Flow Injection Spectrophotometric Determination of Total Iron in Fresh Waters using 1,10-Phenanthroline Reagent

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Summary: A simple flow injection method is reported for the determination of total iron in fresh waters based on spectrophotometric detection of reddish orange iron (II)-1,10-phenanthroline complex measured at wavelength 510 nm. The system permits high throughput of 120 samples per hour. A linear calibration ($r^2 = 0.9981$) graph was obtained over the concentration range 0.2-1.0 mg L^{-1} with relative standard deviation ($n = 4$) 0.6-1.0 %. The limit of detection (3σ blank) was 0.01 mg L^{-1}. The proposed method has been applied successfully for the determination of iron in fresh water samples and the results obtained (0.04 ± 0.003-0.09 ± 0.01 mg L^{-1}) were in reasonable agreement with the results obtained using a spectrophotometric method (0.05 ± 0.008-0.1 ± 0.005 mg L^{-1}) as the reference method.

Introduction

Iron is a major component as well as the fourth most abundant element [1] of the earth's crust, but its dissolved concentration in water is at trace level. It plays an important role in the metabolic and fermentation processes [2, 3]. Iron is an important element in natural water [4, 5] *e.g.,* it binds organic matter to clays and its humic aggregates adsorb/absorb other trace metallic ions. Iron and its compounds have widespread industrial applications; hence large quantities of iron are discharged into the environment. The speciation of iron in aquatic system is very important for biogeochemical studies because of the influence of its chemical form on availability and mobility and its toxicological properties [6, 7]. The increasing importance of the determination of iron in different fields, particularly in environmental samples, has made it necessary to develop simple, inexpensive and sensitive methods for the determination of traces of these analytes in different samples.

Numerous methods based on gravimetric, titrimetric, polarographic, optical, radiochemical and UV-vis spectrophotometric detections have been reported for the determination of iron dealing with clinical, environmental, food staff and drug samples [3, 8].

Flow injection analysis (FIA), an automated technique based on the injection of a liquid sample into a continuous stream of reagent or carrier appears to hold considerable promise for trace level determinations. The technique is capable of manipulating small sample volumes (10-100 μL) which avoids loss of sensitivity by unnecessary dilution when the original sample size is restricted. In addition, the sample can be carried through various stages of physical and chemical processing without contact with the laboratory environment thus reducing possibilities for contaminations. Finally, the rinsing efficiency of the FI system is high, which eliminates sample carry-over while conserving high-purity solvents and reagents required [9-13].

FIA based on spectrophotometric detection for the determination of metal ions has gained much interest in recent years because of their simplicity, high throughput, and good reproducibility. Several flow injection spectrophotometric methods have been reported for the determination of iron using various chelating agents. Table-1, shows the comparison of several spectrophotometric methods for the determination of iron (II) in terms of sample matrix and sensitivity [14-23]. These methods are accurate and selective, but some of these methods involve complicated FIA manifolds, required sample pretreatment, and time consuming as well as low sample throughput.

In the present work, we report, a single channel Flow injection spectrophotometric method for rapid determination of iron (II) in fresh waters using two chelating agents; 1,10-phenanthroline and 2,2-bipyridine coupling with various reducing agents

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Table-1: Comparison among several spectrophotometric methods for the determination of iron (II)/total iron.

Analyte	Matrix	Calibration range	Limit of detection	R^2 value	Sample rate h^{-1}	Ref.
Fe (II)	Tap & mineral water	0–0.5 $\mu g\ L^{-1}$	0.01 $\mu g\ L^{-1}$	0.9998	25	14
Fe (II)	standards	0.02–2.0 $\mu g\ mL^{-1}$	10 ng mL^{-1}	0.9992	N/G	15
Fe (II)	Water	10.3–207 ppb	10 ppb	N/G	70	16
Fe (II)	Standard solutions	3.6 ppb–0.71 ppm	0.7 ppb	0.99984	N/G	17
Total Iron	Fresh waters	50–1000 $\mu g\ L^{-1}$	15 $\mu g\ L^{-1}$	0.997	25	18
Fe (II)	Water	0.195–120 ng mL^{-1}	0.044 ng mL^{-1}	0.999	N/G	19
Fe (II) & Fe (III)	Drinking & natural waters	0–25 $\mu g\ L^{-1}$	2.1 $\mu g\ L^{-1}$	0.9991	N/G	20
Fe (II)	River and tap waters	0–20 $\mu g\ L^{-1}$	0.2 $\mu g\ L^{-1}$	0.9999	N/G	21
Total Iron	Natural waters	0–0.5 mg L^{-1}	0.01 mg L^{-1}	0.9994	144	22
Fe (II) & Fe (III)	Snow, serum & wine	1.0–10 ng mL^{-1}	0.2 ng mL^{-1}	0.9998	N/G	23
Total Iron	Tap water	0.2–1.0 mg L^{-1}	0.01 mg L^{-1}	0.9984	120	This method

N/G = not given

for reduction of iron (III) to iron (II). Manifold parameters have been optimized and the method is applied to fresh water samples.

Results and Discussion

The flow injection spectrophotometric manifold for the determination of iron (II) was optimized by investigating the effect of various parameters including reagents concentration, sample volume, flow rate and reaction coil. All studies were carried out with a 1.0 mg L^{-1} iron (II) solution.

To select the concentration level of 1,10-phenanthroline for complex formation, different concentrations of 1,10-phenanthroline over the range from 0.001-0.2 % were used. The results are shown in Table-2. The peak absorbance increased with increasing the concentration up to 0.005 % and decreased as the concentration was further increased; therefore, 1,10-phenanthroline solution 0.005 % was selected and used for subsequent studies.

Table-2: Ranges investigated and optimized conditions for FI-spectrophotometric manifold parameters.

Parameter	Range studied	Optimized values
1,10-phenanthroline (%)	0.001-0.2	0.005
Hydroxylamine HCl (%)	0.01-0.25	0.1
Flow rate (mL min^{-1})	0.3-1.8	0.9
Sample volume (μL)	30-180	120
Mixing coil (cm)	10-80	20

The response of various reducing agents on the reduction of iron (III to iron (II) was investigated including hydroxylamine hydrochloride, ascorbic acid, stannous chloride and sodium sulfite in the range 0.05-0.25 %. Hydroxylamine hydrochloride showed a maximum peak absorbance at

concentration level of 0.1 % comparatively and therefore was selected for further studies.

The other physical parameters i.e. flow rate (0.3-1.8 mL min^{-1}), sample volume (30-180 μL) and reaction coil length (10-80 cm) were also investigated in terms of sensitivity, speed and reagent consumption and the results are shown in Table-2. A flow rate of 0.9 mL min^{-1} gave maximum peak absorbance with a steady baseline and reproducible peak height and was used subsequently. Similarly a sample injection volume 120 μL gave almost the highest peak absorbance and was used for economy of sample consumption. The reaction coil length of 20 cm gave suitable response and was selected for further studies.

Analytical Figures of Merit

From the stock solution of $Fe(NH_4)_2(SO_4)_2.6H_2O$, a series of standard solutions were treated according to the developed method under the optimum conditions. The calibration graph of peak absorbance vs. iron (II) was linear over the range 0.2-1.0 mg L^{-1} ($r^2 = 0.9981$; $n = 5$) with regression equation y = 0.0885x + 0.0087, [y = peak absorbance, x = concentration mg L^{-1}) as shown in Fig. 1. The limit of detection (3 σ blank) was 0.01 mg L^{-1} with a sample throughput of 120 h^{-1} with relative standard deviation (RSD, $n = 4$) 0.6-1.0 %.

The standards of iron (II) over the range 0.2-1.0 mg L^{-1} were injected (using the manifold shown in Fig. 2) into the stream of 2,2-bipyridin (0.1 %, optimized, range studied 0.01-0.25 % in deionized water) containing hydroxylamine hydrochloride (0.1 %) for reduction of iron (III) to iron (II) measuring the absorbance at 522 nm [8, 24]. A linear calibration graph (Fig. 1) was obtained ($r^2 = 0.9984$; $n = 5$) over
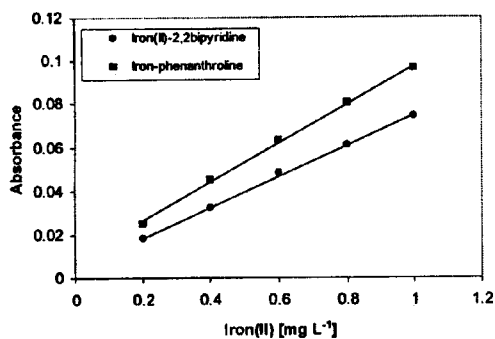
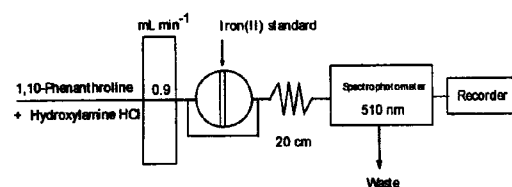Fig. 1:   Calibration graphs for the determination of iron (II).

Fig. 2:   FI-Spectrophotometric manifold for the determination of iron.

the range 0.2-1.0 mg L^{-1} with RSD 0.25-1.2 % ($n = 4$) over the range studied with a limit of detection (3σ blank) 0.01 mg L^{-1}.

Interferences

The influence of major ions in fresh water on the determination of iron (II)-1,10-phenanthroline and iron (II)-2,2-bipyridin complexes (at 0.4 mg L^{-1} respectively) is given in Table-3. With regard to cations, at the concentration level given in Table-2 had no significant effect on Iron (II)-phenanthroline and iron (II)-2,2-bipyridin complexes except that Ca^{2+} had enhancing effect on iron (II)- 2,2-bipyridin complex. No any significant effect found by anions studied for both complexes.

Determination of Total Iron in Fresh Waters

The proposed method was applied to the determination of total iron in fresh water samples. Samples were collected from various locations of the Quetta valley, Pakistan, into acid washed (10 % v/v HCl) HDPE bottles. After collection, samples were filtered through a Whatman filter paper No. 42, kept refrigerated in the dark at 4°C and analyzed within 10 h of sampling. The result obtained by addition

Table-3: The effect of interferences on Fe (II)-1,10-phenanthroline and iron (II)-2,2-bipyridin complexes. All the results are the mean of four replicate injections.

Interferents	Concentration mg L^{-1}	Response on Fe (II)-phenanthroline complex	Response on Fe (II)-2,2-bipyridine complex	Normal range mg L^{-1}
Blank	UHP water	0.003	0.005	-
Fe^{2+}	0.4	0.042	0.030	0.3
Fe^{3+}	0.4	0.056	0.044	-
Ca^{2+}	100	0.044	0.052	100
Mg^{2+}	40	0.042	0.031	30
K^{+}	25	0.042	0.03	-
NH$_4^{+}$	25	0.043	0.032	0.
Pb^{2+}	1.0	0.041	0.032	<0.05
Zn^{2+}	1.0	0.042	0.030	5.5
Cu^{2+}	1.0	0.044	0.031	1.0
Cl^{-}	200	0.042	0.030	250
NO$_3^{-}$	25	0.042	0.028	<10
NO$_2^{-}$	1.0	0.042	0.030	0.1
SO$_4^{2-}$	200	0.045	0.032	250
PO$_4^{3-}$	1.0	0.041	0.030	0.1

Table-4: FI-spectrophotometric determination of total iron in freshwater samples and comparison with spectrophotometric reference method.

Sample	pH	Conductivity mS	CaCO$_3$ mg L^{-1}	Proposed method mg L^{-1} iron	Reference method mg L^{-1} iron
1.	7.37	0.5	0.64	0.04±0.003	0.05±0.008
2.	7.30	0.7	0.97	0.09±0.01	0.1±0.005
3.	7.60	1.1	1.0	0.07±0.003	0.06±0.015

method for the three samples (range: 0.04±0.003-0.09±0.01 mg L^{-1} iron) together with other physicochemical parameters and data obtained by using the phenanthroline reference method [25] are shown in Table-4. The results from the proposed FI-spectrophotometric method and the standard reference method are in reasonable agreement.

Experimental

Materials and Methods

All glassware used during the experiments was precleaned with 25 % HCl for 48 h, thoroughly rinsed with ultra high purity (UHP) deionized water (Elga, Purelab Option, UK), and stored in plastic bags to prevent contamination. All reagents and standards were of analytical grade (Merck, BDH) were prepared in UHP water and further diluted prior to use.

The iron (II) stock solution (0.01 M) was prepared by dissolving 0.196 g of reagent grade ferrous ammonium sulfate hexahydrate, (NH$_4$)$_2$Fe(SO$_4$)$_2$.6H$_2$O in 50 mL of HCl (0.01 M). The standard solutions were prepared daily in UHP

water. The 1,10-phenanthroline stock solution (0.1 %, w/v) was prepared by dissolving 0.1 g of compound to 100 mL of UHP water. A working 1,10-phenanthroline solution (0.005 %) was prepared daily afresh by diluting 5.0 mL of the stock solution in 100 mL of UHP water. The hydroxylamine hydrochloride stock solution (5 % w/v) was prepared by dissolving the compound to 100 mL of UHP water. Stock solutions (100 mg L^{-1}) of various metals ions from atomic absorption standards (Spectrosol, BDH, UK) in 0.01 M HNO_3 and anions from their respective salts were prepared in UHP water (Table-2) and various working solutions were prepared from these stock solutions for interference studies.

Instrumentation and Procedure

The FI-spectrophotometric manifold used for the determination of iron (II) is shown in Fig. 2. A peristaltic pump (Ismatec, Reglo 100, four channels, Switzerland) was used to deliver the reagents solution at desired flow rate. A rotary injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject Fe (II) and Fe (III) standards into reagent carrier stream using a reaction coil of 20 cm. A UV/Visible Spectrophotometer (Jenway, Model 6305, UK) with a flow cell (1.0 mm i.d, 100 μL internal volume) was used to measure the absorbance of iron(II)-phenanthroline complex at 510 nm. The detector output was recorded using a chart recorder (Kipp & Zonen BD 40, Holland).

Conclusions

The proposed single channel flow injection-spectrophotometric method for total iron determination is simple and rapid (sample throughput 120 h^{-1}) and limit of detection (0.01 mg L^{-1}). Common interfering cations and anions present in fresh water at their concentration have no significant effect. The results from the proposed FI-spectrophotometric method and the standard reference method are in reasonable agreement.

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