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SEPARATION OF ANIONIC CHLORO COMPLEXES BY CAPILLARY TUBE ISOTACHOPHORESIS IN NON-AQUEOUS SYSTEMS

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SUMMARY

A method for the separation of anionic chloro complexes by capillary tube isotachophoresis using dimethylformamide is described. ClO_4^-, Cl^- and H^+ are used as the leading ion, terminating ion and counter ion, respectively. The order of effective mobilities of the chloro complexes is Fe(III) > Hg(II) > Cd(II) > Zn(II) > Co(II) > Pb(II) > Mn(II), which follows the order of the stability constants. The migration mechanism of the chloro complexes is explained on the basis of the complex-forming equilibria involved. In this system, the terminating zone acts as a supplier of chloride and hydrogen ion from the leading zone induces the dissociation of the chloro complexes.

INTRODUCTION

Although a number of studies have been carried out on the separation of ionic species by use of capillary tube isotachophoresis (CITP), little work has been done in non-aqueous solvents. However, an appropriate choice of a non-aqueous solvent for CITP often makes it possible to give a better separation of particular species, because the solubilities and effective mobilities of sample species may be affected by the dielectric constant and solvation properties of the solvent chosen. Some investigations have been made on this aspect, for example, the separation of inorganic cations and anions[1,2] and the separation of conjugated bile acids[3], which are slightly soluble in water, which were carried out in methanol.

In order to improve the separability of sample species, not only the "solvent effects" but also the complex-forming equilibria or ion-pairing equilibria must be considered in both non-aqueous and aqueous solvents[4–7]. In choosing a suitable solvent for the separation of metal ions, special consideration should be given to whether the metal species can exist in it as charged species or not. In general, solvents are classified into protic solvents, with strong hydrogen-donor properties, such as water, and dipole aprotic solvents, with dielectric constants higher than 15 but containing no labile hydrogen, such as dimethylformamide and acetonitrile, on the basis of the Lowry–Brønsted theory[8]. As hydrogen-bonding ligands such as the chloride ion are not solvated in dipole aprotic solvents, metal complexes $[MCl_n]^{(n-2)-}$ are consequent-

ly much more stable in these than in protic solvents such as water. This suggests that the appropriate choice of the solvent as a CITP electrolyte will make it possible to effect the migration of charged chloro complexes that are unstable in water.

Lederer and Ward[9] reported the separation of chloro complexes, *e.g.*, those of Hg(II), Cd(II), Bi(III) and Cu(II), by means of paper electrophoresis with filter-paper moistened with 0.5 *M* hydrochloric acid. In isotachophoretic separations in aqueous media, the use of such high concentration of hydrochloric acid as the leading and terminating electrolytes is not permissible. However, if a dipole aprotic solvent is used as the CITP electrolyte, it may make it possible to effect the migration of the anionic chloro complexes even if the concentration of hydrochloric acid is low, because the stability constants of chloro complexes increase much more in dipole aprotic than in protic solvents such as water.

Previously, we reported the isotachophoretic separation of 1,10-phenanthroline (phen) metal chelates using acetonitrile as the solvent[10]. The effective mobilities of the metal chelates were influenced by counter anionic species of the leading electrolyte owing to ligand replacement equelibria. Under the experimental conditions used, the order of interaction for cationic phen metal chelates was $Cl^- > Br^- > I^- > ClO_4^-$. The effective mobilities of Ni(II)–, Fe(II)–, Co(II)–, Zn(II)–, Cd(II)– and Mn(II)–phen chelates were similar when perchlorate was used as the counter ion. The separation of Ni(II)–, Fe(II)–, Co(II)– and Cu(II)–phen chelates was achieved by the use of chloride as the counter ion, and under the same conditions Zn(II)–, Cd(II)– and Mn(II)–phen chelates were not detected. This behaviour of the complexes on CITP also suggests that many metal ions readily form chloro complexes in dipole aprotic solvents and the chloro complexes obtained may migrate isotachophoretically as anionic species.

In this paper we describe the simultaneous separation of anionic chloro complexes such as Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II) by CITP using dimethylformamide (DMF) as the solvent in CITP and the elucidation of the mechanism of migration of the complexes under the recommended operating systems. The most striking characteristic of this method is that the terminating ion simultaneously acts as a complexing agent for metal ions.

EXPERIMENTAL

Apparatus

A Model IP-1B capillary tube isotachophoretic analyser equipped with a potential gradient detector (Shimadzu, Kyoto, Japan) was used. The PTFE isotachophoretic tube for the separation consisted of a main capillary column (150 mm × 0.5 mm I.D.) and a pre-column (40 mm × 1.0 mm I.D.). The current was stabilized at 10–200 μA for 1.0–20 m*M* leading electrolyte. Carrier gas (nitrogen) was passed through the silica gel column to remove traces of water. The cap of the reservoir tank, made of poly(vinyl chloride) resin, was replaced with one made of polyethylene resin, because the former is dissolved by DMF.

Reagents

Perchloric acid, hydrochloric acid, metal salts, DMF and tetraethylammonium (TEA) perchlorate (Wako, Osaka, Japan) were of analytical-reagent grade and were used without further purification.

Stock standard solutions of metal chloro complexes such as Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II) were prepared by dissolving their chlorides in DMF, adding concentrated hydrochloric acid and diluting with DMF to 0.01 M with respect to the metal ion and 0.02 M with respect to hydrochloric acid. Working standard solutions were obtained by diluting the stock standard solutions to 0.002 M with DMF and mixed standard solutions were also prepared from the same stock standard solutions by diluting with DMF to 0.001 M with respect to each metal ion.

Leading and terminating electrolytes were prepared by dissolving suitable volumes of concentrated perchloric acid (*ca.* 60%) and hydrochloric acid (35%) with an Eppendorf Varipett 4710 (volume range 10–100 μl) and diluting with DMF.

RESULTS AND DISCUSSION

Operating systems

The operating system is specified in Table I. Because perchloric acid is a strong acid in DMF, perchlorate was used as the leading ion. On the other hand, chloride was used as the terminating ion in the presence of H^+ because hydrochloric acid is a weak acid in DMF (stability constant, log K = 3.75[11]). In this system chloride as the terminating ion also acts as a complexing agent.

Isotachopherogram

Fig. 1 shows the isotachopherograms obtained at leading ion concentrations of 0.0025 M (Fig. 1a) and 0.02 M (Fig. 1b) and at amounts of Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II) in the chloro complexes of 1.5 nmol (Fig. 1a) and 12 nmol (Fig. 1b). The zone length of the Mn(II) chloro complex increased with decreasing concentration of leading electrolyte compared with that of other chloro complexes. When the metal complexes were present in equimolar concentrations in this separation, *ca.* 20% of the Fe(III) chloro complex was mixed in the Hg(II) zone. However, a satisfactory separation of the above chloro complexes was achieved when Fe(III) or Hg(II) was eliminated.

Effect of concentration of leading electrolyte

The concentration of both the terminating and sample zones is adjusted with the concentration of the leading electrolyte used. In this system, the stability of the chloro complexes may be affected by the concentration of chloride ion that is supplied from the terminating zone and acts as a complexing agent. Therefore, the effect of the concentration of the leading electrolyte on the effective mobilities of the chloro

TABLE I

CITP OPERATING SYSTEM

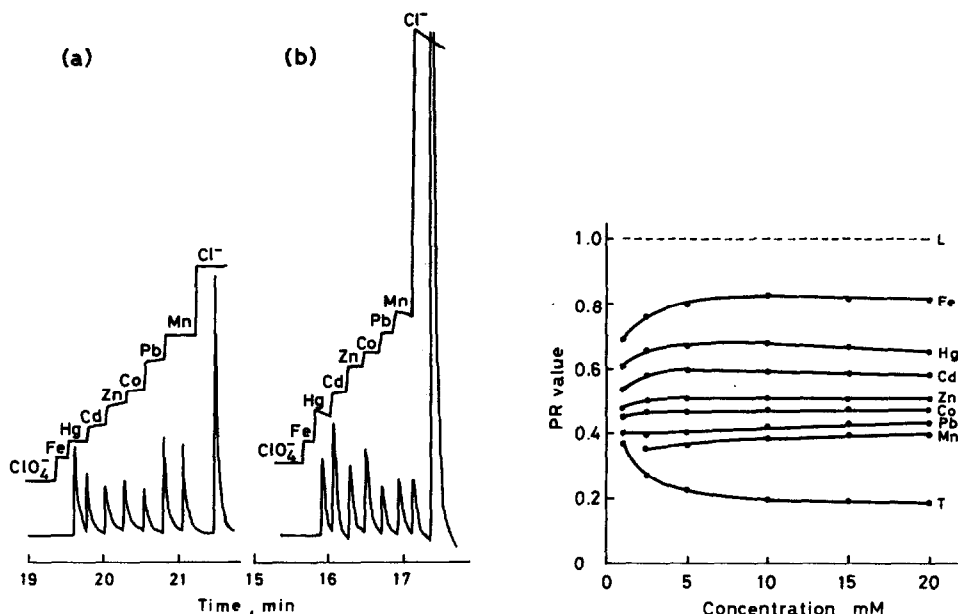| Parameter	Leading electrolyte	Terminating electrolyte
Solvent	N,N-Dimethylformamide	N,N-Dimethylformamide
Anion	ClO_4^-	Cl^-
Counter ion	H^+	H^+
Concentration	1.0–20 mM	
Current	10–200 μA	

Fig. 1. Isotachopherograms of the anionic chloro complexes of Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II). Concentration of leading ion: (a) 0.0025; (b) 0.02 M. Amount of each chloro complex: (a) 1.5; (b) 12 nmole. Driving current: (a) 25; (b) 200 μA. Chart speed: 20 mm/min.

Fig. 2. Effect of concentration of leading ion on the PR values of the chloro complexes. The driving current was stabilized at 10–200 μA for 1.0–20 mM leading ion.

complexes was investigated, as shown in Fig. 2. The PR value in CITP represents the ratio of the potential gradient of the leading zone to that of the sample zone, and is equal to the ratio of the effective mobility of the sample ion to that of the leading ion:

$$\text{PR value} = h_{\text{L}}/h_{\text{S}} = E_{\text{L}}/E_{\text{S}} = \bar{m}_{\text{S}}/\bar{m}_{\text{L}}$$

where h, E and $\bar{m}$ denote step height of isotachopherogram, potential gradient and effective mobility, respectively and the subscripts L and S denote the leading and the sample zones or ions, respectively. The PR value of chloride as the terminating ion increased with decreasing concentration of the leading electrolyte, particularly below 0.005 M. The PR values of metal chloro complexes were almost constant at concentrations of the leading electrolyte in the range 0.005–0.02 M. The PR value of the Mn(II) chloro complex at a leading electrolyte concentration of 0.001 M could not be measured, because the effective mobility of the chloride ion was larger than that of the Mn(II) chloro complex. It seems that a variation of the concentration of leading electrolyte does not lead to a change in the effective mobilities of chloro complexes or a change in the species themselves.

Effect of the concentration of H^+ in the leading electrolyte

The relationship between the concentration of H^+ ion added as a counter ion and the PR values of the chloro complexes was investigated, keeping the sum of the molar concentrations of the two counter cations, H^+ and TEA^+, constant as their
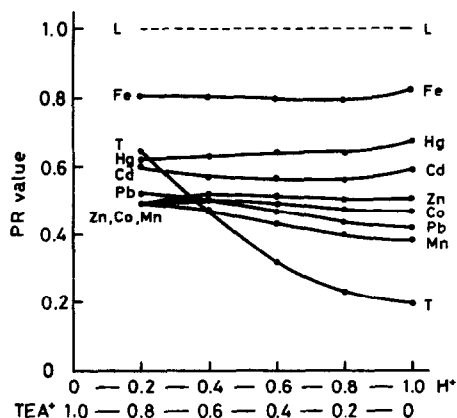
Fig. 3. Effect of the concentration of H^+ in the leading electrolyte on the PR values of the chloro complexes. The concentration of leading ion (ClO_4^-) was constant at 0.01 M. $[H^+] + [TEA^+] = 0.01$ M. Driving current: 100 μA.

ratio was varied. As shown in Fig. 3, the PR values of the Co(II) and Pb(II) complexes at 0.004 M H^+ were almost identical. The PR values of chloro complexes such as Fe(III), Hg(II), Cd(II) were relatively high and almost constant, regardless of the concentration of H^+, but those of Zn(II), Co(II), Pb(II) and Mn(II) were relatively low, and decreased with increasing concentration of H^+ in the leading electrolyte. Also, the differences in the effective mobilities among the latter chloro complexes increased with increasing concentration of H^+, which indicates that H^+ plays an important role in the separation of chloro complexes that have relatively low effective mobilities in this system.

Effect of chloride ion in the leading electrolyte

Fig. 4 shows the isotachopherograms obtained by injecting a mixed sample of seven chloro complexes, Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II) (6 nmol of each metal ion), and varing the addition of hydrochloric acid in the leading electrolyte (0.01 M $HClO_4$) to 0.002 M (Fig. 4a), 0.004 M (Fig. 4b), 0.006 M (Fig. 4c) and 0.01 M (Fig. 4d). Evidently the differences in the effective mobilities of the chloro complexes decreased with increasing concentration of hydrochloric acid, and two mixed zones, one consisting , of Hg(II) and Cd(II) and the other of Zn(II), Co(II) and Pb(II) chloro complexes, were formed. In spite of the increase in H^+ concentration, which increases the differences in the effective mobilities of the chloro complexes as shown in Fig. 3, the mixed zones were formed. These results suggest that the chloride ion acts strongly in the opposite sense to H^+ to decrease the differences in effective mobilities, because the chloride ion added to the leading electrolyte depresses the dissociation of the chloro complexes. In other words, the effective mobilities of chloro complexes are mainly decided by the degree of dissociation of the complexes in individual zones and are related to their stability constants.

In addition, in order to confirm whether a free metal ion injected into this system migrates as a chloro complex or not, DMF solutions that contained only an Fe(III) or Hg(II) perchlorate salt were injected as sample solutions. The resulting isotachopherograms gave a step of either Fe(III) or Hg(II) chloro complex with a PR
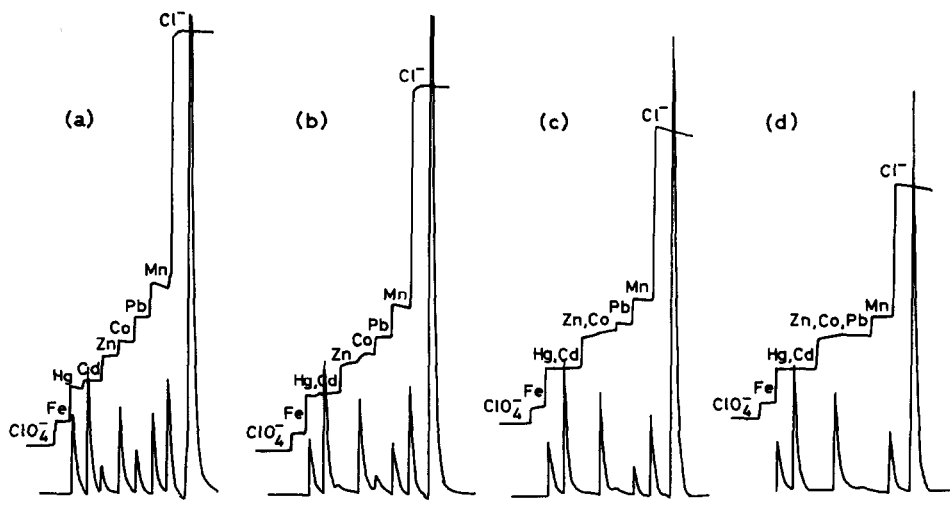
Fig. 4. Isotachopherograms of the anionic chloro complexes of Fe(III), Hg(II), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II). The leading electrolyte was prepared by adding HCl [(a) 0.002; (b) 0.004; (c) 0.006; (d) 0.01 M] to a 0.01 M solution of perchloric acid in DMF solution. Driving current: 100 μA. Chart speed: 20 mm/min.

value identical with that obtained by injecting their chloro complexes initially. This result indicates that the free metal ions that were initially injected in this system migrate in the direction of the terminating zone and then form chloro complexes with terminating Cl^-.

The order of stability constants of the chloro complexes in DMF are Hg(II)
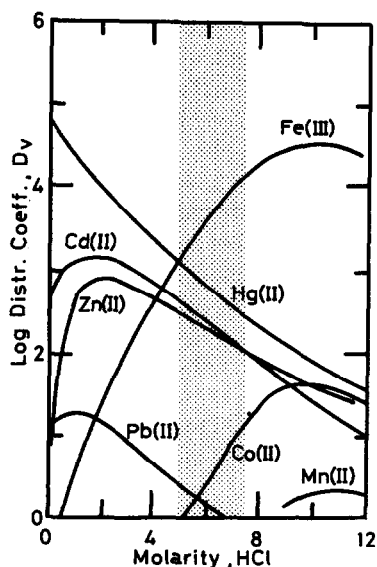

Fig. 5. Distribution coefficients of metal ions on anion-exchange resin. This figure was taken from ref. 14 and rearranged in a single graph.

TABLE II

REGRESSION LINES OF THE CALIBRATION GRAPHS FOR SEVEN COMPLEXES

Chart speed, 40 mm/min; driving current, 25 μA; amount of sample injected, 1-4 nmol; Y, step length (mm); X, concentration of chloro complex (nmole).

Metal ion	Regression line	Correlation coefficient
Fe(III)	$Y = 5.90X$	0.9990
Hg(II)*	$Y = 5.56X$	0.9999
Cd(II)	$Y = 6.97X$	0.9999
Zn(II)	$Y = 6.60X$	0.9995
Co(II)	$Y = 7.28X$	0.9993
Pb(II)	$Y = 7.45X$	0.9999
Mn(II)	$Y = 12.1 \ X$	0.9998

* Single sample was used.

> Cd(II) > Pb(II)[12], and that in acetonitrile (AN), which is also a dipole aprotic solvent, is Hg(II) > Cd(II) > Zn(II)[13]. These correspond to the order of effective mobilities obtained in this investigation, viz., Fe(III) > Hg(II) > Cd(II) > Zn(II) > Co(II) > Pb(II) > Mn(II).

The distribution coefficients of seven metal ions in hydrochloric acid medium with a strongly basic anion-exchange resin reported by Kraus and Nelson[14] are shown in Fig. 5. Their order in the vicinity of 6 M hydrochloric acid is in fair agreement with that of the effective mobilities obtained in this work. In addition, the order of the distribution coefficients in 8–10 M hydrochloric acid reported by Sugii et al.[15] using macroreticular-type poly(4-vinylpyridine) resin is also in fair agreement. From these results, it seems that the extent of the formation of anionic chloro complexes in the dipole aprotic solvent DMF with low concentrations of chloride ion may corresponding to that at high concentrations of hydrochloric acid.

Calibration graphs

As the concentration of all zones varies in proportion to the concentration of leading electrolyte, the sensitivity of CITP increases with decreasing concentration of leading electrolyte when the driving current is varied in proportion to the concentration of leading electrolyte. However, there is a limit to the use of lower concentrations of leading electrolyte because the Mn(II) chloro complex could not be detected by increasing the effective mobility of the terminating ion, as shown in Fig. 2. To confirm the separability of this system, the regression lines and the correlation coefficients were obtained by injecting a mixed sample solution composed of equimolar amounts of Fe(III), Cd(II), Zn(II), Co(II), Pb(II) and Mn(II). Linear relationships were obtained over the ranges 1–4, 2–10 and 4–16 nmole of each metal for concentrations of the leading electrolyte of 0.0025, 0.005 and 0.01 M, respectively, but a mixed zone was obtained on injecting more than the upper limits of 4, 10 and 16 nmole, respectively. Some of the results are presented in Table II.

Migration mechanism of anionic chloro complexes

The presumed model for the isotachophoretic separation of anionic chloro
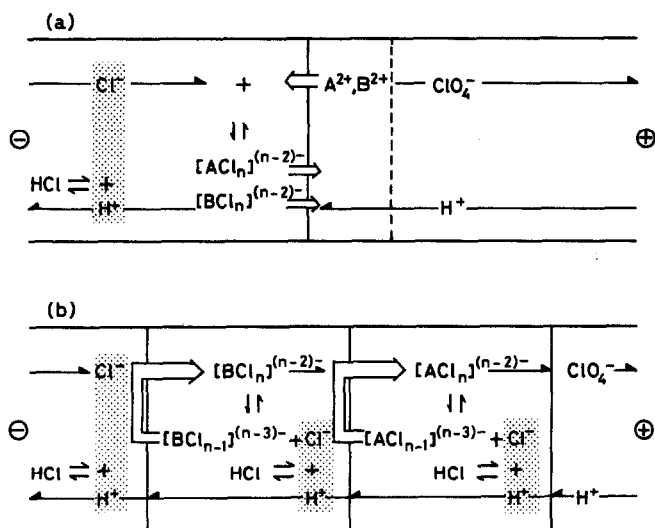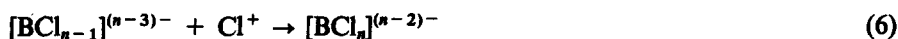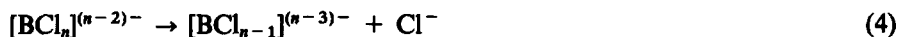
(a)

(b)

Fig. 6. Separation mechanism of the anionic chloro complexes.

complexes is illustrated schematically in Fig. 6. This model is based on the assumption that all chloro complexes dealt within this investigation are kinetically labile, and only one zone is obtained for each metal ion in this isotachophoretic system. Fig. 6a illustrates the state of the system at a certain moment after the solution of divalent metal ions A^{2+} and B^{2+} is injected between the leading and terminating electrolytes. These metal ions, at the beginning, migrate in the direction of the terminating zone and form chloro complexes as follows:

$$A^{2+} + nCl^- \rightarrow [ACl_n]^{(n-2)-} \tag{1}$$

$$B^{2+} + nCl^- \rightarrow [BCl_n]^{(n-2)-} \tag{2}$$

When the effective mobilities of the chloro complexes are higher than that of the terminating ion (Cl^-) and are lower than that of the leading ion (ClO_4^-), well defined sample zones are formed.

Fig. 6b depicts the steady state of zones of chloro complexes already formed, $[ACl_n]^{(n-2)-}$ and $[BCl_n]^{(n-2)-}$. These dissociate to some extent in the individual zones, depending on their stability constants because there is no chloride ion in their direction of migration. When the effective mobilities of the complexes dissociated in each zone become lower, the mixed zones must be formed with a rear complex zone with a low stability constant or with a terminating zone, Cl^-. Then high coordinated complexes are reformed and returned immediately to the former zone:

$$[ACl_n]^{(n-2)-} \rightarrow [ACl_{n-1}]^{(n-3)-} + Cl^- \tag{3}$$

$$[BCl_n]^{(n-2)-} \rightarrow [BCl_{n-1}]^{(n-3)-} + Cl^- \tag{4}$$

$$[ACl_{n-1}]^{(n-3)-} + [BCl_n]^{(n-2)-} \rightarrow [ACl_n]^{(n-2)-} + [BCl_{n-1}]^{(n-3)-} \tag{5}$$

$$[BCl_{n-1}]^{(n-3)-} + Cl^+ \rightarrow [BCl_n]^{(n-2)-} \tag{6}$$

The H^+ as a counter ion of the leading electrolyte also plays an important role in this system. The H^+ ions react with Cl^- derived from dissociation of chloro complexes to form HCl, and then the dissociation of chloro complex can proceeds.

As mentioned above, the mechanism of the separation of chloro complexes can be explained successfully on the basis of the dissociation of the complexes, the supplementation of chloride from the side of the rear zone and the effect of H^+.

The complex-forming equilibria usually adopted in CITP are based on an interaction between the sample species and the counter ion of the leading electrolyte, resulting in a decrease in the mobility of the sample species. This can be referred to as a negative effect for the effective mobility. On the other hand, the complex-forming equilibria between the sample species and the terminating ion, described in this paper, can be referred to as a positive effect, that is, the conversion of sample species into more negative species by complexing with an anionic terminating ion, Cl^-.

This method may be effective not only with ionic species but also with neutral sample species if the following relationships are satisfied:

(i) in the first stage, the effective mobility of the sample species is lower than that of the terminating ion;

(ii) complex-forming equilibrium occurs with the sample species regardless of the charge (cation, anion or neutral) to form a charged labile complex bearing same charge as that of terminating ion;

(iii) the resulting charged complexes have higher effective mobilities than that of the terminating ion and lower than that of the leading ion.

CONCLUSION

The utility of non-aqueous solvents, particularly dipole aprotic solvents in CITP for the separation of metal chloro complexes has been demonstrated. The most striking characteristic of this method is the use of the terminating ion as a complexing agent. In this system, the order of effective mobilities of chloro complexes seems to be defined by the order of the stability constants. Aqueous solutions are unsuitable for use as sample solutions in this system. However, as mentioned previously[10], the use of non-aqueous solvents for the CITP electrolyte may permit the combined use of solvent extraction, e.g., the extraction of metal chloro complexes from hydrochloric acid into diethyl ether.

The application of this technique to the determination of trace metal ions in aqueous solution is now under investigation.

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