

Highly Sensitive Simultaneous Detection of Lanthanide(III) Ions as Kinetically  
Stable Aromatic Polyaminocarboxylato Complexes by Capillary  
Electrophoresis Using Resolution Enhancement with Carbonate Ion.

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## ABSTRACT

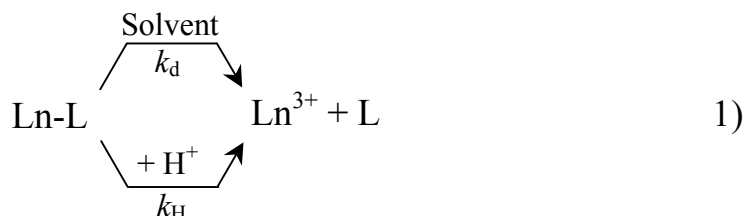
An aromatic polyaminocarboxylate ligand, 1-(4-aminobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetate, (ABEDTA) are proposed as a complexing reagent in the pre-capillary mode to form the kinetically inert Ln(III) - complexes, so that no added ligand is necessary in an alkaline carrier buffer solutions. In addition, highly sensitive detection is possible through a light-absorbing moiety of an aminobenzyl group in the ligand. The fine-tuning of the electrophoretic mobilities of the Ln – abedta complexes is successfully achieved by adding an auxiliary ligand of carbonate ion which alters the charge to size ratio of the complexes through the fast exchange equilibria in a carrier buffer. In fact, all the complexes are detectable with an almost similar analytical sensitivity and the quite acceptable resolution except for Sm-Eu-Gd is obtained using the NaOH – borate carrier buffer solution at pH 12.35 containing 20 mM ( $M = \text{mol dm}^{-3}$ ) of  $\text{Na}_2\text{CO}_3$ . Typically a detection limit for Tb(III) ion on a  $3 \sigma$  basis is as low as 0.94  $\mu\text{M}$ , which is translated to that on an absolute amount basis, 9.4 fmol in a  $1.0 \times 10^{-8} \text{ dm}^{-3}$  (10 nL) injection.

Capillary electrophoresis (CE) is now considered one of the most powerful analytical methodologies for inorganic species including lanthanide(III) ions (Ln(III)).[1-4] The CE separation of 14 Ln(III) ions serves a good model to judge the resolution power of the CE systems designed in terms of separation chemistry and instrumental achievement. The CE separation scheme for Ln ions has actually been limited to the on-capillary mode using weak complexing reagents such as hydroxyisobutyric acid (HIBA).[5-9] So far, the full resolution of Ln(III) ions with the pre-capillary complexation scheme has been still a challenging task.

Complexes favorable for the pre-capillary scheme (with no added ligand in a carrier buffer) should be considered on a kinetic basis. The dissociation processes which metal complexes inevitably undergo during CE migration have been discussed and the concept of a capillary electrophoretic reactor for the kinetic study has been established.[10,11] The success of the pre-capillary complexation scheme has been exemplified in terms of the kinetic selectivity unique to certain metal ions and the high sensitivity at low ppb levels by photometric detection.[12-17]

The dissociation reactions of the Ln(III) complexes possibly taking place during CE migration is bifurcated into solvolysis and proton-assisted paths,[18]

where L denotes a ligand and  $k_d$  and  $k_H$  indicate rate constants of the individual paths.



The dissociation degree of a complex at a particular pH is a sum of the results of these two processes. The solvolysis path is in general predominant under more alkaline pH conditions whereas in neutral solutions the proton-assisted process often prevails. The CE separations of Ln(III) ions as the EDTA or CyDTA complexes under neutral to slightly alkaline pH conditions have been reported by Timerbaev et al. (CyDTA = 1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic acid).[1] In fact, they were compelled to add the ligand in a carrier buffer solution (on-capillary mode) avoiding the proton-assisted decomposition of the complexes encountered even in slightly alkaline solutions.

In this report, 1-(4-aminobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetic acid (ABEDTA, or abedta in complexes) is proposed as a pre-capillary complexing reagent for Ln(III) ions because this ligand forms the kinetically stable complexes with the ions in strongly alkaline solutions. This commercially available ligand has been known as a bifunctional reagent used for labeling of

biopolymers (Meares' Reagent).[19] It is also stressed that the ternary complex formation equilibria with an auxiliary ligand such as carbonate ion in a carrier buffer solution give rise to the great resolution enhancement among Ln(III) ions. Figure 1 shows our strategy to produce the fine-tuned resolution among Ln(III) ions with the pre-capillary complexation scheme.

## EXPERIMENTAL

### Chemicals

The reagent, ABEDTA (Dojindo Lab., Kumamoto, Japan), was dissolved in doubly distilled water with a minimum amount of sodium hydroxide. Standard solutions of Ln(III) were prepared by dissolving the chloride salts (99.9% purity, Wako Pure Chemical Industries, Osaka, Japan) in doubly distilled water with a few drops of concentrated hydrochloric acid. The Ln(III) solutions were standardized by EDTA titration with Xylenol Orange as an indicator. The Ln(III)-abedta complexes were prepared by mixing the equimolar solutions of Ln(III) ions and the ligand. All other chemicals used were of analytical-reagent grade or better and doubly distilled water was employed throughout this study.

## **Apparatus**

The electrophoresis was performed with a BioFocus 3000 capillary electrophoresis equipment (Bio Rad Laboratories Japan, Tokyo, Japan) under constant voltage operation mode typically at 20 kV (80 - 110  $\mu$ A). The experiments were made at 293 K with the thermostating device of the CE equipment. A fused-silica capillary tube (0.05 mm I.D.; total length, 50.0 cm; effective length to the detection window, 43.4 cm) was purchased from Scientific Glass Engineering Inc. (Austin, TX). Solution pH was recorded with an HM 26s pH meter from TOA Electrics (Tokyo, Japan).

## **Sample Preparation and Electrophoresis**

To a mixed solution of 14 Ln(III) ions, the solution of ABEDTA is added and pH of the reaction mixture is adjusted at 11 – 12 by adding dropwise 1.0 M NaOH solution. The final concentration of ABEDTA at 1.0 mM is set in a large excess to those of Ln(III) ions, typically 50  $\mu$ M. This sample solution was injected from the cathode end of a capillary tube by hydrodynamic injection mode at a pressure of 5 psi sec on the sample vial. The injection volume of 10 nL was calculated from this pressure value.[20] The electrophoresis was performed using the thermostated operation mode of the equipment at 293 K.

On-capillary absorbance detection was made at 242 nm. The capillary tube was first pre-conditioned using the ‘pressure diagnostic sequence’ of the CE instrument, that is, 1.0 M NaOH solution (10 min) – distilled water (15 min) – a carrier buffer solution (15 min). Because strongly alkaline carrier buffer solutions were employed for separation, washing the capillary between CE runs was made with distilled water only. The electrophoretic mobility of the complex species,  $\mu_{ep}$ , was calculated from the following equation,

$$t_m = l / [(\mu_{ep} + \mu_{eo})E] \quad 2)$$

where  $t_m$ ,  $l$ ,  $E$ , and  $\mu_{eo}$  are the migration time of the complex species, capillary length, an electric field, and the electroosmotic mobility estimated from the migration time of solvent.

## RESULTS AND DISCUSSION

### **CE Separation of Ln(III) - abedta Complexes in a Pre-capillary Complexation Scheme.**

A pre-capillary complexation mode for the CE of the Ln(III)-abedta complexes was tested with no added auxiliary ligands in a carrier buffer solution. As can be seen from the typical electropherogram shown in Figure 2, all the Ln(III) complexes are detectable with the alkaline carrier, though the mutual

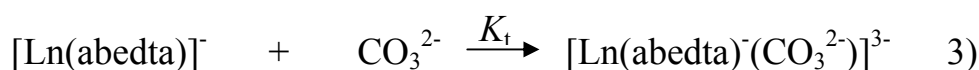
resolution is still unsatisfactory. The Ln(III) complex peaks are somewhat broadened at lower pH ( $\sim 10$ ) possibly owing to the proton-assisted dissociation reactions. This result clearly shows that these ABEDTA complexes are sufficiently inert towards the simple solvolytic processes predominant in more alkaline solutions.

### **Resolution Enhancement Using Auxiliary Ligands via Ternary Complex Formation Reactions.**

It was found that the elution times of the complexes were often irreproducible owing to absorption of atmospheric carbon dioxide into the alkaline sample solutions in spite of no actual pH change on standing. This fact gave a clue for the resolution improvement using certain ionic reactions where seventh or eighth coordination site in the Ln(III) complexes are involved. Such reactions taking place in a carrier buffer solution probably lead a dynamic change in charge/size ratio of the complexes *via* ternary complex formation. The thorough examination based on the experience stated above led discovery of the resolution enhancement with carbonate ion; a set of anions of  $\text{OH}^-$ ,  $\text{B}(\text{OH})_4^-$ , and  $\text{CO}_3^{2-}$  ions in a carrier buffer solution was found to be most effective whereas various candidates for auxiliary ligands such as  $\text{PO}_4^{3-}$  ion, acetate ion, and tartrate ion did not seem to work for this purpose. Some candidate ligands possessing considerable UV absorption were ruled out because of the deterioration of the detection limits for Ln ions. Ternary complex formation



of Gd(III)-polyaminocarboxylate complexes with some anions such as carbonate, phosphate and citrate has been reported by Burai et al.[21] in terms of the contrasting agent employed in magnetic resonance imaging techniques. They demonstrated that even in the presence of 25 mM of carbonate ion, a [Gd(edta)]<sup>-</sup> complex forms the ternary complex having only the stoichiometry of [Gd(edta)CO<sub>3</sub><sup>2-</sup>]<sup>3-</sup>, estimating the equilibrium constant of  $K_t = \frac{[\text{Gd(edta)CO}_3^{2-}]}{[\text{Gd(edta)}][\text{CO}_3^{2-}]} = 10^{2.60}$ . The effect of the CO<sub>3</sub><sup>2-</sup> ion concentration on the mobility of the complexes is shown in Table 1. The fact that the negative  $\mu_{ep}$  values of the Ln - abedta complexes increase with an increase in CO<sub>3</sub><sup>2-</sup> ion concentration indicates the interchange of the complex, [Ln(abedta)]<sup>-</sup> to the ternary complex in the carrier buffer solution as shown in eq 3), which gives rise to the increasing negative charge on the Ln(III) complex species.



The differences in the  $\mu_{ep}$  values between La-Nd and Gd-Er are considerably expanded with the increasing concentration of CO<sub>3</sub><sup>2-</sup> ion. If the complexes of ABEDTA have the  $K_t$  values similar to those of the EDTA ones, the calculation using the  $K_t$  for [Gd(edta)CO<sub>3</sub><sup>2-</sup>]<sup>3-</sup>, gives a rough estimation of the equilibrium composition as 88 % of [Gd(abedta)CO<sub>3</sub><sup>2-</sup>]<sup>3-</sup> and 12 % of [Gd(abedta)]<sup>-</sup> in the carrier buffer at pH 12.35 with 20 mM of CO<sub>3</sub><sup>2-</sup>. This type of equilibrium mixture subjected to the CE separation is most probably responsible for the fine-tuning of the Ln ion resolution. Generally, the

resolution is remarkably improved by the simultaneous addition of  $\text{OH}^-$ ,  $\text{B}(\text{OH})_4^-$ , and  $\text{CO}_3^{2-}$  ions as shown in Figure 3, except for co-elution of Sm(III), Eu(III) and Gd(III) ions. These three Ln ions have been often unresolved in CE.[2] Verna, et al. reported that this problem can be solved by lowering the CE operation temperature to 288 K.[22] Besides the temperature effect on ionic mobility, the judicious choice of the ternary complex systems is possibly an alternative approach to solve this co-elution problem associated with Sm(III), Eu(III) and Gd(III) ions.

### **Detection and Interferences.**

Another advantage of our method is that actually no baseline drift is found since the detection wavelength (242 nm) is longer than that of commonly employed for the direct detection of Ln ions, typically at 190 - 200 nm. It is stressed that the height of the peaks due to the Ln – abedta complexes seems to be nearly identical from La to Lu as can be seen from Figure 3. This indicates that the complexes having the similar composition with respect to ABEDTA ligand actually do not decompose along the CE migration.

Under the same conditions as those shown in Figure 3, the linear calibration graph for Tb(III) ion can be drawn over the range of (2 – 200)  $\mu\text{M}$ . Typically a detection limit (DL) for Tb(III) ion on a  $3\sigma$  basis is as low as 0.94  $\mu\text{M}$ , which is translated to that on an absolute amount basis, 9.4 fmol in a  $1.0 \times 10^{-8} \text{ dm}^{-3}$  (10 nL) injection. An approach to lower the absorbance DL of Ln(III) ions in the

CE methodology has been the on-capillary scheme using light-absorbing complexing reagents dissolved in a carrier buffer solution; the DLs of 1.4 – 1.7  $\mu\text{M}$  was achieved by the combined use of HIBA and UV absorbing cupferron.[23] Also, a bisazo dye, Arsenazo III has been employed for such purpose, giving the DL of 0.35  $\mu\text{M}$  for La(III) ion.[7] Other Ln(III) ions, however, produced rather small peaks with Arsenazo III, giving the ordinary DL at several  $\mu\text{M}$  levels. Recently, Collins and Lu reported the low detection limits at sub- $\mu\text{M}$  range for Ln(III) ions with a microchip electrophoresis device.[24]

No interferences of 100-fold excess of other Ln(III) ions over  $\mu\text{M}$  of Tb(III) ion were observed. An electropherogram of Tb(III) – abedta complex in the presence of a 100-fold molar excess of Gd(III) ion is given in Figure 4. The clear-cut resolution of the CE peaks at the base-line enables us to detect a particular Ln (III) ion without influence from the neighboring ions. The influences of other hard cations,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Co}^{3+}$  on the  $\text{Ln}^{3+}$  detection were investigated; regarding  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ , small broadened peaks were detected at near 19 min between La and Ce at the high concentrations ( $\sim 200 \mu\text{M}$ ) of these metals. These broadened peaks are completely separated from Ln peaks. It is presumed that a complex decomposition caused by the attack of  $\text{OH}^-$  ion in a strongly alkaline buffer leads to the peak broadening. In contrast, a sharp peak of  $\text{Co}^{3+}$ -abedta complex was obtained at near 22 min just behind Ce peak with

baseline resolution, causing no interference at 200  $\mu\text{M}$ . It was consequently proved that triply charged cations do not actually interfere with the Ln measurements.

The method proposed here is essentially different from the traditional on-capillary CE methods for Ln(III) ions in terms of compatibility between light-absorbing labeling and kinetic inertness. In addition, the resolution enhancement using ternary complex formation equilibria in which the residual coordination sites of the complexes partake is indeed valuable. The successful pre-capillary complexation scheme described in this paper indicates that the kinetic aspect of metal complexes employed in CE is of increasing importance even for multielement detection as exemplified by the case for Ln(III) ions in this work. Experiments aiming at improving of the antenna moiety for the detection at visible region and the chemistry for complete resolution of 14 lanthanide complexes by the pre-capillary scheme are now underway.

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## Legends for Figures

Figure 1. Scheme for pre-capillary complexation CE of lanthanide ions ( $\text{Ln}^{3+}$ ) described in this work.  $\text{L}'$ ; an auxiliary ligand.

Figure 2. Typical electropherogram of Ln(III) – abedta complexes with no auxiliary ligands in a carrier buffer.

Sample;  $[\text{Ln}] = 50 \mu\text{M}$ ,  $[\text{ABEDTA}] = 1.0 \text{ mM}$ ,  $[\text{NaOH}] = 10 \text{ mM}$ . Carrier buffer (pH 12.35 with NaOH);  $[\text{Borate}] = 50 \text{ mM}$ . Applied voltage; 20 kV (80  $\mu\text{A}$ ). Injection volume; 10 nL.

Figure 3. Typical electropherogram of Ln(III) – abedta complexes under the optimized conditions with a carrier buffer containing carbonate ion.

Sample;  $[\text{Ln}] = 50 \mu\text{M}$ ,  $[\text{ABEDTA}] = 1.0 \text{ mM}$ ,  $[\text{NaOH}] = 10 \text{ mM}$ . Carrier buffer (pH 12.35 with NaOH);  $[\text{Borate}] = 50 \text{ mM}$ ,  $[\text{Carbonate}] = 20 \text{ mM}$ . Applied voltage; 20 kV (110  $\mu\text{A}$ ). Injection volume; 10 nL.

Figure 4. Electropherogram of Tb(III) - abedta complex at 5.0  $\mu\text{M}$  in the presence of a 100-fold molar excess of Gd(III) ion.

Sample;  $[\text{Tb}] = 5.0 \mu\text{M}$ ,  $[\text{Gd}] = 500 \mu\text{M}$ ,  $[\text{ABEDTA}] = 1.0 \text{ mM}$ ,  $[\text{NaOH}] = 10 \text{ mM}$ . Carrier buffer (pH 12.45 with NaOH);  $[\text{Borate}] = 50 \text{ mM}$ ,  $[\text{Carbonate}] = 20 \text{ mM}$ . Applied voltage; 20 kV (110  $\mu\text{A}$ ). Injection volume; 10 nL.

Insert; electropherogram magnified for the absorbance sensitivity.



Keywords: capillary electrophoresis, pre-capillary derivatization, lanthanide ion, aromatic polyaminocarboxylate, kinetically inert complex, ternary complex

Table 1. The electrophoretic mobilities of Ln(III) – abedta complexes at different concentrations of carbonate ion in the carrier buffer solutions.<sup>a)</sup>

[CO <sub>3</sub> <sup>2-</sup> ] / mM	$\mu_{ep} / 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$				
	La	Nd	Gd	Er	Lu
0	-6.5	-6.6	-6.8	-6.8	-7.1
4.0	-6.5	-6.8	-6.9	-7.1	-7.3
10	-6.4	-6.9	-7.1	-7.3	-7.5
20	-7.0	-7.4	-7.5	-7.9	-8.1

a) Applied voltage at 20 kV. Carrier buffer, [Borate] = 50 mM, at pH 12.30 with NaOH.

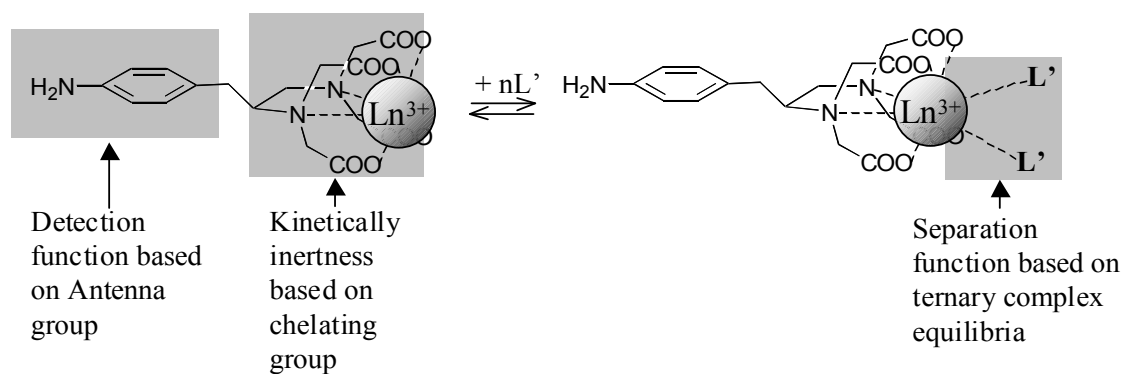


Figure 1. Scheme for pre-capillary complexation CE of lanthanide ions ( $\text{Ln}^{3+}$ ) described in this work.

L'; an auxiliary ligand.

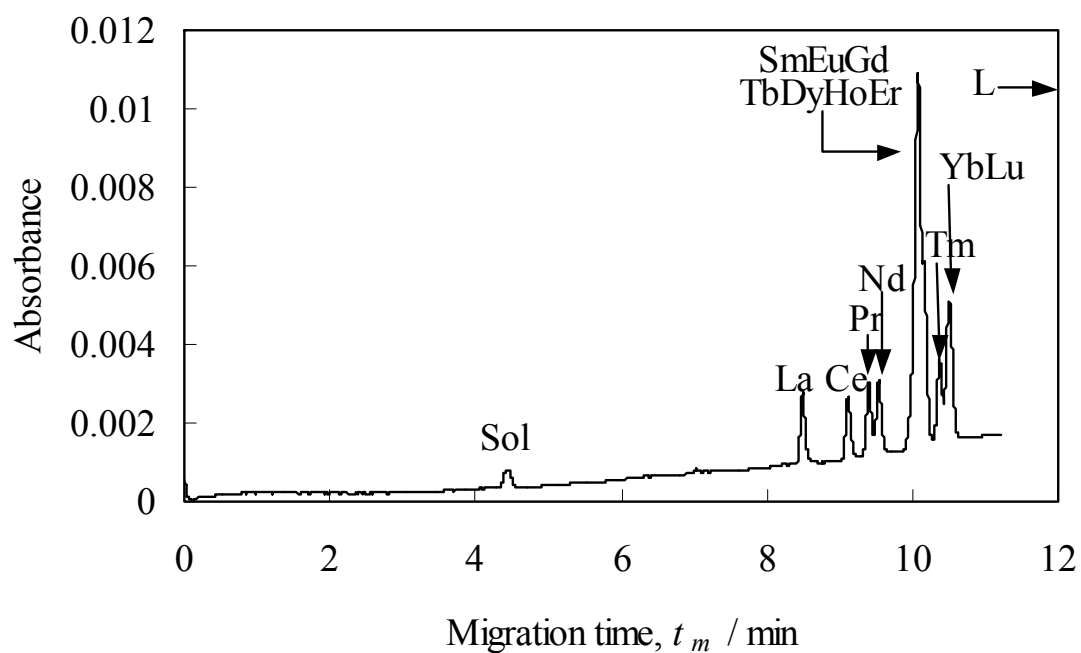


Figure 2. Typical electropherogram of Ln(III) – abedta complexes with no auxiliary ligands in a carrier buffer.

Sample; [Ln] = 50  $\mu$ M, [ABEDTA] = 1.0 mM, [NaOH] = 10 mM. Carrier buffer (pH 12.35 with NaOH); [Borate] = 50 mM. Applied voltage; 20 kV (80  $\mu$ A). Injection volume; 10 nL.

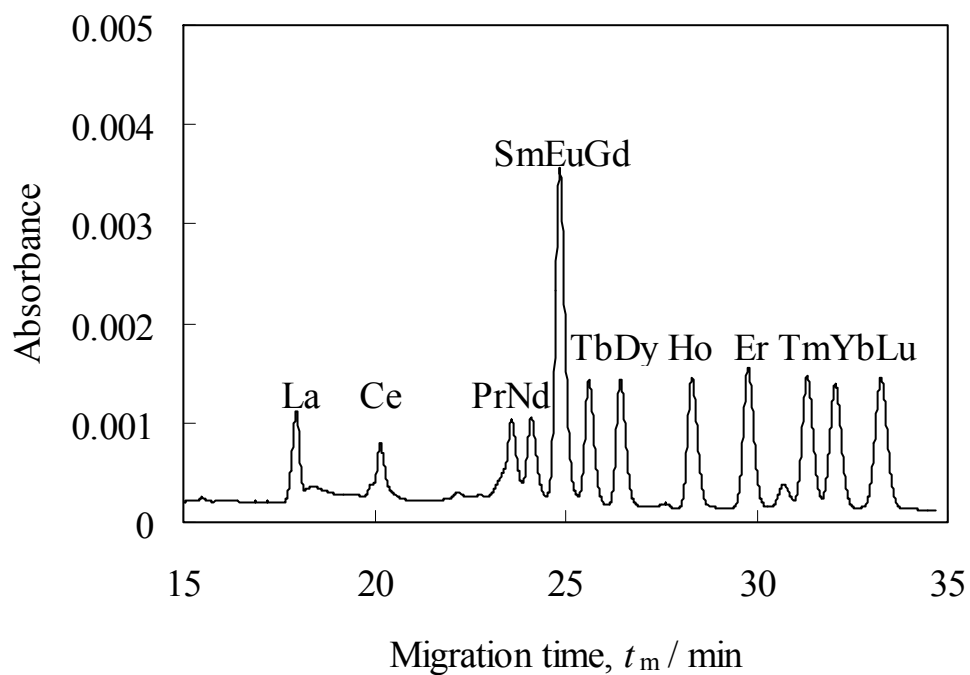


Figure 3. Typical electropherogram of Ln(III) – abedta complexes under the optimized conditions with a carrier buffer containing carbonate ion.

Sample; [Ln] = 50  $\mu$ M, [ABEDTA] = 1.0 mM, [NaOH] = 10 mM. Carrier buffer (pH 12.35 with NaOH); [Borate] = 50 mM, [Carbonate] = 20 mM. Applied voltage; 20 kV (110  $\mu$ A). Injection volume; 10 nL.

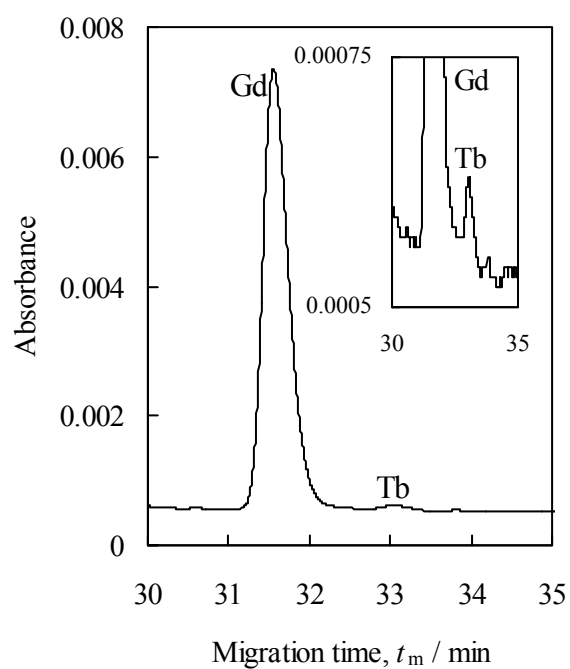


Figure 4. Electropherogram of Tb(III) - abedta complex at 5.0  $\mu\text{M}$  in the presence of a 100-fold molar excess of Gd(III) ion.

Sample; [Tb] = 5.0  $\mu\text{M}$ , [Gd] = 500  $\mu\text{M}$ , [ABEDTA] = 1.0 mM, [NaOH] = 10 mM. Carrier buffer (pH 12.45 with NaOH); [Borate] = 50 mM, [Carbonate] = 20 mM. Applied voltage; 20 kV (110  $\mu\text{A}$ ). Injection volume; 10 nL.

Insert; electropherogram magnified for the absorbance sensitivity.