Solid-Phase Extraction, Separation, and Visible Spectrophotometric Determination of Trace Amounts of Iron in Water Samples

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A simple and reliable method is presented for the rapid extraction, separation, preconcentration, and determination of iron as its bathophenanthroline complex by the use of octadecylsilica membrane disks and spectrophotometry. We evaluated extraction efficiency, the influence of sample matrix, type and optimum amount of extractant, flow rates of sample solution and eluent, pH, amounts of bathophenanthroline and hydroxylamine hydrochloride, breakthrough volume, and limit of detection. We also studied the effects of various cationic interferences on percent recovery of iron. Complete elution of the complex from disks was obtained with a minimal amount of solvent. The limit of detection of the proposed method is 0.080 ppb. The method was applied to the recovery and determination of iron in natural waters.

The determination of trace elements, particularly heavy metals such as iron, has received increasing attention in pollution studies. In filtered samples of oxygenated surface water, iron concentrations seldom reach 1 mg/L. However, iron in water can cause staining of laundry and porcelain. Thus, the determination of trace amounts of iron is important. Flame, Zeeman, and graphite-furnace atomic absorption spectrometry (AAS; 1) and spectrometric methods (2, 3) are the most commonly used techniques for iron estimation. Although in some cases the sensitivity of a method is suitable for determination of trace amounts of iron, extraction and preconcentration steps can improve the sensitivity, accuracy, and detection limit of the system. Consequently, a concentration and matrix-elimination step is usually required.

Liquid–liquid extraction (LLE) with the separation of iron in the presence of coextractant ligands such as 4-acyl-5-pyrazolones (4), crown ethers (5, 6), pyrrolidine-dithiocarbamate (7), pentamethylene dithiocarbamate (8), and di-2-pyridylmethanene-2-(5-nitro)pyridylhydrazone (9) has attracted considerable attention. However, LLE suffers from several drawbacks such as long and labor-intensive extraction, requiring strict control of extraction conditions such as temperature, pH, and ionic strength. Nevertheless, several other techniques for the preconcentration of iron have been proposed including preconcentration on a microcolumn packed with a chelating resin (10), preconcentration on a membrane filter (11, 12), and solid-phase extraction (SPE; 13–16).

Through SPE, octadecyl (C18)-bonded silica cartridges loaded with different ligands offer the possibility of concentrating trace amounts of metal ions by elution with a volume smaller than the volume loaded on the cartridge (17–19). In this application, volumes up to 1 L water are pulled through a cartridge, although the volume for the elution is <5 mL. Thus, concentration factors of up to 1000 are attained. The major disadvantages of the SPE cartridges and tubes (20), which are caused by the small cross-sectional area of the extraction cartridge, are slow sample-processing rates and a low tolerance to blockage by particles and adsorbed matrix components; channeling reduces the capacity of the cartridge to retain analytes and results in contamination of the isolated analytes with impurities originating from the manufacturing and packing process. However, the use of flat disks may greatly reduce these problems. Moreover, the separation and preconcentration step can be achieved through addition of reagent to the sample (17–19, 21–23) or through immobilization of reagent (13, 15, 24–28) on the SPE cartridges or disks. Addition of reagent to the sample is appropriate for the fixation of usable metal species such as Cu(I) or Fe(II), whereas immobilization can provide the convenience of having a prepared SPE system before analysis.

The aim of this work was the development of a simple, sensitive, and efficient method for the selective extraction, concentration, and determination of trace amounts of iron in aqueous media as its bathophenanthroline complex by the use of octadecylsilica membrane disks and spectrophotometry. We studied various experimental conditions, e.g., the type and volume of eluting solvent, and the effects of pH, sample and eluent flow rates, and the amounts of bathophenanthroline and hydroxylamine hydrochloride on extraction efficiency, breakthrough volume, and limit of detection.

Experimental

Reagents

All chemicals used were analytical grade. Double-distilled water was used throughout. Standard Fe(III) solution (1 mg/mL) was prepared by dissolving 0.8635 g ferric alum, FeNH4(SO4)2·12H2O, in water containing 0.5 mL concentrated H2SO4 and diluting to volume with water in a 100 mL
volumetric flask. A 0.1 mg/mL solution of Fe(III) was prepared by appropriate dilution of the stock solution. Bathophenanthroline ($\lambda_{\text{max}} = 284.5$ nm in ethanol) was obtained from Merck (Darmstadt, Germany) and used without any further purification.

**Apparatus**

(a) **Spectrophotometer.**—All absorbance measurements were obtained by using a Shimadzu (Tokyo, Japan) UV-2100 spectrophotometer.

(b) **pH Meter.**—A Model 691 digital Metrohm pH meter equipped with a combined glass-calomel electrode was used for the pH adjustments (Metrohm, Herisau, Switzerland).

(c) **Inductively coupled plasma (ICP) emission spectrometer.**—Model Varian Liberty 150AX Turbo (Varian, Melborn, Australia). Used for determination of iron concentration in real water samples.

**Sample Extraction**

Extractions were performed with Empore membrane disks, 47 mm diameter × 0.5 mm thickness, containing octadecyl-bonded silica (8 μm particles, 60 Å pore size) from J.T. Baker (Phillipsburg, NJ). The disks were used in conjunction with a standard Millipore 47 mm filtration apparatus (Millipores, A., Yvelines, France).

To remove small, suspended particles before extraction, water samples were filtered through cellulose acetate filters, 0.45 μm pore size (Millipore). After each filtration, the residue in the filter was washed with 10 mL deionized water. To 150 μL Fe(III) solution (0.1 mg/mL) containing 15 μg Fe(III) were added 40 mL double-distilled water, 0.5 mL 6M HCl, 1 g sodium acetate, 0.1 g hydroxylamine hydrochloride, and 0.4 mg bathophenanthroline, and the solution was heated to boiling. The cooled solution was transferred to a 50 mL volumetric flask and diluted to volume with water.

Before extraction, each membrane was washed with eluting solvent. After the membrane was placed in the extraction apparatus, 10 mL solvent was introduced into the reservoir of the apparatus and drawn slowly through the disk by application of a slight vacuum. After the disk had been completely dried by having air drawn through it for a few minutes, 10 mL methanol was again drawn through the disk. A thin layer of methanol was left on the surface of the disk, and the disk was washed with 10 mL deionized water. This step prewets the surface of the disk before the extraction of the iron–bathophenanthroline complex from water. It is important to note that the surface of the disk did not become dry from the time that methanol was added until the extraction was completed. Then the sample solution containing iron, bathophenanthroline, and buffer was passed through the membrane. To keep the surface of the disk wet during extraction, 0.5 mL methanol was added to all samples during each extraction. After the sample had passed through the disk, full vacuum was used for a few minutes to remove excess water. A 25 × 100 mm test tube was then placed under the extraction funnel. Approximately half of a 10 mL portion of the final elution solvent was then drawn through the membrane, and the vacuum was interrupted at this point to allow the solvent to soak the disk for several minutes. The remaining portion of the final elution solvent was then drawn through the disk. The concentration of the iron-bathophenanthroline complex was determined by spectrophotometry at 533 nm.

**Results and Discussion**

Some preliminary experiments were performed in order to investigate the quantitative retention of iron ions by the octadecyl silica membrane disk in the absence and presence of bathophenanthroline. It was found that, although the membrane disk itself does not show any tendency for the extraction of iron ions, it is capable of retaining the iron–bathophenanthroline complex in the sample solution (the test solution used contained 15 μg iron and 0.4 mg bathophenanthroline in 50 and 250 mL water, respectively). It is noteworthy that bathophenanthroline is well known as an excellent ligand for the solvent–solvent extraction of iron from water samples (21).

**Choice of Eluent**

In order to choose a proper eluent for the retained iron complex after its extraction from water, 5 mL of various organic solvents such as methanol, ethanol, propanol, chloroform, and isopentyl alcohol; methanol, ethanol, and propanol containing 0.2 g NaClO$_4$; and isopentyl alcohol saturated with NaClO$_4$ were tested as eluents, and the absorbance of each solution was obtained versus that of each solution blank at 533 nm. The data in Table 1 show that 5 mL ethanol or methanol containing 0.2 g NaClO$_4$ is a proper eluent. Because of safety considerations, subsequent elution of the complex was performed with ethanol containing NaClO$_4$. From the data given in Table 1, it is immediately obvious that pure solvents wash only a part of the complex from the disk. Further experiments showed that the complex could not be quantitatively eluted by increasing the volume of the solvents, but in the presence of a small amount of NaClO$_4$, elution of the complex was quantitative. Most probably, various interaction forces between the complex and the octadecyl silica are responsible for these results.

Octadecyl silica-bonded phases are formed by the reaction of siloxane groups on the surface of the silica support with

<table>
<thead>
<tr>
<th>Table 1. Effect of type of eluent on extraction efficiency</th>
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<tr>
<td><strong>Eluent</strong></td>
</tr>
<tr>
<td>Ethanol</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>Propanol</td>
</tr>
<tr>
<td>Isopentyl alcohol</td>
</tr>
<tr>
<td>Chloroform</td>
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</tbody>
</table>

| $a$ The volume of eluent is 5 mL. |
| $b$ NaClO$_4$. |
octadecyl chlorosilane. It is interesting to note that the fraction of the silanol groups that react with the silane reagent is usually between 50 and 60%; thus, acidic Si-OH groups are present on the surface of the silica (29). Therefore, interactive forces between the octadecyl silica-bonded phase and Fe(bathophenanthroline)_3^{2+} in the presence of acetate counter ions may be dispersive or ion–ion in nature. Thus, for quantitative elution of the complex, the presence of a salt in the eluent is necessary. The sorption of cations or cationic complexes on silica gel, based on the cation-exchange properties of silanol groups, was reported previously (30–32).

To choose the proper volume of eluent, we eluted the complex with various volumes of ethanol containing NaClO_4; data showed that 10 mL ethanol containing 0.4 g NaClO_4 quantitatively eluted the complex from the disk.

To investigate the optimum amount of NaClO_4 in 10 mL ethanol for the elution of the complex from the disk, we performed elution with various amounts of NaClO_4 from 0 to 0.4 g. The elution of the complex was found to be quantitative when >0.2 g NaClO_4 was used. Consequently, subsequent elution experiments were performed with 10 mL ethanol containing 0.2 g NaClO_4.

**Effect of Amounts of Ligand and Reducing Agent**

The optimal amount of bathophenanthroline for maximum extraction of iron was investigated (Figure 1). We found that the extraction of (15 μg) iron was maximized when >0.3 mg ligand was used. Thus, 0.4 mg bathophenanthroline was used for further studies. o-Phenanthroline forms stable complexes with iron(II). The parent compound has a pair of nitrogen atoms located in positions that allow each to form a covalent bond with the iron(II) ion. Three o-phenanthroline molecules combine with each iron ion to yield the Fe(phen)_3^{2+} complex, where phen indicates o-phenanthroline. The color of this complex at λ_max = 533 nm is very intensive (ε = 2.24 × 10^4). On the other hand, the color of the oxidized form of the complex Fe(phen)_3^{3+} is very low (2, 33). Thus, iron(III) is reduced with hydroxylamine before SPE as follows:

\[ 2\text{NH}_2\text{OH} + 4\text{Fe}^{3+} = \text{N}_2\text{O} + 4\text{Fe}^{2+} + 4\text{H}^+ + \text{H}_2\text{O} \]

**Effect of pH and Flow Rates**

The influence of pH on the extraction of iron ions was studied in the pH range of 1.5–7 by using sodium acetate/HCl for pH adjustment (Figure 2). Percent recovery was constant in the pH range of 4–7. It is interesting to note that a similar pH effect has already been reported for the separation of iron by solvent extraction using bathophenanthroline (34). At pH values of <2.5, however, percent recovery decreased, most probably because of the competition of H^+ with Fe(II) for reaction with bathophenanthroline. Higher pH values (>7) were not tested because of the possibility of the hydrolysis of octadecyl silica in the disk. In this study, a buffer solution of pH 5.4 was adopted.

The influence of the flow rates of the sample and eluting solutions on the retention and recovery of iron ions was investigated. It was found that the retention of iron ions by the membrane disk was not significantly affected by flow rates of 8–75 mL/min for the sample solution. However, quantitative elution of iron ions from the disk was achieved by a flow rate in the range of 1.0–3.5 mL/min. Similar results were also reported for organic pollutants (35, 36) as well as for various cations (26, 27).

**Analytical Performance**

When solutions of 15 μg iron in 50, 100, 150, 250, 500, 600, 750, and 1000 mL water were passed through the disks, the iron–bathophenanthroline complex was quantitatively retained from 600 mL and smaller volumes. Thus, breakthrough volume for the method was 600 mL. By increasing the amounts of ligand to 1 mg, breakthrough volume reached 1000 mL. The limit of detection (LOD) of the method for the determination of iron was studied under the optimal experimental conditions. The LOD obtained from 3σ of the blank was 0.080 ppb (for 500 mL sample solution). The dynamic linear range of the method was in the range of 10–700 ng/mL (for 50 mL sample solution).
The influence of several ions such as VO$_3^-$ and MoO$_4^{2-}$ on the SPE and determination of iron ions (15 $\mu$g iron in 50 mL solution) was studied. A relative error of twice the standard deviation of the measurements (i.e., 3% concentration) was considered tolerable. The results are summarized in Table 2. Most of the cations and anions examined did not interfere with the extraction and determination of iron or were tolerated at very high levels. However, some of the species tested such as Co$^{2+}$, Ni$^{2+}$, and VO$_3^-$, and especially Cu$^{2+}$, interfered with the determination of the iron ion. The interference effect of Cu$^{2+}$ is due to the preferential formation of a very stable, almost colorless, complex between copper(I) and bathophenanthroline (34). The interference effect of copper ion was eliminated by the addition of excess amounts of thiourea, as a masking agent, to the sample solution. On the other hand, the interfering effect of Co$^{2+}$, Ni$^{2+}$, and VO$_3^-$ was eliminated by using excess amounts of bathophenanthroline (4:1 ratio of ligand to cation).

To assess its applicability to real samples, the method was applied to the extraction and determination of iron in 50 mL of different water samples. For this purpose, the real samples were first passed through a cellulose acetate membrane filter (0.45 $\mu$m pore size). Then 50 mL of each chosen sample was used to measure the amount of iron, as described in the Experimental section. As shown in Table 3, the amount of iron is measured when the values are located in the dynamic linear range of the calibration curve. Obviously the volume of the samples depended on the amount of iron in each sample. The volume should be adjusted for the dynamic linear range. Tap water samples were taken from different cities in Iran and analyzed (Table 3). The results of 3 analyses of each sample by the proposed method and by ICP emission spectrometry were in satisfactory agreement.

### Conclusions

The proposed method has several advantages. The method is rapid in comparison with previously reported procedures for the separation and determination of iron (2, 3). The time needed for the separation and determination of iron in a 500 mL water sample is $\leq$30 min. The method can selectively separate iron ions from other associated metal ions, even those present at much higher concentrations. The volume of organic solvents in the proposed method is much smaller than that consumed at much higher concentrations. The advantage of this method in comparison with AAS and ICP emission spectrometry methods is its simplicity and applicability to field determination of iron. It is interesting to note that by combining the SPE procedure with sensitive iron determination methods such as that ICP emission spectrometry method, the detection limit of the method can be lowered by a factor of about 50. On the other hand, the field measurement of iron can be undertaken because of the simplicity of the proposed method, which does not need sophisticated equipment.

### Acknowledgment

We acknowledge the Atomic Energy Organization of Iran for the ICP emission spectrometry analysis of real water samples.

### Table 2. Tolerance limits of diverse ions in the recovery of 15 $\mu$g iron from 50 mL sample solution

<table>
<thead>
<tr>
<th>Foreign ion</th>
<th>Tolerated ratio of foreign ion to iron ion</th>
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<tbody>
<tr>
<td>K$^+$, Li$^+$, Na$^+$, Sr$^{2+}$, Ba$^{2+}$, Ca$^{2+}$, Be$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Se(IV), Ce(III), Ti$^{4+}$, Pb$^{2+}$, Rb$^+$</td>
<td>500$^a$</td>
</tr>
<tr>
<td>VO$_3^-$</td>
<td>5</td>
</tr>
<tr>
<td>MoO$_4^{2-}$, Zn$^{2+}$</td>
<td>30</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>15</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>2</td>
</tr>
<tr>
<td>Ni$^{2+}$, Cu$^{2+}$</td>
<td>Interference</td>
</tr>
</tbody>
</table>

$^a$ Highest ratio tested.

### Table 3. Results for the determination of iron in water samples

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Fe found, ppb (RSD, %)$^a$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SPE</td>
</tr>
<tr>
<td>Tehran tap water</td>
<td>61.7(5.2)</td>
</tr>
<tr>
<td>Ahvaz tap water</td>
<td>50.1(9.7)</td>
</tr>
<tr>
<td>Islamshahr tap water</td>
<td>32.3(9.4)</td>
</tr>
<tr>
<td>Robatkarim tap water</td>
<td>50.6(1.4)</td>
</tr>
<tr>
<td>Well water</td>
<td>32.1(2.8)</td>
</tr>
</tbody>
</table>

$^a$ RSD = relative standard deviation (based on 3 replicate analyses).

### References
