Available online at www.sciencedirect.com
SCIENCE DIRECT®

Talanta 68 (2006) 969–973

Talanta

www.elsevier.com/locate/talanta

Determination of total and dissolved amount of iron in water samples using catalytic spectrophotometric flow injection analysis

S. Lunvongsa, M. Oshima, S. Motomizu*

Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan

Received 15 March 2005; received in revised form 21 June 2005; accepted 28 June 2005

Abstract

A flow injection spectrophotometric method has been developed for the determination of dissolved and total amounts of iron in tap and natural water samples. The method for the determination of iron employs a sample acidification step in order to decompose iron hydroxide and iron-complexes into free iron, Fe(III) and Fe(II). The amounts of free iron were detected using a catalytic action of Fe(III) and Fe(II) on the oxidation of *N*,*N*-dimethyl-*p*-phenylenediamine in the presence of hydrogen peroxide. Increase in absorbance of oxidized product was detected spectrophotometrically at 514 nm. The proposed method allows 0.02 and 0.06 $\mu g\, l^{-1}$ of LOD and LOQ, respectively, with relative standard deviation (RSD) below 2%. The accuracy and the precision of the method were evaluated by the analysis of the standard reference material, river water. The developed method was successfully applied to real water samples.

Keywords: Iron; Dissolved iron; Flow injection analysis; Natural water; Catalytic reaction; Spectrophotometry

1. Introduction

In general, natural waters contain various chemical forms of iron depending on a geological area and other chemical components. Iron(II) is normally less present in river water [1], and Fe(III) can precipitate rapidly by the formation of hydrous iron oxide and hydroxides, which can absorb other trace metals [2]. Thus, iron ion controls the mobility, bioavailability and toxicity of other trace metals in the natural water system [3]. Therefore, methods for the determination of iron are needed to predict the fate and mobility of metals in natural waters.

A variety of well-established methods for quantitative analysis of iron have been developed; they are inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), electrochemistry, and ion chromatography (IC). Though all of these methods are highly sensitive, main disadvantages are the necessity of expensive and sophisticated instrumentation. Flow injection analysis (FIA) system equipped with a simple detector such as a UV–vis spectrophotometric detector is one of the most effective and suitable approach for routine analysis, mainly owing to its simplicity, low instrumentation cost, high sample throughput and robustness.

Kinetic methods based on catalytic reactions have been applied to trace elemental analysis because of their high sensitivity and low limit of detection. A FIA system equipped with a spectrophotometric detector seems to be a suitable technique for the catalytic method of analysis, which can lead to many advantages such as higher precision and high sampling frequency, compared to a batch-wise method [4]. Therefore, a number of flow injection-catalytic spectrophotometric methods have been proposed for the determination of trace amount of iron [5–9]. Flow injection-catalytic methods based on the oxidation of *N*,*N*-dimethyl-*p*-phenylenediamine (DPD) with hydrogen peroxide and of *p*-phenetidine with periodate couple with in-valve microcolumn separation/preconcentration on a diethylenetriamine, DETA, sorbent have been proposed for the determination of dissolved iron in seawater samples [5]. An iron preconcentration step involving an in-line 8-hydroxy-quinoline chelating resin column followed by the

---

* Corresponding author. Tel.: +81 86 251 7846; fax: +81 86 251 7846.
E-mail address: motomizu@cc.okayama-u.ac.jp (S. Motomizu).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.06.067

spectrophotometric detection of the oxidation product of *N*,*N*-dimethyl-*p*-phenylenediamine have been applied to seawater samples [6]. Ohno et al. applied FIA to a catalytic method based on oxidative coupling reaction of *p*-anisidine with *N*,*N*-dimethylaniline in the presence of hydrogen peroxide for the determination of copper and iron [9]. Hirayama and Unohara recommend *N*,*N*-dimethyl-*p*-phenylenediamine as a sensitive reagent for the catalytic determination of iron, and a batch-wise procedure with DPD was applied to water samples [10]. Later, the FIA method with DPD gave a highest sensitivity reported so far: LOD was reported to be 0.08 $\mu g\,l^{-1}$ [5].

In this paper, the authors want to develop a highly sensitive flow injection spectrophotometric method for the determination of iron based on a catalytic oxidation of DPD with hydrogen peroxide by optimizing the experimental conditions in details. The proposed method was very simple and the sensitivity is the highest of the other spectrophotometric methods reported so far: the method allows the determination of sub-ppb levels of iron without any preconcentration procedure. The proposed method was applied to the determination of iron in water samples. The sample pretreatment procedure (acidified prior to filtration and after filtration) permits the fractionation of iron in natural water, total and dissolved iron. To decompose iron hydroxides and iron-complexes into free iron, the added concentration of acid and the effective releasing time was examined. The information on total and dissolved amount of iron could be used for the mobility assessment of metals in natural waters.

2. Experimental

All lab-wares were cleaned by soaking them in 10% (v/v) hydrochloric acid before use, followed by rinsing them thoroughly with ultrapurified water. All standard and sample solutions were prepared using the ultrapurified water by using Elix 3/Milli-Q element system (Nihon Millipore, Japan).

2.1. Reagents

Iron standard solutions for the preparation of calibration graph were prepared daily by diluting of a 1000 $mg\,l^{-1}$ standard iron solution for atomic absorption spectrometry (Wako Pure Chemicals, Japan) with 0.1 M hydrochloric acid. A carrier solution, CS, was a 0.1 M hydrochloric acid solution prepared from a concentrated hydrochloric acid (electronic grade, Mitsubishi Chemicals, Japan). An oxidizing solution, OS, was 0.5 M hydrogen peroxide solution prepared from a 30% hydrogen peroxide (electronic grade, Cica Kanato Chemical, Japan). A reagent solution, RS, was a 0.012 M *N*,*N*-dimethyl-*p*-phenylenediamine (DPD: Wako Pure Chemicals, Japan) was dissolved in a diluted hydrochloric acid. A buffer solution, BS, was an ammonium acetate buffer (pH 5.7) prepared by mixing appropriate amounts of 2 M acetic acid prepared from a glacial acetic acid (electronic grade, Mitsubishi Chemicals, Japan) and 4 M ammonia solution prepared from a concentrated ammonia solution (electronic grade, Mitsubishi Chemicals, Japan). In the interference study, all stock standard solutions of metal ions were prepared by appropriate dilution of stock solutions of metal ions with 0.1 M hydrochloric acid.

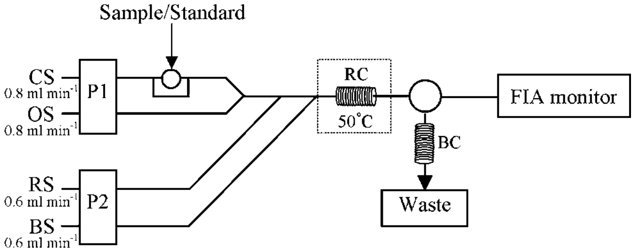

Fig. 1. Flow injection system for the determination of iron in water samples: CS, 0.1 M HCl; OS, 0.5 M H_2O_2; RS, 1.2×10^{-2} M *N*,*N*-dimethyl-*p*-phenylenediamine (DPD); BS, 2 M NH_4OAc buffer, pH 5.7; RC, reaction coil (400 cm × 0.5 mm i.d.); BC, back pressure coil. Dotted box is refers to thermostated bath.

2.2. Sample pretreatment

River water samples were collected in cleaned PTFE bottles, and then were split into two portions: the one was kept as it is, and the other was acidified immediately to 0.1 M hydrochloric acid by adding a small portion of concentrated hydrochloric acid. After standing it overnight, the acidified samples were filtered through a quantitative filter paper with pore size <5 μm (Toyo Roshi, Japan). The filtered water samples were analyzed for pseudototal iron containing particulate-bound fraction. The samples not acidified were immediately filtered through the filter paper, and then the filtered water samples were acidified to 0.1 M hydrochloric acid. The acidified water samples were stood for 30 min. After that, the samples were analyzed for the iron content, which includes the aggregation of iron hydroxide and humic complex iron.

2.3. FI manifold

A schematic diagram of FIA system used in this work is shown in Fig. 1. The system contained two double plunger pumps (F.I.A. Instrument, model 201, Tokyo), which were used to propel CS, RS, BS and OS. The samples and the standard solutions were introduced into the carrier stream using a six-way injection valve with a 500 μl sample loop. The absorbance was measured by a Soma model S-3250 spectrophotometer, and the data acquisition was accomplished with FIA monitor (F.I.A. instrument). A TCI model GAS DIF dry thermostat bath was used to kept the reaction coil temperature at 50 °C.

3. Results and discussion

3.1. Optimization of FIA variables

A standard Fe(III) solution, 2 μg l^{-1} was injected into the proposed flow system for the optimization of a FIA system and a detection reaction in order to achieve high sensitivity.

The effect of DPD concentration was examined in the range from 2.4×10^{-3} to 2.4×10^{-2} M. Peak height sharply increased with increasing the concentration of DPD up to 1.2×10^{-2} M, and at the concentrations above it the peak height was almost constant. Therefore, a 1.2×10^{-2} M of DPD was selected as an optimum concentration. The effect of hydrogen peroxide concentration was examined in the range from 0.1 to 0.5: the increase in the hydrogen peroxide concentration improved the height of the peak. The sharp increase was obtained with increasing the concentration of hydrogen peroxide from 0.1 to 0.5 M, and at the concentrations above 0.5 M the peak height slightly increased. The hydrogen peroxide concentration was fixed at 0.5 M for further experiments.

The effect of flow rate on peak height was examined by varying each flow rate from 0.4 to 1.0 ml min^{-1} for each pump: the flow rate of individual lines of each pump is the same flow rate. When the flow rate of the pump 1 was varied, the flow rate of the pump 2 was fixed at 0.8 ml min^{-1}. The decrease of peak height with increasing the flow rate was observed though sampling frequency increased: 0.8 ml min^{-1} of the flow rate of the pump 1 was chosen as a compromise between sensitivity and sampling frequency. The flow rate of the pump 2 was varied in the range from 0.4 to 1.0 ml min^{-1}, while the flow rate of the pump 1 was fixed at 0.8 ml min^{-1}. The peak height slightly decreased and the sampling frequency increased with increasing flow rate. The 0.6 ml min^{-1} was selected as the optimum flow rate of the pump 2. The effect of reaction temperature was examined over the range of 27–60 °C. The result shows that the temperature greatly influences the sensitivity: 50 °C was selected as the reaction temperature. The effect of sample volume on the peak height was studied. An increase in the injection volume from 100 to 700 μl improved the peak height, though sampling frequency decreased. The injection volume of 500 μl was chosen as a compromise between the sensitivity and the analysis time. The length of the reaction coil was varied from 0.5 to 7.0 m. Signal increased with increasing coil length. A 4.0 m of reaction coil length was chosen as a compromise between the sensitivity and the analysis time. The optimum conditions obtained in this work were summarized in Table 1.

3.2. Effect of HCl concentration on releasing of iron from iron complexes

Chemical forms of iron in natural waters mainly exist as Fe(III) hydroxide and humic complexes. Such iron complexes are less reactive than the free iron, Fe(III) and Fe(II), and therefore cannot be determined accurately by the catalytic methods [11]. The elemental analysis of water samples usually requires acidification prior to the analysis. Acidification of real samples can change the chemical forms of iron, dissociation of unreactive iron(III) hydroxides and complexed iron species to free iron [12]. Kawakubo et al. [11] reported that for the determination of soluble iron in river water samples, the samples must be acidified to 0.1 M hydrochloric acid and equilibrated for 1 day.

In this work, the concentration of acid and the effective releasing time was examined. Various amounts of the concentrated hydrochloric acid were added to water samples to give final concentration of 0.05, 0.1, 0.2 and 0.3 M. All sample solutions were injected into the flow injection system, and iron contents were measured. The releasing of iron was expressed as a function of time as is shown in Fig. 2. The initial rate of the iron releasing increased which increasing the concentrations of hydrochloric acid, and the peak height remained constant after 15 min, though the slight increase was observed for the sample treated with 0.05 M hydrochloric acid. This is probably because 0.05 M hydrochloric acid was not enough for releasing bound iron.

The acidified water sample, 0.1 M HCl, was also assayed over several hours and a few days were necessary to ensure that there was no further increased in the release amount of iron detected over time. There is no significant increased in free iron concentration after the acidification for 25 min to 1 day. This result indicates that all of iron was released by the treatment procedure. Therefore, the acidification of water sample was fixed to 0.1 M hydrochloric acid, and 30 min for releasing time was chosen for the water sample pretreatment.

3.3. Interference study

The effects of potential interfering ions were examined by using the solutions containing 1.0 μg l^{-1} Fe and one of other

Table 1
Selected conditions for the determination of iron

Variables	Range studied	Optimum conditions
H_2O_2 concentration, OS	0.24 to 2.4×10^{-2} M	1.2×10^{-2} M
DPD concentration, RS	0.1–1.5 M	0.5 M
Injection volume	100–750 μl	500 μl
Flow rate of pump 1	0.8–2.0 ml min^{-1} (total flow rate)	1.6 ml min^{-1}
Flow rate of pump 2	0.8–2.0 ml min^{-1} (total flow rate)	1.2 ml min^{-1}
Reaction coil length	50–700 cm	400 cm
Reaction temperature	30–60 °C	50 °C

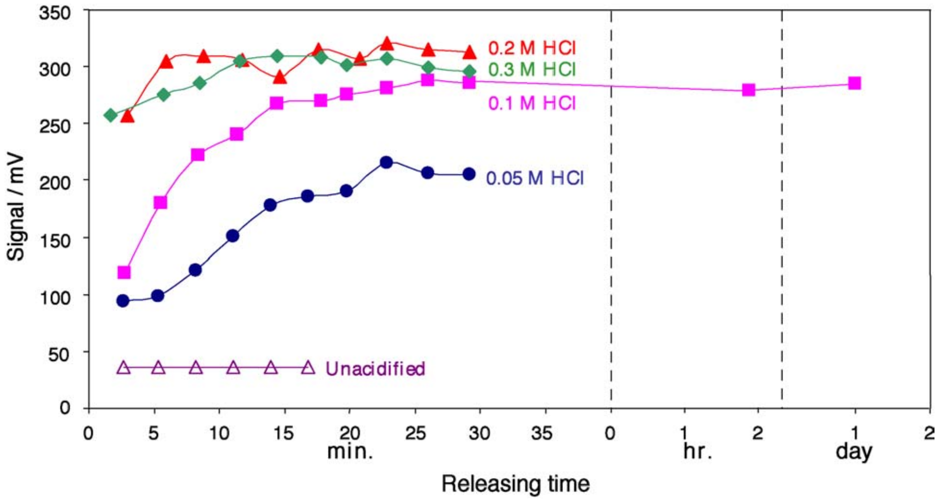

Fig. 2. Releasing profile of iron as a function of time after initial acidification of a water sample to 0.05, 0.1, 0.2 and 0.3 M with concentrated hydrochloric acid compared with unacidified sample.

Table 2
Maximum tolerance limits of diverse ions on the determination of 1.0 μg l^{-1} Fe

Diverse ions	Maximum tolerance concentration (mg l^{-1})
Li^+	600
Na^+, K^+, Ca^{2+}	200
Mg^{2+}	15
Mn(II), V(V)	4.0
Al^{3+}	2.0
Cd(II), Ni(II), Pb(II), Zn(II), Co(II)	1.0
Cr(III), Mo(VI)	0.25
$PO_4{}^{3-}$	20
$SO_4{}^{2-}$	50
Cu(II)	0.40[a]

[a] The value obtained in the presence of 6.7 × 10^{-4} M triethylenetetramine.

ions. The tolerable concentration of each diverse ion was taken as a highest concentration causing an error of ±5%. The results were summarized in Table 2. Most of the ions examined did not interfere with the determination of iron. Copper was found to seriously interfere with the determination of iron. And the interference from copper can be eliminated by adding triethylenetetramine as a masking agent. The added amount of triethylenetetramine, 6.7 × 10^{-4} M, has been tested for its affecting sensitivity of iron. The result indicated that at 6.7 × 10^{-4} M of triethylenetetramine is the maximum concentration which does not affect the sensitivity. The interference from copper up to 400 μg l^{-1} was completely removed by adding 6.7 × 10^{-4} M triethylenetetramine.

3.4. *Validation of the proposed method*

Under the optimum conditions, a linear response was observed for iron concentrations up to 2 μg l^{-1} with a correlation coefficient of 0.998. The equation of this line is $I = 99.07C + 0.6901$, where I and C are the signal intensity and iron concentration in μg l^{-1}, respectively. The detection limit was estimated to be 0.02 μg l^{-1} for a signal-to-noise ratio of 3. In the proposed method the detection limit is much improved than the method [5], in which the detection limit was reported to be 0.08 μg l^{-1}, and is the best of the spectrophotometric and fluorometric methods reported so far. Therefore, the present system will be an alternative method besides of the chemiluminescence techniques that are presently favored. The precision of the proposed method, in terms of relative standard deviation, was less than 2%. The sample throughput of the proposed method was 20 samples h^{-1}.

In order to evaluate the accuracy using the proposed method, the determination of iron in standard reference materials, river water (JAC 0032 and JAC 0031) was carried out. The analytical results obtained by the proposed method are in good agreement with the certified values as is shown in Table 3.

Table 3
Determination of iron in standard reference material, river water

Sample[a]	Taken (g)	Total (g)	Dilution factor	Found[b] (μg l^{-1})	Certified value (μg l^{-1})
JAC 0032	0.5025	20.02	39.85	56 ± 0.4	57 ± 2
JAC 0031	4.0215	20.00	4.975	6.5 ± 0.1	6.9 ± 0.5

[a] Purchased from The Japan Society for Analytical Chemistry.

[b] Average values of three replicates.

Table 4
Determination of iron in tap water and river water

Sample	Dilution factor	Added (μg l^{-1})	Found[a] (μg l^{-1})	Concentration in sample (μg l^{-1})[a]	Recovery (%)
Tap water[b]	1	0	0	0.55 ± 0.01	–
Tap water[b,c]	1	0	0	0.54 ± 0.02	–
Average				0.55 ± 0.01	
Tap water[d]	15	0	1.58 ± 0.02	23.7 ± 0.2	–
Tap water[c,d]	15	0	1.58 ± 0.02	23.7 ± 0.3	–
Tap water[d]	15	0.507	2.09 ± 0.02	23.8 ± 0.2	99
Tap water[d]	10	0	2.30 ± 0.02	23.1 ± 0.2	–
Tap water[c,d]	10	0	2.31 ± 0.02	23.1 ± 0.2	–
Average				23.5 ± 0.4	
River water[e]	60	0	1.04 ± 0.02	63 ± 1	–
River water[e]	60	0.515	1.55 ± 0.03	62 ± 2	99
River water[c,e]	60	0	1.05 ± 0.02	63 ± 1	–
River water[e]	30	0	2.10 ± 0.03	63 ± 1	–
Average				63 ± 1	
River water[f]	160	0	1.12 ± 0.01	179 ± 2	–
River water[f]	160	0.485	1.61 ± 0.03	180 ± 4	101
River water[c,f]	160	0	1.05 ± 0.00	167 ± 0	–
River water[f]	80	0	2.24 ± 0.01	179 ± 1	–

[a] Three replicates of determination.
[b] Unacidified samples.
[c] With the presence of 6.7×10^{-4} M triethylenetetramine.
[d] Acidified samples.
[e] Acidified prior to filtration.
[f] Acidified after filtration.

3.5. Analytical applications

The proposed method was applied to the determination of iron in tap water and river water samples to evaluate the applicability. The river water sample was sampled at the Zasu River, and the tap water sample was obtained from local pipe line. The tap water was discarded for 30 min before collection. Table 4 shows the analytical results of total iron, the aggregation of Fe(III) hydroxides, humic iron and iron which loosely bound to particulate matter (acidified prior to filtration) [13] and dissolved amount of iron (acidified after filtration) of river water samples. Acidified river water sample before filtration without triethylenetetramine gave higher amounts of iron than that of river water sample with triethylenetetramine. This result indicates that trace amounts of copper reaching from particulate matter interfere with the determination of iron. For tap water, free iron and bound iron form, which is probably present as Fe(III) hydroxide, were obtained when analyzed unacidified and acidified sample, respectively [11]. The results of recovery test, masking effect of triethlylenetetramine were also summarized in Table 4, and which shows that quantitative recoveries were obtained.

4. Conclusions

A highly sensitive method for the determination of iron was developed with DPD by optimizing the experimental variables in details. The proposed FIA method for iron determination gave higher sensitivity over spectrophotometric FIA methods reported so far. It is simple and robust with good reproducibility and accuracy which satisfy the requirements of trace analysis. The method was applied to the iron determination in surface waters. The analytical results of iron using the proposed sample pretreatment procedure provide useful information of total and dissolved amounts of iron in natural water, which could be used for the environmental assessment of the iron in term of potentially liability and/or bioavailability.

References

[1] M.R. Sangi, D. Jayatissa, J.P. Kim, K.A. Hunter, Talanta 62 (2004) 924.
[2] E. Bruninx, E. Van Meyl, Anal. Chim. Acta 80 (1975) 85.
[3] T.M. Forence, C.E. Batley, CRC Crit. Rev. Anal. Chem. 9 (1980) 259.
[4] T. Kawashima, S. Nakano, Anal. Chim. Acta 261 (1992) 167.
[5] I.Y. Kolotyrkina, L.K. Shpigun, A. Malohoff, Y.A. Zolotov, Analyst 120 (1995) 201.
[6] D.A. Weeks, K.W. Bruland, Anal. Chim. Acta 453 (2002) 21.
[7] J.M.T. Carneiro, A.C.B. Dias, E.A.G. Zagatto, R.S. Honorato, Anal. Chim. Acta 455 (2002) 327.
[8] S. Kawakubo, Y. Okazaki, M. Iwatsuki, Anal. Sci. 19 (2003) 373.
[9] S. Ohno, M. Tanaka, N. Teshima, T. Sakai, Anal. Sci. 20 (2004) 171.
[10] K. Hirayama, N. Unohara, Anal. Chem. 60 (1988) 2573.
[11] S. Kawakubo, Y. Hagihara, Y. Honda, M. Iwatsuki, Anal. Chim. Acta 388 (1999) 35.
[12] S. Kawakubo, M. Iwatsuki, Anal. Sci. 16 (2000) 945.
[13] S. Kawakubo, K. Tachikawa, M. Iwatsuki, J. Environ. Monit. 4 (2) (2002) 263.