

Study of isotachophoretic separation behaviour of metal cations by means of particle-induced X-ray emission

I. Separation of twenty metal cations using an acetic acid buffer system and α -hydroxyisobutyric acid complex-forming system

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ABSTRACT

Twenty metal ions were separated isotachophoretically and the zones were fractionated and analysed off-line by particle-induced X-ray emission (PIXE). The recovery, migration order and separation efficiency were studied in relation to the use of a complexing agent, α -hydroxyisobutyric acid (HIB). The migration order using the WNH_4Ac system [aqueous solution (W) of NH_4^+ as the leading ion and acetate (Ac) ion as the pH-buffering counter ion, pH = 4.8] was Ba, Sr, Ca, Na, Mg, (Mn, Fe^{II} , Co), Ni, Zn, La, Ce, Cd, Y, Li, Lu, Gd, (Cu, Pb) and ($\text{Zr}^{\text{IV}}\text{O}$, Fe^{III}). The order using the WNH_4Ac -HIB system was Ba, Sr, Ca, Na, Mg, Mn, Fe^{II} , Cd, Co, Li, Ni, Zn, La, Pb, Ce, Gd, Cu, Y, $\text{Zr}^{\text{IV}}\text{O}$, Lu. The recovery of the metal cations was 100% with both electrolyte systems, except for Fe^{II} and $\text{Zr}^{\text{IV}}\text{O}$. The low recovery of $\text{Zr}^{\text{IV}}\text{O}$ was due to hydroxyl complex formation and that of Fe^{II} to hydroxyl complex formation of Fe^{III} , the oxidation product of Fe^{II} . For these ions, the recovery with the WNH_4Ac -HIB system was lower than that with the WNH_4Ac system.

INTRODUCTION

Preparative capillary isotachopheresis (ITP) is a useful technique for the separation and purification of ionic substances. A dilute component in a sample is concentrated according to Kohlrausch's regulating function and correspondingly the concentrated sample is diluted. Such a separation mode is advantageous especially for the fractionation of minor components in a sample.

The other important feature of preparative isotachopheresis is that the recovery of sample components is in principle 100% as the components migrate between the leading and terminating zones, because no packings are used in the separation tube and the separation is carried out in a free electrolyte solution. In fact this is valid for many

ionic substances, as exemplified for several organic acids by the agreement between the observed zone lengths and the theoretical estimates [1]. However, in the analysis of metal ions which usually form variously charged ion pairs with a complex-forming reagent and hydroxyl ions, the recovery depends on the reagent selected, its concentration and the pH of the leading solution. As discussed by Kaniansky and Everaerts [2], the reactions in complex-forming equilibria must be rapid enough to allow migration as a homogeneous zone, and the ion pair formed must be sufficiently soluble in the solvent used. If not, part of the sample will not migrate isotachophoretically and consequently the recovery will be reduced. This results in an unsuitable sample for isotachopheresis, *e.g.*, Fe^{III}, which easily forms hydroxyl complexes at isotachophoretically safe pH values. In order to confirm the recovery of metal cations, the separated zones must be fractionated and analysed directly by another independent method. This has not yet been carried out, as far as we know.

In addition to the recovery, the isotachopheretic separability of metal ions and the order of migration are also important from the viewpoint of the preparative strategy in isotachopheresis. The separation behaviour and the step heights eighteen metal cations were reported by Everaerts *et al.* [3] using a thermometric detector and a conductivity detector. They discussed the separability of the metal cation using the step heights with several leading electrolytes. Strictly, however, as exemplified by Mikkers *et al.* [4], the same effective mobility at the steady state does not always mean that no separation is possible. Thus the separability should not be simply discussed on the basis of the difference in the step heights at the steady state (*i.e.*, the difference in the effective mobilities), although the difference is usually a good measure of separation. We found this situation for some metal ions, as discussed later.

In order to study such a separation behaviour of metal ions, a new detection method should be applied, because universal detectors and a UV detector are not useful for the present purpose. We utilized an off-line combination of ITP and particle-induced X-ray emission (PIXE), which is a multi-elemental method with high sensitivity [5]. As the latter method is based on the characteristic X-rays emitted by target elements, it has a high specificity for the determination of the elements even if they are not separated. Amount of sample necessary depends on the element but it is usually of the order of submicrograms.

By the use of a preparative isotachopheretic analyser [6], sample zones were fractionated dropwise (one drop \times 5 μ l) and the fractions were analysed by PIXE. In this paper, the separation behaviour is discussed in relation to the use of a complexing agent, α -hydroxyisobutyric acid (HIB), which is the most effective agent for the separation of rare earth ions [7,8] and other heavy metal ions [9,10].

EXPERIMENTAL

Samples

The metal ions treated were monovalent Li and Na, bivalent Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, Zr^{IV} O, Ba and Pb and trivalent Y, La, Ce, Gd and Lu. Stock solutions were prepared using the chlorides, which were obtained from Tokyo Kasei (Tokyo, Japan). The solution analysed was a mixture of twenty metal ions (0.30–0.5 mM each). The water used was purified with a PURIC-R ion exchanger (Japan Organo, Tokyo, Japan). The specific resistance of the water was $18.3 \cdot 10^6 \Omega/\text{cm}$. The

TABLE I

ELECTROLYTE SYSTEM USED IN ISOTACHOPHORESIS

HIB = α -Hydroxyisobutyric acid; CARH = carnitine hydrochloride; HPC = hydroxypropylcellulose.

Component	WNH ₄ Ac	WNH ₄ Ac-HIB
Leading electrolyte	20 mM ammonia solution	20 mM ammonia solution
Complexing agent	None	10 mM HIB
pH-Buffering agent	Acetic acid	Acetic acid
pH of leading electrolyte	4.8	4.8
Terminating electrolyte	10 mM CARH	10 mM CARH
Additive	0.2% HPC	0.2% HPC

pH of the sample mixture was adjusted to 3 by adding hydrochloric acid to suppress the oxidation of Fe^{II} to Fe^{III}. The sample was prepared just before fractionation. Small amounts of two cationic dyes, toluidine blue (TB) and astrazon pink (AP) were added to the sample solution as position markers in fractionation.

Electrolyte system

The electrolyte systems used are summarized in Table I. The notations proposed by Everaerts *et al.* [3] are used in this paper. For example WNH₄Ac defines an aqueous solution (W) of NH₄⁺ as the leading ion and acetate ion (Ac) as the pH- buffering counter ion. The WNH₄Ac system was essentially the same as that of WKAc [3], as the absolute mobility of K⁺ and NH₄⁺ are approximately equal ($m_K = 79.1 \cdot 10^{-5}$, $m_{NH_4} = 76.8 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). The WNH₄Ac system was used owing to the practical requirement for the rapid collection of the sample signals in PIXE measurement, which will be detailed later.

The use of a complex-forming reagent is a traditional technique for improving separability, especially that of metal ions. We used HIB, which is useful for the separation of rare earth elements [7,8]. The concentration of the complex-forming agent (HIB) is very important for obtaining good separations. Taking into account the results of preliminary experiments and the results of simulation, a leading electrolyte containing 10 mM HIB was used in this work (WNH₄Ac-HIB). The leading electrolyte was 20 mM ammonia solution and pH_L was adjusted to 4.80 by adding acetic acid. The terminating electrolyte was a 10 mM solution of carnitine hydrochloride. The actually migrating terminator was H⁺.

Hydroxypropylcellulose (HPC) was added to the leading electrolytes and the terminating electrolyte (0.2%) in order to suppress electroendosmosis. It was obtained from Tokyo Kasei and the viscosity of a 2% solution was given as 1000–4000 cP at 20°C.

Preparative equipment

The preparative isotachophoretic analyser used and the method of fractionation have been reported in a previous paper [6]. The principle was of the Arlinger type [11], except for the dropwise fractionation. Dropwise fractionation (*ca.* 5 μ l) was carried out by applying a counter-flow of the leading electrolyte with a syringe pump. A coupled separation tube was used (16 cm \times 1 mm I.D. \times 2 mm O.D. and 30 cm \times 0.5 mm I.D.

× 1 mm O.D.), which connected the injection valve and a potential gradient detector. The volume of the coupled tube was 0.184 cm³. A typical single run took about 50 min. The migration current was 300 μA for the first 22 min, then it was decreased to 150 μA. The potential gradient detector and the fractionating compartment were connected by a PTFE tube of (16 cm × 0.5 mm I.D. × 1 mm O.D.). After being monitored by the potential gradient detector, sample zones were sliced by the counter-flow of the leading electrolyte applied by a syringe pump. The drops fell onto target holders, where a Nuclepore filter (thickness 5 μm, pore size 0.1 μm) was mounted on an aluminium frame. In a typical experiment 50 fractions were obtained in 9 min. The amount of metal elements in each fraction was 3–5 nmol. The fractions were dried in a desiccator. The residues were analysed individually as the PIXE target.

PIXE analysis

PIXE spectra of the fractions were measured by bombarding the targets with a 2-MeV H⁺ beam energized by a Van de Graaff accelerator (Nisshin High Voltage, Model AN-2500). The beam current was typically 40 nA. The detector used was a high-purity Ge detector (Ortec, Model GLP-10180) and the multi-channel analyser was a Laboratory Equipment Model AMS-1000. A single run took ca. 230 s. Owing to the limitations of the detection system used, the light elements with atomic numbers less than 14 (Si) could not be detected. In the present experiment, therefore, Li, Na and Mg were not analysed. The assignment of these ions was carried out isotachophoretically.

The element with maximum sensitivity in the PIXE measurement is Cl or K.

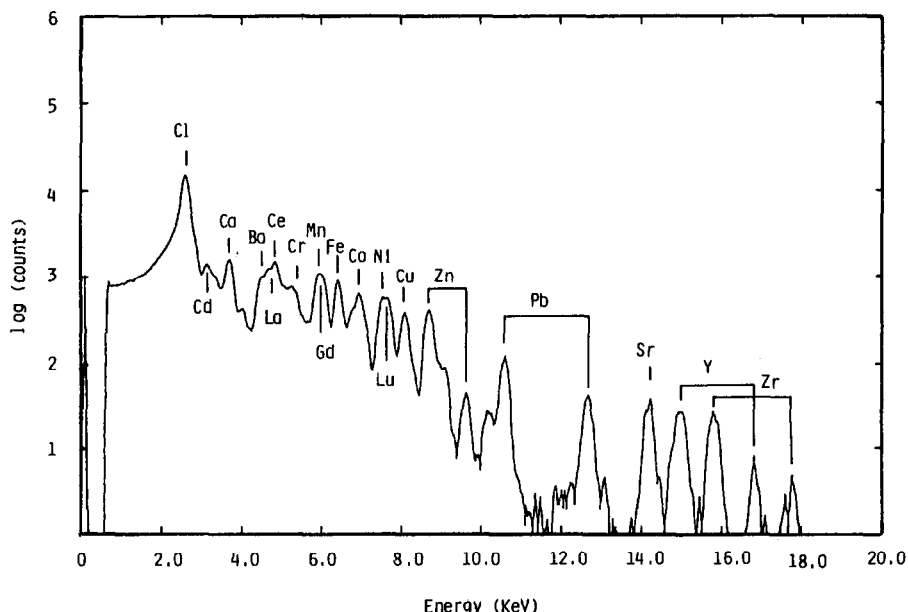


Fig. 1. PIXE spectrum of a mixture of chlorides of Ca, Mn, Fe, Co, Ni, Cu, Zn, Sr, Y, Zr, Cd, Ba, La, Ce, Gd, Lu and Pb. $E_p = 2$ MeV, 40 nA, 10 μC. The total sample amount was 200 nmol.

Both elements appear frequently in ITP as the leading ions. When the WKAc system was used, each fraction contained considerable amounts of K^+ , because the counter-flow used to slice the sample zones should be the same as the leading electrolyte. Not only did it disturb the accurate determination of the neighbouring Ca which was present in our sample, but it also reduced the data acquisition speed owing to the limit of the X-ray counting rate of the detection system. Fig. 1 shows the PIXE spectra of the present sample before separation.

The data reduction software PIXS (developed by the authors) with a relative intensity database of characteristic X-rays was used in the analysis of the PIXE spectra [12]. The calculations were carried on an NEC PC-9801RA microcomputer (80386–80387, 16 MHz). It took *ca.* 100 s to deconvolute the spectra.

RESULTS AND DISCUSSION

Separability

First a mixture of twenty metal cations, Li, Na, Mg, Ca, Mn, Fe^{II} , Co, Ni, Cu, Zn, Sr, Y, $Zr^{IV}O$, Cd, Ba, La, Ce, Gd, Lu and Pb, was separated and fractionated using the WNH_4Ac system ($pH_L = 4.8$). The sample volume was $25 \mu l$ (0.3 – 0.5 mM each) and the total sample amount was *ca.* 200 nmol. Fig. 2 shows the isotachopherogram obtained with the use of the potential gradient detector. The total time-based zone length of the sample zones was 9 min (migration current = $150 \mu A$). The electric charge integrated through the migration process was 0.594 C. Apparently from Fig. 2, the

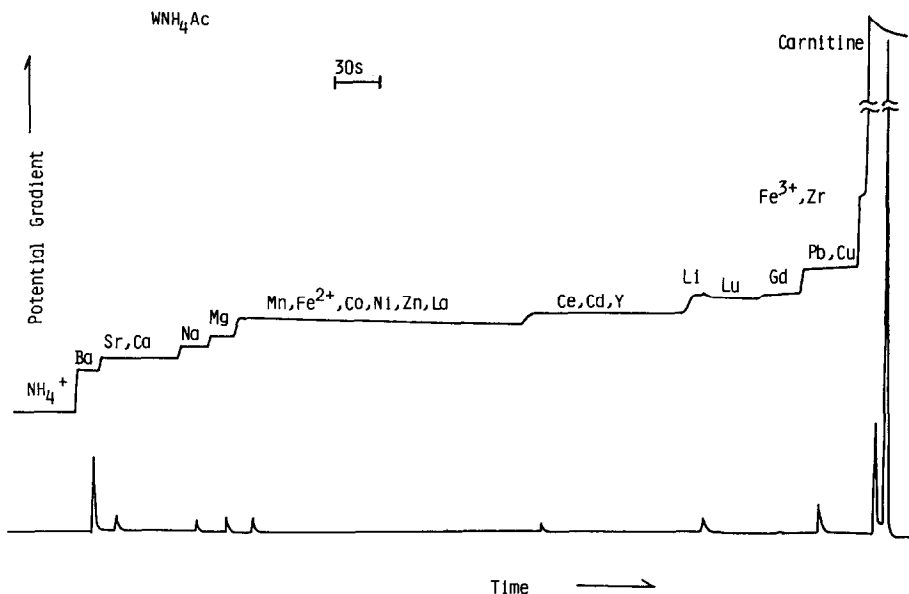


Fig. 2. Isotachopherogram of twenty metal cations (Li, Na, Mg, Ca, Mn, Fe^{II} , Co, Ni, Cu, Zn, Sr, Y, $Zr^{IV}O$, Cd, Ba, La, Ce, Gd, Lu and Pb) with the WNH_4Ac system ($pH_L = 4.8$). Concentration of leading NH_4^+ = 20 mM. Migration current = $150 \mu A$. Coupled separation tube: 16 cm \times 1 mm I.D. \times 2 mm O.D. and 30 cm \times 0.5 cm I.D. \times 1 mm O.D.

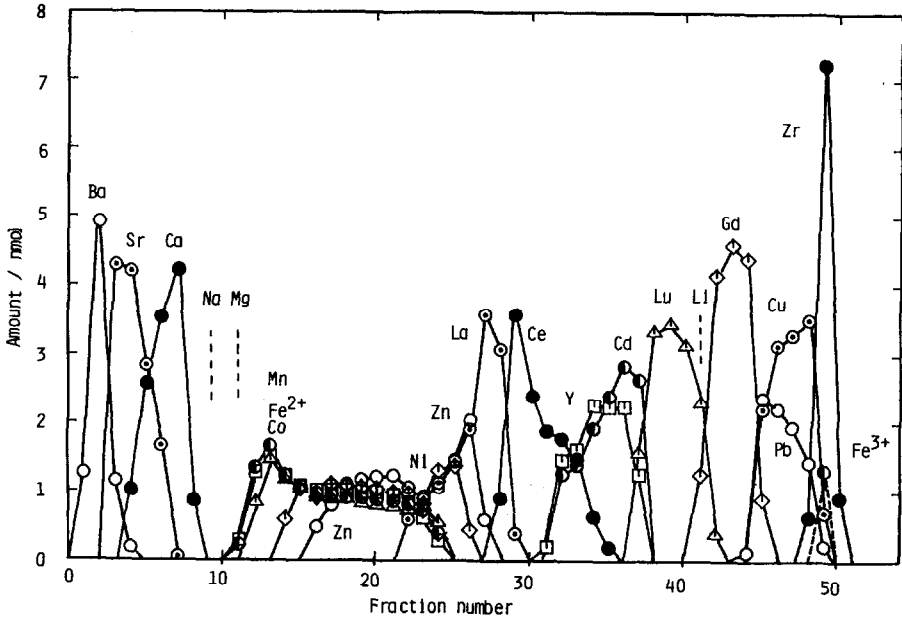


Fig. 3. Analytical result for the fractions with the WNH_4Ac system using PIXE. For the electrolyte conditions, see Fig. 2. The electric charge integrated through the migration process was 0.594 C.

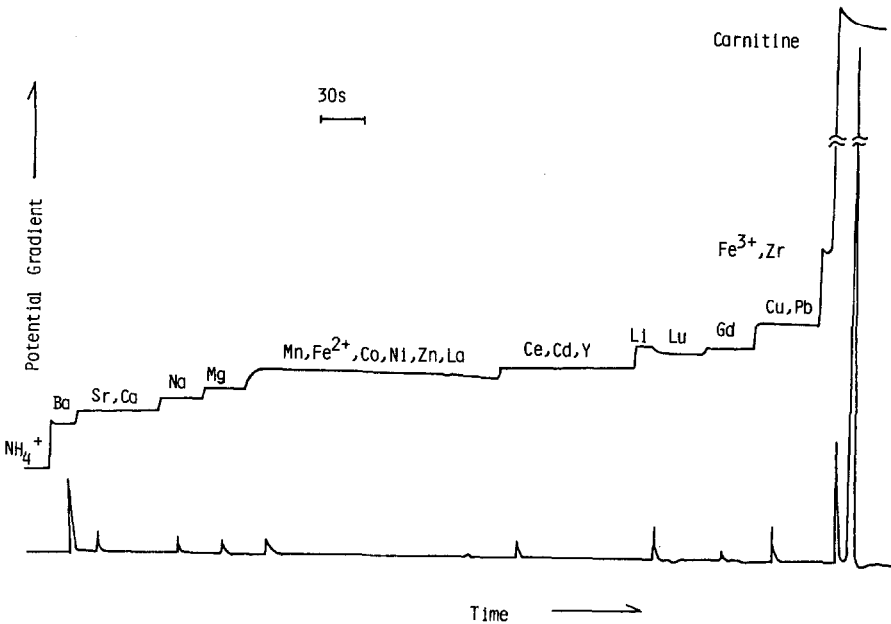


Fig. 4. Isotachopherogram of twenty metal cations (as in Fig. 2) with the WNH_4Ac system ($\text{pH}_1 = 4.8$) with extended separation tube. Concentration of leading NH_4^+ = 20 mM. Migration current = 150 μA . Coupled separation tube: 32 cm \times 1 mm I.D. \times 2 mm O.D. and 30 cm \times 0.5 mm I.D. \times 1 mm O.D.

potential gradients of several components were almost identical, suggesting that the separability was not good. Fig. 3 shows the result of PIXE analysis for the fractions except for Li, Na and Mg. Several mixed zones were observed in the fractions, suggesting that the separation was in the transient state.

As it seemed that the applied electric charge was insufficient for separation, the separation tube was extended to give a better resolution for the same amount of sample. The coupled tube was 32 cm \times 1 mm I.D. \times 2 mm O.D. and 30 cm \times 0.5 mm I.D. \times 1 mm O.D. Figs. 4 and 5 show the isotachopherogram and the analytical results for the fractions. Although the electric charge integrated was twice as large as in the previous experiment (1.053 C), no distinct difference was observed between the isotachopherogram in Figs. 4 and 2. In contrast to the pessimistic estimation of poor separability from the agreement of the potential gradient trace, as shown in Fig. 5, PIXE analysis revealed that an extended capillary was very effective in the separation of Ni, Zn, La, Ce, Cd and Y. The separation of Mn, Fe^{II} and Co was not improved, although the length of the mixed zone was shortened. This suggested that the effective mobilities of these elements in the mixed zone were almost identical under the electrolyte condition used. Similarly, the separation of Cu and Pb was not improved. It was confirmed that the same step heights in an isotachopherogram with a potential gradient detector at the steady state does not always mean that no separation is achieved [4].

It is interesting that two Fe zones with different effective mobilities were detected in this system. The first Fe zone to appear was undoubtedly due to Fe^{II}. The second

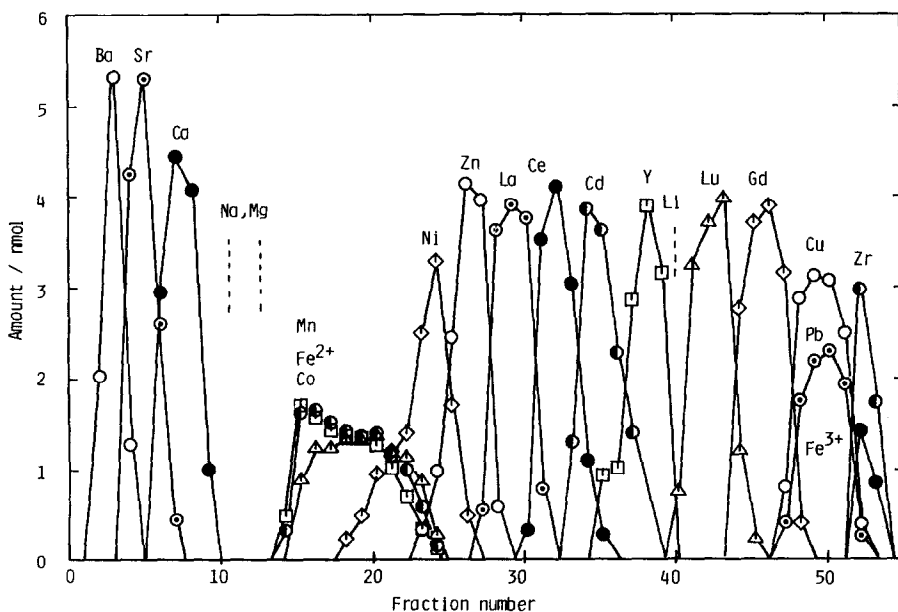


Fig. 5. Analytical result for the fractions with the WNH_4Ac system using PIXE, with extended separation tube. For the electrolyte conditions, see Fig. 4. The electric charge integrated through the migration process was 1.053 C.

zone can probably be assigned to Fe^{III} , as we made similar observations for an Fe^{II} sample. However, as the injected iron sample was nominally Fe^{II} , the Fe^{III} zone found represented the oxidation product of Fe^{II} . It has been accepted that Fe^{III} ion cannot be measured by isotachopheresis because it forms a hydroxyl complex and is deposited at isotachophoretically safe pH values [3]. As suggested, we could not observe the step corresponding to Fe^{III} when its solution was injected. However, by means of PIXE analysis we confirmed that Fe^{III} migrated just at the front end of the terminating zone.

The migration order with the WNH_4Ac system was Ba, Sr, Ca, Mg, Na, (Mn, Fe^{II} , Co), Ni, Zn, La, Ce, Cd, Y, Li, Lu, Gd, (Cu, Pb) and ($\text{Zr}^{\text{IV}}\text{O}$, Fe^{III}). In general, the quality of separation with the WNH_4Ac system was not good, but the system is still useful for the separation of a considerable number of metal ions.

It may be interesting to compare the migration order with that at pH_L 5.4 (WKA system, 10 mM K^+) [3]. The order was Ag (18), Ba (59), Sr (73), Ca (86), Na (100), Mg (102), Fe^{II} (108), La (109), Mn (114), Co (114), Ni (115), Zn (130), Cd (142), Cu (169), Cr (175), Pb (179), Li (194), Sn^{II} (1083), where the figures in parentheses are the reported relative step heights using a conductivity detector. The difference in the migration order is due to the differences in the pH_L values and the leading ion concentrations. The latter factor is not so small because the effective mobility of polyvalent ions is affected more than that of monovalent ions by a change in ionic strength.

As discussed previously, good separability of metal cations was achieved by adding HIB to the leading electrolyte of an acetic acid buffer system [7–10]. Figs. 6 and

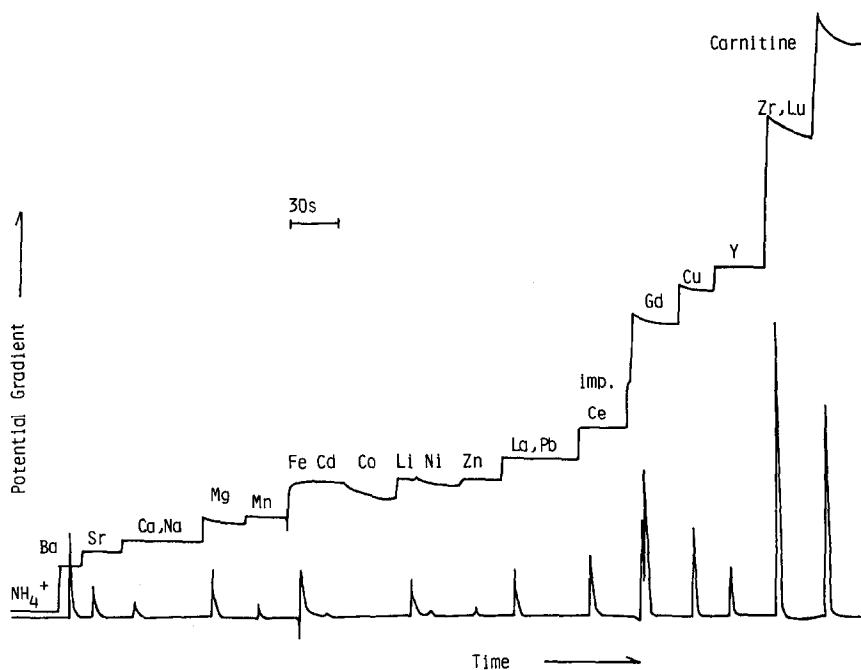


Fig. 6. Isotachopherogram of 20 metal cations (as in Fig. 2) with the WNH_4Ac -HIB system ($\text{pH}_L = 4.8$). Concentration of HIB = 10 mM and leading NH_4^+ = 20 mM. Migration current = 150 μA .

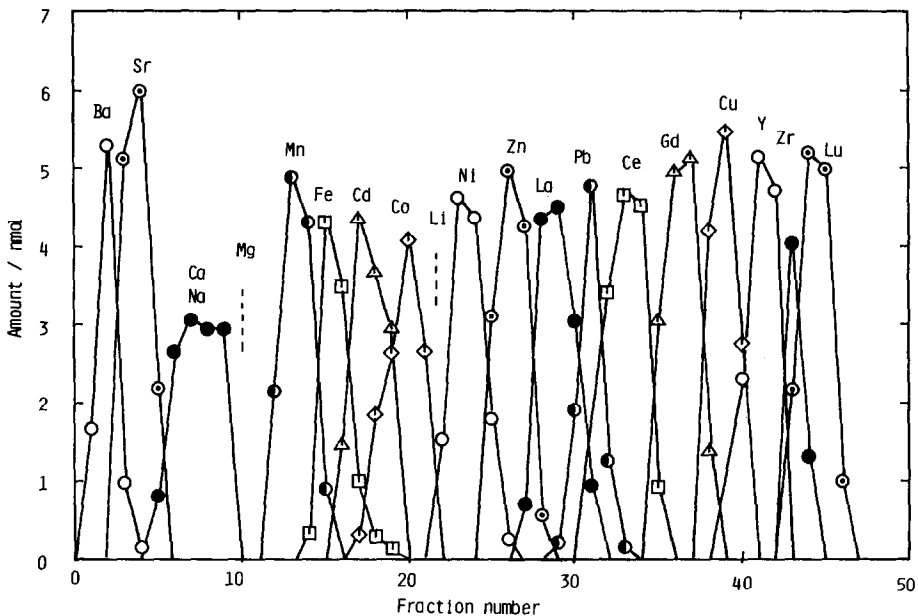


Fig. 7. Analytical result for the fractions with the $\text{WNH}_4\text{Ac-HIB}$ system using PIXE. For the electrolyte conditions, see Fig. 6. The electric charge integrated through the migration process was 0.567 C.

7 show the isotachopherogram and the analytical results for the fractions. The migration time and the applied current were the same as those with the first WNH_4Ac system. The electric charge integrated through the migration process was 0.567 C, which was similar to that for Figs. 2 and 3. Apparently from Figs. 6 and 7, the separability was considerably improved by adding HIB. Good separation was achieved even for Fe^{II} , Co, Ni, Pb and Cu, although they were difficult to separate with the WNH_4Ac system by applying twice as much electric charge as with the $\text{WNH}_4\text{Ac-HIB}$ system.

The migration order with the present $\text{WNH}_4\text{Ac-HIB}$ system was Ba, Sr, (Ca, Na), Mg, Mn, Cd, Fe^{II} , Co, Li, Ni, Zn, La, Pb, Ce, Gd, Cu, Y, $\text{Zr}^{\text{IV}}\text{O}$ and Lu. In this system the Fe^{III} zone was not detected, in accordance with the previous report [9]. It should be noted that the step heights of La and Pb are identical but the separation was again complete.

Recovery

The recovery of metal cations was evaluated on the basis of PIXE analysis, defined as

$$\text{recovery (\%)} = \frac{\text{analysed amount of element in fractions}}{\text{analysed amount of element injected}} \cdot 100$$

where the analysed amount was obtained using PIXE.

In isotachopheresis, the sample should migrate between the leading and the terminating zones forming homogeneous zones. If not, isotachophoretic migration

TABLE II
RECOVERY (%) OF FRACTIONATED METAL CATIONS EVALUATED BY MEANS OF PIXE
For the electrolyte system used, see text.

Z	Metal ion	Electrolyte system		
		WNH ₄ Ac (0.594 C)	WNH ₄ Ac (1.053 C)	WNH ₄ Ac-HIB (0.567 C)
20	Ca	98	104	100
25	Mn	103	102	97
26	Fe ^{II}	100 ^a	102 ^b	71
27	Co	99	102	103
28	Ni	102	102	98
29	Cu	104	102	99
30	Zn	98	103	101
38	Sr	101	98	99
39	Y	100	99	101
40	Zr ^{IV} O	67	37	40
48	Cd	100	100	104
56	Ba	100	102	98
57	La	101	104	103
58	Ce	102	103	104
64	Gd	103	102	102
71	Lu	102	100	97
82	Pb	100	100	100

^a 10% was found as Fe^{III}.

^b 17% was found as Fe^{III}.

does not take place and then complete recovery cannot be expected. In these experiments the concentration of complexing agent and the pH of the leading solution were determined to obtain good recoveries considering the above restriction of isotachophoretic migration. With the WNH₄Ac electrolyte system, most of metal cations considered form weak 1:1 ion pairs with acetate ion. The stability constants (log *K*) of the ion pairs are small for most of the metal ions treated, *e.g.*, those of BaAc⁺, CaAc⁺ and MgAc⁺ are < 1 and at most *ca.* 2 for CuAc⁺. On the other hand, with the WNH₄Ac-HIB electrolyte system, metal ions form multi-coordinated ion pairs with HIB ion, including mixed ligand ion pairs, *e.g.*, LuHIB, Lu(HIB)₂, Lu(HIB)₃ and LuAc(HIB)₂. The abundance of such ion pairs is considerably higher than that with the WNH₄Ac system as simulated previously [8].

Table II summarizes the evacuated recovery for three runs. Apparently, the recovery of almost all the metal cations treated could be regarded as 100%, taking into account the possible error of a few percent in PIXE analysis. However, the recovery of ZrO was affected by both the electric charge (or migration time) and the electrolyte system used. This observation suggested that the abundance of the ion pairs with low mobility increased gradually and the complex-forming equilibria were not rapid enough to allow migration as a homogeneous zone. Although we have no direct information of the chemical form of the lost Zr compound, the most plausible explanation is that the formation of immobile hydroxyl complexes reduced the recovery. From Table II, it is apparent that the oxidation of Fe^{II} to Fe^{III} took place even

during migration. It should be noted that the recovery of Fe as a whole was 100% with the WNH_4Ac system. On the other hand, the recovery of Fe was reduced when the $\text{WNH}_4\text{Ac-HIB}$ system was used, because the Fe^{III} zone migrating at the front end of the terminating zone in the WNH_4Ac system no longer migrated, suggesting that the hydroxyl complex of Fe^{III} interacted more strongly with HIB ion than acetate ion.

In conclusion, PIXE is a useful method for revealing the separation behaviour of metal cations in isotachopheresis. The migration order and the recovery of metal ions are very important, especially from the viewpoint of the preparative strategy, as confirmed by the use of a PIXE detector. More detailed studies by means of PIXE of the separation of metal cations which easily form hydroxyl complexes, e.g., Fe^{III} and Ga^{III} , are in progress.

It should be noted that the difference in mobilities at the steady state is a good measure of separation but it is not always necessary for separation. The separation is successful when the mobility of the sample differs sufficiently at the transient mixed zones. However, a problem is that this situation is difficult to detect when universal detectors or a UV detector are used.

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