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Isotachophoretic separation of Fe(II) and Fe(III) by using 1,10-phenanthroline as a complex-forming agent

Takeshi Hirokawa*, Kazuhiro Nishimoto, Fumitaka Nishiyama

Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, Kagamiyama 1, Higashi-hiroshima 739, Japan

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Abstract

A mixture of Fe(II) and Fe(III) phenanthroline (phen) chelate complexes was isotachophoretically separated and analyzed by using a conventional electrolyte system for cation analysis (leading electrolyte: 20 m*M* ammonia solution, acetic acid buffer, pH 4.8; terminating electrolyte: β-alanine, pH 3.6). The migration order was Fe(III) chelate, Fe(II) chelate, phen. The recovery was 100% both for Fe(II) and Fe(III) according to PIXE analysis of the isotachophoretic (ITP) fractions. The sample mixture used (pH 2) was stable for a few days at room temperature. The sensitivity of Fe(III) chelate was 60% of that of Fe(II) chelate. This is because the ionic charge of Fe(II) chelate (+2) was larger than that of Fe(III) chelate, in which the coordination number of phen was 1.8±0.1 due to the addition of at least one pH buffer anion. The ionic charge of Fe(III) chelate was estimated as +1.4 by simulation of the concentration and effective mobility at the ITP steady state.

Keywords: Isotachophoresis; Iron; 1,10-Phenanthroline

1. Introduction

Since the electrophoretic mobility of Fe(III) ions is very small at a medium pH range due to the formation of hydroxyl complexes, electrophoretic analyses of Fe have usually been done in the form of Fe(II) ions [1–3]. Although Fe(III) ions are stable at a very low pH range (<1), electrophoretic analysis, especially isotachophoresis, is not very successful at such low pH [1]. This may be the main reason why simultaneous isotachophoretic separation of Fe(II) and Fe(III) has not yet been reported.

In the conventional analysis of Fe(III) by spectrophotometry, Fe(III) is reduced to Fe(II) to form a stable red chelate complex with 1,10-phenanthroline (phen). Yoshida and Hirama studied the isotachophoretic separation of several phen chelates including Fe(II), Co(II), Cu(II), etc., demonstrating that such phen chelate complexes were suitable for electrophoretic analysis [4]. However, they did not mention the migration behavior of the Fe(III)-phen chelate.

Although the stability of the Fe(III)-phen chelate ($\log K=14.1$ [5]) is much less than that of the Fe(II)-phen chelate ($\log K=24.3$ [5]), formation of Fe(III)-phen chelate may suppress the production of hydroxyl complexes even in the medium pH region. If the charge of the central metal ion is preserved in

*Corresponding author.

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these chelate complexes, they will be easily separated by isotachophoresis (ITP). Based on such a strategy, this paper focusses on the simultaneous isotachophoretic determination of Fe(II) and Fe(III) after formation of their phen chelate complexes.

2. Experimental

2.1. Chemicals

$Fe(II)SO_4(NH_4)_2SO_4 \cdot 6H_2O$, $Fe(III)Cl_3 \cdot 6H_2O$ and 1,10-phenanthroline hydrochloride were of guaranteed grade (GR) obtained from Katayama Chemical (Osaka, Japan). Hydrochloric acid and nitric acid were of ultrapure grade purchased from E. Merck (Darmstadt, Germany). Hydroxypropylcellulose (HPC, extra pure) was obtained from Tokyo Kasei (Tokyo, Japan). The viscosity of a 2 wt.% HPC aqueous solution was 1000–4000 cP at 20°C according to the specification. A standard solution of Fe for atomic absorption spectroscopy (1 mg/l, Tokyo Kasei) was used as an analytical standard of PIXE.

2.2. Samples

Stock solutions (20 m*M*) of $Fe(II)SO_4(NH_4)_2SO_4 \cdot H_2O$, $Fe(III)Cl_3 \cdot 6H_2O$ and 1,10-phenanthroline hydrochloride were prepared with water purified by ion-exchange resin (Japan Organo, Model PURIC-R, Tokyo, Japan). The pH of the stock solutions of $Fe(II)SO_4(NH_4)_2SO_4$ and $Fe(III)Cl_3$ was adjusted to 2 by adding H_2SO_4 and HNO_3, respectively. The pH of 20 m*M* 1,10-phenanthroline hydrochloride was 3.1 as prepared. A Horiba Model F7-AD expanded pH meter (Tokyo, Japan) was used for pH measurement.

Test mixtures of Fe(II) and Fe(III)-phen chelates were prepared by appropriately mixing these stock solutions before use. A hydroxylamine hydrochloride solution (1 wt.%) was prepared just before use. When Fe(III) was reduced to Fe(II), the hydroxylamine solution was mixed with the test mixtures of Fe(II) and Fe(III) chelate before analysis.

2.3. Operational electrolyte systems

Table 1 shows the operational electrolyte systems used. The leading electrolyte mainly used in this study was 20 m*M* ammonia solution buffered by adding acetic acid to pH 4.8. Several leading electrolytes were prepared in the same manner by using different organic acids as the pH buffer, propionic acid, *n*-butyric acid, *n*-valeric acid and *n*-caproic acid, respectively. The terminating electrolyte was a 20 m*M* solution of HCl buffered by adding β-alanine, where the β-alanine cation plays the role of a cationic terminator. All the electrolytes contained 0.1 wt.% hydroxypropylcellulose (HPC) to suppress electroendosmosis.

2.4. Isotachophoretic apparatus and R_E measurement

The detector used for ITP was a high-frequency contactless conductivity detector (HFCCD) [6] newly designed and constructed by Dr. Zuska (Charles Univ., Prague). Since a fused-silica capillary was used as the detection cell, the reproducibility of the signal was very good. The detector was used in combination with the separation unit of a LABECO ZKI-001 isotachophoretic analyzer (Sp.

Table 1
Operational electrolyte systems for isotachophoresis

Leading electrolyte	20 m*M* Ammonia solution
pH Buffer	Acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid, *n*-caproic acid
pH	4.80
Additive	0.1 wt.% hydroxypropylcellulose (HPC)
Terminating electrolyte	20 m*M* Hydrochloric acid
pH Buffer	β-Alanine
pH	3.60
Additive	0.1 wt.% HPC

Nova Ves, Slovakia). The separation tube was a PTFE capillary (30 cm×0.25 mm I.D.). The migration current was 40 μA. The high-voltage power supply was that for a Shimadzu IP-2A (Kyoto, Japan). All the measurements were carried out at 25°C in a temperature-controlled room.

The qualitative index used was R_E, which is defined as the ratio of the potential gradient (E/V cm^{-1}) of the sample zones (E_S) to that of the leading zone (E_L) [7]. When a conductivity detector is used, it is equal to the ratio of the specific resistance of each zone. Na^+ and Li^+ were used as the internal standard: the simulated R_E values were 1.495 and 1.963, respectively. The in-house developed computer program SIPS was used for simulation. The program can be used for the evaluation of qualitative and quantitative indices and steady-state quantities such as concentrations of zone components.

The separation unit of the preparative isotachophoretic analyzer used was made in our laboratory [8]. The separation column used consisted of a preseparation capillary (40 cm×1 mm I.D.) and a main capillary (16 cm×0.5 mm I.D.). The apparatus and its operation were detailed in [7]. Analysis time was 1 h, and the amount of electric charge applied during separation was 1.2 C. The zones of the separated components were fractionated by applying a counter flow of the leading electrolyte. The dropwise fractions were separately sampled on a Nuclepore filter backing (thickness 5 μm and pore size 0.1 μm, Nuclepore, USA) supported by an aluminum flame. The volume of one fraction was 5 μl, and contained several nmoles of Fe.

2.5. PIXE analysis

For the PIXE measurement [9], a Van de Graaff accelerator at our faculty was used (Nisshin High Voltage, Model AN-2500, Tokyo, Japan). The energy of the H^+ beam was 2 MeV, the beam current was 50 nA, and the beam diameter was 6 mm. The detector was a high pure Ge detector (an ORTEC Model GLP-10180, USA), used in combination with a Model AMS-1000 multichannel analyzer (Laboratory Equipment, Tokyo, Japan). A typical single run for an ITP fraction took 200 s (10 μC). The amount of Fe in the fractions was determined by comparing the X-ray counts with those of a reference standard.

3. Results and discussion

3.1. Migration behavior of Fe(II) and Fe(III) chelates

Fig. 1a and b show the isotachopherograms obtained for the Fe(II) chelate and the Fe(III) chelate, respectively. In the case of Fe(II), only one zone was observed, when the concentration ratio of

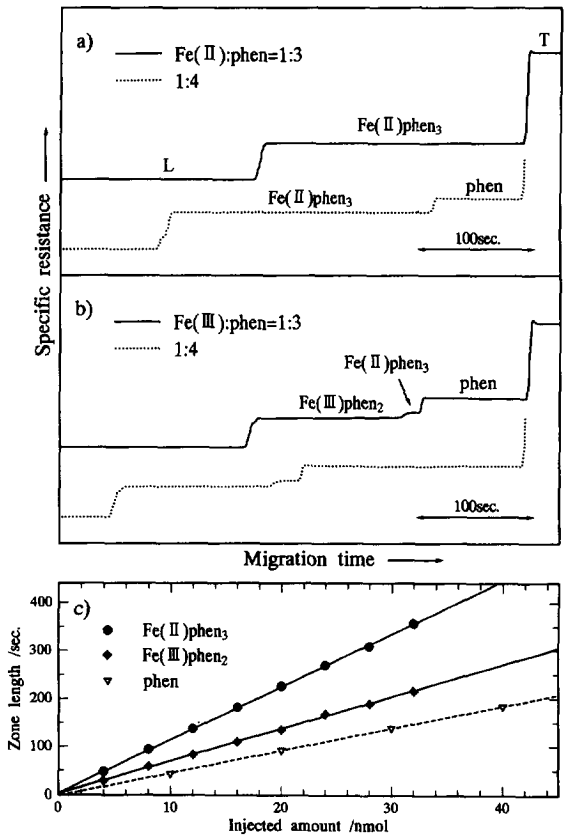

Fig. 1. Isotachopherograms of Fe(II) and Fe(III) chelate complexes with 1,10-phenanthroline. (a) Fe(II) chelate; solid line=5 m*M* Fe(II)–15 m*M* phen (injected volume 4 μl); dotted line=4 m*M* Fe(II)–16 m*M* phen (5 μl). (b) Fe(III) chelate; solid line=5 m*M* Fe(III)–15 m*M* phen (4 μl); dotted line=4 m*M* Fe(III)–16 m*M* phen (5 μl). (c) Calibration lines of Fe(II) chelate, Fe(III) chelate and phen; migration current 40 μA; operational electrolyte system as given in Table 1 (acetic acid buffer).

Fe(II) and phen was 1:3 (Fig. 1a). When the amount of phen was increased to 1:4, a zone of excess phen was observed just after the chelate zone. This observation confirmed that the coordination number of phen is 3 for Fe(II). The ionic charge was estimated as 2+. Table 2 shows the observed R_E values and absolute mobilities of Fe(II) chelate, Fe(III) chelate and phen: the mobilities were determined to fit the observed R_E values.

On the other hand, in the case of Fe(III), three zones were observed even when the concentration ratio of Fe(III) and phen was 1:3 (Fig. 1b). From comparison of R_E values, the first zone was assigned as the Fe(III) chelate. The zone length of phen increased with increase of the concentration ratio to 1:4. According to isotachophoretic determination of the excess phen, the apparent coordination number of Fe(III) chelate was 1.8±0.1. Fe(II) and Fe(III) chelates are referred to as Fe(II)-phen$_3$ and Fe(III)-phen$_2$, hereafter.

A short zone between Fe(III)-phen$_2$ and phen in Fig. 1b was assigned as Fe(II)-phen$_3$ on the basis of its R_E value and red color. Fe(II)-phen$_3$ was probably formed during sample preparation.

Fig. 1c shows calibration lines of Fe(II)-phen$_3$, Fe(III)-phen$_2$ and phen. Obviously, they were quite normal. It should be noted that the zone length of Fe(II)-phen$_3$ was 1.7 times longer than that of Fe(III)-phen$_2$. In other words, the sensitivity of Fe(III)-phen$_2$ was 60% of that of Fe(II)-phen$_3$. Since the zone length of the isotachophoretically separated zones depends on the sample amount, effective mobility and effective charge of the sample ions [1], this experimental result suggested the following possibilities. (1) If the recovery of Fe(III)-phen$_2$ is 100%, the ionic charge of Fe(III)-phen$_2$ may not be 3+, but at least less than 2+. (2) On the other hand, if the recovery is insufficient, the ionic charge might be 3+. In order to obtain the recovery, then, an equimolar mixture of these chelates was analyzed by ITP-PIXE.

3.2. Recovery of Fe(II) and Fe(III) chelates by ITP-PIXE

An amount of 10 μl of an equimolar mixture of Fe(II) and Fe(III) chelates [2 m*M* Fe(II)–2 m*M* Fe(III)–16 m*M* phen] was analyzed by ITP-PIXE. Fig. 2a shows the observed isotachopherogram. The separated zones of Fe(II)-phen$_3$ and Fe(III)-phen$_2$ were fractionated by using a counter flow of a leading electrolyte [8]. Consequently, the recovery of both chelates was 100%, respectively.

Fig. 2b shows the analytical results for each fraction. Obviously, the amount of Fe in Fe(III)-phen$_2$ fractions (average=3.99 nmol/fraction) was higher than that of Fe(II)-phen$_3$ fractions (2.78 nmol/fraction, ratio=1.44). This indicated that the ionic charge of Fe(III)-phen$_2$ in the ITP zone was less than that of Fe(II)-phen$_3^{2+}$, i.e. Fe(III)-phen$_2$ had another ligand, a hydroxyl ion or a pH buffer anion.

3.3. Ionic charge and chemical form of Fe(II)phen$_3$ chelate

Concentration of Fe(II)-phen$_3^{2+}$ in its own zone was 6.60 m*M*, according to computer simulation for the isotachophoretic steady state. On the basis of the simulated concentration and ITP-PIXE experiment, the concentration of Fe(III)-phen$_2$ in the steady state was estimated as 6.60×1.44=9.5 m*M* (C^1). Then, the ionic charges and the mobilities of Fe(III)-phen$_2$ chelate components were determined to fit with the observed concentration and the R_E value. Consequently, a divalent cation with pK_1=4.23 and

Table 2
Observed R_E values and absolute mobilities of Fe-phen chelates and phen (25°C)

Sample	R_E (obs.)	Charge	Absolute mobility/10^{-5} ($cm^2\ V^{-1}\ s^{-1}$)	pK_a
Fe(II)-phen$_3$	2.44	2+	38.2	–
Fe(III)-phen$_2$AcO	2.18	2+	55.3	4.23
		1+	27.7	–
phen	3.01	1+	32.3	4.96[a]

[a] See Ref. [5].

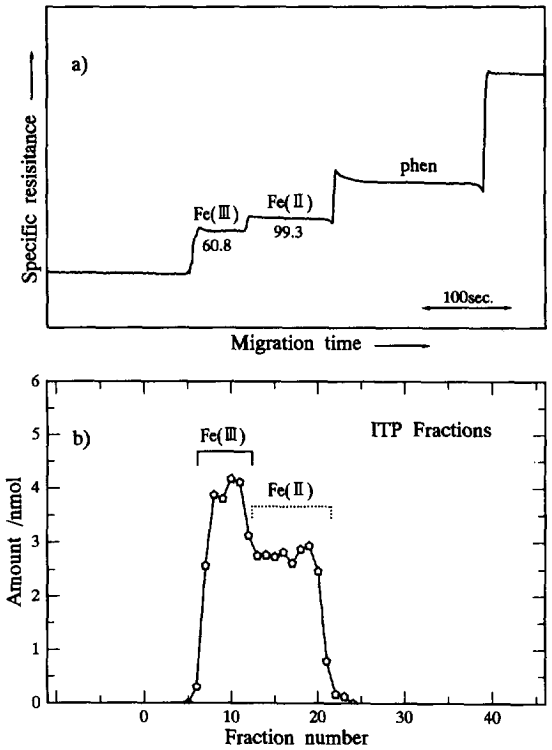

Fig. 2. (a) Isotachopherogram of an equimolar mixture of Fe(II)-phen$_3$ and Fe(III)-phen$_2$: 2 m*M* Fe(II)–2 m*M* Fe(III)–16 m*M* phen (injected volume 5 μl); migration current 40 μA. (b) Analytical result of the same mixture by ITP-PIXE; injected volume 10 μl; operational electrolyte system as given in Table 1 (acetic acid buffer).

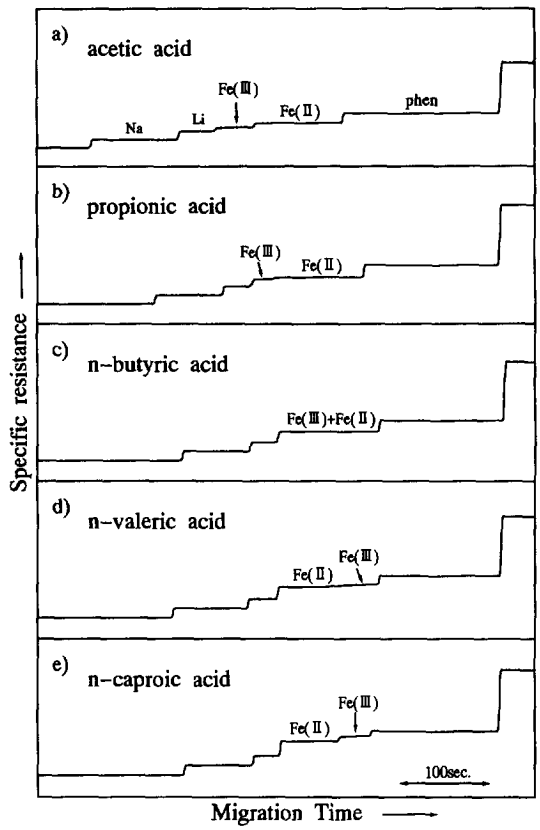

Fig. 3. Isotachopherogram of an equimolar mixture of Fe(II)-phen$_3$ and Fe(III)-phen$_2$: 1.67 m*M* Fe(II)–1.67 m*M* Fe(III)–13.3 m*M* *phen* (injected volume 6 μl); Na and Li internal standard; migration current 40 μA; pH buffer. (a) Acetic acid, (b) propionic acid, (c) *n*-butyric acid, (d) *n*-valeric acid, (e) *n*-caproic acid.

pK_2≫4.23 and m_1=55.3·10^{-5} cm^2 V^{-1} s^{-1} and m_2=27.7·10^{-5} cm^2 V^{-1} s^{-1} gave R_E=2.18 and C^t= 9.50 m*M*. The effective charge was 1.40.

This simulation strongly indicated that Fe(III)-phen$_2$ contained the other anionic ligand. In order to confirm this, the separation behavior of Fe(III)-phen$_2$ and Fe(II)-phen$_3$ was studied by using other pH buffers with different molecular mass. If a pH buffer in the leading electrolyte interacts with the chelate, the R_E value of the Fe(III)-phen$_2$ zone may increase according to the increase of the molecular mass of the pH buffers.

Fig. 3 shows the isotachopherograms observed with several leading electrolytes the pH of which was adjusted to 4.8 by adding various organic acids H(CH$_2$)$_n$COOH (n=2–5). The observed R_E values of Fe(III)-phen$_2$ were 2.18, 2.41, 2.58, 2.77 and 3.04 for acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid and *n*-caproic acid, respectively. Obviously, the R_E of Fe(III)-phen$_2$ increased with increasing molecular mass of the pH buffer. However, that of Fe(II)-phen$_3$ did not change significantly (R_E=2.44, 2.51, 2.58, 2.65 and 2.77, respectively), causing an inversion of migration order of Fe(III)-phen$_2$ and Fe(II)-phen$_3$ when valeric acid and caproic acid were used as the pH buffer. This indicates that Fe(III)-phen$_2$ had at least a pH buffer anion as its ligand.

3.4. Analysis of Fe(II) and Fe(III) mixture

Obviously, from the above discussion, Fe(II) and Fe(III) can be determined simultaneously by ITP when complex-formation with phen is utilized. Even when the molar ratio of Fe(II) and Fe(III) was

changed from 1:3 to 3:1, analytical results agreed with the prepared values within experimental errors, confirming the analytical utility of the method for practical samples. Separability of equimolar Fe(II)-phen$_3$ and Fe(III)-phen$_2$ was 130 nmol/C according to the calibration line method [10].

Finally, the stability of equimolar Fe(II)-phen$_3$ and Fe(III)-phen$_2$ mixture was studied at room temperature. No significant change of the isotachopherogram was observed until two days after preparation. After ten days, however, the zone length of Fe(II)-phen$_3$ had increased 15% (from 99.3 s to 114.5 s) indicating that Fe(III)-phen$_2$ was reduced to Fe(II)-phen$_3$ (Fig. 4a). After sixty days, almost all of Fe(III)-phen$_2$ zone was decomposed. Not only the mixtures but also Fe(III)-phen$_2$ itself showed a similar behavior. For accurate analysis of Fe(III), therefore, the sample should be used within a few days after preparation.

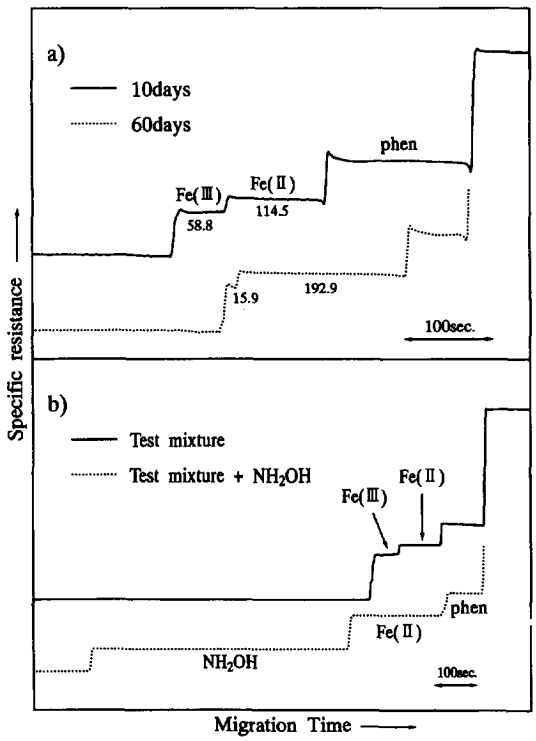

Fig. 4. Isotachopherogram of an equimolar mixture of Fe(II)-phen$_3$ and Fe(III)-phen$_2$. (a) Solid line=2 m*M* Fe(II)–2 m*M* Fe(III)– 16 m*M* phen (injected volume 5 μl), 10 days after preparation; dotted line=60 days after preparation; figures show zone passing time in seconds. (b) Solid line (before addition of hydroxyl amine)=2 m*M* Fe(II)–2 m*M* Fe(III)–16 m*M* phen (5 μl); dotted line (after addition of 1/5 portion of 1 wt.% hydroxylamine hydrochloride solution)=1.67 m*M* Fe(II)–1.67 m*M* Fe(III)–13.3 m*M* phen (6 μl); operational electrolyte system as given in Table 1 (acetic acid buffer); migration current 40 μA.

Fe(III) can be completely reduced to Fe(II) by adding 1 wt.% hydroxyl amine. Fig. 4b shows the isotachopherogram of a mixture [2 m*M* Fe(II)–2 m*M* Fe(III)–16 m*M* phen] just after adding a 1/5 portion of 1 wt.% hydroxylamine solution. If very accurate analysis of total Fe is necessary, such an operation may be useful.

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