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The Capillary-tube Isotachophoretic Determination of Sodium(I), Magnesium(II), Calcium(II), Strontium(II), and Barium(II) Using Complex-forming Equilibria between CyDTA and the Metal Ions

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The capillary-tube isotachophoresis technique was used to determine sodium(I), magnesium(II), calcium (II), strontium(II), and barium(II). The complex-forming equilibria between CyDTA and the metal ions were used to expand the differences among their effective mobilities. The mobilities of IIa metal ions decreased with increases in the pH of the leading electrolytes and in the CyDTA concentration in the leading electrolytes. The simultaneous determination of the above five cations could be performed when the pH of the leading electrolyte was adjusted at 5.5 or above. The relative standard deviations were 1.7—2.2% for determining 15.0—25.0 nmol of the cations when the pH of the leading electrolyte was 5.70. Magnesium(II) and calcium(II) ions underwent "enforced isotachophoretic" migration.

It is useful to apply the capillary-tube isotachophoresis technique to the analysis of small amounts of samples which contain several constituents. This method requires only a few μl of the sample solutions.

It is necessary for performing a satisfactory separation that there be enough difference among the effective mobilities of the sample ions. If the difference is not effective enough, we have to expand the difference among the mobilities.

To change the mobilities is to alter the environment of the sample constituents in the leading electrolyte. There are three main methods of doing so:[1] (1) the change in the pH of the leading electrolyte, (2) the replacement of the solvent of the leading electrolyte, and (3) the use of complex-forming equilibria.

In Method (3), various factors, *e.g.*, the kind of complexing agent and the concentration and pH of the leading electrolyte, affect the mobilities of the sample ions and may further complicate the condition. Because of the variation in the mobilities of the sample ions, the separability of the sample ions might be enhanced in some cases.

Although this method is analogous to that which is carried out in zone electrophoresis, adding complexing agents to supporting electrolyte, zone electrophoresis has little quantitative ability, whereas capillary-tube isotachophoresis has a good quantitative ability. This method has been presented in several papers,[2–4] there has, however, been little work in which this method has been applied to the determination of metal ions.

In this paper, we studied the utility of the complex-forming equilibria in determining the metal ions by capillary-tube isotachophoresis. Magnesium(II), calcium(II), strontium(II), and barium(II) were used as sample ions, and CyDTA was added to the leading electrolyte as a complexing agent. The above four cations and sodium(I) were separated and determined simultaneously, and the importance in this method of the choice of the terminating electrolyte was pointed out.

Experimental

Apparatus. Isotachopherograms were recorded with a Capillary-tube Isotachophoretic Analyzer (Shimadzu, model IP-1B, with a potential gradient detector, model PGD-1). The migration current was stabilized at 100 μA. The length of the capillary tube (PTFE, I.D. 0.5 mm) was 20 cm, and the oven temperature was 20 °C. Measurements of pH were made with an expanded-scale pH-meter (Horiba, model F-7ss). 1.0—7.0 μl portions of sample solutions were introduced into the instrument with a micro syringe.

Materials. The 1,2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid (CyDTA), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris), and Triton X-100 were purchased from the Wako Pure Chemical Industry Co., Ltd. The other chemicals used were of a reagent grade and were dissolved in deionized water.

Sodium(I), Magnesium(II), Calcium(II), Strontium(II), and Barium(II) Solutions: The stock solutions were prepared by dissolving their nitrates in water. The standard solutions containing some or all of the above metal ions were obtained by mixing the stock solutions and then diluting them to the desired concentrations.

NH_4OH Solution: The NH_4OH solution was diluted to give a 1-mol dm^{-3} solution with water.

CyDTA Solution: The CyDTA was dissolved to give a 0.1-mol dm^{-3} solution in 0.4 mol dm^{-3} of an NH_4OH solution.

The leading electrolytes were obtained by mixing the NH_4OH solution and the CyDTA solution, followed by their dilution to the desired concentrations. For adjusting the pH of the leading and terminating electrolytes, acetic acid or succinic acid was used. The compositions of the leading and terminating electrolytes are shown in Table 1.

Results and Discussion

Effect of CyDTA Concentration in the Leading Electrolyte on the PR Value.[†] Figure 1 shows the relations between the PR values of the metal ions [magnesium(II), calcium(II), strontium(II), and barium(II)] and the CyDTA concentration in the leading electrolyte. When CyDTA is not added to the leading electrolyte, the order of the PR values *i.e.*, the order of mobilities of the metal ions, was: barium(II) > stron-

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[†] The PR value[4] (potential gradient ratio value) represents the ratio of the potential gradient of the leading ion to that of the sample ion (PG_L/PG_S). Therefore, this corresponds to the ratio of the mobility of the sample ion to that of the leading ion (m_S/m_L).

TABLE 1. ELECTROLYTE SYSTEMS

	Leading electrolyte[a)]		Terminating electrolyte
(I)	0.01 mol dm^{-3} NH_4OH, 0—2 mmol dm^{-3} CyDTA, acetic acid, pH 4.30	(IV)	0.01 mol dm^{-3} Tris, acetic acid, pH 4.40
(II)	0.01 mol dm^{-3} NH_4OH, 1 mmol dm^{-3} CyDTA, acetic acid, pH 4—6	(V)	0·01 mol dm^{-3} β-alanine, acetic acid, pH 4.40
(III)	0.01 mol dm^{-3} NH_4OH, 1 mmol dm^{-3} CyDTA, succinic acid, pH 5.70		

a) 0.1% Triton X-100 was added.

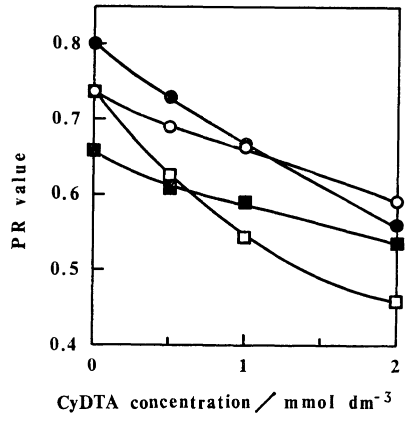

Fig. 1. Relation between the PR value and the concentration of CyDTA in the leading electrolyte. Electrolyte system: (I) and (IV); ■: Mg^{2+}, □: Ca^{2+}, ○: Sr^{2+}, ●: Ba^{2+}.

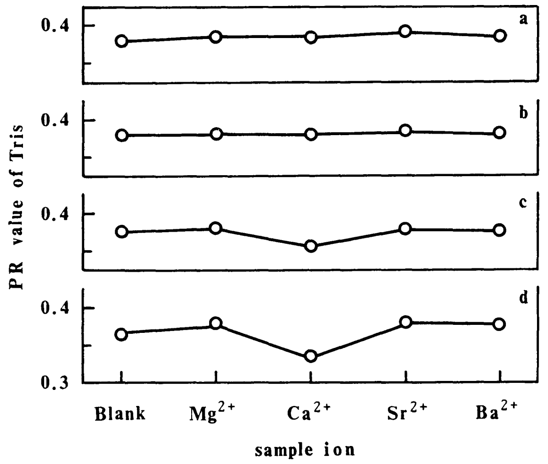

Fig. 2. Effect of pH of the leading electrolyte and sample ions on the PR value of Tris.
Electrolyte system: (II) [a) pH 3.98, b) pH 4.30, c) pH 4.50, d) pH 4.70] and (IV).

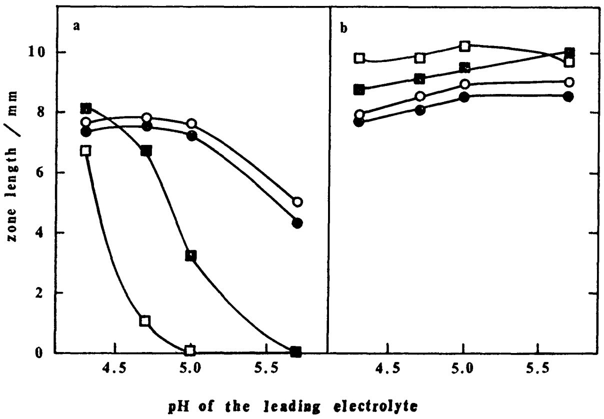

Fig. 3. Effect of terminating ions and pH of the leading electrolyte on the zone length.
Electrolyte system: a) (II) and (IV), b) (II) and (V); migration current: 100 μA; chart speed: 5.0 mm min^{-1}; sample taken: 30 nmol; other symbols are the same as in Fig. 1.

tium(II)≈calcium(II)>magnesium(II). It is not possible to separate calcium(II) from strontium(II). When CyDTA was added to the leading electrolyte, the PR value of the metal ions decreased with an increase in the CyDTA concentration. This indicates that the interaction between CyDTA and metal ions increases with an increase in the CyDTA concentration and that it makes possible the mutual separation between the alkaline earths.

The slopes of the curves show that the effect of an increasing CyDTA concentration on the PR value of calcium(II) and barium(II) is greater than on that of magnesium(II) and strontium(II). The variations in the mobilities of the metal ions, which result from the interaction between CyDTA and metal ions, depend on the overall stability constants of the CyDTA complexes.

Comparison of Tris and β-Alanine as Terminating Ions. As is shown in Fig. 2, the PR value of the terminating ion (Tris) became abnormally small with an increase in the pH of the leading electrolyte in the case of calcium(II), which had a rather larger complexing ability than the other IIa elements. Further, the zone length of the metal ions decreased with an increase in the pH of the leading electrolyte, and in the pH region over 5.0, first the calcium(II) ion, and next the magnesium(II) ion were unable to be detected (Fig. 3a), in spite of the fact that the PR values of the metal ions were larger than that of Tris, as is shown in Fig. 4.

On the other hand, when β-alanine was used as the terminating ion, the zone length of the metal ions varied little, even higher pH's of the leading electrolyte (Fig. 3b).

Protons released from CyDTA by a complexing reaction with sample ions cause the pH of the sample-ion zones to decrease, and the pH of Tris zone is kept near that of the leading electrolyte. Therefore, the pH of the Tris zone seems to be higher than that of the sample-ion zones. In this case, if sample ions are transfered into the Tris zone by diffusion or any

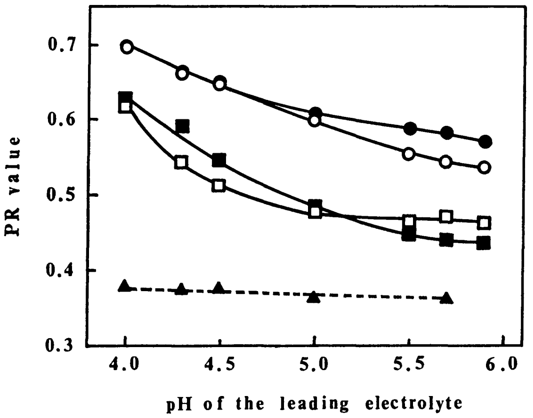

Fig. 4. Relation between the PR value and pH of the leading electrolyte.
Electrolyte system: (II) and (IV) (pH of the leading electrolyte below 4.5) and (V)(above 5.0); ▲: Tris (these values were obtained using electrolyte system (IV) even if pH of the leading electrolyte was above 5.0); other symbols are the same as in Fig. 1.

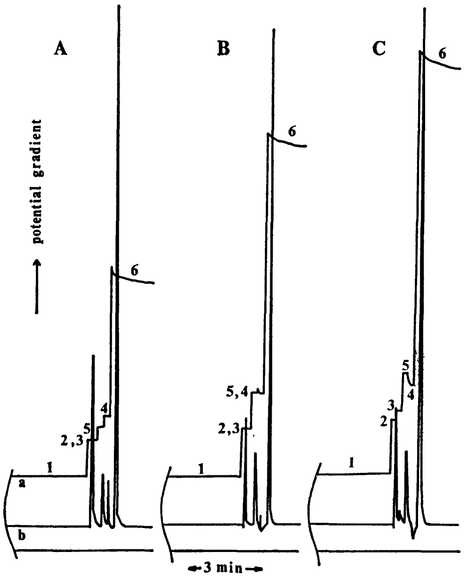

Fig. 5. Isotachopherograms.
Electrolyte system: (II) [(A) pH 4.30, (B) pH 5.00, (C) pH 5.70] and (V); a) potential gradient, b) differential potential gradient; 1) NH_4^+, 2) Ba^{2+}, 3) Sr^{2+}, 4) Ca^{2+}, 5) Mg^{2+}, 6) β-alanine.

other factors, they can not return to their own zones because their effective mobilities are smaller in higher pH zones than in lower pH zones. As a result, the zone lengths of these sample ions decrease and the PR value of Tris becomes smaller to form a mixed zone, as is shown in Figs. 2 and 3a. On the other hand, as β-alanine has a small pK_a value ($pK_{a1}=3.6$), the pH's of this zone decrease, and even if sample ions are transfered into this zone, they can return to their own zones (see Ref. 1).

Consequently, in the IIa metal-ion analysis using complex-forming equilibria, β-alanine should be used as a terminating ion, while Tris can not be applied; nevertheless, the later has a lower effective mobility than IIa metal ions under the complex-forming equilibria.

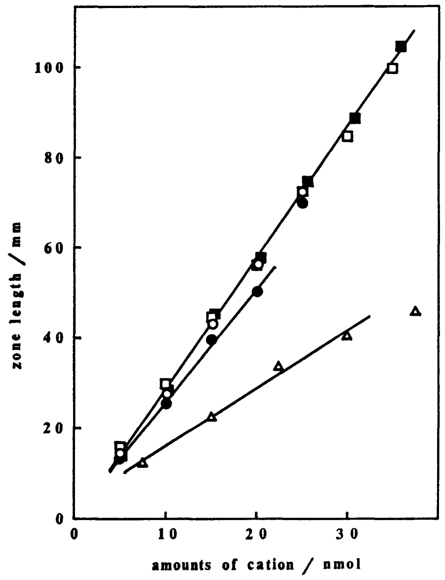

Fig. 6. Calibration curves.
Electrolyte system: (III) and (V); migration current: 100 μA; chart speed: 40 mm min^{-1}; △: Na^+; other symbols are the same as in Fig. 1; the curves of Ca^{2+} and Sr^{2+} were much the same as that of Mg^{2+}.

TABLE 2. SIMULTANEOUS DETERMINATION OF Na(I), Mg(II), Ca(II), Sr(II), AND Ba(II) IONS

Ion	Taken nmol	Found nmol	Difference %	R.S.D. %
Sodium(I)	22.5	22.4	−0.44	2.2
Magnesium(II)	20.4	20.1	−1.5	1.9
Calcium(II)	25.0	25.2	0.80	1.9
Strontium(II)	20.2	19.8	−2.0	1.9
Barium(II)	15.0	15.1	0.67	1.7

The conditions are the same as in Fig. 6.

The PR values of the sample ions are almost the same whether Tris or β-alanine is used as the terminating ion.

Relation between the PR Values and the pH of the Leading Electrolyte. The PR values of the sample ions decreased with an increase in the pH of the leading electrolyte (Fig. 4). The strontium(II) ion and the barium(II) ion were separable at a higher pH of the leading electrolyte; they were, however, not separable at a lower pH of the leading electrolyte. The magnesium(II) ion and the calcium(II) ion were also separable at a higher pH of the leading electrolyte. Four cations of IIa elements could be separated simultaneously when the pH of the leading electrolyte was adjusted to 5.5 or above. The separability of this method was larger in the higher-pH range of the leading electrolyte than in the lower range examined.

Figure 5 shows isotachopherograms of the sample ions at various pH's of the leading electrolytes, indicating that, at pH 5.00 and 5.70, the magnesium(II) and calcium(II) ions undergo "enforced isotachophoretic"[5) migrations. This phenomenon results from the fact that the magnesium(II)-ion zone has a higher pH than the calcium(II)-ion zone, as is to be expected from the fact that the stability constant of the magnesium(II) ion is smaller than that of the calcium(II) ion.

Simultaneous Determination of Sodium(I), Magnesium(II), Calcium(II), Strontium(II), and Barium(II) Ions. Calibration curves were made by using a mixed solution of 7.5×10^{-3} mol dm^{-3} sodium(I), 5×10^{-3} mol dm^{-3} magnesium(II), calcium(II), strontium(II), and barium(II) nitrates, while varying the sampling volume from 1.0 to 7.0 μl.

For the adjusting pH of the leading electrolyte, succinic acid was used, because the buffer action of succinic acid is greater than that of acetic acid at pH 5.70.

Although the pH of the terminating electrolyte is rather lower than that of the leading electrolyte, in order to decrease the initial potential, it was adjusted to 4.40. The results are shown in Fig. 6. The linear region of the curve of the sodium(I) ion was 7.5—30.0 nmol (1.0—5.0 μl), while that of the barium(II) ion was 5—20.0 nmol (1.0—5.0 μl). When 6.0 μl of the mixture was introduced into the instrument, the sodium(I) ion exceeded the maximum load (about 30 nmol), thus making the zone length of the barium(II) ion (25 nmol) longer. If the amount of the sodium(I) ion is less than the maximum load, the linear region of the calibration curve of the barium(II) ion may be longer.

Table 2 shows the results of the simultaneous determination of the above five cations. The sample solution used was a artificial mixture of 4.5×10^{-3} mol dm^{-3} sodium(I), 4×10^{-3} mol dm^{-3} magnesium(II), 5×10^{-3} mol dm^{-3} calcium(II), 4×10^{-3} mol dm^{-3} strontium(II), and 3×10^{-3} mol dm^{-3} barium(II) nitrates; a 5.0-μl portion of the sample solution was introduced into the instrument.

The relative standard deviations were 1.7—2.2%, and the differences were −2.0—0.80% (mean values of five determinations). The time needed for an analysis was about 16—20 min.

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