 0.8 ml min^{-1} .

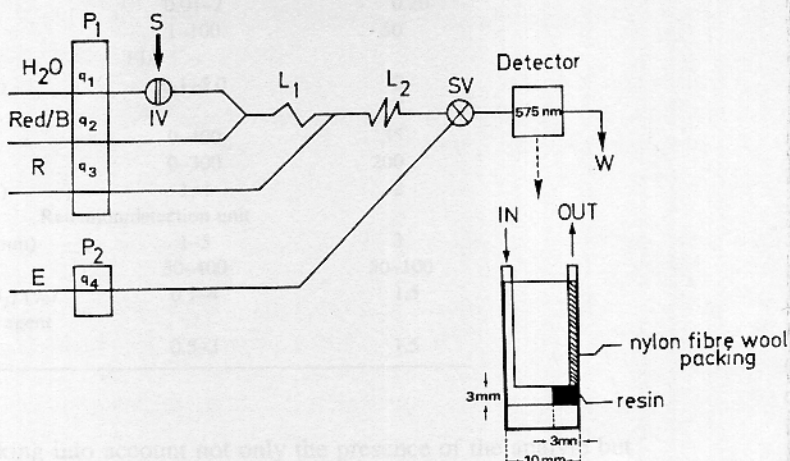


FIG. 1. Manifold used and the schematic diagram of the resin-filled flow-through cell. P = peristaltic pump; S = sample; Red/B = reductor in buffer system; R = reagent (ferrozine); E = eluent (1.5% sodium peroxide); q = flow rate; IV = injection valve; L = reaction coil; SV = switching valve; W = waste.

The reaction product formed (Fe(II)-Ferrozine complex) was retained by the flow cell, packed with Dowex 1-X1 anion exchanger, when the switching valve was in position 1. The retention signal was monitored at 575 nm. When the signal reached the maximum height (i.e., the tail of the sample plug), 65 s after injection, the concentrated reaction product was readily eluted with 1.5% Na_2O_2 , which was inserted via position 2 of SV, thereby restoring the baseline.

RESULTS AND DISCUSSION

Study of Variables

This study was aimed at finding the optimum conditions (sensitivity, stability, reproducibility, rapidity, and regenerability) for the chemical flow-through sensor. The variables were optimized by the univariate method. The optimum values of the variables and ranges over which they were investigated are given in Table 1.

Chemical and FIA Variables

In the spectrophotometric determination of iron using ferrozine (or another ferrozine derivative) as the chromogenic reagent the analytical reaction always requires the presence of a reductant (Fe(II) active species) and a buffer system to give a pH value between 3-8. In the present method, an optimum signal was obtained for concentrations of 0.1% (w/w) hydroxylamine even when all the analyte in the sample was in the Fe(III) form. Similarly, as the analytical signal was practically independent of pH over the range 3-9, a diluted 0.2 M acetate solution was sufficient to keep the pH in the center of the range. Therefore a salient advantage of the proposed method compared with its corresponding manual conventional (17) and batch ion-exchange absorptiometric (18) counterparts (which use the same derivatization reaction) was the lower concentration of these ingredients required.

Although a maximum signal was obtained over 1×10^{-4} M ferrozine, a concentration

TABLE I
 Study of Variables

Variable	Range studied	Optimum value
Chemical		
Hydroxylamine (%)	0.01-10	0.10
CH ₃ COONa (M)	0.01-2	0.20
Ferrozine (10 ⁻⁴ M)	1-100	50
FIA		
Injected volume (ml)	0.1-5.0	2
Reactor length (cm)		
R ₁	0-400	35
R ₂	0-300	200
Flow rate (ml min ⁻¹)	1-4	2
Retention/detection unit		
Resin (path length, mm)	1-5	3
Particle size (mesh)	50-400	50-100
Eluting agent (Na ₂ O ₂) (%)	0.1-4	1.5
Flow rate of eluting agent (ml min ⁻¹)	0.5-3	1.5

of $5 \times 10^{-3} M$ was selected, taking into account not only the presence of the analyte but also that foreign cations can be present in the sample with the evident consumption of reagent.

With respect to the FIA manifold, the lengths of the reactors had little influence on the analytical signals as the reduction and complex formation reactions are fast. Nevertheless, reactors of lengths at least 50 (L₁) and 200 cm (L₂) were necessary in order to increase the dispersion of the reaction products, thus facilitating retention by fitting the concentration of the reaction product in the solution reaching the flow cell to the retention kinetics.

Similarly, the flow rate also influenced the signal. Increased flow rates decreased dispersion of the reaction products and consequently the absorbance signal because the retention kinetics was not instantaneous. An optimum value of 2.0 ml min⁻¹ was chosen as a compromise between sensitivity and sample throughput.

A variation in the sample volume from 0.1 to 5 ml resulted in a proportional increase in the analytical signal. The portion of resin has a capacity large enough to concentrate all the analyte injected. The sample volumes can be selected depending on the concentration level of the analyte in the sample. When an unknown sample is analyzed, a 2-ml injection volume was always used. However, if the analytical signal is sufficiently higher than 0.023 absorbance units (which corresponds to the determination limit signal), smaller injection volumes can be used in order to increase the sample frequency.

Variables of the Retention/Detection Unit

Using the FIA and chemical conditions given above the reaction product (Fe-ferrozine complex) was easily and strongly retained in an anionic Dowex 1-X1 resin, showing a maximum absorption at 575 nm. A weakly basic anion exchange resin (Ion-Exchanger II, Merck) was also tested as support; however, not only was the retention considerably

slower, but also the baseline was higher than those observed when strongly a basic Dowex 1-X1 resin was used as support.

The absorbance increased in proportional to the volume of resin packed in the light path of the flow cell. The signal was optimum when only between 2.5 and 3.5 mm of the light-path portion of the flow cell was filled with resin which produced a baseline absorbance of 1.2 ± 0.2 absorbance units. Above 5 mm the detector capacity (2.5 absorbance units) was saturated.

The diameter of the flow-through cell was also an important factor with respect to sensitivity. Hence a flow cell with a diameter of 3 mm was selected.

The particle size of the resin packed in the cell affected the baseline more than the analytical signal. Decreasing the particle size resulted in an increase in the absorbance of the resin itself through increased compaction of the solid. A resin of 50–100 mesh was chosen as optimum for further experiments.

In order to make the retention/detection unit regenerable, a study of the potential of different species as eluting agents was carried out. It was observed that the anionic complex Fe (II)–ferrozine was very strongly sorbed in the resin and elution was negligible with a number of anions tested. Similarly, organic solvents were not efficient for fast elution because the complex was slightly soluble in organic media. An attempt to dissociate the complex in a strong acid medium was lost because the Fe(II)–ferrozine complex is kinetically inert to dissociation (decoloration after 24 h). However, oxidation into the Fe(III)–ferrozine complex by a Na_2O_2 solution was the key for an extremely effective elution. This Fe(III) species, formed at the resin, was displaced easily only with the components of the buffer, resulting in faster restoration of the baseline.

Analytical Properties of the Method

A series of standards of concentrations between 5.0 and 500 ng ml^{-1} were inserted into the manifold under the optimum working conditions. The calibration graph obtained was linear over the determination range 10.0–200 ng ml^{-1} . The linear relation between retention peak height absorbance and iron concentration was expressed by the least squares method as

$$A = 2.51[\text{Fe}(\mu\text{g ml}^{-1})] - 0.002 \quad (r = 0.9998).$$

The detection limit, determined by the 3σ criterion, was 4.3 ng ml^{-1} . The repeatability of the method, expressed as rsd of 11 replicate injection of solutions containing 15, 100, and 180 ng ml^{-1} , was 2.9, 1.3, and 2.1%, respectively. At least 400 sequential determinations can be carried out with the same sensor and the sampling frequency (0.5 ml injection volume) was found to be 50 h^{-1} .

Interference Studies

The effects of several foreign ions on the determination were studied by adding known quantities of a desired foreign ion to solutions of 25 ng ml^{-1} iron. The tolerance limit was taken as being the amount causing an error of $\pm 5\%$ in the analytical signal. As can be seen in Table 2, cationic species were largely tolerated. Similarly, common anions were found not to interfere at up to a 1000 (w/w) interferent-to-analyte ratio. On the other hand, EDTA and CN^- interfere negatively, at a 1 (w/w) ratio, by inhibition of the complex

TABLE 2
 Study of Interference

Foreign species	Tolerance limit (ng/ml) ^a
Al(III), Mg(II), Ca(II), Sr(II)	10,000 ^b
Cd(II), Pb(II), Mn(II)	6,000
Cr(III)	4,500
Cu(II), Co(II), Zn(II)	1,600

^a 25 ng ml⁻¹ of iron.

^b Maximum tested.

formation reaction. According to these tolerance limits the method proposed here has a selectivity higher than both the batch ion-exchange method and the sensor based on Fe-SCN reaction. This high selectivity of the proposed flow sensor resulted not only from the analytical reaction but also from the *in situ* concentration kinetics. The influence of the ionic strength of the sample on the retention of the anionic iron-ferrozine complex was tested by injecting samples, of 100 ng ml⁻¹ iron, in the presence of increasing concentrations of NaCl. It was observed that concentrations below 1M NaCl did not affect this process.

Application of the Method

In order to test the analytical validity of this approach, the method has been applied to the determination of iron in tap and river water. The tap water samples were collected and analyzed without a previous treatment. The river water samples were collected at the Maipo river in Santiago, Chile (in March, 1994). They were instantly drawn through a membrane filter (pore size 0.45 μm). The determination of iron for the filtrate was carried out using the proposed method (Table 1). The results were quite consistent with those provided by atomic absorption spectrophotometry (Table 3).

 TABLE 3
 Determination of Iron in Tap and River Water

Sample	No.	Iron content found, ng ml ⁻¹ (rsd,%)			
		Proposed method	(rsd)	AAS method	(rsd)
Tap water	1	36.5	(1.9)	37.0	(2.7)
	2	36.4	(2.0)	37.0	(2.6)
	3	37.2	(1.9)	36.9	(2.4)
	4	39.5	(1.8)	38.9	(2.6)
River water	1	59.2	(1.6)	58.9	(2.8)
	2	58.5	(1.6)	59.1	(2.6)
	3	58.8	(1.7)	59.2	(2.8)
	4	60.0	(1.8)	59.5	(2.8)

Note. Average of eight determinations.

CONCLUSIONS

A comparison of the method proposed here with its corresponding batch ion exchange absorptiometric counterpart (17) shows that the present sensor integrated approach is much faster (25 times) and less complicated because the batch method requires manual mixing of the reagents; addition of the resin to the medium; washing, filtration, and collection and collection of the resin with the retained product in a conventional cell; and measurement. The simplicity of the proposed method is reflected in the fact that all the steps are performed in the flow system (automation capability). In addition, the sensor is reusable; i.e., it can be regenerated by elution of the immobilized reaction product (in the batch method, the resin is disposable after each assay). Compared with a normal flow injection method, the proposed method is more sensitive and selective resulting from the *in situ* concentration kinetics, though less rapid. Finally, a comparison with the flow-through sensor based on the Fe(III)-SCN complex (8) shows that the present sensor is more selective (Cu does not interfere) and offers a similar sensitivity and precision, though it requires a selecting valve to introduce the eluting agent.

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