Separation of alkali and alkaline-earth metal and ammonium cations by capillary electrophoresis using poly(ethylene glycol) and tartaric acid

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Abstract

The separation behavior of alkali and alkaline-earth metal and ammonium cations was investigated by capillary electrophoresis (CE) using poly(ethylene glycol) (PEG) and tartaric acid (H_2Tar) as electrolyte systems. Creatinine was used as a visualized co-ion for indirect UV detection (254 nm). Separation of all the analyte cations increased with the volume fraction of PEG in water and the decrease in mobility for alkaline-earth metal cations was larger than that for alkali metal cations. The separation of alkaline-earth metal cations was also enhanced by complex formation with tartarate ions, resulting in decreased mobility. Addition of H_2Tar in water–PEG (50:50, v/v) had a larger effect than addition to water alone, suggesting larger stability constants with alkaline-earth metal cations. The separation pattern of alkaline-earth metal cations by H_2Tar in water was in good agreement with the results calculated. Thus, the group separation of monovalent and divalent cations, respectively, and also the complete separation of all cations were shown for the electrolyte systems of PEG and H_2Tar.

Keywords: Electrolyte systems; Alkali metals; Alkaline-earth metals; Tartaric acid; Poly(ethylene glycol)

1. Introduction

Capillary electrophoresis (CE) is a technique to separate ionic species in a small-diameter (<100 μm) capillary column containing electrolyte solution, applying electric field strength of 10–30 kV at both ends of the column. Because of its higher resolution, shorter analysis time, lower buffer consumption, and greater simplicity in operation compared to ion chromatography (IC) [1,2], CE has recently received a great deal of attention for the determination of low-molecular-mass ions. However, as the separation of ions by CE is based on the difference in electrophoretic mobilities of the cations, ions with identical charge, size, and structure in solutions are difficult to separate. Thus, the electrolyte system must be selected to modify the mobilities of the analyte ions.

The separation of alkali and alkaline-earth metal and ammonium cations by CE is such a case, because some of them have very similar ionic mobilities [3], as shown below. Three possible

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factors have been reported to modify the mobilities of alkali and alkaline-earth metal and ammonium cations: (a) complex formation of the cations with electro-neutral cyclic polyethers [4–11], (b) solvation (or complexation) by organic solvents [10–13] and (c) ion-pair formation with negatively charged ligands [8,11–19]. These factors were examined by isotachophoresis (ITP) [4,5,10,14–16,19] and CE [6–9,11–13,17,18]. Cyclic polyethers, for example 18-crown-6, which have electron donor atoms, oxygen atom in the cyclic structure, are suitable for forming complexes with the cations. The complexing ability, viz. the stability constant, depends on the cavity of the crown compounds relative to the diameter of the cations [20,21], thus the mobilities of the cations can be modified due to the change in their structure and size.

As for (b), although the separation is usually achieved in aqueous solutions, the volume fraction of organic solvents such as methanol (MeOH) and poly(ethylene glycol) (PEG) in water changes the selectivity of separation. MeOH solvates cations through ion–solvent dipole interaction and thus the mobilities of the cations decrease with the volume fraction of MeOH [12,13]. As PEG is a linear analogue of cyclic polyether and is flexible in structure, the complex formation with the cations is not specific and is weak compared to that of their corresponding cyclic polyether. A volume fraction of 40–45% (v/v) of PEG in water was effective for the separation of alkali and alkaline-earth metal cations [10].

The third factor, (c), is the use of negatively charged ligands such as ionic forms of tartaric acid (H_2Tar) and α-hydroxyisobutyric acid (HIBA). They form cationic and neutral ion-pairs with alkaline-earth metal cations, resulting in a decrease in mobility of the cations. However, the formation of ion-pair complexes of alkali metal cations is weak in water and, for example, the mobilities of Li^+ and Na^+ are almost constant with respect to the addition of H_2Tar [19]. Although the separation as anionic complexes is also possible for alkaline-earth metal cations [17], it is difficult to convert monovalent cations to anionic species.

Tazaki et al. [4] showed the possibilty of complete separation by ITP using 60 mM 18-crown-6. Šimuni-čová et al. [8] showed the separation of the analyte cations, except for NH_4^+, by ITP using water–PEG (60:40, v/v) and 3 mM maleic acid (pH 5.0), where NH_4^+ was used as a leading cation. Further, they [8] showed the separation by CE using 40 mM 18-crown-6 and 5 mM tartaric acid (pH 5.2) (FEP capillary column, 25 cm×0.3 mm I.D., driving current 75 μA). However, the effects of addition of 18-crown-6, maleic acid, and tartaric acid are not clear, due to lack of the concentration dependence data. For control of the separation of the title cations, it is necessary to address and optimize the effect of each factor.

In this study, the separation behavior of alkali and alkaline-earth metal and ammonium cations by CE was examined in electrolyte systems containing poly(ethylene glycol) (PEG) and/or negatively charged ligand, tartaric acid (H_2Tar). Detection of each cation was accomplished by an indirect UV absorption method using creatinine as a visualized co-ion. As PEG is not volatile, it is easy to handle, and it is cheaper. As the stability constants of alkaline-earth metal cations with H_2Tar in water are known [14,15], the contribution of H_2Tar can be estimated using the constants.

Thus, the separation behavior by H_2Tar in water and water–PEG mixtures will become more clear and the contribution of each factor can be addressed.

2. Experimental

2.1. Chemicals

All solutions of alkali (Li^+, Na^+, K^+, Rb^+, Cs^+) and alkaline-earth metal (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) cations and NH_4^+ were prepared from their chloride salts of reagent grade (Wako Chemicals, Tokyo, Japan or Katayama Chemicals, Osaka Japan). Stock standard solutions (2–5 mM) of each cation were prepared with deionized water, and were mixed and diluted to the specified concentration for the measurement of migration times and electropherograms of all cations.

2.2. Electrolyte systems

The chemicals used for electrolyte systems were poly(ethylene glycol) (PEG, average molecular mass

200), tartaric acid, creatinine, hydrochloric acid (1 M) of reagent grade from Katayama Chemicals. The electrolyte systems without tartaric acid were 30 mM creatinine and 15 mM hydrochloric acid in water–PEG (100:0–40:60, v/v). The electrolyte systems with tartaric acid in water and in water–PEG (50:50, v/v) were 30 mM creatinine, 15−2x mM hydrochloric acid, x (x=0–7.5) mM tartaric acid [pH 4.5 and 4.9–5.0, respectively, for water and water–PEG (50:50, v/v), respectively].

2.3. Apparatus and electrophoretic procedures

CE experiments were carried out using a Jasco Model CE-875 capillary electrophoresis system with a UV–Vis detector (Jasco, Tokyo, Japan). The capillary column used (bare synthetic silica from Otsuka Electronics, Tokyo, Japan) was 75 μm I.D. (375 μm O.D.)×50 cm (30 cm to the detector).

Prior to first use, a new capillary column was cleaned, passing through ca. 0.5 ml of 0.2 M NaOH by aspiration using a vacuum pump and was washed with ca. 1 ml of deionized water. This procedure was repeated twice. Before CE experiments, the column filled with electrolyte system was conditioned, applying the same voltage as for the experimental run. If not in use for a while, the electrolyte solution was kept in the column and the column condition was checked by comparing the new electropherogram with the previous one.

Sample injection was carried out in electrokinetic mode as specified in the figures. The applied voltages of sample runs (also specified in the figures) were in the range +10–30 kV with currents of 4–12 μA at 20±1°C. Indirect UV detection (254 nm) was employed by use of creatinine as a visualized co-ion.

3. Results and discussion

3.1. Electrophoresis with complex-forming equilibria

The ionic migration of cations in an electric field is the sum of the electrophoretic mobilities of the cations and the electroosmotic flow. As electroosmotic flow is constant for all ions under the same CE conditions, the selectivity of cation separation in

Table 1
Absolute mobilities of alkali and alkaline-earth metal and ammonium cations

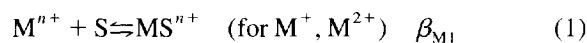| Cations	m_0^+ ($10^5 \cdot cm^2 \cdot V^{-1} \cdot s^{-1}$)[a]
Lithium (Li$^+$)	40.1
Sodium (Na$^+$)	51.9
Ammonium (NH$_4^+$)	76.2
Potassium (K$^+$)	76.2
Rubidium (Rb$^-$)	80.6
Cesium (Cs$^+$)	80.1
Magnesium (Mg^{2+})	55.0
Calcium (Ca^{2+})	61.7
Strontium (Sr^{2+})	61.6
Barium (Ba^{2+})	66.0

[a] Ref. [3].

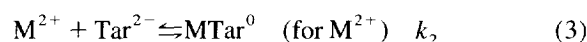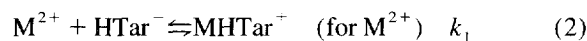CE can be attributed to the difference in electrophoretic mobilities.

Table 1 shows the absolute mobilities of the title cations in aqueous solution without complexing reagent [3]. As the ionic mobilities of some cations (K$^+$ and NH$_4^+$, Rb$^+$ and Cs$^+$, and Ca^{2+} and Sr^{2+}) are very close, complete separation of the cations by CE is difficult. In addition, as the difference in mobility among all the cations is small, it is necessary to consider not only the separation of the two pairs but also total separation.

Polyethylene glycol (S) as organic solvent and tartaric acid (H$_2$Tar) as negatively charged ligand were added in electrolyte systems in this study. The title cations interact with poly(ethylene glycol) through oxygen atoms. The ion-pair complex-forming equilibria take place between alkaline-earth metal cations (M^{2+}) and tartarate ions (HTar$^-$, Tar^{2-}), whereas the equilibria for M$^+$ are weak with low concentrations of H$_2$Tar. Thus, the complex-forming equilibria of the cations can be written as follows:
(1) with poly(ethylene glycol) (S)

$$M^{n+} + S \rightleftharpoons MS^{n+} \quad (\text{for } M^+, M^{2+}) \quad \beta_{M1} \quad (1)$$

(2) with tartaric acid (H$_2$Tar)

$$M^{2+} + HTar^- \rightleftharpoons MHTar^+ \quad (\text{for } M^{2+}) \quad k_1 \quad (2)$$

$$M^{2+} + Tar^{2-} \rightleftharpoons MTar^0 \quad (\text{for } M^{2+}) \quad k_2 \quad (3)$$

where β_{M1} is the constant for complex formation by PEG and k_1 and k_2 are the stability constants for MHTar$^+$ and MTar^0, respectively.

The mobilities of cations complexed with PEG are affected not only by the change in cation size but also by changes in dielectric constant and viscosity of water–PEG mixtures, due to the addition of volume fraction of PEG. However, as the mobilities of the ion pairs formed with tartarate ions are smaller than those of noncomplexed cations, due to the decrease in effective charge, the effective mobilities of alkaline-earth metal cations decrease with increasing concentration of the complexing counterions, tartarate ions ($HTar^-$, Tar^{2-}).

3.2. Effect of poly(ethylene glycol)

Fig. 1 shows the effect of addition of poly-(ethylene glycol) (PEG, average molecular mass, 200) on the ratios of migration time of each cation to that of Li^+ in water–PEG (100:0–40:60, v/v) mixtures. The applied potential was $+10$ kV for 0–20% (v/v) PEG, $+20$ kV for 30–35% PEG, and $+30$ kV for 40–60% PEG. Fig. 2 shows the electropherograms of 0.1 mM solution of ten cations for 35, 50 and 60% (v/v) PEG, respectively.

The addition of PEG enhanced the separation of the analyte cations and decreased the mobility of the cations. For monovalent cations (M^+), PEG has a differentiating effect for the ion pairs NH_4^+/K^4 and Rb^+/Cs^+ and the mobility of NH_4^+ was higher than that of the alkali metal cations in the PEG range examined (Fig. 1). Although PEG has no clear solvent dipole such as MeOH, PEG interacts through oxygen atoms. However, the interaction is very weak and a volume fraction of 40–60% (v/v) PEG is necessary for the mutual separation of M^+. This is in good agreement with ITP measurement using PEG [10].

For alkaline-earth metal cations, the addition of PEG retarded the migration in the order Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}. The retardation effect by PEG was larger than that for the alkali metal cations, suggesting a stronger complexiation by PEG for alkaline-earth metal cations with divalent charge. This order is in good agreement with ITP measurements using PEG [10] and also with CE using 18-crown-6 [9]. Thus, the possibility of group separation of the alkali and alkaline-earth metal cations was suggested with further increase in PEG content ($\geq60\%$, v/v). However, as both the mobility and the electric current were very low at higher PEG contents and also the separability of the Na^+/K^+ pair decreases,
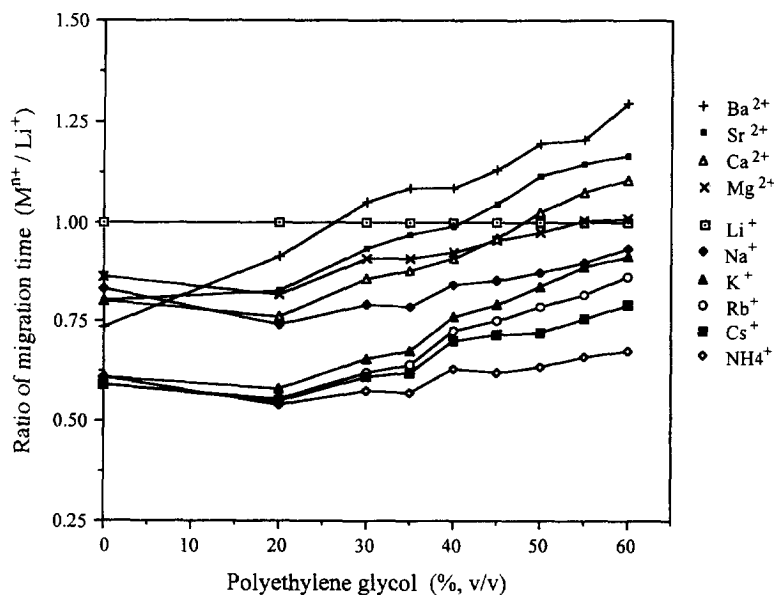

Fig. 1. Effect of poly(ethylene glycol) on the ratios of migration time of M^{n+} to Li^+. Electrolyte system: 30 mM creatinine, 15 mM HCl in water–PEG (100:0–40:60, v/v) mixtures.
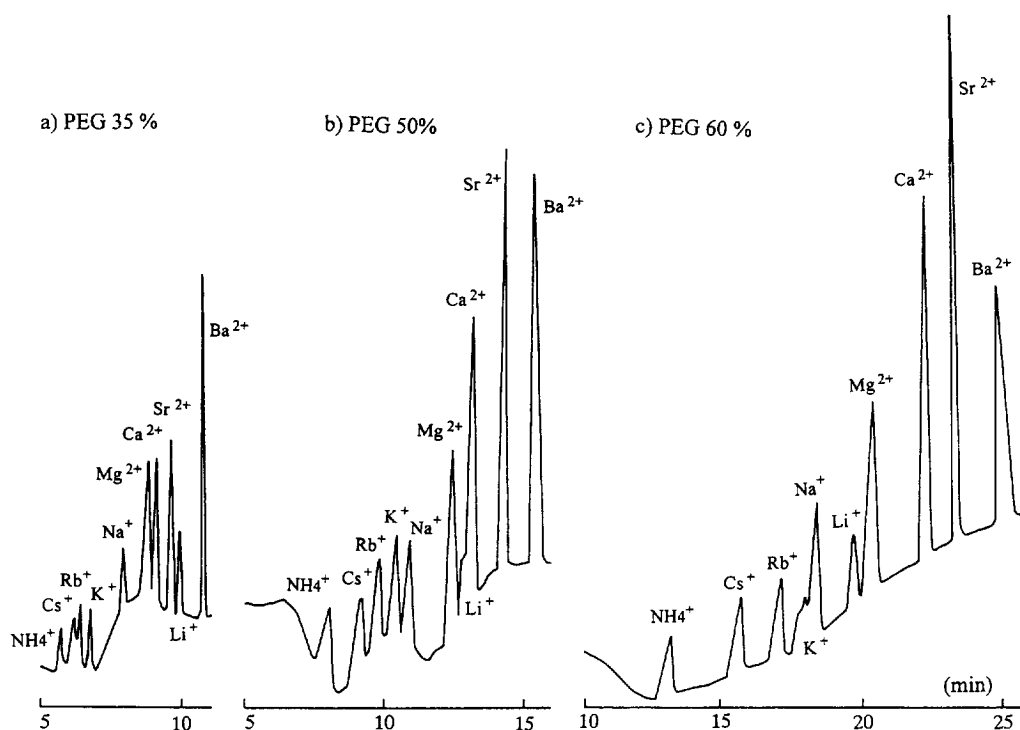
Fig. 2. Effect of poly(ethylene glycol) on the CE separation of a mixture of alkali and alkaline-earth metal and ammonium cations (10^{-4} mol/l, each). Conditions: Electrolyte system, 30 mM creatinine and 15 mM HCl in water–PEG [(a) 65:35, (b) 50:50, (c) 40:60, v/v] mixtures. Applied voltage, (a) +20 kV, (b,c) +30 kV. The capillary column was 75 μm I.D., 50.0 cm overall length, and 30.0 cm from anode to detector. Electromigration injection was (a) 5 s at +20 kV; (b,c) 20 s at +20 kV.

it is necessary to use another reagent for complete separation at lower PEG content.

3.3. Effect of tartaric acid

Fig. 3a shows the effect of addition of tartaric acid (0–7.5 mM) in water on the ratios of migration times of alkaline-earth metal cations to that of Li$^+$. Fig. 4 shows the electropherograms of ten cations, where tartaric acid was added at 0, 2, 3 and 7.5 mM. As is clear from Fig. 3a and Fig. 4, both the migration ratios to Li$^+$ and migration times increase with the addition of tartaric acid; however, the increments are different among the divalent cations. In general, the larger the formation constant, k, the lower the apparent charge on the cations and thus the lower the mobility of the cations are expected to be. Table 2 shows the stability constants of alkaline-earth metal cations with tartaric acid and acidity

constants of tartaric acid. Table 2 and Fig. 3a indicate that the larger k_2 for Ca^{2+} results in a larger decrease in mobility and the smaller k_2 for Mg^{2+} in the smaller decrease. Sr^{2+} and Ba^{2+} with medium k_2 values gave intermediate results. However, the migration times of the alkali-metal cations and NH$_4^+$ without complexing ability were almost constant, in spite of the addition of tartaric acid.

Fig. 5 shows the effect of addition of 0–2.5 mM tartaric acid in water–PEG (50:50, v/v). Fig. 6 shows the electropherograms of ten cations in the mixture containing tartaric acid at 0, 1.0 and 1.5 mM, respectively. The addition of tartaric acid resulted in the increase of the migration time for alkaline-earth metal cations in the same way as in water (Fig. 3a), however, the dependence was larger in water–PEG (50:50, v/v), suggesting higher formation constants. However, the migration times of monovalent cations (M$^+$) were almost constant in spite of addition of
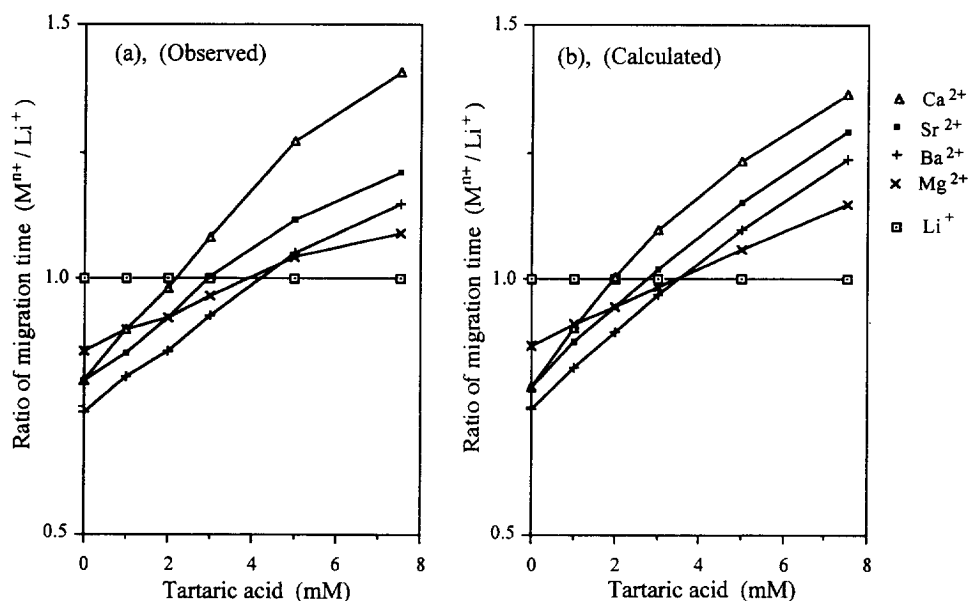
Fig. 3. Effect of tartaric acid on the ratios of migration time of M^{n+} to Li^+. (a) Observed values, (b) calculated values. Electrolyte system, 30 mM creatinine, 15–2x mM HCl and x mM H_2Tar in water (pH 4.5).
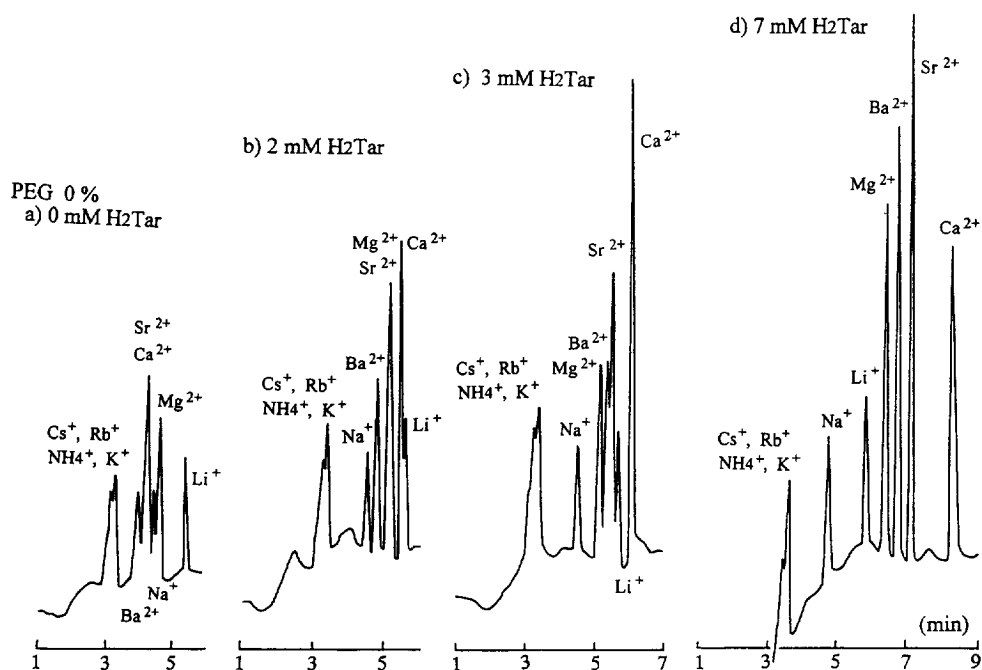

Fig. 4. Effect of tartaric acid on the CE separation of a mixture of alkali and alkaline-earth metal and ammonium cations (10^{-4} mol/l, each). Electrolyte system; 30 mM creatinine, 15–2x mM HCl and x mM H_2Tar in water (pH 4.5). Applied voltage, +10 kV. Electromigration injection, 5 s at +10 kV. Other conditions as in Fig. 2.

Table 2
Acidity constants of tartaric acid (K_1 and K_2) and stability constants (k_1 and k_2) of alkaline-earth metal cations with tartarate ions (HTar$^-$, Tar^{2-})

Ligand	$\log K_1$	$\log K_2$
Tartaric acid	3.036	4.366
Cations	$\log k_1$	$\log k_2$
Calcium (Ca^{2+})	1.59	2.83
Magnesium (Mg^{2+})	–	2.349
Strontium (Sr^{2+})	–	2.690
Barium (Ba^{2+})	–	2.686

Refs. [14,15].

tartaric acid, indicating no interaction with tartaric acid even in water–PEG mixtures with higher PEG content.

3.4. Simulation of effect of tartaric acid in water

The total concentration of each alkaline-earth metal cation in water containing tartaric acid is the sum of the free and complex-forming cations, as
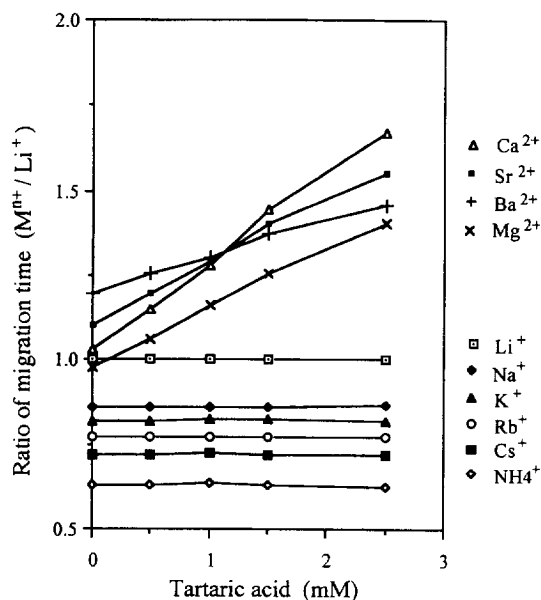

Fig. 5. Effect of tartaric acid on the ratios of migration time of M^{n+} to Li$^+$. Electrolyte system, 30 mM creatinine, 15–2x mM HCl and x mM H$_2$Tar in water–PEG (50:50, v/v) (pH 4.9–5.0).

shown in Eqs. (2,3). Thus, the effective electrophoretic mobility of alkaline-earth metal cations can be expressed as Eq. (4)

$$\bar{m}_M = \frac{m_1 + m_2 k_1 [\text{HTar}^-]}{1 + k_1 [\text{HTar}^-] + k_2 [\text{Tar}^{2-}]} \tag{4}$$

where m_1 and m_2 are the mobilities of M^{2+} and MHTar$^+$, respectively and the mobility, m_3, of MTar^0 is zero. [HTar$^-$] and [Tar^{2-}] are the concentration of tartarate ions.

Fig. 3b shows the calculated values of ratios of migration time to Li$^+$ for the tartaric acid in water system. The migration times of alkaline-earth metal cations can be calculated as the sum of their electrophoretic and electroosmotic flows. The former was calculated by Eq. (4) using the acidity constants of tartaric acid and stability constants of M^{2+} with tartarate ions (Table 2) and the absolute mobilities of M^{2+} (Table 1) and MHTar$^+$. The absolute mobility of CaHTar$^+$ is 25 (10^5 cm^2 V^{-1} s^{-1}) [14]. The effective mobilities, m_1 and m_2 at finite ionic strengths were corrected using Onsager's equation [14,15]. The latter was calculated from a linear function (osmotic flow vs. the migration time), which was obtained for Li$^+$ and Na$^+$. The osmotic flows of Li$^+$ and Na$^+$ were obtained by subtracting the effective mobilities of Li$^+$, Na$^+$, corrected using Onsager's equation [14,15], and the absolute mobilities of Li$^+$ and Na$^+$ (Table 2), from the measured cation mobilities.

As is clear from Fig. 3a and b, the effect of addition of tartaric acid for Ca^{2+} is in good agreement with experimental data. Also, the pattern of the addition effect of tartaric acid was well simulated for other alkaline-earth metal cations, although differences were found between calculated and observed data. Although k_1 and m_2 of MHTar$^+$, except for Ca^{2+}, are not available, the fraction of HTar$^-$ is small at pH 4.5 and k_1 is smaller than k_2, and thus the pattern of separation does not change largely.

In conclusion, the separation of alkali and alkaline-earth metal and ammonium cations was achieved by CE using PEG and tartaric acid. PEG was effective for all the cations examined and tartaric acid was effective for alkali-earth metal cations. The next step is optimization of the CE system for determination.
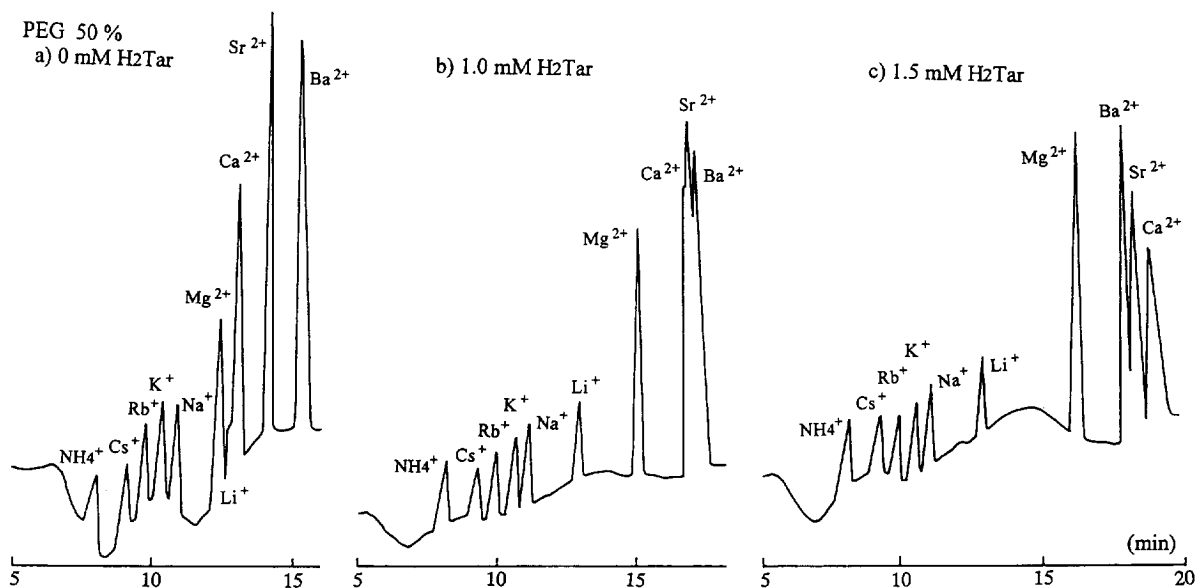
Fig. 6. Effect of tartaric acid on the CE separation of a mixture of alkali and alkaline-earth metal and ammonium cations (10^{-4} mol/l, each). Electrolyte system; 30 mM creatinine, 15–2x mM HCl and x mM H$_2$Tar in water–PEG (50:50, v/v) (pH 4.9–5.0). Applied voltage, +30 kV. Electromigration injection, 20 s at +20 kV. Other conditions as in Fig. 2.

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