Separation and Determination of Alkaline-Earth Metal Ions as UV-Absorbing Chelates with EDTA by Capillary Electrophoresis. Determination of Calcium and Magnesium in Water and Serum Samples

Shoji MOTOMIZU*, Mitsuko OSHIMA*, Shin-ya MATSUDA*, Yoshimitsu OBATA and Hiroshi TANAKA****

**Department of Chemistry, Faculty of Science, Okayama University, Tsushimanaka, Okayama 700, Japan*
***Applied Biosystems Japan, Inc., 3-3-6, Minamisuna, Koto, Tokyo 136, Japan*

Capillary electrophoresis of alkaline-earth metal ions was examined with a UV-absorbing chelating agent. The metal chelates separated in a capillary were measured by on-column UV-absorptive detection. When ethylenediaminetetraacetic acid (EDTA) was used as an chelating agent in a carrier solution (pH 9.2), the order of the migration time (t_m) of metal ions was as follows: $Ba^{2+}<Sr^{2+}<Ca^{2+}<Mg^{2+}$ (Be^{2+} could not be detected). These four ions were separated within 16 min using a capillary (50 μm i.d.) of 75-cm effective length (L_D) at an applied voltage of 30 kV, and calcium and magnesium ions were separated within 4 min using a 25-cm capillary at an applied voltage of 20 kV. By using a 50-cm or 25-cm capillary, Ca^{2+} and Mg^{2+} in river, tap and underground water samples were determined; the detection limits of metal ions were about 10^{-5} M (1 M=mol dm^{-3}), and the relative standard deviations for the determination of around 10^{-4} M of the metal ions were less than 2.0%. The method was also applied to the determination of Ca^{2+} and Mg^{2+} in a standard serum sample.

Keywords Alkaline-earth metal ion, water sample, serum sample, EDTA, capillary electrophoresis, on-column UV detection

Capillary zone electrophoresis (CZE) has become a powerful and important technique for the separation and analysis of charged substances.[1-6] The majority of CZE applications have aimed at separating biological macromolecules, such as proteins and polynucleotides. The CZE technique, however, seems to be highly promising for the separation and determination of charged small molecules, in competition with other electrophoretic techniques, such as paper electrophoresis (PE), gel electrophoresis (GE) and capillary isotachophoresis (CIP). By CIP techniques, simple organic and inorganic ions have been separated and determined: some examples are the CIP applications to inorganic anions and cations.[7-10] Equilibrium studies on the formation of metal complexes have been made by PE[11,12] and CIP.[13,14] By coupling the electrophoresis with metal complex formation, the separation and the detection sensitivity of metals have been enhanced.[15-21] Yoshida *et al.*[15], Gebauer *et al.*[16] and Nakabayashi *et al.*[20,21] used EDTA as a complex forming agent for analysis of metal ions by CIP techniques.

Several studies on the separation of metal ions by CZE have been done without any chelating agent[22-24] and with 4-(2-pyridylazo)resorcinol[25], β-diketones[26], cyanide ion[27], 8-quinolinol[28] and porphine analogues.[29] These studies aimed at utilizing the high separation ability of CZE. However, CZE techniques are useful for the simultaneous determination of metal ions, if the sensitivity and reproducibility are enough for the quantitative analysis of metals.

In a serum analysis by HPLC, some endogenous organic substances in serum often adsorb irreversibly on column packing materials, which results in lowering the functions of the columns. On the other hand, in the analysis by CZE, regeneration of capillaries is very easy and reproducible, and sample sizes necessary for analysis are very small, compared with other methods such as HPLC, flame photometry, or AAS. In such respects, CZE is promising for serum analysis.

In this work, the authors aim at developing the simultaneous determination of alkaline-earth metal ions as their chelates with EDTA.

Experimental

Apparatus

The CZE system was an Applied Biosystems Model 270A with a UV detector. Sampling was carried out by a hydrodynamic (vacuum) injection method: a vacuum was applied to the detector end of the capillary. Separations were performed in untreated fused-silica capillaries (50 μm i.d.), obtained from GL Science (Tokyo, Japan). Capillaries used are 47 cm, 72 cm and 97 cm in total length (L_T), and 25 cm, 50 cm and 75 cm in separation length from an anode to a detector (L_D). On-column

flow cells were created by removing a small portion of the capillary's polyimide coating by burning. Capillary, reservoirs and sample vials were kept in a temperature-controlled room: here the room was kept at 35°C. Absorbances were displayed on a Hitachi D-2500 Chromato-Integrator and a System Instruments Lab-chart 180.

Reagents

EDTA (disodium salt) was purchased from Dojin Laboratories. Sodium tetraborate (borax) was used for the preparation of buffer or carrier solutions. The pHs of the carrier solutions were adjusted by adding a dilute boric acid or a sodium hydroxide solution to the borax solution. The chlorides or nitrates of alkaline-earth metal ions were used, and the accurate concentrations were determined by EDTA titration method.

All the reagents used were of analytical reagent grade, and were dissolved in water purified with both a deionizing-distilling apparatus and a Milli Q apparatus.

Procedure

Carrier solutions containing 0.02 M $Na_2B_4O_7$ and EDTA were used. The capillary tubing was filled with a carrier solution for 3 min under vacuum after being washed with 1 M NaOH and water each for 5 min under vacuum for each measurement. In the case of river, well and tap water, the washing with 1 M NaOH and water was unnecessary. After that, a sample solution was introduced into the anodic end of the capillary by the vacuum injection, and each end was immersed in the cathode and the anode reservoir containing the same carrier solution. Then, the voltage was applied and electrophoresis started. These procedures were performed automatically. All solutions, including sample solutions, were used after filtering them through a membrane filter, 0.20 μm pore size.

Results and Discussion

Detection of metal chelates

Most alkaline-earth metal chelates of EDTA show absorption maxima at wavelengths around 190 nm, where molar absorptivities are 2000 – 3000 $dm^3\ mol^{-1}\ cm^{-1}$. Though the spectrophotometric detector of the CZE system works at wavelengths above 190 nm, the noise level of the detector and the absorbance of inorganic anions such as halide ions increased rapidly at wavelengths below 200 nm. Therefore, the detection was carried out at 200 nm. The detector can be operated at the range of the absorbance unit full scale (AUFS) above 10^{-3}. Therefore, we can expect by a simple calculation that alkaline-earth metal chelates at concentrations of several 10^{-6} M are determined with a capillary flow cell of 0.05-mm path length, provided the molar absorptivities are about 2000 $dm^3\ mol^{-1}\ cm^{-1}$.

Table 1 Electrophoretic mobilities (μ_{ep}) of divalent metal chelates with EDTA

Metal	$\mu_{ep}/10^{-4}\ cm^2\ V^{-1}\ s^{-1}$	Metal	$\mu_{ep}/10^{-4}\ cm^2\ V^{-1}\ s^{-1}$
Fe^{3+}	4.00	Mn^{2+}	3.86
Ba^{2+}	3.13	Pb^{2+}	3.95
Sr^{2+}	3.18	Pd^{2+}	4.00
Ca^{2+}	3.19	Co^{2+}	4.02
Mg^{2+}	3.39	Ni^{2+}	4.02
Be^{2+}	ND	Zn^{2+}	4.04
Cd^{2+}	3.75	Cu^{2+}	4.11
Hg^{2+}	3.84		

a. Carrier, 0.02 M borax buffer+2×10^{-3} M EDTA (pH 9.2); sample, metal ion+2×10^{-3} M EDTA (pH 9.2); 20 kV; 35°C; capillary length, 50 cm (total length, 72 cm).

Electrophoretic mobilities of alkaline-earth metal chelates

Table 1 shows the electrophoretic mobilities, μ_{ep}, of alkaline-earth metal chelates and other common metal ions at pH 9.2, where most of the divalent metal ions are present as chelates or divalent chelate anions. Be(II) ion reacts with EDTA to form a chelate: its stability constant (log K) is about 9.3. In the present procedure, however, Be(II) could not be detected. This is probably because Be(II) is present as hydroxide at pH 9.2. In a series of alkaline earth metals, the order of electrophoretic mobilities of chelates is as follows: (absolute μ_{ep} values) $Mg^{2+}>Ca^{2+}>Sr^{2+}>Ba^{2+}$. This order is the same as that obtained by PE.[12] Electrophoretic mobilities (absolute values) of other divalent metal ions examined are larger than those of alkaline-earth metals, and therefore did not interfere with the determination of alkaline-earth metal ions. Iron(III) chelate showed a μ_{ep} value similar to those of divalent metal ions. This indicates that the chelate of iron(III) is a divalent ion, and possibly iron(III) forms a mixed chelate such as $Fe(OH)Y^{2-}$ (Y^{4-}: EDTA anion).

In general, the relationship between electrophoretic mobility (μ_{ep}) and the reciprocal of the square root of molecular mass ($1/\sqrt{m}$) is linear, when very little hydration of ions occurs.[15,30] This is true in alkaline-earth metal chelates, though the magnesium chelate shows the abnormally large mobility. In other metal ions, there is no obvious relationship between μ_{ep} and ($1/\sqrt{m}$). However, we can see that the mobilities (absolute values) of chelates of metal ions belonging to the same period increase with an increase in atomic number, and in the fourth period the order of ionic mobilities are in good agreement with that of Irving–Williams series.[31] The difference in the hydration of chelates probably affected ionic mobilities more than that in molecular mass.

Factors affecting the separation of metal ions

Effect of capillary length. In general, the chemical nature of calcium is very similar to that of strontium, and the ion exchange HPLC separation of these two metals is sometimes difficult. In this work, the separation of four alkaline-earth metal ions was achieved by using a 75-cm

capillary within 16 and 24 min at 20 kV and 30 kV, respectively (Fig. 1). In a shorter capillary, though the efficiency of the separation was not enough, retention times were shorter and the peaks were sharp and high.

Effect of the potential field strength on electrophoretic mobility. Figure 1 also shows the effect of potential field strength, E, on the mobility of four alkaline-earth metal chelates. By applying a higher voltage, the separation of the four alkaline-earth metals was improved, and the retention time was shortened. Figure 2 shows the effect of E on the velocity of electroosmotic flow, u, and μ_{ep} of metal chelates. As expected from the following Helmholtz's equation, u is proportional to E:

$$u = \varepsilon \cdot \zeta \cdot E/4\pi\eta \quad (1)$$

where ε, ζ and η are dielectric constant, zeta potential and viscosity, respectively. Essentially, the value μ_{ep} of an ion does not vary, even though E is varied. However, the values μ_{ep} (absolute values) increased with increasing E. This is probably because the temperature of the carrier in the capillary was elevated by the Joule heat: as a rule, the μ_{ep} value increases by 2% for each 1°C increase in temperature.[32] Assuming that the changes in μ_{ep} values depend on the temperature increase, the temperature of the carrier in the capillary seems to increase by 2°C for each 100 V of E. For short analysis time, a high potential field is preferable. However, the background becomes noisy with an increase in E. Therefore, an applied voltage around 20 kV is recommended when L_T is about 70 cm.

Effect of the amount of EDTA in carrier. EDTA can form stable chelates with many multivalent metal ions. Some of the chelates, however, will dissociate into metal ions and EDTA at low concentrations of excess EDTA: barium and strontium chelates can dissociate during the migration in the capillary by using the carrier containing only borax buffer. In this work, the carrier containing borax and 2×10^{-3} M EDTA was used in order to complete the chelation. At this concentration of EDTA in the carrier, calcium and magnesium ions formed their chelates quantitatively with EDTA during the migration, whereas barium and strontium ions did not. Therefore,

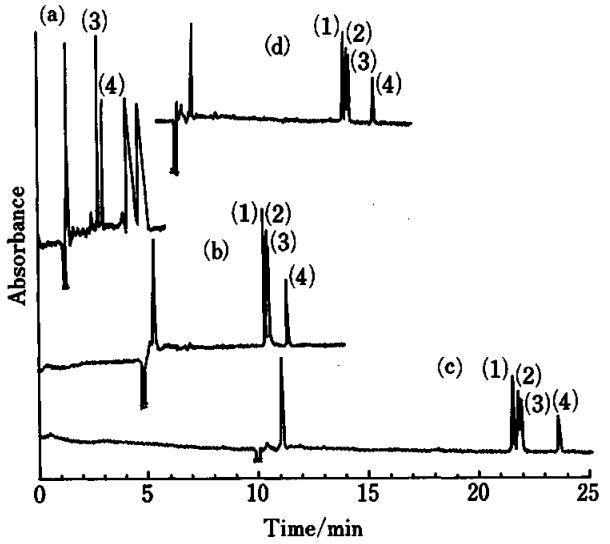

Fig. 1 Effect of capillary length (L_D) and potential field strength (E) on the separation of alkaline-earth metal chelates. (a)–(c) 20 kV; (d) 30 kV; (1) Ba^{2+}; (2) Sr^{2+}; (3) Ca^{2+}; (4) Mg^{2+}. L_D (cm): (a) 25; (b) 50; (c) and (d) 75. Sample, 2×10^{-4} M metal ion+2×10^{-3} M EDTA; carrier, 2×10^{-3} M borax+2×10^{-3} M EDTA (pH 9.2); sampling time, 3 s.

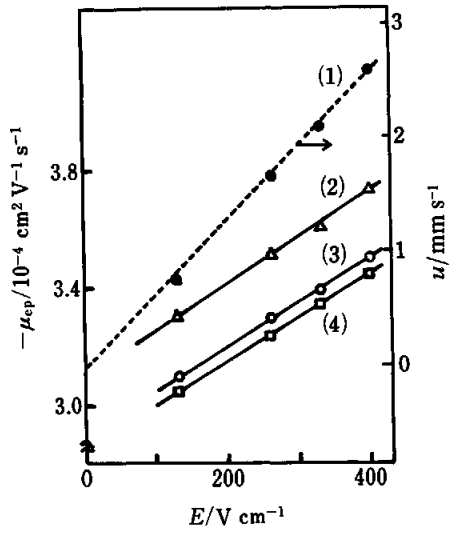

Fig. 2 Effect of potential field strength (E) on the velocity of electroosmotic flow (u) and electrophoretic mobilities of alkaline earth metal chelates (μ_{ep}). (1) u *vs.* E; (2)–(4) μ_{ep} *vs.* E; (2) Mg^{2+}; (3) Ca^{2+}; (4) Ba^{2+}. L_D=50 cm; L_T=72 cm. Sample and carrier solutions are the same as in Fig. 1.

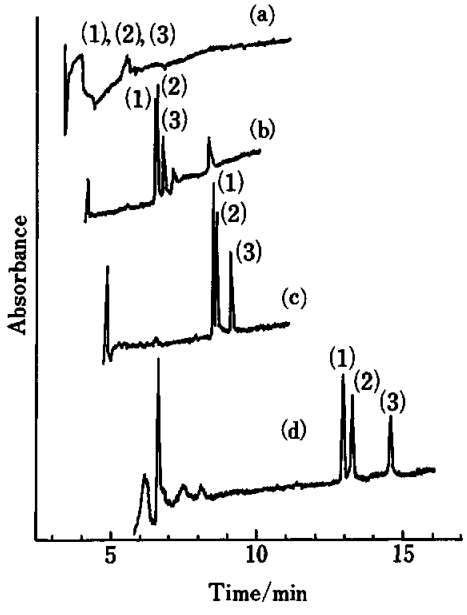

Fig. 3 Effect of borax concentration in carrier solutions on the peak shape of chelates. (1) Ba^{2+}; (2) Ca^{2+}; (3) Mg^{2+}; borax (mM): (a) 0; (b) 2; (c) 10; (d) 30. L_D=50 cm; E= 20 kV; sampling time, 1 s. Sample is the same as in Fig. 1.

we can determine only calcium and magnesium ions by injecting the water samples without any special pretreatments.

Effect of salts in a carrier and a sample. Figure 3 shows the electropherograms obtained with and without borax in the carriers. The lower concentrations of borax resulted in more rapid electroosmotic flow. This is because the effective charge density of the inner surface of a silica capillary increases with decreasing concentrations of borax. Electrophoretic mobilities of the chelates also became larger with decreasing concentrations of borax in the carrier, as is shown in Table 2. Figure 4 shows the electropherograms obtained with and without 20 mM borax in sample solutions. The peaks of metal chelates were narrow and high without borax. This is because the concentration of sample ions by a so-called "stacking effect" is more effective in sample solutions with a low concentration of borax than with a high concentration of borax. When sodium chloride at concentrations up to 0.1 M was added to sample solutions containing 0.002 M EDTA, it did not cause any effect on the electropherograms. This is probably because monovalent chloride ions do not affect the stacking of the sample ions due to their electrophoretic mobilities being smaller than those of divalent chelates. In practical analyses of water samples, the carrier containing 10^{-2} M borax and 2×10^{-3} M EDTA was used. Since the samples, except for non-diluted seawater and serum, usually contain less than 0.1 M sodium chloride, there is no interference from coexisting salts.

Effect of pH value of carrier on electrophoretic mobilities of chelates. The pHs of carriers were varied by adding a sodium hydroxide solution or solid boric acid to the carriers containing 2×10^{-2} M borax and 2×10^{-3} M EDTA. Over the region from pH 8.0 to pH 10.5, the electrophoretic mobilities for alkaline earth chelates were identical within the experimental errors: $-\mu_{ep}/10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ for Ba^{2+}, Sr^{2+}, Ca^{2+}, and Mg^{2+} were 3.13±0.01, 3.18±0.01, 3.19±0.01 and 3.39±0.01, respectively, with a 50-cm (L_D) capillary and a voltage of 20 kV. This indicates that in 2×10^{-3} M EDTA solution of pH 8 to pH 10.5, these four alkaline-earth metal ions form stable chelates and free metal ions are very few.

Table 2 Effect of borax concentration in carrier solution on electroosmotic flow and the electrophoretic mobilities of chelates

Borax/mM	u[a]	μ_{ep}[b]			
		Ba^{2+}	Sr^{2+}	Ca^{2+}	Mg^{2+}
0	2.55	—	—	—	—
2	2.27	−3.43	−3.43	−3.48	−3.62
5	2.09	−3.30	−3.30	−3.35	−3.53
10	1.90	−3.24	−3.28	−3.29	−3.48
20	1.63	−3.12	−3.16	−3.17	−3.37
30	1.52	−3.10	−3.14	−3.16	−3.36

Concentration of EDTA in carrier solution: 2×10^{-3} M.
a. $u=50/(t_0\times60)$ (cm s^{-1}); t_0: times necessary for water zone of the samples to reach the detector (min).
b. Electrophoretic mobility (10^{-4} $cm^2\ V^{-1}\ s^{-1}$).

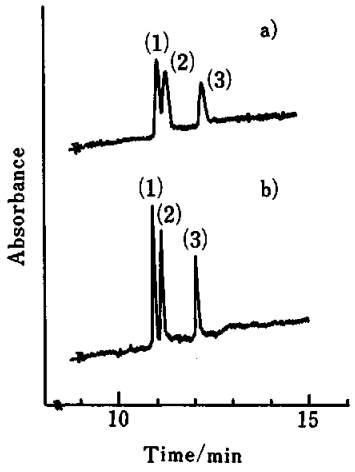

Fig. 4 Effect of borax concentration in sample solution on the peak shape of chelates. (1) Ba^{2+}; (2) Ca^{2+}; (3) Mg^{2+}; (a) 20 mM borax; (b) without borax. Sample, 2×10^{-4} M metal ion+2×10^{-3} M EDTA+borax; carrier, 2×10^{-3} M borax+2×10^{-3} M EDTA. Other conditions are the same as in Fig. 3.

Reproducibility

In a quantitative analysis using CZE, the reproducibility of electroosmotic flow and sample size are very important to obtain a reproducible peak height and peak area. Table 3 shows the reproducibility of migration times (t_m), $-\mu_{ep}$, peak heights (PH) and peak areas (PA) of chelates. Electrophoretic mobilities of chelates were very reproducible, though t_m and t_0 (the time necessary for the water zone of a sample to reach the detector) showed a little daily variation; the reproducibilities of PH and PA are enough for the quantitative determination.

Effect of sample size

The sample size was varied by varying sampling time from 1 s to 8 s using a vacuum system (−127 mmHg): by the vacuum sampling method adopted here, 1 s sampling time corresponds to about 3 nl. Figure 5 shows the effect of sample size on PH and PA. PA increased linearly over the sampling time from 1 to 8 s. PH also increased linearly over the sampling time from 1 s to 6 s. In the regions of sampling time above 6 s, the so-called "stacking effect" does not act proportionally, and therefore PH does not increase linearly and peaks become broader. This broadening of peaks results in poor separation of each peak. The reproducibility of PH and PA obtained by 8 s sampling time was enough for the quantitative analysis; the relative standard deviations (RSD) for the seven repeated measurements of PH and PA of 2×10^{-4} M alkaline earth metal ions were 0.5 to 1.5%, respectively.

Table 3 Results for reproducibilities

Sample[a]	t_0[b]	t_m[c]	$-\mu_{ep}$[d]	PH[e]	PA[f]
I Ba		10.61±0.01	3.12±0.01	2.99±0.06	1.40±0.06
Ca	5.05±0.01	10.81±0.01	3.17±0.01	2.96±0.05	1.68±0.05
Mg		11.67±0.01	3.37±0.01	2.04±0.05	1.28±0.07
II Ba		11.05±0.11	3.22±0.01	3.03±0.06	1.50±0.06
Ca	5.16±0.03	12.26±0.12	3.27±0.01	2.86±0.03	1.56±0.02
Mg		12.19±0.15	3.49±0.01	2.10±0.03	1.18±0.03

Capillary: 50 cm (effective length) and 72 cm (total length); 20 kV; λ, 200 nm; sampling time, 1 s; metal ion, 2×10^{-4} M; carrier, 2×10^{-3} M borax+2×10^{-3} M EDTA. a. Experiments for samples I and II were carried out on different days. b. Times necessary for water zone of the samples to reach the detector (min). c. Migration times of chelates necessary to reach the detector (min). d. Electrophoretic mobilities (10^{-4} cm^2 V^{-1} s^{-1}). e. Peak height (cm). f. Peak area (10^4 μV s). Each value is a mean of three replicate determinations, and a plus or minus value shows the deviation from the mean value.

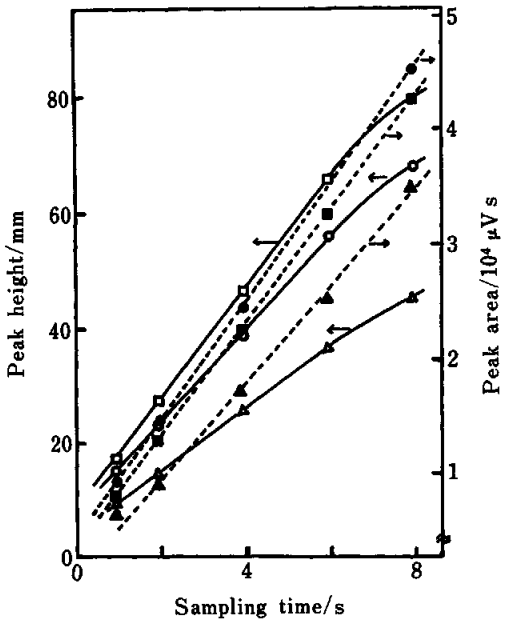

Fig. 5 Effect of sample size on the peak height and peak area of chelates. Solid line, peak height; dotted line, peak area. □, ■: Ba^{2+}; ○, ●: Ca^{2+}; △, ▲: Mg^{2+}. Sample, 2×10^{-4} M metal ion+2×10^{-3} M EDTA. Other conditions are the same as in Fig. 4.

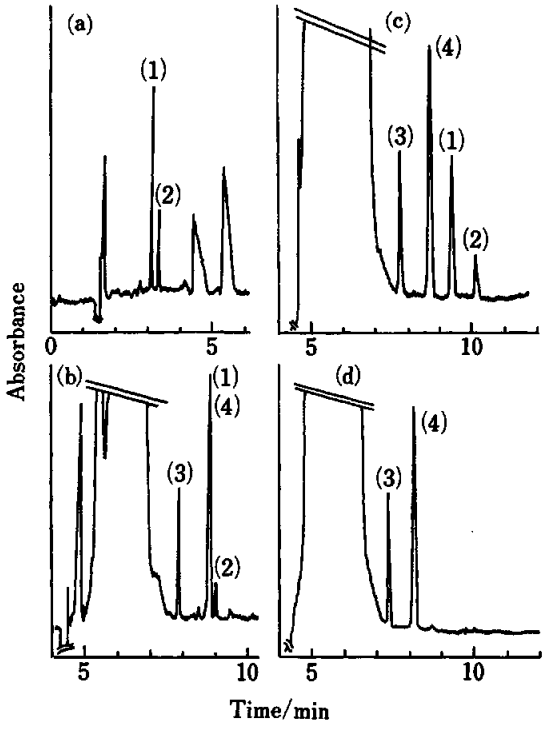

Fig. 6 Electropherograms of water and serum samples. (a) well water, L_D=25 cm, L_T=47 cm, sampling time 3 s, carrier: 20 mM borax+3 mM EDTA; (b) 50-fold diluted serum (Wako control serum I), L_D=50 cm, L_T=72 cm, sampling time 5 s, carrier: 20 mM borax+3 mM CyDTA; (c) 50-fold diluted serum (Dade Mori-Trol I), L_D=50 cm, L_T=72 cm, sampling time 10 s, carrier: 20 mM borax+2 mM EDTA; (d) the same as (c) except carrier: 20 mM borax. (1) Ca^{2+}; (2) Mg^{2+}; (3) and (4) unknown substances originating from the serum. 20 kV, 35°C, λ=200 nm.

Application to the determination of calcium and magnesium in practical samples

This method can be applied to the determination of calcium and magnesium ions in the aqueous solution at concentrations above 10^{-5} M. The calibration graphs of calcium and magnesium were linear over the ranges from 1×10^{-5} M to 1×10^{-3} M using PH and PA, when sampling time was 3 s, capillary length (L_D) 50 cm and applied voltage 20 kV. RSDs for PH of 2×10^{-4} M, 4×10^{-4} M, 6×10^{-4} M, 8×10^{-4} M and 1×10^{-3} M standard calcium were 1.54, 1.35, 0.85, 0.87 and 0.99% (n=7), respectively, and RSDs for PA were almost the same as those for PH. The RSDs for magnesium determination were almost the same as those for the calcium determination. When a capillary of L_D=25 cm was used, the peaks of calcium and magnesium chelates appeared within 3.5 min and were very sharp, as shown in Fig. 6(a). In this case, the calibration graphs of calcium and magnesium using PH were a little curved, whereas those using PA were linear over the range from 1×10^{-5} M to 1×10^{-3} M. However,

Table 4 Analytical results for water samples

Sample[a]	Found/10^{-4} M					
	Peak area		Peak height		EDTA titration	
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
Tap water	1.98 (4.4)	0.78 (8.7)	1.85 (1.6)	0.72 (5.7)	1.91	0.69
Asahi River	1.92 (4.9)	0.70 (7.6)	1.83 (1.1)	0.66 (1.2)	1.81	0.65
Well water	3.55 (9.1)	1.97 (5.7)	3.51 (1.6)	1.97 (1.2)	3.38	1.93

Obtained with a 25-cm capillary. The values in the parentheses are the relative standard deviations (%) for 5 replicates. a. These were sampled on May 5, 1991.

Table 5 Determination of calcium and magnesium in serum

		t_m/min	Peak area, ×10 μV s	Peak height, ×0.2 mm	Found/10^{-5} M		Ref./10^{-5} M
					Area	Height	
Ca	Mean	9.59	1374	212	4.44	3.50	4.5–5.0[c]
	SD	0.09	40	50	0.16	0.11	
	RSD (%)	0.9	2.9	2.3	3.6	3.1	
Mg	Mean	10.35	413	63	1.93	1.69	1.5–1.8[c]
	SD	0.10	20	23	0.11	0.07	
	RSD	0.97	5.0	3.6	5.7	4.4	

Sample: 50-fold diluted Dade Moni-Troll; sampling: 10 s. Capillary was washed with 1 M NaOH for 5 min and water for 5 min in every measurement. Data were treated with a SIC Labchart 180, using 4 replicates. a. These are calculated from the indicated values for the 50-fold diluted sample.

the reproducibilities were worse than those obtained using a capillary of L_D=50 cm. Table 4 shows the results of the reproducibilities for PH with 25-cm capillary. The reproducibilities for PA were worse than those for PH, and the relative standard deviations for PA were 5 to 10%.

The method was applied to the determination of calcium and magnesium in the practical water samples and in serum sample. In the previous preliminary work with 1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic acid (CyDTA), calcium in serum could not be separated from the peaks due to endogenous substances in the serum.[33] Figure 6(c) shows the electropherogram of a control serum. The peaks of calcium and magnesium were well separated from the matrices. In the serum analysis, the washing of the capillary with 1 M NaOH and water was necessary. Otherwise, osmotic flow was less reproducible because of the adsorption of components of serum. By washing the capillary with NaOH and water, the reproducibilities became good. Table 5 shows the analytical results: the contents of calcium and magnesium were in good agreement with the indicated values. The contents obtained by using PH were smaller than those using PA. This is because metal ions interact with the matrices of serums. The analytical results for water samples in Table 4 are in good agreement with those obtained by EDTA titration method.

In conclusion, CZE of alkaline-earth metals was studied with use of running buffer solutions containing EDTA. The method was applied to the determination of Ca^{2+} and Mg^{2+} in serum and water samples. In the serum analysis, Ca^{2+} and Mg^{2+} could be determined reproducibly even by introducing a small size of a 50-fold diluted sample solution into a capillary without any special pretreatments. The results obtained in this work suggest that CZE is a promising method for the determination of metals in the aqueous samples.

References

1. J. W. Jorgenson and K. D.Lukacs, *Science* [Washington, D.C.], **222**, 266 (1983).
2. J. W. Jorgenson, *Anal. Chem.*, **58**, 743A (1986).
3. M. J. Gordon, X. Huang, S. L. Pentoney, Jr. and R. N. Zare, *Science* [Washington, D.C.], **242**, 224 (1988).
4. A. G. Ewing, R. A. Wallingford and T. M. Olefirowicz, *Anal. Chem.*, **61**, 292A (1989).
5. R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr.*, **29**, 1 (1989).
6. W. G. Kuhr, *Anal. Chem.*, **62**, 403R (1990).
7. J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, **51**, 339 (1970).
8. J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, **68**, 207 (1972).
9. H. Yoshida, M. Hida and M. Taga, *J. Chromatogr.*, **325**, 179 (1985).
10. H. Yoshida and M. Hida, *J. Chromatogr.*, **351**, 388 (1986).
11. Y. Kiso, *J. Sci. Hiroshima Univ. ser. A-II*, **27**, 17 (1963).

12. Y. Kiso, *J. Sci. Hiroshima Univ. ser. A-II,* **27**, 23 (1963).
13. E. Jercan and G. Popa, *J. Chromatogr.,* **42**, 89 (1969).
14. Y. Kiso and T. Hirokawa, *Chem. Lett.,* **1980**, 745.
15. H. Yoshida, I. Nukatsuka and S. Hikime, *Bunseki Kagaku,* **28**, 382 (1979).
16. P. Gebauer, P. Bocek, M. Deml and J. Janák, *J. Chromatogr.,* **199**, 81 (1980).
17. I. Nukatsuka, M. Taga and H. Yoshida, *J. Chromatogr.,* **205**, 95 (1981).
18. Y. Hirama and H. Yoshida, *J. Chromatogr.,* **322**, 139 (1985).
19. S. Tanaka, T. Kaneta and H. Yoshida, *J. Chromatogr.,* **447**, 383 (1988).
20. Y. Nakabayashi, K. Nagaoka, Y. Masuda and R. Shinke, *Talanta,* **36**, 639 (1989).
21. Y. Nakabayashi, K. Nagaoka, Y. Masuda and R. Shinke, *Analyst* [London], **114**, 1109 (1989).
22. T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.,* **264**, 385 (1983).
23. L. Gross and E. S. Yeung, *Anal. Chem.,* **62**, 427 (1990).
24. T. Tsuda, *Nippon Kagaku Kaishi,* **1986**, 937.
25. T. Saitoh, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr.,* **469**, 175 (1989).
26. K. Saitoh, C. Kiyohara and N. Suzuki, *Twelfth International Symposium on Capillary Chromatography,* p. 725 (1990), Kobe.
27. M. Aguilar, X. Huang and R. N. Zare, *J. Chromatogr.,* **480**, 427 (1989).
28. D. F. Swaile and M. J. Sepaniak, *Anal. Chem.,* **63**, 179 (1991).
29. T. Saitoh, H. Hoshino and T. Yotsuyanagi, *Anal. Sci.,* **7**, 495 (1991).
30. Y. Kiso, "*Zone Electrophoresis* (in Japanese)", p. 60, Nankodo, Tokyo, 1972.
31. H. Irving and R. J. P. Williams, *J. Chem. Soc.,* **1953**, 3192.
32. R. J. Wieme, "*A Laboratory Handbook of Chromatographic and Electrophoretic Methods*", ed. E. Heftmann, Chap. 10, Van Nostrand Reinhold, New York, 1975.
33. S. Motomizu, S. Nishimura, Y. Obata and H. Tanaka, *Anal.Sci.,* **7** (Supplement), 253 (1991).

(Received April 17, 1992)
(Accepted July 1, 1992)