Notes

Determination of Fe(II) and Fe(III) in Water by Flame Atomic Absorption Spectrophotometry after Their Separation with *Aspergillus niger* Immobilized on Sepiolite

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Introduction

The speciation of iron in aquatic systems is very important for environmental and biological studies because of the influence of its chemical forms on the bioavailabilty of iron and physico-chemical and toxicological properties of other trace elements and organic substances.[1] Iron is present in bivalent and in trivalent states in the natural environment. The changes between these two forms of iron are also important in various biological[2] and geochemical[3] processes. The low concentration of iron present in natural waters (at μg l^{-1} levels) necessitates the selection of a suitable preconcentration procedure.[4] Numerous methods, such as spectrophotometric,[5-8] potentiometric titration,[9] flame[10] and electrothermal[11] atomic absorption spectrophotometric methods, have been widely used for the determination of iron species.

In recent years, microorganisms such as yeast, bacteria and fungi have often been proposed for the preconcentration and speciation of trace metals.[12-19] Elmahadi and Greenway[16] used two types of algae: *Chlamydomonus reinhartii* and *Selenestrum capricornitum*, by immobilizing covalently to the controlled-pore glass for the preconcentration of Cu^{2+}, Ag^{+}, Cr^{3+} and Cr^{6+}. They also used these reagents for the speciation of Cr(VI) and Cr(III) using a flow system. Neidhart *et al.*[17] used human erytrocytes for the speciation of Cr(III) and Cr(VI) as free cells and by immobilizing them onto calcium alginate. They found that the kinetics of chromate uptake by erytrocytes alginate beads are slightly slower than mobile free erytrocytes due to the diffusion of chromate from the solution through the gel into the beads. They also investigated Cr(VI) uptake by erytrocytes as a function of constant incubation time, concentration of chromate, incubation temperature and pH of the solution. Robles *et al.*[19] have described a reliable method for speciation of soluble inorganic selenium ions, Se(IV) and Se(VI), which combines an uptake process by using living bacterial cells and electrothermal atomic absorption spectrometry.

This paper describes the preconcentration of Fe(II), separation of Fe(II) from Fe(III) and flame atomic absorption spectrometric determination of each iron species by using an adsorbent, *Aspergillus niger*, immobilized on sepiolite.

Experimental

Instrumentation

A GBC 933 Model flame atomic absorption spectrophotometer with deuterium lamp background correction was used for the determination of iron ions in the aqueous phase. The measurement conditions were as follows: 10 cm slit air–acethylene burner; air(10 l/min)–acetylene(2 l/min) flame; 0.2 nm spectral bandwidth; 248.3 nm wavelength; 7.0 mA lamp current. All pH measurements were made with a JENWAY 3010 Model pH meter and combination glass electrode. A Cole-Parmer microfiltration apparatus with membrane filter (0.45 μm pore size, manufactured by Microfiltration Systems (MFS)) was used for the filtration of water samples.

Reagents

All chemicals used were of analytical reagent grade unless otherwise specified. Triply distilled water was used throughout the experiments. Fe(II) and Fe(III) stock solutions (1000 mg l^{-1}) were prepared from $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Merck) and $FeCl_3 \cdot 6H_2O$ (Merck), respectively, by dissolving in 0.1 mol l^{-1} HCl solution. HCl (36%, Merck), HNO_3 (65%, Merck) and hydroxylamine hydrochloride (Merck) were used. Working iron standard solutions were prepared just before use by diluting the stock standard solution with water.

Materials

The sepiolite used as a substrate for the immobilization of *Aspergillus niger* in this paper was collected from the trances dug in the Turktaciri sepiolite deposit, which is a sedimentary type located to the west of Ankara, Turkey. It was ground and sieved to 35 – 60 mesh. The characterization of sepiolite, and the cultivation and immobilization of *Aspergillus niger* onto sepiolite, were identical with those reported elsewhere.[20,21]

Column preparation

A glass column (10 mm i.d. and 200 mm length) with a glass-wool plug over its stopcock was used. A 0.3-g of sepiolite on which *Aspergillus niger* immobilized was put into the column

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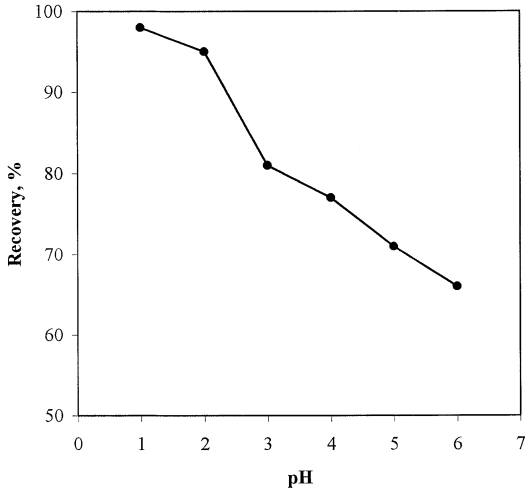

Fig. 1 The effect of pH on the recovery of iron(II) (0.50 μg ml^{-1}, eluent: 10 ml of 1.5 mol l^{-1} HNO_3, 0.3 g of adsorbent, flow rate 4 ml min^{-1}) by *Aspergillus niger* immobilized on sepiolite.

(about 1.5 cm height). A peristaltic pump was connected to the bottom end of the column. The connections of the peristaltic pump was made of Teflon. Before use, 1 mol l^{-1} HCl solution and doubly distilled deionized water were passed through the column in order to condition and clean it. Then, the column was conditioned to the studied pH.

General separation, preconcentration and determination procedures

For the optimization of column separation and preconcentration methods, 100 ml of spiked sample solutions containing 50 μg of iron species (Fe(II) or Fe(III)) were used. The pH of the solution was adjusted to the desired value (pH = 1) at which the recovery of one species (Fe(II)) is the highest and the other one (Fe(III)) is the lowest. The resulting solution was drawn through the column by using a peristaltic pump adjusted to the desired flow rate (4 ml min^{-1}). After washing the column with distilled water, retained species (Fe(II)) was eluted with 10 ml of 1.5 mol l^{-1} nitric acid solution. By using the calibration graph obtained by plotting absorbances against the concentrations of standard Fe(II) solutions, iron(II) was determined in the eluate by flame atomic absorption spectrometry (FAAS). Total iron was determined as Fe(II) by the method described above after reducing Fe(III) to Fe(II). The reduction of Fe(III) to Fe(II) was performed by the addition of excess amounts of hydroxylamine hydrochloride.[22] Then, the concentration of Fe(III) was calculated by subtracting the concentration of Fe(II) from the total iron concentration. The optimum conditions for separation of Fe(II) from Fe(III) and for preconcentration of Fe(II) have been determined by using the general procedure given above.

Results and Discussion

Effect of pH

The retention of Fe(II) onto the column as a function of pH has been investigated. Fe(III) was studied in previous research.[21] The pH of the solution was adjusted in a range of 1 to 6 by hydrochloric acid or ammonia solution, and passed through the column (0.3 g of adsorbent). Retained ions were eluted by 10 ml of 1.5 mol l^{-1} nitric acid. Fe(II) ions have been determined in the eluate by FAAS. As can be seen in Fig. 1, the highest recovery for Fe(II) was obtained at the pH value of 1. In a previous study,[21] the retention of Fe(III) on *Aspergillus niger* immobilized on sepiolite was low (<5%) at pH 1. The recovery of Fe(II) is quantitative at pH 1 and that of Fe(III) is rather low (<5%). This could make it possible to separate Fe(II) from Fe(III) and to determine Fe(II) by adjusting the pH to1.

Table 1 Effect of the type and volume of elution solution on the recovery of iron(II) (50 μg Fe(II), 100 ml sample)

Type of elution solution	Concentration/ mol l^{-1}	Volume/ ml	Recovery[a], %
HCl	0.5	5	34
		10	41
		15	44
HCl	1.0	5	47
		10	51
		15	58
HCl	1.5	5	54
		10	63
		15	67
HNO_3	0.5	5	64
		10	71
		15	74
HNO_3	1.0	5	72
		10	83
		15	88
HNO_3	1.5	5	89
		10	98
		15	98

a. Mean of 3 determinations.

Effect of amount of adsorbent (bed height)

The effect of the amount of adsorbent on the retention of Fe(II) ions was examined. For this purpose, the amounts of adsorbent were tested in a range of 0.1 to 0.5 g. It was found that the retention of Fe(II) ions increased with increasing the amount of the adsorbent up to 0.3 g. Above 0.3 g amount, it was practically not changed, and reached a plateau. Therefore, a 0.3-g volume of the adsorbent was found to be optimum for preconcentration and separation purposes.

Effect of type and volume of elution solutions

The regeneration of the adsorbent (*Aspergillus niger* immobilized on sepiolite) for the reuse in multiple biosorption–desorption cycles is important for the nondestructive recovery. For that reason, the concentration of acid solution used for the stripping metals bound to the cell surface must be as low as possible. To obtain a higher preconcentration factor, the volume of the elution solution must be also as small as possible. The elution studies were performed with 0.5, 1.0 and 1.5 mol l^{-1} hydrochloric and nitric acid solutions. The eluate volume was 5, 10 and 15 ml. As can be seen in Table 1, a 10-ml volume of 1.5 mol l^{-1} nitric acid solution was found to be satisfactory. Although the same results were obtained with 15 ml of solution, this volume was not preferred due to the low preconcentration factor.

Effect of flow rate of sample solution

The retention of Fe(II) on *Aspergillus niger* immobilized on sepiolite and the duration of complete analysis are affected by the flow rate of the sample solution. Therefore, the effect of the flow rate of sample solution was examined under the optimum

Table 2 Effect of interfering ions on recovery of iron(II) (50 μg Fe(II), 100 ml sample)

Interfering ion	Concentration ratio (μg ml^{-1}/μg ml^{-1}) (Ion:Fe(II))	Recovery[a], %
Pb^{2+}	20	98.2
Zn^{2+}	20	98.0
Cu^{2+}	20	96.6
Cd^{2+}	20	96.9
Ni^{2+}	20	97.2
Cr^{3+}	20	96.5
Cr^{6+}	20	98.1
Co^{2+}	20	97.4
Mn^{2+}	20	97.8
Na^{+}	100	95.8
K^{+}	100	94.5
Ca^{2+}	100	95.6
Mg^{2+}	100	95.3

a. Mean of 3 determinations.

conditions (pH, bed height and eluent type) by using a peristaltic pump. The flow rate of the sample solution was adjusted in a range of 1 – 7.5 ml min^{-1}. It was found that the retention of Fe(II) was practically not changed up to 4 ml min^{-1} flow rate. Above this value, it gradually decreased. For that reason, 4 ml min^{-1} was chosen as the optimum flow rate.

Effect of volume of sample solution

For the investigation of the usefulness of the proposed method for the preconcentration of very dilute analyte solutions, 100, 250, 500, 750 and 1000 ml of sample solutions containing 50 μg of Fe(II) were passed through the column under the optimum conditions (pH, bed height, flow rate and eluent type) after the preconcentration procedure described above has been applied. It was found that Fe(II) ions could be recovered quantitatively up to 750 ml of the sample solution. Above 750 ml of the sample solution, the recovery decreased gradually with increasing volume of sample or with decreasing the concentration of the analyte. Because a 10-ml volume of acid solution was used as an eluent, 75-fold preconcentration factor was obtained.

Precision of the method

The precision of the determination of iron(II) was evaluated under the optimum conditions mentioned above. For this purpose, seven successive retention and elution cycles (with 50 μg of Fe(II) in 100 ml of solution) were performed. It was found that the recovery of Fe(II) was 98.2 ± 0.3% at 95% confidence level. In conclusion, the precision of the method is very good, and the recovery of the analyte is quantitative (>95%).

Detection limit

The detection limit was evaluated as the concentration corresponding to three times the standard deviation of the blank signal and was found to be 113 ng ml^{-1} for Fe(II) by using a synthetic sample solution blank.

Capacity studies

The breakthrough capacity was used in this work to evaluate the amount of metal adsorbed onto *Aspergillus niger* immobilized on sepiolite. The breakthrough capacity of the adsorbent is defined as the amount of metal ions that can be extracted per unit mass under the operating conditions prevailing, prior to being detected in the column effluent.[23] The procedure for capacity study has been given in Ref. 24. The breakthrough capacity was found as 96 μmol/g for Fe(II). On comparing the former results[21] related to breakthrough capacity for Fe(II) obtained with same adsorbent, we see that *Aspergillus niger* immobilized on sepiolite, Fe(II) has greater breakthrough capacity. The difference in breakthrough values may be due to the presence of the different types of binding sites on the fungus cell wall[14] and that capacity may depend on the nature and oxidation state of the metal.

Table 3 Determination of Fe(II) and Fe(III) in spiked sample solutions[a]

Added/ μg ml^{-1}		Found/ μg ml^{-1}		Recovery[b], %		Relative error, %	
Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)
0.2	0.2	0.195	0.193	98	97	–3	–4
0.3	0.2	0.290	0.194	97	97	–3	–3
0.2	0.3	0.195	0.293	98	98	–3	–2
0.3	0.4	0.291	0.395	97	99	–3	–1

a. Sample volume 100 ml.
b. Mean of 5 determinations at 95% confidence level.

Effect of interfering ions

The effect of the presence of other metal ions on the retention of iron(II) was investigated. For this purpose, Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Mn^{2+}, Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} ions were added individually to a 100-ml of solution containing 50 μg of Fe(II) and the general procedure was applied. As can be seen in Table 2, although the ratio of interfering ions to iron was 20 for Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+} and Mn^{2+} ions and 100 for Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} ions, they did not significantly affect the retention of iron. In our previous work[21] the retention of some of these ions were investigated and we found that it was very low at pH 1. Particularly, the interfering effects of nickel and cobalt were serious. However, the interference could be sufficiently overcome by the separation in the column.

Column reuse

To test the long-term stability of the biocolumn, the column containing *Aspergillus niger* immobilized on sepiolite was subjected to succesive binding and stripping cycles by passing 100 ml of a 0.5-μg ml^{-1} solution of Fe(II) at pH 1, and then stripping the metal with 10 ml of 1.5 mol l^{-1} HNO_3 solution. The procedure was carried out twenty times. There was no observable deterioration of column performance with repetitive usage.

Application

The proposed method was applied to the separation of Fe(II) from Fe(III) in spiked sample solutions and in Kızılırmak river water (located in Kırıkkale, Turkey). The river water samples were filtered through celulose membrane filter (pore size 0.45 μm; manufactured by Microfiltration Systems) and analyzed as soon as possible after sampling. As shown in Tables 3 and 4, the proposed method could be applied successfully for the separation, preconcentration and speciation of trace amounts of iron in both spiked and river water samples. As shown in the tables, the accuracy of the results were quite satisfactory. The

Table 4 Determination of Fe(II) and Fe(III) in river water[a]

Added/ μg		Found/ μg		Recovery[b], %		Relative error, %	
Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)
—	—	4.7	9.6	—	—	—	—
5.0	5.0	9.3	13.9	96	96	–4	–5
10.0	10.0	14.0	18.7	95	96	–5	–5
15.0	15.0	18.8	23.6	95	97	–5	–4

a. Sample volume 100 ml.
b. Mean of 5 determinations at 95% confidence level.

relative error was lower than 5% for both iron(II) and iron(III).

Conclusion

The proposed method for the separation, preconcentration and speciation of iron is simple, sensitive and accurate. Iron can quantitatively be recovered by the collector studied with a high precision. Repeated use of the column is possible. It can be concluded that the columns are also relatively stable up to 20 runs.

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