

Highly Sensitive Determination of Lanthanides by Capillary Electrophoresis with Direct Visible Detection after Pre-capillary Complexation with Aromatic Polyaminocarboxylate and Additionally Applying Dynamic Ternary Complexation with Nitrilotriacetic Acid

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Running title: inert Ln complex separation using ternary complexation with vis detection

A list of abbreviations:

| | |
|--------|--|
| ABEDTA | 1-(4-aminobenzyl)ethylenediamine- <i>N,N,N',N'</i> -tetraacetic acid |
| CyDTA | <i>trans</i> -1,2-diaminocyclohexane- <i>N,N,N',N'</i> -tetraacetic acid |
| DOTA | 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid |
| DO3A | 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid |
| DPA | 2,6-pyridinedicarboxylic acid |
| DTPA | diethylenetriamine- <i>N,N,N',N'',N''</i> -pentaacetic acid |
| IDA | iminodiacetate |
| Ln | lanthanide |
| NBD-F | 4-fluoro-7-nitrobenzofran |
| NTA | nitrilotriacetic acid |
| Quin2 | 8-amino-2-[(2-amino-5-methylphenoxy)methyl]-6-methoxyquinoline- <i>N,N,N',N'</i> -tetraacetate |
| TTHA | triethylenetetramine- <i>N,N,N',N'',N''',N'''</i> -hexaacetic acid |

Key words: lanthanide ion, aromatic polyaminocarboxylate, dynamic ternary complex formation, kinetically inert complex

Abstract

A newly synthesized aromatic polyaminocarboxylate (H_4L) was applied to pre-capillary derivatizing capillary electrophoresis as a chelating reagent for lanthanide ions (Ln^{3+}). The Ln-L complexes provide both kinetic stability on dissociation due to their methyl-EDTA coordinating structure, and high light-absorptivity ($\epsilon_{\max} = 2.4 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ dm}^3$) in the visible region at 469 nm thanks to their nitrobenzofurazan moiety. A ligand was employed for capillary zone electrophoresis based on a unique concept: both **pre-capillary and dynamic on-capillary complexation** is carried out on one center-metal ion to achieve high resolution. As a ternary complex formation agent, iminodiacetate (IDA), bound to the mother complex (Ln-L), was added to the carrier buffer solution. **The carrier buffer solution of 9.5 mmol dm⁻³ (pH 9.45) borate and 33.5 mmol dm⁻³ IDA**, drastically improved the resolution among Ln^{3+} ions. **Each of the Ln complexes were effectively separated, except Pr-Sm**. Furthermore, the absence of L from the carrier solution, which stabilizes the baseline fluctuation, provided **low detection limits** (typically $4.2 \times 10^{-7} \text{ mol dm}^{-3}$). This strongly suggests that Ln-L complexes are kinetically stable even with a large excess of IDA. Quite unexpectedly, the order of migration differs from that of the atomic number, inverting at Nd. This is due to the effect of the cavity size of the residual coordination sites on the ternary complexation and the electronic density of Ln^{3+} .

Introduction

Capillary electrophoresis (CE) is an analytical method with excellent resolution, currently being widely and successfully applied to various organic and inorganic compounds [1-5]. With respect to the detection of metal ions, a number of systems have been reported [6-8]. In most of them, complexing reagents are included in the carrier buffer solutions, playing a role both in the detection and the separation of the analyte metal ions. Containing the detection reagents in the carrier solution allows for excellent resolution based on the dynamic complexation equilibrium [9], but it also gives baseline fluctuations due to the light-absorptivity of the reagents themselves, which results in compromised detection limits (10^{-5} - 10^{-6} mol dm⁻³ in general). In addition, although both direct and indirect detection were employed in the UV region in most CE systems, a number of organic compounds in samples frequently interfere with UV detection.

To improve the baseline fluctuations in on-capillary methods, pre-capillary complexing methods without a detection agent in the carrier buffer were developed [10-15]. In contrast to on-capillary complexing CE, in which signals of metal complexes are superimposed on a large background signal, the pre-capillary technique makes full use of detector potential. In those pre-capillary systems, it is reported that the use of chelating reagents with a chromophore possessing light-absorptivity in the visible region provides low detection limits in 10^{-7} - 10^{-8} mol dm⁻³ levels. However, those systems require kinetic inertness of the metal complexes on dissociation for detection. When the complex bands are spatially separated from the ligand band in the capillary tube, a concentration jump takes place, i. e. the absence of the ligand around the complexes. This provides a strong driving force to decompose the complexes during migration. Therefore, only kinetically inert complexes could be detected. Moreover, unlike on-capillary techniques, metal complexes must be separated without a dynamic complexing equilibrium. Generally, since the large metal complexes are of almost the same size and charge, they migrate to each other in a very similar fashion. This frequently results in low resolution among metal ions in the pre-capillary technique. It is something of dilemma as to whether to accept a poor detection limit in on-capillary complexing mode with auxiliary ligands or accept low selectivity in the pre-capillary complexing mode without an on-capillary complexing equilibrium.

Although there have been reports in the past about the mutual separation of lanthanide ions (Ln³⁺), at present there is a high level of interest in the complete separation of those ions with high detectability using CE. Ln³⁺ is also characterized by a high detection limit (above several μ mol dm⁻³) and an unpractical UV detection due to its on-capillary complexation. While it was difficult to find an appropriate ligand with the requirements for Ln³⁺ ions for use in the above-mentioned pre-capillary derivatizing method, we used the pre-capillary complexing system with UV direct detection with 1-(4-aminobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetic acid (ABEDTA) as a light-absorption ligand for Ln³⁺ [16] based on dissociation kinetic studies [17, 18]. In this system, the

Ln^{3+} complexes were detected as kinetically stable complexes. In addition, mutual separation among Ln^{3+} ions was carried out based on the concept of utilizing the dynamic ternary complex formation with the carbonate ion, $[(\text{Ln-abedta})\text{-CO}_3]^{3-}$, though a complete separation among 14 Ln^{3+} ions was not achieved; Sm, Eu and Gd complexes were detected at the same migration time. Since Ln^{3+} ions have generally 8-10 coordinate sites, 2-4 coordination sites on Ln^{3+} probably remained hydrated (EDTA was 6-fold coordination) [19-22]. Those residual first-coordination sites in the mother complexes were used for dynamic coordinating with an auxiliary ligand in the carrier buffer. This concept is completely unique in terms of the system design: it involves kinetically inert and labile complexation reactions on one center-metal ion. Its attractiveness stems from the fact that it compensates for the disadvantages of the direct detection method without the addition of ligands in the carrier buffer; that is, both high **detectability** and high resolution are accessible. There have been no reports about the analytical utilization of dynamics on the residual coordination sites in the mother complex. There are, however, two problems with the ABEDTA method: a) the detection wavelength was in the UV region ($\lambda_{\text{abs}} = 242 \text{ nm}$), which was not practical for real samples, b) the auxiliary ions for ternary complex formation were limited to the use of inorganic anions since the addition of organic ligands caused a fluctuation in the baseline due to UV-absorptivity. In this paper we introduce a newly synthesized ABEDTA derivative with a high molar absorption coefficient in the visible region, L (Figure 1), and will report on the use of adding organic ternary complex agents to a pre-capillary complexing CE.

Materials and methods

Chemicals

All solutions were prepared using deionized water (over 18 M Ω) by Milli-Q SP. TOC. System (Millipore Co., Bilerica, MA, USA). All standard solutions of lanthanide ions were prepared by dissolving chloride salts (99.9 % purity, Wako Pure Chemical Industries, Japan) with a few drops of concentrated hydrochloride solution to $1.0 \times 10^{-2} \text{ mol dm}^{-3}$. The factors of the concentration of the solutions were determined by EDTA titration using Xylenol Orange as an indicator. The reagents for the synthesis of L, 1-(4-aminobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetic acid (ABEDTA, more than 90 % purity) and 4-fluoro-7-nitrobenzofran (NBD-F) were purchased from Dojindo Lab. (Kumamoto, Japan). The following ternary complex reagents were employed: iminodiacetic acid (IDA), 2,6-pyridinedicarboxylic acid (DPA, 98 % purity, Tokyo Kasei Kogyo Co., LTD., Japan), nitrilotriacetic acid (NTA), Tiron (98 % purity, Dojindo Lab., Japan), salicylic acid (SA, 99.5 % purity), sodium carbonate (99.7 % purity, Wako Pure Chemical Industries, Japan) and glycine (99.0 % purity, Kanto Kagaku, Tokyo, Japan). Boric acid (Superpure, 99.8 % purity, Merk, Darmstadt, Germany) as pH buffer in carrier solutions was dissolved in deionized water and the pH was adjusted to desirable values of 9-10 with 3 mol dm $^{-3}$ ultra-pure sodium hydroxide solution

(Kanto Kagaku, Tokyo, Japan).

Synthesis of NBD-ABEDTA

A reagent, NBD-ABEDTA (L or H₄L, Fig. 1) was easily synthesized by mixing a 25 mL aqueous solution of 1×10^{-3} mol dm⁻³ ABEDTA with a 2.5 mL solution of 1×10^{-2} mol dm⁻³ NBD-F solution and then made up to 50 ml at pH 5.0 with acetate buffer (2×10^{-3} mol dm⁻³). The mixed solution was left in a thermostat at 298 K for 24 hours. At higher (8-10) and lower pH value (2-3) in mixture solutions, the addition reaction seems either not to proceed or to produce byproducts. The reaction was completed in about 17 hours, which was ascertained by fluorescent measurements at 355 nm and 550 nm (excitation wavelength at 235 and 469 nm) corresponding to emissions originating from aminobenzyl moiety in ABEDTA and nitrobenzofrazan moiety in NBD-ABEDTA, respectively. The color of the mixture turned from pale pink to dark red during the reaction. The mixture was acidified with drop-wise concentration HCl at pH 3.0. A dark red precipitate of L was obtained after allowing the mixture to stand for 24 hours in the dark at 277K, and then filtered and dried. The yield of L was more than 81 % (0.0114g). Purity of above 98 % was confirmed using CE and TLC. ¹H NMR data (500 MHz, D₂O/NaOD, 293 K): δ (ppm) 2.28-2.35(m, 2H), 2.53 (m, 1H), 2.73 (s, 1H), 2.76 (s, 1H), 2.87 (m, 1H), 3.04-3.18 (m, 5H), 3.28 (s,1H), 3.31 (s, 1H), 6.43 (d, 1H, J = 10.7 Hz), 7.05 (d, 2H, J = 8.3 Hz), 7.32 (d, 2H, J = 8.3 Hz), 7.72 (d, 2H, 10.2 Hz). FAB-MS ([M-H]⁻): m/e = 559.08 (Calculated for C₂₃H₂₃N₆O₁₁: 559.14). The stock solution of NBD-ABEDTA was prepared by simply dissolving the powder in ultra pure water.

Capillary electrophoresis

Experiments were performed on a Bio-Focus 3000 capillary electrophoresis instrument (Bio Rad Laboratories Japan, Tokyo, Japan) equipped with UV/Vis absorbance detector under constant voltage mode. We employed a fused-silica capillary tube (an inner diameter, 50 μ m; a outer diameter, 375 μ m; total length, 60.0 cm; effective length to the detection window, 53.4 cm) obtained from Scientific Glass Engineering Inc. (Austin, TX). The temperature of the capillary column was set at 293 K using a thermostat device equipped with the CE apparatus. New capillary tubes were pretreated by rinsing with 1 mol dm⁻³ solution of NaOH for 20 minutes and deionized water for 20 minutes. Absorption spectra were measured using a SHIMADZU UV2400PC spectrophotometer. Solution pH values were measured with a HM26s pH meter (TOA Electrics, Tokyo, Japan).

Typical procedure

A stock solution of NBD-ABEDTA was added to standard sample solution of Ln³⁺, and then the solution was mixed with a borate pH buffer solution to adjust the pH value to 9-10. A prepared solution of 3.5×10^{-4} mol dm⁻³ of L and 1.0×10^{-3} mol dm⁻³ of borate was left to stand for 5 minutes,

followed by a hydrostatic injection with nitrogen gas pressure (5 psi × sec) at the capillary cathodic end. From the pressure value, it was calculated a volume of 8 nL was injected. The complexes were detected with on-capillary absorbance at 469 nm. The capillary tube was rinsed with a carrier buffer solution for 5 minutes between each CE run. A constant voltage mode was employed at 20 kV.

Results and Discussion

Spectroscopic property of L and Ln-L complexes

The synthesized L was complexed with each of the Ln³⁺ ions. The absorbance spectra of L and Ln-L complexes were almost the same (Fig. 2). The molar absorption coefficients of L and Ln-L complexes were obtained as $2.4 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ dm}^3$ at 469 nm. It is noted that larger coefficients and the longer absorption wavelengths were observed than those of other polyaminocarboxylate reagents used in previously reported CE experiments, such as EDTA [9, 23-26], CyDTA [27], NTA [28], TTHA [29] and Quin2 [14], which made the absorbance CE detection more sensitive and selective.

Electrophoresis of Ln-L complexes

The electrophoresis of Ln-L complexes was carried out under various pH conditions. The detection of Ln complexes without the addition of L to the carrier indicates that the Ln-L complexes are a kinetically stable species in terms of migration time. All the Ln-L complexes were detected in a pH range from 8-11, though the peaks were strongly diminished when pH values exceeded 12.3. In our previous study, the solvolytic dissociation process of Ln-abledta complexes did not take place even at pH 12.2 [16]. This implies that the collapse was not due to the dissociation of Ln ion from the complex but to the decomposition of light-absorption moiety in the ligand as a result of hydrolysis. Below pH 7, the peaks were broader. It is strongly suggested that a proton-assisted dissociation reaction occurs. While the dissociation kinetics of some Ln-polyaminocarboxylate has been studied [17, 18, 30-36], slow solvolytic dissociation processes have been shown to take place predominantly at relatively neutral-high pH. For example, the half-lives of solvolysis for Ce-edta complex and Ce-cydtA were about 1 hour [37] and 21 minutes [17], respectively, and it was sufficiently inert for detection in CZE.

Without a ternary complexing reagent in the carrier buffer, poor mutual separation was achieved, as shown in Fig. 3. The electropherogram at pH 12 was rather similar to that of Ln-abledta complexes (data not shown) [16]. The order of migration was found to be from light to heavy rare earth ions, which was reasonable in terms of the ionic radii of Ln³⁺, and this tendency has been observed in other reports about Ln³⁺ separation [27, 38-41]. Regarding the ligand design for CE, a large ligand frame, such as NBD-ABEDTA, is generally undesirable because the increase of the ligand size hardly makes any difference in the size of the complexes if the center-metal ions is different, which

play a central role in controlling the mobility of the complexes. In the use of Ln-L complexes, a high resolution based on differences in molecular size is not expected.

Employment of ternary complex reagents.

In order to improve resolution among Ln ions, various ternary complex systems were examined. There are several studies with respect to ternary complex formation with Ln-polyaminocarboxylate. In those studies, formation reactions of Ln-polyaminocarboxylate-L' complexes (L' is an abbreviation for a ternary complex reagent) were found using NMR, CE and fluorometry; carbonate, citrate, phosphate [42], iminodiacetate [43, 44], nitrilotriacetate [43], fluoride [45], 8-hydroxyquinoline-5-sulfonate (HQS) [46] and catecholamines [47, 48]. Horrocks et al. reported on the dynamics of the ternary Eu-dcta complex with iminodiacetate using a selective laser excitation method and determined the rate of formation and dissociation reaction of the ternary complex to lie at the sub millisecond level [49]. As additives to the carrier buffer for ternary complex formation, the following reagents were investigated; carbonate (a bidentate with one four-membered ring), EDTA, CyDTA (hexadentates with five five-membered rings), dipicolinic acid (a tridentate with two five-membered rings), Tiron (a bidentate with one five-membered ring) and iminodiacetate (a tridentate with two five-membered rings). Halides, HQS and catecholamines were not employed in this study due to the low stability of complex formation and the redox. It was expected that a number of residual coordination sites and a number of atoms participating in the chelate ring and the electric charge of the auxiliary ligand provide different charges and sizes of ternary complexes, which have altered mobility depending on the Ln ion involved. Although the addition of a carbonate ion gave a similar resolution to that previously reported [16], peak heights were very small due to ligand decomposition at high pH values. It was found that all the Ln peaks broadened considerably when the multidentates EDTA and CyDTA were added to the carrier as auxiliary ligands. Since these agents were highly nucleophilic, a ligand exchange reaction ($\text{Ln-L} + \text{L}' \rightarrow \text{Ln-L}' + \text{L}$) was very likely to take place. The rate of ligand exchange reaction of Ln-polyaminocarboxylate complex with other polyaminocarboxylates is in an order higher than that of the solvolytic dissociation process [17]. The use of Tiron merely resulted in a slightly improved resolution. Although finding an appropriate ternary complexing agent was difficult due to the involvement of dissociation kinetics and formation equilibrium, the auxiliary agents, IDA and DPA, showed prominent separation performance. It seems that doubly negative charged and tridentate ligands are best suited for Ln separation. In the DPA system, all the detected Ln complexes were completely separated. However, no peaks for the two light rare earth complexes, La and Ce, were observed. By contrast, the employment of IDA as an additive to the carrier buffer allowed for the detection of all the Ln complexes. Since the stability constants of the Ln-dpa complexes [50] exceed those of the Ln-ida complexes [51] by more than four orders of magnitude, it is very probable that the ligand-exchange reaction of the DPA system is

more active, though one should not illogically relate thermodynamics with kinetics. These results show that ternary complex agents with multiple negative charges and weak but sufficient coordinating ability are the most desirable.

Optimization and determination of Ln ions

The optimum conditions for mutual resolution on the Ln-L-ida system were investigated as shown in Fig. 4. The mobility of Ln-L complexes decreased with increasing IDA concentration. This strongly suggests that a dynamic ternary complex formation equilibrium takes place. It is possible to estimate the population of the Ln-L-L' ternary complex based on the stability constants for Ln-edta-ida complexes; $10^{3.48}$, $10^{4.23}$, $10^{2.55}$, $10^{2.51}$ mol⁻¹ dm³ for Pr, Eu, Yb and Lu [43], respectively, and the Ln-L complexes of 91-99 % formed a ternary complex when an IDA of 33.5 mM was added to the carrier. The difference of population depending on the center metal ion seemed to control the resolution. In contrast to IDA concentration, borate concentration was influential in migration in a positive direction (Fig. 4 b). This fact implied that borate ion did not form a ternary complex with Ln-L, and the marked influence of sodium ion in the buffer probably altered the mobility of the complexes in a positive direction due to the weak ion-pair or ion atmosphere of sodium cations. The effect of pH value on mobility was also investigated. The mobility has a minimum value at pH 9.5. It is likely that the conditional stability constant of the ternary complex by the proton dissociation of IDA ($pK_{a2} = 9.33$) [52] governs the mobility of complexes at pH 9.3-9.5 and the effect of the sodium ions (as mentioned above) is predominant beyond pH 9.5. The pH value was set at 9.5, at which the mobility was most negative.

A typical electropherogram under optimum conditions is shown in Figure 5. High resolution among Ln ions was achieved with the exception of Pr and Sm, while baseline Pr-Sm separation was possible at different IDA concentrations and pHs, but the separation of an alternative pair of Ln ions was not as clear. The detection limit of this system was typically determined as 4.2×10^{-7} mol dm⁻³ (73 ppb, 3.4 fmol as amount basis) for Lu³⁺. This **detection limit** is remarkable without pre- or on-column concentration (typically μ M level) [38], though the detection of low nano molar levels was successfully achieved by combining an on-capillary complexation system and an electrokinetic supercharging technique [53]. One might presume that the collapse of the peaks of metal complexes in the concentration range around the limit of detection takes place, which is a phenomenon known as the bleeding effect (the complex gradually decomposes, breaking down into its components). This effect causes significant problems in the field of quantitative analysis, especially when the injected amount is small. In our system, however, the calibration line typically for Lu³⁺ was observed. Good linearity was obtained in the range from 5×10^{-7} to 1.2×10^{-5} mol dm⁻³ ($R^2 = 0.9966$) and was retained around the detection limit. Apparently, the bleeding effect did not take place in this system. This fact further supports the supposition that spontaneous dissociation of

the Ln complex hardly proceeds in small concentration ranges. With respect to the interference of other common metal ions (Ca^{2+} , Mg^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} , Pb^{2+} , Cd^{2+} and Hg^{2+}), no peak was observed. It is likely that these metal complexes decomposed through a ligand-exchange reaction with a large excess of IDA. It is noted with interest that Ln complexes can resist the nucleophilic attack of IDA through the formation of the ternary complex.

Migration order of Ln

It is a matter of great interesting that the order of migration in the IDA ternary complex system was different from that of the atomic number such as in the carbonate system [16]. The migration of Ln complexes in the IDA system was in order of the atomic number from ^{57}La to ^{60}Nd and in reverse order of atomic number from ^{60}Nd to ^{71}Lu (show Fig. 5 and 6). This inversion is unusual in the separation science of Ln ions [38]. To our knowledge, the inverted separation was observed only in the reversed-phase HPLC system of Ln complexes with Quin2, an octadentate aromatic polyaminocarboxylate ligand [54]. In that research, inversion was observed at the Ho complex. This unique migration behavior could be related to the density of the electric charge and the number of the residual coordination sites on the center-metal ions. The relationship among the mobility of the Ln-L complexes, the number of coordinated water molecules of Ln-edta complexes [19-22] and stability constant for ternary complex formation of Ln-edta-ida [43, 44] is depicted in Fig. 6. The density of the electric charge generally increases with a decrease in the Ln ion radius, i.e. the density increases according to the atomic number. It is expected that the larger charge density on hard metal ions, such as Ln ions, provides a stronger bond to ligands with charge-charge interaction, which leads to a larger stability constant. Separation has been carried out on the basis of the difference of the complexing stability among Ln ions in most separation systems [27, 38]. In those studies, heavier (harder) Ln^{3+} , which more strongly forms an anionic complex, migrates more swiftly toward the positive end when anionic ligands are employed. This results in detection in the order of the atomic number. In our ternary complexing system, a similar principle probably was at work when carbonate ions were employed as auxiliary ligands, because the molecular size of the carbonate ion is small (a bidentate, a four-membered ring) enough to bind to the residual sites on the Ln ions (the number of sites is 2-4) without strain or electric repulsion in order to form the $[\text{Ln-L-CO}_3]^{3-}$ ternary complex. On the other hand, the employment of IDA (a tridentate, two five-membered rings), probably caused structural hindrance and electric repulsion to binding to the heavier Ln mother complex since the cavity of the residual coordination sites (the number is minimally two) is too small for IDA to bind to the mother complex. Therefore, in the formation of the ternary complexes, $[\text{Ln-L-ida}]^{3-}$, the effect of the charge density of the center metal ion probably competes with that of hindrance and repulsion. It is likely that electric charge effect predominates in light Ln ions (La-Nd), and the structural and electric repulsion effect is predominant in heavier ones (Sm-Lu), which results in the inversion of the

migration order. This tendency corresponds to that of the equilibrium constant of $[\text{Ln-edta-ida}]^{3-}$ complex, $K_{\text{Ln-edta-ida}}$, and the number of coordination of the water molecule, q , in $[\text{Ln-edta}(\text{H}_2\text{O})_n]^{3-}$ complex; the value of $K_{\text{Ln-edta-ida}}$ turns up at Eu ion, and q decreases with an increase in the atomic number.

Concluding remarks

We developed a new simultaneous direct detection system for Ln ion with a low detection limit in the visible region. The resolution in this system was inferior to some on-capillary complexing systems in this state, such as α -hydroxyisobutyric acid (HIBA) system [55], in which complete separation of Ln^{3+} was achieved. However, the uniqueness of the concept in our system is noteworthy; the residual coordination sites on a center-metal in a kinetically inert mother complex are utilized in order to manipulate resolution. This concept utilizes the advantages of both on-capillary and pre-capillary complexation methods. Other chemical systems using other polyaminocarboxylate frames will be available since some ternary complex systems have been reported, such as Ln-dtpa-PO_4^{3-} , $-\text{CO}_3^{2-}$ [42], $-\text{F}^-$ [45] and Ln-dota-PO_4^{3-} , $-\text{CO}_3^{2-}$ [42] Ln-d03a-ATP , -phosphocreatine system [56].

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Legends

Figure 1 Synthesis of NBD-ABEDTA (abbreviated to L or H₄L).

Figure 2 Absorbance spectra of Ln-L complexes and L.

[L] = 1.0×10^{-5} mol dm⁻³, [Ln³⁺] = 1.0×10^{-5} mol dm⁻³, [Borate] = 1.0×10^{-3} mol dm⁻³ (pH 9.50). ×, La and Pr-Lu complexes; ●, Ce complex; —, L.

Figure 3 Typical electropherogram of Ln-L complexes without an auxiliary ligand in the carrier buffer. Sample, [Ln³⁺] = 2×10^{-5} mol dm⁻³, [L] = 4×10^{-3} mol dm⁻³, [Borate] = 10 mmol dm⁻³. Carrier buffer, [Borate] = 20 mmol dm⁻³ (pH 11.7 with NaOH).

Figure 4 Effect of electrolyte concentration in the carrier on electrophoretic mobility of Ln-L complex. ○, La; ●, Ce; ▲, Nd; □, Tb; ■, Lu complex. Left: [Borate] = 15 mmol dm⁻³ (pH 9.50), [IDA] = 32-40 mmol dm⁻³. Middle: [Borate] = 5-15 mmol dm⁻³ (pH 9.50), [IDA] = 30 mmol dm⁻³. Right: [Borate] = 15 mmol dm⁻³, [IDA] = 40 mmol dm⁻³, pH 9.3-9.8.

Figure 5 Typical electropherogram of Ln-L complexes with IDA as an auxiliary agent in carrier buffer. Sample, [Ln³⁺] = 5×10^{-6} mol dm⁻³, [L] = 1×10^{-2} mol dm⁻³, [Borate] = 1.0 mmol dm⁻³. Carrier buffer; [Borate] = 9.5 mmol dm⁻³ (pH 9.45), [IDA] = 33.5 mmol dm⁻³.

Figure 6 Mobility of Ln-L complexes, stability constant for Ln-EDTA-ida ternary complexes and a number of metal-bonded water molecule. ○, mobility of Ln-L complexes; ■, stability constant of Ln-edta complex with IDA [43, 44]; ▲, a number of water molecules bound to center metal ion [19-22].