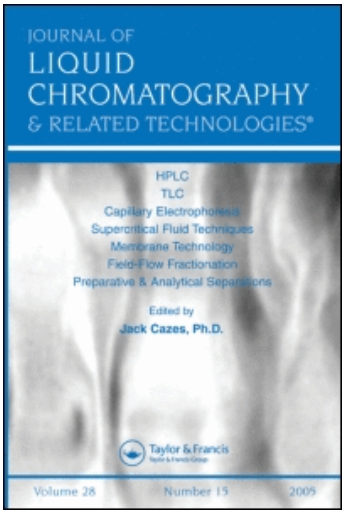

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Liquid Chromatography – Catalytic Analysis Detection as a Highly Sensitive and Automated Fractional Determination Method: Determination of Iron(II) and (III)

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Abstract: Liquid chromatographic separation and catalytic analysis detection was investigated for automated fractional determination of iron(II) and (III). Chromatographic separation between these species was performed with a deoxygenated mixed solution (pH 3.5) of ethylenediamine and citric acid as the eluent and a separation column containing sulphonate groups. The eluent was introduced to an air segmented continuous flow analysis based on the catalytic reaction with Bindschedler's green leuco base. The detection limits of this method for iron(II) and (III) were 8.2 nmol dm^{-3} and 1.7×10^2 nmol dm^{-3}, 3, respectively. This method was applied to the analysis of lake and pore waters.

Keywords: 4,4′-bis(dimethylamino)diphenylamine, Air segmented continuous flow analysis, Catalytic analysis detection, HPLC, Iron

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INTRODUCTION

A highly sensitive and automated fractional determination method can be developed by connecting liquid chromatographic separation and catalytic analysis detection.[1] Catalytic analysis is very sensitive, if a suitable reaction system is selected. For example, 10 pmol dm^{-3} of lead is analyzed by using the oxidation reaction of Pyrogallor red with peroxodisulfate[2] and 50 pmol dm^{-3} of cobalt is measured by the Tiron and hydrogen peroxide system.[3] Most of these catalytic analysis methods are based on the catalytic effect of the redox-active element to be analyzed on the oxidation reaction of a coloring agent. Therefore, as more than one species with different oxidation states of the objective element (for instance, iron(II) and (III)) can be determined with the same sensitivities by the methods,[4] catalytic analysis is adequate as a detection technique for a fractional determination according to the oxidation states of the element.

When the catalytic method is automated, this method can be connected to a liquid chromatographic system. Automation of this method can be easily achieved by applying a flow system to the method.[3–6] As the sensitivity and accuracy of the catalytic method are heavily affected by the reaction time, the time must be strictly regulated for reproducible detections. Automation of the catalytic method can also satisfy this request. The catalytic method is often interfered with by coexisting elements having redox potentials similar to that of the element to be analyzed. However, this interference can be removed by separating those elements from the analyte element with a liquid chromatograph. When such interfering elements can be quantitatively separated, the elements will be treated not as interfering but as analyte elements, because we can sequentially determine not only the original analyte element but also the elements, which were formerly considered to be interfering, by using the method connecting liquid chromatographic separation and catalytic analysis detection.

Based on the above, we previously investigated liquid chromatography-catalytic analysis detection (LC-CA) for vanadium(IV) and (V), and developed a highly sensitive and automated fractional determination method for them.[1] In that work, we utilized the catalytic effect of vanadium on the oxidation reaction of Bindschedler's green leuco base (BGL, 4,4′-bis(dimethylamino)diphenylamine) by potassium bromate. The catalytic method was automated with air segmented continuous flow analysis (ASCFA).[4] As a catalytic method usually requires a long reaction time, the analytical signals broaden and overlap, due to the dispersion of the sample to the mobile phase in a reaction coil during flow injection analysis.[7,8] However, when using ASCFA, which segments the reaction solution with air, such an effect is very slight.

Iron(II) and (III) also have a catalytic effect on the oxidation reaction of BGL.[9] The effect of iron is well known to be very active in the use of hydrogen peroxide as an oxidizing agent for BGL. On the other hand, the catalytic effect of vanadium is weak in this reaction system.[9,10] In the present work, automated fractional determination of iron(II) and (III) was studied with LC-CA using the reaction system.

EXPERIMENTAL

System for Liquid Chromatograph – Catalytic Analysis Detection

Figure 1 shows a schematic diagram of the LC-CA system for the determination of iron(II) and (III). This system's constitution is essentially the same as that previously reported.[1] The liquid chromatograph consisted of a Hitachi L-7120 polymer made double plunger pump, Reheodyne polymer made sample injection valve, and a Tosoh TSK gel IC-Cation-SW Column (5.0 cm × 4.6 mm i.d.). This column containing hydrophilic silica gel (particle size: 5 μm) with sulphonate groups was thermostated at 40°C. All components (tubes, pump, injection valve, and column ware) of the liquid chromatograph in contact with the eluent, sample, or

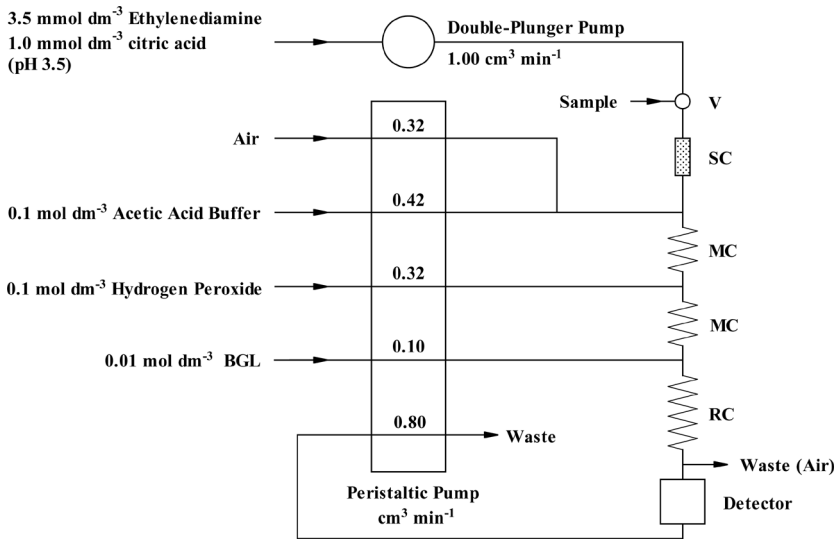

Figure 1. Schematic diagram of liquid chromatograph – catalytic analysis detection for the automated fractional determination of iron(II) and (III). V: injection valve; SC: separation column; MC: mixing coil; RC: reaction coil; Detector: UV-Visible spectrophotometer.

standard solutions, were plastic made in order to prevent contamination of the iron from their components. Under a constant flow rate of the eluent at 1 cm^3 min^{-1}, a 0.1 cm^3 portion of each sample was injected. The eluent was prepared as a mixture (pH 3.5) of 3.5 mmol dm^{-3} ethylenediamine and 1 mmol dm^{-3} citric acid.

An ASCFA system consisting of a Bran Leube model II autoanalyzer was used for catalytic analysis detection and was connected with the liquid chromatograph. The eluent from the liquid chromatograph, the 0.1 mol dm^{-3} hydrogen peroxide solution as an oxidizing agent, and the solution of BGL (0.01 mol dm^{-3}) as a coloring reagent, were added, in turn, into the flow of 0.1 mol dm^{-3} acetic acid buffer solution (pH 4.0) segmented with air. The flow rates were regulated to be 0.32 cm^3 min^{-1} for air, 0.42 cm^3 min^{-1} for acetic acid buffer solution, 0.32 cm^3 min^{-1} for hydrogen peroxide solution, and 0.10 cm^3 min^{-1} for BGL solution. In the reaction coil, BGL was well oxidized at 40°C for about 4 min. After the removal of the air to segment the solution flow, the absorbance of the solution was measured at 720 nm with the detector, an UV-VIS spectrophotometer.

Reagents

Highly purified water produced by the Milli-Q water system (Millipore) was used for preparation and dilution of standard solutions and washing of bottles and tubes. Stock standard solutions (20 mmol dm^{-3}) of iron(II) and (III) with 0.1 mol dm^{-3} hydrochloric acid were prepared from ferrous and ferric chlorides, respectively. These solutions were freshly prepared every 1 month. Working standard solutions of iron(II) and (III) with 0.01 mol dm^{-3} hydrochloric acid were prepared by diluting the respective stock solutions everyday.

The solution of BGL at 0.01 mol dm^{-3} level was prepared from a commercial reagent (Dotite, Kumamoto, Japan) and was acidified to pH 1.5 by the addition of hydrochloric acid. This solution was freshly prepared every week. All other chemicals used were of reagent grade.

RESULTS AND DISCUSSION

Liquid Chromatographic Separation Between Iron(II) and (III)

Liquid chromatographic separation between iron(II) and (III) was examined under various operating conditions for the chemical composition and pH of the eluent. However, their quantitative separation expected from previous works[11–14] was not achieved. It was suggested that

iron(II) was oxidized to iron(III) in the separation column and tubes of the liquid chromatograph system, and that such oxidation interfered with the quantitative separation. For example, liquid chromatograms with a mixture (pH 3.5) of 3.5 mmol dm^{-3} ethylenediamine and 10 mmol dm^{-3} citric acid as the eluent are shown in Figure 2. The chromatogram of iron(III) showed a single peak (Figure 2b), whereas that of iron(II) had two peaks not separated from each other (Figure 2a). As the retention time of iron(II) is longer than that of iron(III) in this operating condition,[11,12,15] it is inferred that the second peak in Figure 2a was due to iron(II) and that the preceding first broad one was formed by the oxidation of iron(II) to iron(III) within the liquid chromatographic system. In order to reduce this interference, the entire system was preconditioned by flushing 0.1 mol dm^{-3} sodium sulphite solution (pH 7) into the system at 1 cm^3 min^{-1} for 1 hour.[16] In addition, dissolved oxygen was eliminated from the eluent by bubbling nitrogen gas for 2 hours and the eluent was preserved under nitrogen gas atmosphere during liquid chromatographic measurements. Thus, iron(II) and (III) could be completely separated from each other. Figure 3 shows chromatograms under the operating condition mentioned above under "Experimental". No peak of iron(III) was detected with the injection of iron(II) solution alone (Figure 3c).

We examined the effects of ethylenediamine and citric acid concentrations in the eluent on the chromatographic separation between iron(II) and (III) (Figures 4 and 5). The pH value was fixed to 3.5 in order to prevent the oxidation of iron(II). The retention time of iron(III) was independent of ethylenediamine and citric acid concentrations and was

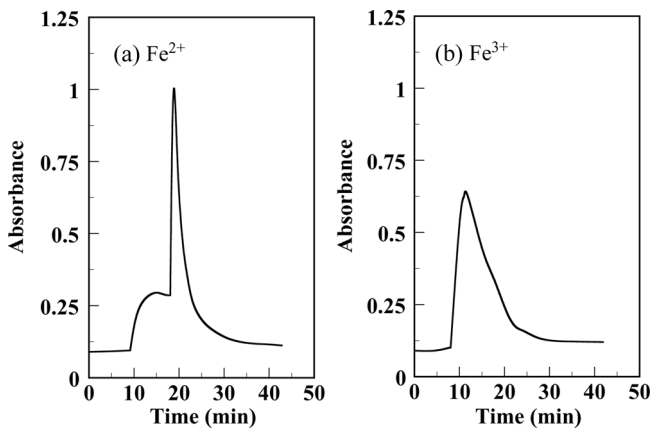

Figure 2. Chromatograms of iron(II) and (III). (a): 100 μmol dm^{-3} of iron(II) alone. (b) 100 μmol dm^{-3} of iron(III) alone. Eluent: mixture (pH 3.5) of 3.5 mmol dm^{-3} ethylenediamine and 10 mmol dm^{-3} citric acid.

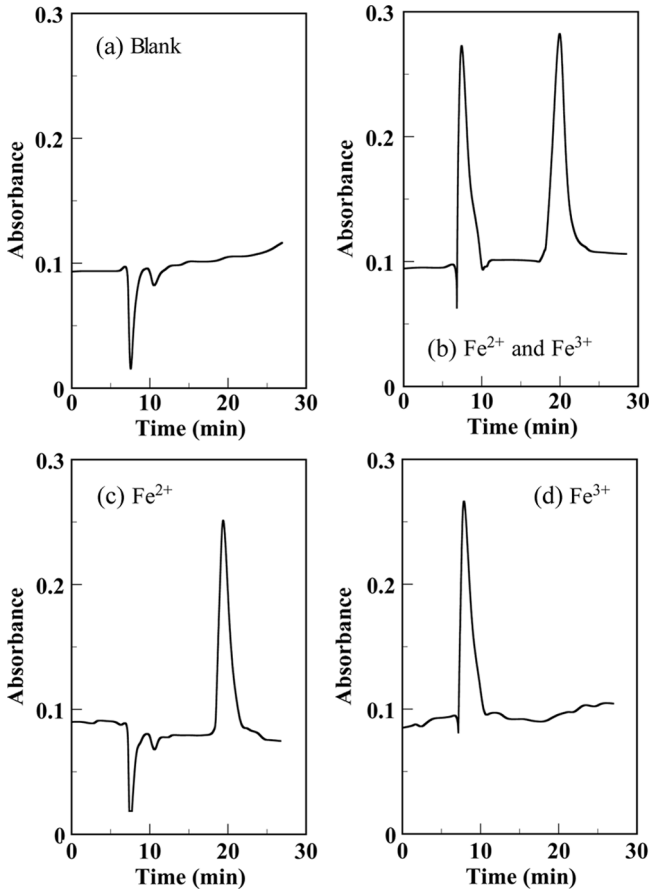

Figure 3. Chromatograms of iron(II) and (III) with the eluent under nitrogen gas atmosphere after deoxygenation of a column and tubes. (a) Blank. (b) 1 μmol mol dm^{-3} of iron(II) and 1 μmol dm^{-3} of iron(III). (c) 1 μmol dm^{-3} of iron(II) alone. (d) 1 μmol dm^{-3} of iron(III) alone. Eluent: mixture (pH 3.5) of 3.5 mmol dm^{-3} ethylenediamine and 1 mmol dm^{-3} citric acid.

almost constant (7.5–9.1 min). These results suggested that iron(III) is not retained on the resin. On the other hand, the retention time of iron(II) decreased with the increases of ethylenediamine and citric acid concentrations. We selected 3.5 mmol dm^{-3} of ethylenediamine and 1 mmol dm^{-3} of citric acid as their optimum concentrations, in order that the difference between retention times of iron(II) and (III) be about 10 min. The citric acid concentration was adjusted to be as low as possible, because it affected the sensitivity in catalytic analysis detection as described later.

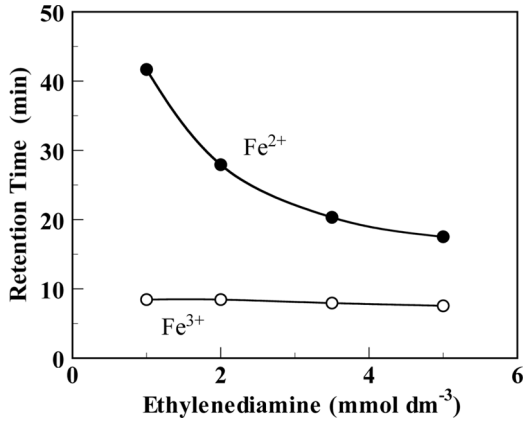

Figure 4. Effect of ethylenediamine concentration on the retention time of iron(II) and (III). pH and citric acid concentration: 3.5 and 1 mmol dm^{-3}, respectively.

Detection of Iron with Catalytic Analysis

Optimum operating conditions were examined for the catalytic detection of iron. Figures 6a–d show the effects of pH, concentrations of hydrogen peroxide and BGL, and reaction temperature on the catalytic signals (absorbance) of 20 μmol dm^{-3} iron(S) and blank (B) solutions and their ratio (S/B ratio). In this examination, the liquid chromatograph was not connected to the ASCFA system. An iron standard (20 μmol dm^{-3}) or blank solution was directly introduced to the ASCFA system and its

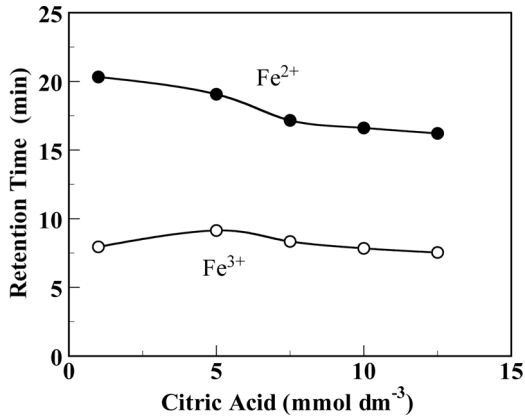

Figure 5. Effect of citric acid concentration on the retention time of iron(II) and (III). pH and ethylenediamine concentration: 3.5 and 3.5 mmol dm^{-3}, respectively.

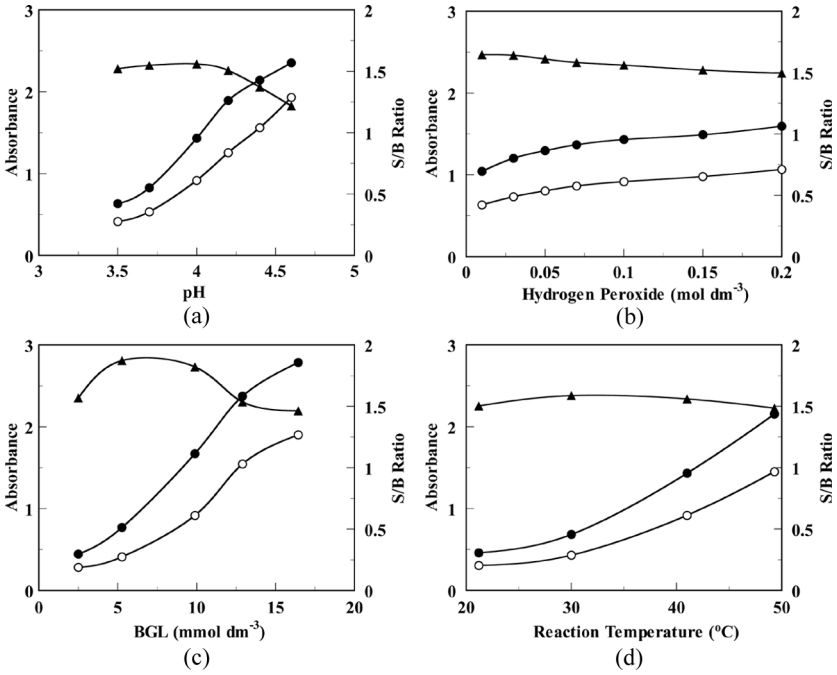

Figure 6. Effects of operating conditions on the absorbance and S/B ratio in air-segmented continuous flow analysis of iron. (a), (b), (c), and (d) effects of pH, hydrogen peroxide and BGL concentrations and reaction temperature, respectively. ●: 20 μmol dm^{-3} of iron(III) (S). ○: Blank (B). ▲: S/B ratio. Standard operating condition: pH 4.0, 0.1 mol dm^{-3} of hydrogen peroxide, 0.01 mol dm^{-3} of BGL and the temperature at 40°C.

absorbance signal was measured. As shown in Figure 6a, both absorbance signals of iron standard and blank solutions increased with pH in the buffer solution, and the S/B ratio showed a maximal value at pH 4.0. Therefore, the pH in the solution was adjusted to this value.

When the hydrogen peroxide concentration increased, the S/B ratio gradually decreased and had no maximal value (Figure 6b). Therefore, we selected 0.1 mol dm^{-3} of hydrogen peroxide as the optimum concentration, considering the absolute signal intensity of iron standard solution and the relative concentration of the oxidizing agent to the analyte element. The optimum concentration of BGL was taken to be 0.01 mol dm^{-3}, giving a relatively high S/B ratio and signal intensity of iron standard solution (Figure 6c). Considering the high S/B ratio, the signal intensity of the iron standard solution, and the ease of temperature control throughout the year, the reaction temperature was maintained at 40°C (Figure 6d).

Detection Limit, Reproducibility, and Calibration Curve

The detection limits (signal to noise ratio = 3) of iron(II) and (III) by the present method were 8.2 nmol dm^{-3} and 1.7×10^2 nmol dm^{-3}, respectively. The value for iron(III) was 20 times higher than that for iron(II). As shown in Figure 3, a water dip or negative peak by the injected sample or standard solution appeared in the chromatograms. As iron(III) is hardly retained on the resin, its peak overlaps with that of the water dip. The detection limit of iron(III) is, thus, very poor compared to that of iron(II). When the water dip is eliminated by the addition of some chemicals to the sample and standard solutions, a similar detection limit to that of iron(II) may be attained for iron(III). Steinmann and Shotyk[13] studied liquid chromatography for iron(II) and (III) using a post-column reaction with 4-(2-pyridylaso)resorcinol. They reported 10 ng g^{-1} (1.8×10^2 nmol dm^{-3}) and 5 ng g^{-1} (9×10^1 nmol dm^{-3}) as the detection limits of iron(II) and (III), respectively. Elsewhere, detection limits of 5 μg L^{-1} (9×10^1 nmol dm^{-3}) for iron(II) and 25 μg L^{-1} (4.5×10^2 nmol dm^{-3}) for iron(III) were reported in liquid chromatography, with thermal lens detection using post-column reagents of ascorbic acid and 1,10-phenanthroline.[14] Although the present value for iron(III) was at the same level as those in the above works, the detection limit of iron(II) in the present study was much lower than those previously reported. This result suggests that catalytic analysis detection is an excellent post-column reaction for liquid chromatography.

The reproducibilities for 10 measurements at the 500 nmol dm^{-3} levels of iron(II) and (III) were 1.7% and 3.7%, respectively. The calibration curves for both iron species showed good linearities with correlation coefficients of 0.997 [iron(II)] and 0.995 [iron(III)] in the range of 0.2–2 μmol dm^{-3} (n = 6). The calibration curves for iron concentrations above 2 μmol dm^{-3} can be drawn by the use of a shorter reaction coil to reduce the reaction time in ASCFA (Figure 1). The calibration curves in the range of 20–600 μmol dm^{-3} also showed good linearities with correlation coefficients similar to those in the lower range.

Interference

The effects of coexisting species on the present method were investigated. Table 1 shows the tolerance limit of the species, which was defined as the concentration that produced 5% error in the determination of 1 μmol dm^{-3} of iron(II) or (III). Determination of iron(II) was interfered with by the coexistence of silver, cadmium, manganese, and lead ions at 10–50 times the iron(II) concentration. As vanadate, chromate, molybdate ions were eluted with no retention on the resin and had a catalytic effect

Table 1. Tolerance limits of interfering species to the liquid chromatograph – catalytic analysis detection of 1 μmol dm^{-3} iron(II) or (III)

Tolerance limit (μmol dm^{-3})	Interfering species	
	To iron(II)	To iron(III)
0.1		VO_4^{3-}, CrO_4^{2-}
0.5		MoO_4^{2-}
1		VO^{2+}
5		Al^{3+}, Citric Acid
10	Ag^+, Cd^{2+}	
20	Mn^{2+}	
50	Pb^{2+}, Mg^{2+}	

on the oxidation of BGL, these ions, even at low concentrations, severely affected the determination of iron(III).

Citric acid, forming a stable complex with iron(III), depressed its peak. Due to this effect, citric acid concentration in the eluent discussed in the section "Liquid chromatographic separation between iron(II) and (III)" was regulated to as low a concentration as possible.

Analysis of Lake and Pore Waters

Lake and pore waters were successfully analyzed using the present method, since the interfering ions exist at lower concentrations than the tolerance limits shown in Table 1.[17–20]

A surface water sample was collected in the southern basin of Lake Biwa, Japan. The sample was filtered through a polyvinylidene fluoride filter with 0.45 μm pore size and the filtrate was analyzed within 5 h after collection. Pore water samples were collected from the bottom sediment, taken at a station at 73 m depth in the northern basin of Lake Biwa. Collection of the sediment samples was made by a core sampler. The sediments were transferred into a 50 mL plastic centrifuge tube in a glove bag filled with nitrogen gas. All the subsequent procedures were conducted in the glove bag under nitrogen atmosphere. Pore water samples were extracted by centrifuging for 10 min at 3000 rpm. The supernatant fraction was filtered through a polyvinylidene fluoride filter with 0.45 μm pore size, and the filtrate was immediately analyzed without dilution.

The results are shown in Table 2. Neither iron(II) nor (III) was detected in the surface lake water. On the other hand, iron(II) was found at high concentrations in pore waters. The iron concentrations in pore water samples were also directly determined by atomic absorption spectrometry. These values agreed well with those obtained by the present

Table 2. Iron(II) and (III) concentrations in lake and pore water samples (μmol dm^{-3})

	Present method		
Sample	Iron(II)	Iron(III)	AAS (total iron)
Lake water	ND	ND	ND
Pore water			
0–2 cm[a]	93	ND	118
2–5 cm	224	ND	238

All samples were collected in March, 2004. AAS: Atomic absorption spectrometry. ND: Not detectable.
[a]Depth from the sediment surface.

method (Table 2). These analytical results for surface lake and pore waters suggest that the difference between redox states of the waters influenced the concentrations of iron(II) and (III).[21,22]

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REFERENCES

1. Sugiyama, M.; Tamada, T.; Hori, T. Liquid chromatography – catalytic analysis detection for highly sensitive and automated fractional determination of vanadium(IV) and -(V). Anal. Chim. Acta **2001**, *431*, 141–148.
2. Anderson, R.G.; Brown, B.C. The determination of lead in mosses by means of its catalytic effect on the persulphate oxidation of pyrogallol red. Talanta **1981**, *28*, 365–368.
3. Isshiki, K.; Nakayama, E. Determination of ultratrace amounts of cobalt by catalysis of the tiron-hydrogen peroxide reaction with an improved continuous-flow analysis system. Talanta **1987**, *34*, 277–281.
4. Sugiyama, M.; Hori, T. Air-segmented continuous-flow analysis for vanadium based on a catalytic reaction with Bindschedler's green leuco base. Anal. Chim. Acta **1992**, *261*, 189–196.

5. Fuge, R. An automated method for the determination of molybdenum in geological and biological samples. Analyst **1970**, *95*, 171–176.
6. Bradfield, E.G.; Stickland, J.F. The determination of molybdenum in plants by an automated catalytic method. Analyst **1975**, *100*, 1–6.
7. Hirayama, K.; Unohara, N. Spectrophotometric determination of trace vanadium by means of flow injection analysis based on the catalytic oxidation of Bindschedler's green leuco base by potassium bromate. Nippon Kagaku Kaishi. **1981**, 98–102 (in Japanese).
8. Zhao-Lun, P.; Shu-Kun, X. Determination of molybdenum at $\mu g\, l^{-1}$ levels by catalytic spectrophotometric flow injection analysis. Anal. Chim. Acta. **1983**, *145*, 143–150.
9. Hirayama, K.; Sawaya, T. Catalytic determination of trace amounts of iron by means of the oxidation of Bindschedler's green leuco base by hydrogen peroxide. Nippon Kagaku Kaishi **1976**, 1401–1408 (in Japanese).
10. Hirayama, K.; Unohara, N. Spectrophotometric determination of ultratrace vanadium based on its catalytic oxidation of Bindschedler's green leuco base by potassium bromate. Bunseki Kagaku **1980**, *29*, 733–737 (in Japanese).
11. Muto, G.; Oikawa, K. *Ion Chromatography*; Kodansha Scientific: Tokyo, Japan, 1983; 162pp (in Japanese).
12. Oikawa, K.; Suzuki, Y.; Sunahara, H.; Tanaka, K.; Futatsuki, Y.; Honma, H.; Matsushita, S.; Muto, G.; Murano, K. *Ion Chromatography*; Kyoritsu Shuppan: Tokyo, Japan, 1988; 246 (in Japanese).
13. Steinmann, P.; Shotyk, W. Ion chromatography of organic-rich natural waters from peatlands V. Fe^{2+} and Fe^{3+}. J. Chromatogr. A. **1995**, *706*, 293–299.
14. Divjak, B.; Franko, M.; Novic, M. Determination of iron in complex matrices by ion chromatography with UV-Vis, thermal lens and amperometric detection using post-column reagents. J. Chromatogr. A. **1988**, *829*, 167–174.
15. Karcher, B.D.; Krull, I.S. The use of complexing eluents for the high performance liquid chromatographic determination of metal species, in *Trace metal analysis and speciation*; Krull, I.S., Ed.; Elsevier: Amsterdam, 1991; 123–166.
16. Cardellicchio, N.; Ragone, P.; Cavalli, S.; Riviello, J. New strategies for determination of transition metals by complexation ion-exchange chromatography and post column reaction. J. Chromatogr. A. **1997**, *770*, 185–193.
17. Rossmann, R.; Barres, J. Trace element concentrations in near-surface waters of the Great Lakes and methods of collection, storage, and analysis J. Great Lakes Res. **1988**, *14*, 188–204.
18. Ward, N.I. Trace elements, in *Environmental Analytical Chemistry*; Fifield, F.W., Haines, P.J., Eds.; Blackie Academic and Professional: London, 1995; 320–351.
19. Sugiyama, M.; Hori, T.; Kihara, S.; Matsui, M. Geochemical behavior of trace elements in Lake Biwa. Limnol. **2005**, *6*, 117–130.
20. Sugiyama, M. Dissolved trace elements in lakes, in *Umi to Mizuumi no Kagaku (Chemistry in the Ocean and Lakes)*; Sohrin, Y., Isshiki, K., Eds.; Kyoto Daigaku Gakujutsu Shuppankai: Kyoto, Japan, 2005; 360–377 (in Japanese).

21. Davison, W. Iron and manganese in lakes. Earth-Sci. Rev. **1993**, *34*, 119–163.
22. Hamilton-Taylor, J.; Davison, W. Redox-driven cycling of trace elements in lakes, in *Physics and Chemistry of Lakes*; Lerman, A., Imboden, D., Gat, J., Eds.; Springer: Berlin, 1995, 217–263.

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