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Bidirectional isotachophoretic separation of metal cations using EDTA as a chelating agent

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Abstract

An operational electrolyte system using EDTA as a chelating agent was developed for the bidirectional isotachopheresis (ITP) of metal ions. The anolyte used was 20 mM HCl buffered by adding β -alanine at pH 3.6. The catholyte was 20 mM ammonia solution buffered by acetic acid at pH 4.8 and containing EDTA in the range 0–1 mM. In relation to the concentration of EDTA, the isotachophoretic separation behaviour of eighteen metal cations containing equimolar EDTA was studied by using with particle-induced X-ray emission (PIXE). They were Na^+ , Li^+ , Ba^{2+} , Sr^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , La^{3+} , Ce^{3+} , Co^{2+} , Zn^{2+} , Gd^{3+} , Pb^{2+} , Y^{3+} , Ni^{2+} , Cu^{2+} , Lu^{3+} and Fe^{3+} in increasing order of chelate stability constants. When a catholyte containing 0.1 mM EDTA was used, Na^+ , Li^+ , Ba^{2+} , Sr^{2+} , Mg^{2+} , Ca^{2+} and Mn^{2+} were completely recovered as cations and La^{3+} , Ce^{3+} , Co^{2+} , Zn^{2+} , Gd^{3+} , Pb^{2+} , Y^{3+} , Ni^{2+} , Cu^{2+} and Lu^{3+} were completely recovered as chelate anions. The recovery was incomplete for a test mixture without adding EDTA, even when the EDTA concentration in the catholyte was 1 mM. Absolute mobilities of several chelates were obtained and the concentration and pH of isotachophoretic zones were simulated to clarify the decomposition during migration.

1. Introduction

When conventional operational systems for isotachopheresis (ITP) [1,2] are used, anions or cations migrate isotachophoretically to form stacked zones. That is, isotachophoretic regulation of separand concentration is restricted to separands of the same charges as the leading ion. However, when an appropriate electrolyte system with dual leading ions and dual terminating ions is used, both anions and cations migrate

isotachophoretically to form stacked zones. That is, isotachopheresis can in principle be bidirectional [3].

We have reported that an operational electrolyte system for bidirectional isotachopheresis (BITP) could be constructed simply by combining a leading electrolyte for anions and that for cations [4]. Considering the limitation of the pH difference between the combined leading electrolytes, we have described fifteen bidirectional electrolyte systems covering the pH range 3–10 [5].

BITP is an effective method for the analysis and fractionation of both anions and cations in a sample. When both components are to be ana-

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lysed, the use of a bidirectional operational electrolyte system may considerably shorten the analysis time, although a dual detector system is necessary. Even if such a system is not available, it is very convenient that an electrolyte system can be applied for the analysis of both anions and cations. Moreover, a new separation system may be developed by utilizing BITP.

We have been studying the isotachophoretic separation behaviour of metal cations, including rare earth ions. Recently we reported the selective analysis of alkali ions and alkaline earth metal ions from a test mixture of twenty metal cations using tartaric acid as the complexing agent [6]. The strongly interacting ions contained in the test mixture presumably formed complexes with low effective mobility or non-ionic ones, as they could not be detected as isotachophoretic zones. If mobile anions were formed using an appropriate complex-forming agent, BITP could be applied for the analysis of such a complex mixture.

In this paper, we describe the selective separation of metal ions by means of BITP utilizing EDTA as a chelate-forming agent. The role of EDTA is to reverse the ionic charges of strongly interacting cations into anions. Taking into account previous studies [7,8] on the ITP analysis of EDTA chelates by using unidirectional electrolyte systems, the dependence of the recovery of chelate anions on pH and EDTA concentration was studied for eighteen metal cations by means of ITP with particle-induced X-ray emission (PIXE) [6]. The cations are Na^+ , Li^+ , Ba^{2+} , Sr^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , La^{3+} , Ce^{3+} , Co^{2+} , Zn^{2+} , Gd^{3+} , Pb^{2+} , Y^{3+} , Ni^{2+} , Cu^{2+} , Lu^{3+} and Fe^{3+} in increasing order of stability constants. The purpose was to clarify how the stability constants should differ for BITP of the metal cations.

The stability of the chelate zone is important from the viewpoint of recovery. The concentrations of the chelate and the free metal ions in the isotachophoretic zone are simulated to describe the stability of the chelate zone. As no mobilities of chelate anions have previously been reported, they were determined by ITP.

2. Theoretical

2.1. Operational electrolyte system for BITP using EDTA

The operational electrolyte system in BITP consists of a leading electrolyte for anions (anolyte) and that for cations (catholyte). The pH buffer in the anolyte plays the role of the terminating cations, and that in the catholyte plays the role of the terminating anions.

Fig. 1 illustrates the electrolyte system for BITP filled in a separation capillary. The anolyte is 20 mM HCl buffered by adding β -alanine at pH 3.6 and the catholyte is 20 mM ammonia solution buffered by adding acetic acid at pH 4.8. The catholyte contains the complexing agent EDTA in the range 0–1 mM. In this system, β -alanine and acetic acid are simultaneously cationic and anionic terminators, respectively.

In previous studies on the ITP separation of EDTA chelates, no EDTA was added to the catholyte [7,8]. As shown later, the addition of EDTA was necessary to obtain complete recovery at pH 3.6.

As in conventional unidirectional ITP, the effective mobility of the terminators should not

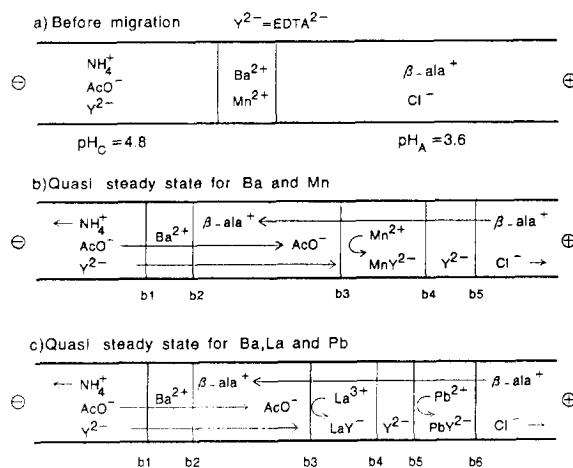
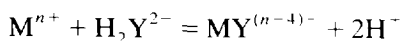


Fig. 1. Schematic diagrams of bidirectional isotachophoresis utilizing EDTA as a chelating agent. The anolyte (anionic leading electrolyte) is HCl buffered by β -alanine and the catholyte (cationic leading electrolyte) is ammonia solution buffered by acetic acid (electrolyte system No. 2 in Table 2).

be too small or too large. Otherwise, ITP is impossible or the effective mobility range of analysable samples become very narrow. Therefore, the pH difference of the electrolyte is not arbitrary [4] and preferably is in the range $0.5 < \text{pH}_C - \text{pH}_A < 1.5$, where pH_C is the pH of the catholyte and pH_A is that of the anolyte.

2.2. Stability of isotachophoretic zone of EDTA chelates

Let us consider Ba^{2+} and Mn^{2+} as the sample, as shown in Fig. 1a and b. The chelate stability constants with EDTA^{4-} at zero ionic strength were 9.62 and 15.63, respectively. Under the pH conditions used in this study, EDTA may exist as divalent anions, EDTA^{2-} (hereafter abbreviated as H_2Y^{2-}). Then the chelate-forming reaction with metal cations M^{n+} that actually occurs in the isotachophoretic zone can be written as



As the conditional stability constants of EDTA chelates are much smaller than the stability constants, weakly interacting Ba^{2+} will be detected as cations forming a steady-state zone, as shown in Fig. 1b. On the other hand, strongly interacting Mn^{2+} is detected as a chelate anion. Accordingly, the recovery as the chelate anion is closely related to the pH of the anolyte (pH_A) and the concentration of EDTA contained in the catholyte.

The other important factor affecting the recovery is the migration order, as discussed in detail by Gebauer et al. [8]. H_2Y^{2-} from the

catholyte overtakes the MnY^{2-} zone, because H_2Y^{2-} has a larger effective mobility than that of MnY^{2-} . This migration order for H_2Y^{2-} and the chelate of metal ions may be reversed, depending on the central metal ion. For example, the effective mobility of PbY^{2-} is slightly larger than that of H_2Y^{2-} as shown in Fig. 1c. Obviously, from the concentration regulation in isotachopheresis, the concentration of EDTA is much higher in the EDTA zone than that in the catholyte. In the case of PbY^{2-} , if the chelate decomposes to release free metal ions, they will return to its original zone forming the chelate zone again reacting with H_2Y^{2-} . It should be noted the effective mobility of a monovalent chelate anion, e.g., LaY^- , is always smaller than that of H_2Y^{2-} and therefore such an effect is not expected.

Since H_2Y^{2-} is fed continuously from the catholyte, the EDTA zone between the boundaries b_4 and b_5 (Fig. 1) cannot reach the steady state, that is, the zone length increases with time. Accordingly, the boundaries b_3 and b_4 moves with a smaller velocity than the isotachophoretic velocity. On the other hand, the boundaries b_1 , b_2 and b_5 show purely isotachophoretic behaviour.

3. Experimental

3.1. Samples

Table 1 lists the eighteen metal ions and the values of the stability constants (ionic strength =

Table 1
Chelate stability constants of metal cations

$\text{MY}^{(n-4)-}$	Log K	$\text{MY}^{(n-4)-}$	Log K	$\text{MY}^{(n-4)-}$	log K
NaY^{3-}	3.46	MnY^{2-}	15.63	PbY^{2-}	19.70
LiY^{3-}	4.61	LaY^-	17.28	YY^-	19.90
BaY^{2-}	9.62	CeY^-	17.76	NiY^{2-}	20.34
SrY^{2-}	10.50	CoY^{2-}	18.02	CuY^{2-}	20.52
MgY^{2-}	10.65	ZnY^{2-}	18.26	LuY^-	21.62
CaY^{2-}	12.43	GdY^-	19.17	FeY^-	26.82

0). The values were obtained by applying an ionic strength correction to the reported values at ionic strength = 0.1 [9]. An equimolar test mixture was prepared from 20 mM stock solutions of the chlorides obtained from Tokyo Kasei (Tokyo, Japan). The concentration of the component was 1 mM. The pH of the sample solution was adjusted to 4.8 by adding acetic acid. The pH of the sample solution was kept constant just before starting migration, because the sample is injected into the catholyte and the pH was adjusted to 4.8.

A test mixture of the eighteen metal cations containing an equimolar amount of EDTA was also prepared by mixing the above test mixture and 20 mM EDTA solution. The pH of the EDTA solution was adjusted to 4.8 by adding ammonia solution. The component concentration was 0.5 mM.

Small amounts of cationic dyes (toluidine blue and astrazon pink) and an anionic dye [4,5-dihydroxy-3-(*p*-sulfophenylazo)-2,7-naphthalene-disulfonic acid (SPADNS)] were co-migrated to monitor the migration process and determine the timing of fractionation.

3.2. Operational electrolyte system

Table 2 summarizes the electrolyte systems used. The anolyte was 20 mM HCl and chloride was the leading ion for anions. The pH of the anolyte (pH_A) was adjusted to 3–6 by using appropriate buffers as shown in Table 1. The catholyte was 20 mM aqueous ammonia solution containing EDTA as the complexing agent, and ammonium was the leading ion for cations. The concentration of EDTA was varied as 0.1, 0.25, 0.5 and 1.0 mM. The pH of the catholyte (pH_C) was adjusted to 4.8 by adding acetic acid. The electrolytes used contained 0.1 mass-% of hydroxypropyl cellulose (HPC) (Tokyo Kasei) to suppress electroosmotic flow. The viscosity of a 2 mass-% HPC solution was 1000–4000 cP at 20°C.

All reagents were purchased from Tokyo Kasei. pH measurements were carried using a Horiba (Tokyo, Japan) Model F-7 AD digital pH meter.

Table 2
Operational electrolyte system used in isotachopheresis

Anolytes	20 mM HCl
pH buffers	(1) Triglycine (pH 3.1) (2) β -Alanine (pH 3.6) (3) ϵ -Aminocaproic acid (pH 4.4) (4) Histidine (pH 6.0)
Catholytes	20 mM ammonia solution
pH buffers	(1) Acetic acid (pH 4.8) (2) Caproic acid (pH 4.8) (3) MOPS (pH 6.0)
Chelating agent	EDTA, 0.1–1.0 mM

Operational electrolyte system for BITP

System No.	Anolyte	Catholyte	EDTA concentration (mM)
1	1	1	
2	2	1	
3	3	2	
4	4	3	
5	2	1	0.1
6	2	1	0.25
7	2	1	0.5
8	2	1	1.0

3.3. Isotachophoretic apparatus used for R_E measurement

The detector used for R_E measurement was a high-frequency contactless conductivity detector (HFCCD) [10]. The separation unit of the apparatus used was that of a Labeco (Spiska Nova Ves, Slovakia) ZKI-001 instrument. The separation column used consisted of a pre-separation capillary (10 cm \times 0.5 mm I.D.) and a main capillary (20 cm \times 0.25 mm I.D.). The migration current when detecting zones was 50 μA , which was supplied by a high-voltage power supply for a Shimadzu (Kyoto, Japan) IP-2A instrument. Measurements were carried out at 25°C in a temperature-controlled room.

The qualitative index R_E is defined as the ratio of the potential gradient ($E \text{ V cm}^{-1}$) of a sample zone (E_S) to that of a leading zone (E_L) [11]. When a conductivity detector is used, it is equal to the ratio of specific resistance (ρ) of each zone. It is also equal to the ratio of the effective

mobility (\bar{m}) of leading ion to that of sample ion in its zone from the equality of the velocity of migrating zones ($v = \bar{m}E$):

$$R_E = E_S/E_L = \rho_S/\rho_L = \bar{m}_L/\bar{m}_S$$

As the output signal of the HFCCD shows a non-linear response to the specific resistance [10], the signal obtained was converted into specific resistance using a fourth-order polynomial expression of output voltage. Picric acid was used as the internal standard to correct the slight drift of HFCCD signals. The simulated R_E value was 2.64 at $\text{pH}_A = 3.6$.

3.4. Micro-preparative isotachoretic analyser

Fig. 2 shows a schematic diagram of the capillary-type micro-preparative analyser modified for BITP. The design and the method of fractionation have already been reported in detail for the unidirectional version [12]. The separated sample zones were fractionated from a nozzle by applying a counter-flow of the anolyte and the catholyte. Two syringe pumps were used for the purpose.

Fig. 2b shows the injection compartment of the analyser. The compartment was made of two blocks of acrylic resin. A semipermeable membrane was inserted between two blocks to prevent hydrodynamic flow in the separation tube.

The separation column used consisted of a PTFE precolumn (46 cm \times 1 mm I.D. for anions, 63 cm \times 1 mm I.D. for cations) and a PTFE main column (20 cm \times 0.5 mm I.D.). The total volume of the column was 0.40 cm³ for anions and 0.53 cm³ for cations. The quantity of electric charge integrated until the first sample zone was 1.1 C.

One drop (ca. 5 μ l) comprised one fraction, which contained ca. 5 nmol of component. The amount depends on the velocity of the counter-flow and migration current. In the present experiments, the former was ca. 30 μ l min⁻¹ and the latter was 150 μ A.

3.5. PIXE analysis

For PIXE spectral measurements, a Van de Graaff accelerator was used (Model AN-2500;

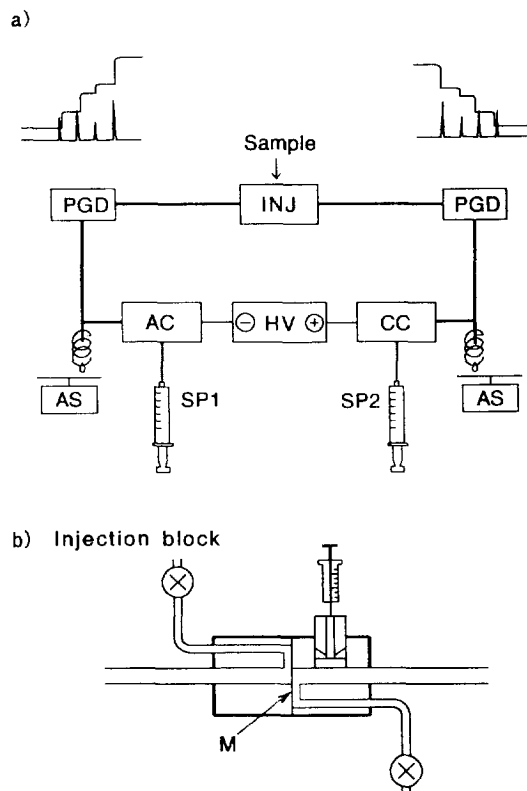


Fig. 2. (a) Schematic diagram of preparative ITP equipment. PGD = potential gradient detector; INJ = sample injection valve; AC = anolyte compartment; CC = catholyte compartment; HV = high-voltage power supply; SP1 and SP2 = syringe pumps making counter flow of anolyte and catholyte; AS = automated sampler. (b) Injection compartment. M = semipermeable membrane.

Nisshin High Voltage, Tokyo, Japan). The energy of the H⁺ beam was 2 MeV, the beam current was ca. 50 nA and the integrated charge was 10 μ C. The detector used was a high-purity Ge detector (an Ortec Model GLP-10180) and the multi-channel analyser was a Laboratory Equipment, (Tokyo, Japan) Model AMS-1000. Owing to the limitations of the X-ray detection system used, light elements below atomic number 14 (Si) could not be detected.

A Nuclepore filter (thickness 5 μ m and pore size 0.1 μ m) was used as the target backing material, which was mounted on an aluminium flame. The separated zones were fractionated dropwise on the Nuclepore filter. After drying in

a desiccator, the fractions were used as PIXE targets. A typical single run took ca. 200 s to obtain a PIXE spectrum of a fraction. The spectra obtained were analysed by using the spectrum deconvolution software PIXS [13].

4. Results and discussion

4.1. Mobility of EDTA chelates

First, absolute mobilities of EDTA and several EDTA chelates were determined by the best-fitting procedure for the observed and simulated R_E using the least-squares method [14].

Table 3 gives the evaluated absolute mobilities of several chelate anions, R_E values obtained by using electrolyte system No. 2 (pH_A 3.6) and the stability constants. The mobility of H_2Y^{2-} was obtained using three R_E values (2.12, 1.96 and 1.86) obtained by using electrolyte systems Nos. 2, 3 and 4 in Table 2, respectively. It was assumed that the mobility of the ionic component of EDTA increased linearly with increasing charge.

Obviously from Table 3, the mobilities of divalent anions are twice those of the monovalent anions. However, the difference in the mobilities among the monovalent or divalent ions is very small. This is natural because the mobility of these chelates is mostly determined by the bulky ligand, suggesting that only a limited number of chelate anions can be separated at once.

4.2. Migration behaviour of EDTA chelates

The isotachopheretic separation behaviour of EDTA chelates was studied by Yoshida et al. [7] for fourteen metal ions, Mg^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Bi^{3+} , Fe^{3+} and La^{3+} . They concluded that an anolyte (leading electrolyte for anions) of low pH should be avoided in order to obtain good recoveries and the use of specific buffering counter ions was effective for obtaining better separability.

First, the migration behaviour of EDTA chelates was observed using electrolyte systems containing no EDTA. Fig. 3 shows a typical isotachopherogram of LaY^- and PbY^{2-} obtained by using electrolyte system No. 2 (pH_A 3.6) and calibration lines obtained by using various electrolyte systems in the pH range 3.1–6. The stability constants of LaY^- and PbY^{2-} were $\log K = 17.3$ and 19.7, respectively. As shown in Fig. 3b, the calibration line at $\text{pH}_A = 3.6$ did not pass through the origin: 5 nmol of LaY^- decomposed during migration with integrated charge 0.33C.

Fig. 3c shows the calibration lines of LaY^- obtained at various pH_A , confirming that the LaY^- chelate decomposed at low pH, releasing La^{3+} and H_2Y^{2-} , and the rate of decomposition was large at low pH. On the other hand, the decomposition of PbY^{2-} and LuY^- ($\log K = 21.6$) could not be detected even at pH 3.6.

As discussed by Gebauer et al. [8] for NTA-metal complexes, such decomposition was due to the small conditional complex stability constants at low pH, where the chelate was labile depend-

Table 3
Absolute mobilities of EDTA chelates obtained by isotachopheretic method

$\text{MY}^{(n-4)-}$	R_E	m_0	$\text{MY}^{(n-4)-}$	R_E	m_0
CeY^-	4.20	20.5	AlY^-	3.62	23.5
LaY^-	4.19	20.6	CaY^{2-}	2.28	42.3 ^a
YY^-	4.04	21.3	NiY^{2-}	2.31	41.5
LuY^-	4.02	21.4	PbY^{2-}	2.18	43.6
FeY^-	3.70	23.1	H_2Y^{2-}	2.26	44.0

The $\text{p}K_a$ values of the EDTA used were 2.11, 3.01, 6.84 and 11.84 at zero ionic strength. These values were obtained by applying an ionic strength correction to the reported values of 2.0, 2.67, 6.27 and 11.0 at ionic strength 0.1.

^a Obtained at $\text{pH}_A = 6$.

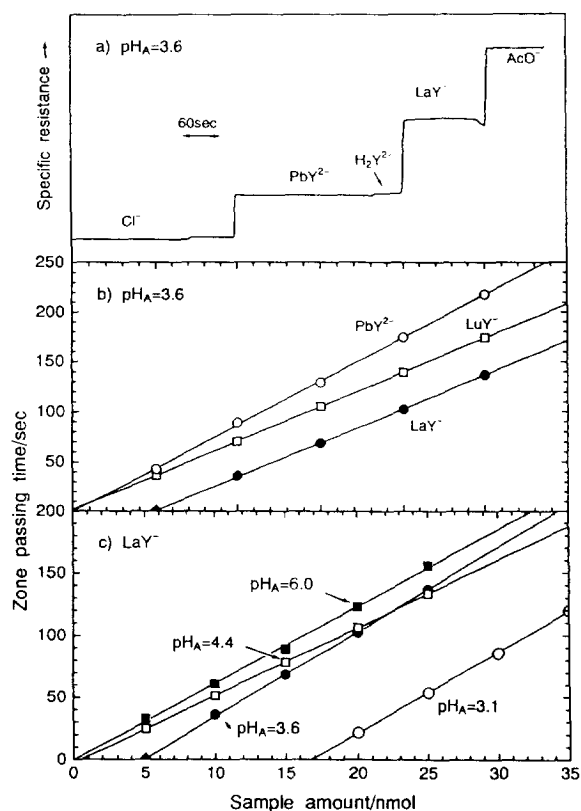


Fig. 3. (a) Isotachopherogram of LaY^- and PbY^{2-} ($Y = \text{EDTA}$) obtained at $\text{pH}_A = 3.6$; (b) calibration lines obtained at $\text{pH}_A = 3.6$; (c) calibration lines obtained by using various electrolyte systems in the pH_A range 3.1–6. The injected sample amount was $1.2 \mu\text{l}$ (12 nmol of metal ions). Operational electrolyte systems as in Table 1. Migration current = $50 \mu\text{A}$. Quantity of integrated charge = 0.28 C .

Table 4

Simulated concentration of some chelates and free metal ions in the isotachophoretic zones

$\text{MY}^{(n-4)}$	Log K^a	pH	Log K'^b	Concentration (mM)	
				Chelate	Free metal
LaY^-	17.28	3.91	-1.36	9.90	0.059
LuY^-	21.62	3.89	2.99	10.4	0.00042
PbY^{2-}	19.70	3.74	1.07	7.40	0.0046

$$^a \text{Log } K = \frac{[\text{MY}^{(n-4)}]}{([\text{M}^{n+}][\text{Y}^{4-}])}$$

$$^b \text{Log } K' = \frac{[\text{MY}^{(n-4)}][\text{H}^+]^2}{([\text{M}^{n+}][\text{H}_2\text{Y}^{2-}])}$$

ing on the stability constants. In this case, LaY^- gradually decomposed to release La^{3+} and H_2Y^{2-} and the released H_2Y^{2-} formed the isotachophoretic zone next to the chelate zone as shown in Figs. 1 and 3a. On the other hand, La^{3+} migrates towards the cathode to form an isotachophoretic zone when a bidirectional electrolyte system is used.

The rate of chelate decomposition must be closely related to the conditional stability constants of the chelate and therefore free metal ions in the zone. Using the absolute mobilities evaluated for the chelate ions, the isotachophoretic chelate zones were simulated. Table 4 shows the total concentration of LaY^- , LuY^- and PbY^{2-} in the isotachophoretic zones and the concentration of free metal ions at pH_A 3.6. In the LaY^- zone, the free metal ion concentration was only 0.6% of that of the chelate. In spite of this low concentration of free ions, the decomposition could not be neglected. On the other hand, the free metal ion contents of the LuY^- and PbY^{2-} zones were 0.004% and 0.06%, respectively.

4.3. Difference of the stability constants necessary for bidirectional isotachophoretic separation

According to preliminary experiments, MnY^{2-} ($\log K = 15.63$) mostly decomposed to form Mn^{2+} zone when pH_A was 3.1 and there was no

EDTA in the catholyte. Therefore, MnY^{2-} was selected as the standard chelate to investigate the difference in the stability constants necessary for bidirectional separation. Several two-component mixtures of MnY^{2-} and (lanthanide) Y^- were analysed and the recovery was investigated.

Fig. 4a shows migration scheme for MnY^{2-} and LaY^- releasing Mn^{2+} , La^{3+} and Y^{2-} . Fig. 4b and c show calibration lines obtained for anions and cations by using a bidirectional electrolyte system. The difference in the stability constants between LaY^- and MnY^{2-} was $\Delta \log K = 1.65$. Obviously from Fig. 4b and c, MnY^{2-} decomposed to migrate as cations and part of the LaY^- and CeY^- also did. When CeY^- ($\Delta \log K = 2.13$) was co-migrated with MnY^{2-} , the re-

covery as CeY^- was better than that of LaY^- . The anionic increased with increase in the stability constants, and finally complete recovery was obtained for GdY^- ($\Delta \log K = 3.54$).

It was revealed from this experiment that Na^+ , Li^+ , Ba^{2+} , Sr^{2+} , Mg^{2+} , Ca^{2+} and Mn^{2+} ($\log K$ of $\text{MnY}^{2-} = 15.6$) were recovered as cations at pH 3.1 and the metal ions Gd^{3+} ($\log K$ of $\text{GdY}^- = 19.2$), Pb^{2+} , Y^{3+} , Ni^{2+} , Cu^{2+} , Lu^{3+} and Fe^{3+} were recovered as chelate anions, when the sample contained an equimolar amount of EDTA but no EDTA was present in the catholyte. Among the other metal ions, La^{3+} and Ce^{3+} were detected as both anions and cations. For CoY^{2-} , NiY^{2-} and ZnY^{2-} , no cationic zones were observed. The zone length of these chelates could not be observed accurately, as the step heights overlapped with that of the H_2Y^{2-} zone.

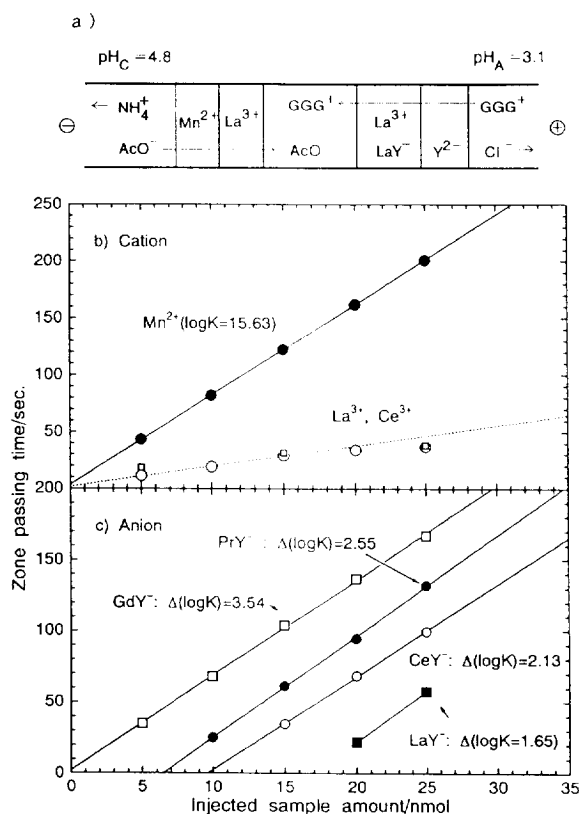


Fig. 4. (a) Migration scheme of MnY^{2-} and LaY^- liberating Mn^{2+} and La^{3+} , and calibration lines obtained for (b) anions and (c) cations by using the bidirectional electrolyte system.

4.4. Recovery of metal cations

Finally, the test mixture of the eighteen metal cations without adding EDTA was analysed by ITP-PIXE using electrolyte systems Nos. 6–8 in Table 2. EDTA was present only in the catholyte. A 10- μl volume of the sample mixture (amount of each component = 10 nmol) was separated and fractionated.

Fig. 5 shows the analytical results for ITP fractions obtained by PIXE. The EDTA concentration was 0.25 mM (Fig. 5a) and 0.5 mM (Fig. 5b). Obviously from Fig. 5, Ba^{2+} , Sr^{2+} and Ca^{2+} were detected as cations and the others were detected as anions. The recoveries of Ba^{2+} , Sr^{2+} and Ca^{2+} were 100%. As Li^+ , Na^+ and Mg^{2+} could not be detected using the PIXE detection system used, they are not shown in Fig. 5. All of these cations can be assigned by using a conventional isotachopheretic method using standard samples. The separability of cations were affected by the EDTA concentration.

Concerning the chelate anions, it was found that the recovery was not so good in general. When the EDTA concentration was 0.25 mM, La, Ce and Mn could not be detected either as anions or as cations. When the EDTA concen-

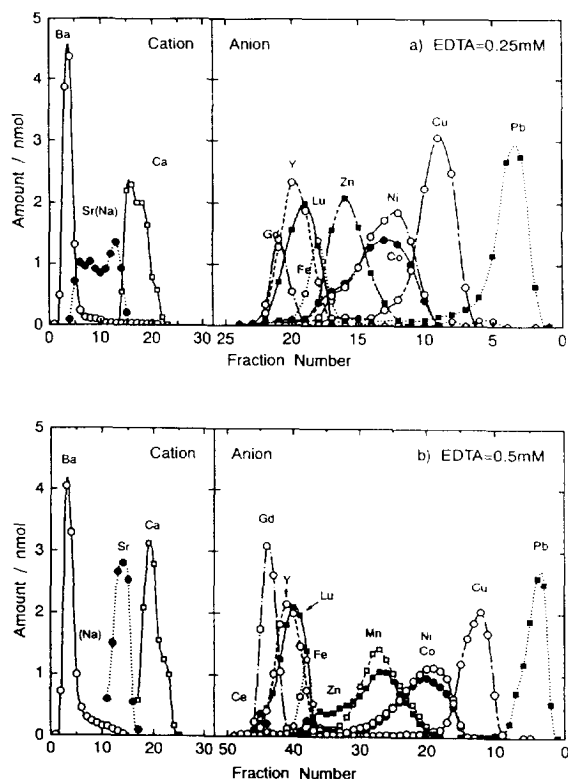


Fig. 5. Analytical results for the fractions of the eighteen metal cations obtained by PIXE. The concentration of EDTA in the catholyte was (a) 0.25 and (b) 0.5 mM. The injected sample amount was 10 μ l (10 nmol metal of ions). Operational electrolyte systems Nos. 5 and 6 in Table 1. Quantity of integrated charge = 1.1 C.

tration was increased to 0.5 mM, a 50% recovery was obtained as MnY^{2-} and a small amount of CeY^- was detected. However, La still could not be detected as the chelate.

In general, the recovery increased when the EDTA concentration was high, as is obvious for, e.g., MnY^{2-} and GdY^- in Fig. 5. However, complete recovery could not be obtained, even when the EDTA concentration was increased to 1 mM. The addition of EDTA at ca. 1 mM was not useful, because non-complexing EDTA disturbed the migration of NiY^{2-} , CoY^{2-} , MnY^{2-} and ZnY^{2-} to form a mixed zone, and the zone length of non-complexing EDTA increased with increase in the EDTA concentration.

Complete recovery was obtained for the chelate anions when the sample solution contained an equimolar amount of EDTA and the catholyte contained EDTA at a low concentration. When a catholyte containing 0.1 mM EDTA was used, Na^+ , Li^+ , Ba^{2+} , Sr^{2+} , Mg^{2+} , Ca^{2+} and Mn^{2+} ($\log K$ of $\text{MnY}^{2-} = 15.63$) were completely recovered as cations, and La^{3+} ($\log K$ of $\text{LaY}^- = 17.28$), Ce^{3+} , Co^{2+} , Zn^{2+} , Gd^{3+} , Pb^{2+} , Y^{3+} , Ni^{2+} , Cu^{2+} and Lu^{3+} were completely recovered as chelate anions.

It should be noted that the addition of 0.1 mM EDTA to the catholyte was sufficient for complete recovery. This is reasonable from the result of simulation that the concentration of free metal ions in the isotachophoretic zone is very small, as shown in Table 4.

Fig. 6 summarizes the recovery of the eighteen metal ions. The sample components were arranged in order of increasing stability constants. Weakly interacting ions of Na^+ to Ca^{2+} were always recovered as cations and the recovery was 100%. The others were detected as anions and the recovery was closely dependent on the EDTA concentration in the catholyte. A 100% recovery was obtained for the eighteen metal cations in the presence of an equimolar amount of EDTA.

It is interesting that the recovery was closely

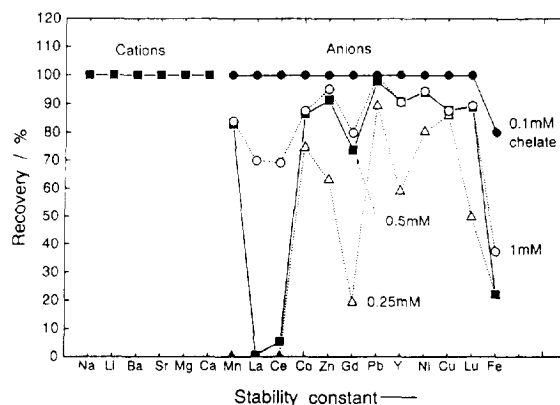


Fig. 6. Recovery of the eighteen metal cations. Operational electrolyte systems Nos. 4, 5, 6 and 7 in Table 1. Quantity of integrated charge = 1.1 C.

dependent on the migration order, confirming the previous study by Gebauer et al. [8]: when the effective mobilities of the chelates were greater than that of EDTA, such as PbY^{2-} in Fig. 3, the observed recovery was much better than the value estimated from the stability constants. Even when the chelate divalent anions have similar effective mobilities to the EDTA anion, the EDTA concentration co-existing with the chelate was high owing to mixed zone formation. Consequently, divalent chelates showed better recoveries than monovalent chelates.

In conclusion, metal cations can be analysed by bidirectional isotachopheresis using EDTA as a complex-forming agent. At pH_A 3.6, the weakly interacting alkali and alkaline earth metal ions were detected as cations and the other metal ions were detected as anions forming chelates. This separation system will be applied to trace element analyses of, e.g., biochemical materials.

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