Simultaneous Determination of Iron(II) and Iron(III) by Micellar
Electrokinetic Chromatography Using an Off-line Selective
Complexing Reaction

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A simultaneous determination with UV detection/capillary electrophoresis for Fe(II) and Fe(III) was achieved using a sulfonated bathophenantholine and desferrioxamine B. When the electrophoretic buffer was 60 mmol l⁻¹ SDS, 10 mmol l⁻¹ acetic acid (pH 4.0) and 10 mmol l⁻¹ ascorbic acid and at −10 kV, the iron(II) and iron(III) could not only be completely separated, but also be sensitively determined.

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Introduction

Iron ion is one of the most common and important metal ions in nature, and its valences are bivalent and trivalent. Simultaneously analyzing the two valences is important not only for biochemistry and environmental chemistry, but also in many scientific and industrial fields. So far, atomic absorption spectrometry (AAS), inductively coupled plasma (ICP) and X-ray fluorescent spectrometry (XRF) have been generally known as analytical techniques for metal ions; however, these methods can not distinguish any difference in the metal valences. Therefore, a general method is achieved by the following procedure: first, Fe(II) is complexed with specific chelating agents, and then measured by spectrophotometry. Fe(III) is subsequently reduced to Fe(II) and the total iron is determined, yielding the concentration of Fe(III) based on the difference in the absorbance. Namely, this method is an indirect distinguishable determination method utilizing the difference in absorbance. In the above method, there are two serious problems which are (i) the sample solution must be measured twice and (ii) a micro-volume sample solution could not be measured. Therefore, this method could not be applied to biological and environmental samples, which are usually in micro-volumes and are rare samples.

On the other hand, capillary electrophoresis (CE) is a high-performance separation technique with more than 100000 theoretical plates, and its injection volume is at microliter levels. Therefore, CE is easily suitable for micro-volume and rare samples. However, there have been few reports concerning the oxidation state analysis of metal ions. Because metal ion analyses by CE generally use only one kind of ligand, CE is not appropriate for analyzing the oxidation state of metal ions. The authors thus considered that, if we could use two selective ligands for Fe(II) and Fe(III), a distinguishable analysis could be made. The described method was then attempted using a bathophenantholine derivative and desferrioxamine B as selective ligands. So far, the use of two selective ligands for Fe(II) and Fe(III) was reported by Pozdniakova et al., in which EDTA and 1,10-phenanthroline (phen) were used with CE. However, a previous report showed very poor results concerning the sensitivity (ppm levels). In CE, the sensitivity during spectrophotometric detection is rather poor due to the micro-light pass length (< 100 µm). The proposed method achieved not only a distinguishable analysis, but also a highly sensitive analysis for the different oxidation states of iron.

Experimental

Reagent

Iron(II) sulfate heptahydrate, iron(III) chloride hexahydrate, 1,10-phenanthroline monohydrate (phen) and 2,2′-bipyridyl (bpy) were from the Kanto Chemical Co., Ltd. (Tokyo, Japan). Bathophenanthroline disulfonic acid, disodium salt (bathophen) was from the Dojin Co., Ltd. (Tokyo, Japan). Desferrioxamine B (DFB) was from Ciba-Geigy as its methanesulfonate salt. Sodium dodecyl sulfate was from Wako Chemical Co. Ltd. (Osaka, Japan). All other chemicals were of analytical reagent grade from Kanto Chemical, unless otherwise noted.

Iron(II) sulfate heptahydrate and iron(III) chloride hexahydrate were dissolved in a 0.1 mol l⁻¹ hydrochloric acid solution, and these solutions were titrated by EDTA chelatometric titration. The solutions of the concentrations which were determined were used as stock solutions. The working solutions were prepared by dissolving the stock solution with distilled water. Bathophen and DFB were dissolved in distilled water and determined at just 10⁻³ mol l⁻¹.

Apparatus

A Nippon Bunko V-570 double beam spectrophotometer and a 1-ml quartz cell (light path length: 1 cm) were used for the spectrophotometry. CAPI-3200 (Otsuka Electronics, Osaka) was used as the capillary electrophoresis system. A 75-µm i.d. fused-silica capillary (Otsuka Electronics) with a 50-cm total length was used. All detections and determinations were performed at 200 nm by a multi-photodiode array detector.

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Procedure
A 0.4 ml sample solution containing the iron ions, 0.4 ml of $0.4 \times 10^{-4}$ mol l$^{-1}$ DFB, 0.4 ml of $1.0 \times 10^{-3}$ mol l$^{-1}$ bathophen and 0.5 ml of $0.1$ mol l$^{-1}$ KH$_2$PO$_4$ solution were placed into a 5-ml measuring flask, and the mixture was dissolved in distilled water to the marked line. This solution was hydrostatically injected into the CE system for 30 s from 25 mm. The components of the running buffer were $60$ mmol l$^{-1}$ SDS, $10$ mmol l$^{-1}$ acetic acid (pH = 4) and $10$ mmol l$^{-1}$ ascorbic acid. The application voltage was $-10$ kV.

Results and Discussion

Off-line complexation prior to CE
First, the complex formations of bathophen and DFB with the iron ions were examined. Bathophen was generally known to form a stable red complex with Fe(II) ($\log \beta_3 = 22.39$), and DFB was also known to form a stable complex with Fe(III) ($\log \beta_3 = 30.06$). In this study, bathophen with Fe(III) and DFB with Fe(II) were not found to react. The spectra are shown in Fig. 1. The bathophen rapidly reacted with Fe(II) and formed a stable complex. The maximum absorbance of this complex was observed at 535 nm. However, bathophen did not react with Fe(III), and the absorption spectra did not change for at least 40 min (Fig. 1-a). On the other hand, DFB rapidly reacted with Fe(III) and formed a stable complex. This complex was detected at 431 nm. Namely, DFB did not react with Fe(III), and the absorption spectra did not change for at least 40 min (Fig. 1-b). The respective Fe-complexes were stable for at least 40 min in the absorbance spectra. Both Fe-complexes were quantitatively formed in the pH range of 1 - 12. Therefore, the two complexes were able to simultaneously form under the same condition. In this study, the condition of pH=4 was established using a phosphoric buffer solution.

Separation of iron(II) and iron(III)
The best separation was performed under the electrophoretic buffer condition of $60$ mmol l$^{-1}$ SDS, $10$ mmol l$^{-1}$ acetic acid (pH 4.0) and $10$ mmol l$^{-1}$ ascorbic acid. The electropherogram is shown in Fig. 2. The figure shows that the elution order was found to be [Fe(III)-H·DFB]$^+$, [H·DFB]$^+$, [Fe(II)-(bathophen)]$_3$$^-$ and [H-bathophen]$.^-$ Here, these chemical species were identified by the following pK$_a$ values, DFB: pK$_{a}$ (amino group) = 8.39, 7 bathophen; pK$_{a1}$ (sulfuric group) = 2.83, pK$_{a2}$ = 5.20 and phen; pK$_{a1}$ (imino group) = 0.70, 10 pK$_{a2}$ (imino group) = 4.98. Regarding the free bathophen, it was assumed to form by monoprotonation as the phen at pH 4.). Considering the positive voltage applied to the capillary end, this elution order was inconsistent. This result is considered to occur for the following reason. The pH of the electrophoretic buffer was pH = 4.0 under this experimental condition; therefore, the electroosmotic flow was negligible. A large quantity of SDS micelle thus exists in the electrophoretic buffer compared with the sample; therefore, [Fe(III)-H·DFB]$^+$ and [H-bathophen]$^-$. migrated due to the electrophoresis based on their charge. On the other hand, when DFB was injected, these two peaks were detected. However, these peaks have not yet been identified.

The phen derivatives are generally known as the Fe(II) ligand for spectrophotometric analysis. In this study, three kinds of ligands (phen, bpy, bathophen) were examined. Bathophen was satisfactory based on the separation between the
Fe(II)-complex and the ligand and the peak intensities at the detection wavelength (200 nm). On the other hand, ascorbic acid was added into the electrophoretic buffer to suppress the oxidation of Fe(II)-bathophen complex.

Simultaneous determination of iron(II) and iron(III)

The calibration curves were shown to be linear. The determination ranges were 5.0 \times 10^{-7} \text{ - } 3.0 \times 10^{-5} \text{ mol l}^{-1} for Fe(II) and 5.0 \times 10^{-8} \text{ - } 4.0 \times 10^{-6} \text{ mol l}^{-1} for Fe(III). The relative standard deviations (RSD) (n = 5) were 7.98\% for Fe(II) and 0.75\% for Fe(III). This difference in the RSD values was considered to be the difference in the complex stabilities. Both Fe-complexes have great stabilities (log $\beta_{\text{Fe(III)-DFB}} = 22.3$); however, there is also a great difference between the two complexes; i.e., Fe(II)-bathophen $\ll$ Fe(III)-DFB ($\beta_{\text{Fe(III)-DFB}} \beta_{\text{Fe(II)-bathophen}} = 10^9$). Therefore, compared with Fe(II)-DFB, a small quantity of complex dissociation in the determination of iron(II) and iron(III) was achieved by using selective and stable ligands. Moreover, when the MEKC was performed under the condition of negligible electroosmotic flow, the Fe-complexes were preconcentrated by micelle sweeping. With this preconcentration, the resulting iron analysis was capable of not only complete separation, but also of a sensitive determination.

Conclusion

A simultaneous determination of Fe(II) and Fe(III) was achieved by using selective and stable ligands. Moreover, when the MEKC was performed under the condition of negligible sensitivity, the proposed method had a sensitivity enhancement of about 70-fold greater than that of UV/CE by techniques. The proposed method was compared with other highly sensitive analyses, such as AAS and XRF. Though the compared AAS and XRF used the preconcentration method, the proposed method was able to determine a lower concentration range of about 1/10 without any preconcentration (for Fe(II)). Accordingly, sensitive detection could be done without a sensitive detector and a preconcentration method, the proposed method was able to determine a lower concentration range of about 1/10 without any preconcentration (for Fe(II)).

References