

Direct Fluorescence Detection of **Ultratrace** Lanthanide(III) Ions Complexed with Aromatic Polyaminocarboxylate Avoiding Quenching of Ligand-Centered Emission Using Capillary Zone Electrophoresis with Ternary Complexing Technique

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Ultratrace level detection and **the** separation of lanthanide ions (Ln^{3+}) was achieved using capillary zone electrophoresis with laser induced fluorescent detection (CZE-LIF) using an aromatic polyaminocarboxylate ligand synthesized in our previous work. The ligand forms kinetically stable Ln complexes at the pre-capillary derivatizing step and effectively avoids quenching processes of the ligand-centered fluorescence through complexation with Ln^{3+} without paramagnetic and heavy atom effects because of the distance between the chelating and the antenna moiety. During the on-capillary separation step, the mother Ln complexes competitively form ternary complexes with the auxiliary ligands, iminodiacetate and citrate, which provide different mobility for each **of the** Ln^{3+} complexes. The emissively labeled Ln^{3+} complexes were efficiently separated based on the ternary complex equilibrium. Since the carrier buffer employed was free from emissive ligands, **a** high signal to noise ratio was obtained by its silent baseline. A **lower** detection limit of 9.1×10^{-11} mol dm^{-3} (15.6 ng dm^{-3} , 0.46 atto mole as an amount basis) was successfully achieved typically for Lu^{3+} with **a** simple CZE mode. We propose a combination mode of a pre-capillary and an on-capillary complexing technique as a method **which provides both** high sensitivity and high resolution.

Introduction

There is still a high level of interest in the separation and detection of lanthanide ions (Ln^{3+}) using capillary electrophoresis (CE).¹⁻⁴ Various modes of CE have been examined, such as on-capillary and pre-capillary techniques for the derivatizing modes, spectrophotometry and fluorometry for the detection modes, and on-line concentration modes for obtaining higher sensitivity. One of the most **effective** systems uses hydroxyisobutylic acid (HIBA) as an on-capillary complexing agent.⁵ When a combination of two on-line concentration techniques

(electrokinetic injection and transient isotachopheresis), known as electrokinetic supercharging, was applied to the HIBA system, complete separation was successfully achieved with nano mol dm^{-3} level sensitivity, the highest among all the CE works for Ln^{3+} to our knowledge.⁶ The disadvantages of this method are the inevitable employment of UV detection and rather low reproducibility when compared to the simple CZE mode. The on-line preconcentration technique using the difference of conductivity between the carrier buffer solution and samples could not avoid fluctuations which occur depending on the nature of samples.

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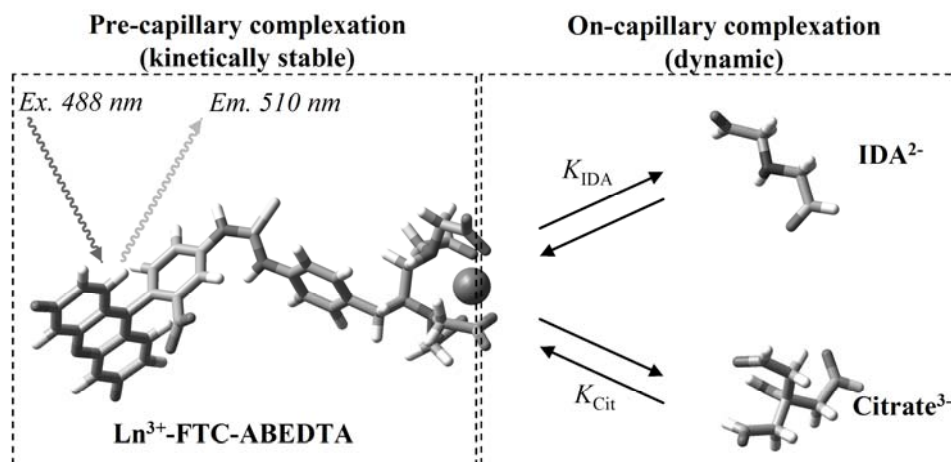


Fig. 1 The chemical system in this work combined between pre-capillary complexation (the Ln-L mother inert complex) and on-capillary ternary complexation (Ln-L and the auxiliary reagents, IDA and citrate).

We developed a CZE method with spectrophotometric detection in the visible region ($\lambda_{\text{abs}}/\text{nm}$ 469) for Ln^{3+} .⁷ In this method, a combination of a pre-capillary technique of Ln^{3+} mother complexes ($[\text{Ln-NBD-ABEDTA}]^+$) with a polyaminocarboxylate (1-(4-aminobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetic acid, abbreviated as ABEDTA) attached to a nitrobenzofurazan (NBD) as a light-absorption moiety and an on-capillary ternary complexation technique of mother complexes with iminodiacetate (IDA) was successfully achieved (the detection limit was typically $4.7 \times 10^{-7} \text{ mol dm}^{-3}$).⁷ After the complexation of Ln-NBD-ABEDTA in the pre-capillary process, the ternary complexation of Ln-NBD-ABEDTA with IDA took place during the on-capillary separation processes. Ternary complexation was possible since the EDTA-like complexes (hexadentate) with Ln^{3+} (octa or nonacoordinate) had 2-3 residual coordination sites to be bound with other small ligands.⁸⁻¹¹ In this system, the net charge and size of each of the Ln mother complexes was controlled through a ternary complexing equilibrium, which led to high separation efficiency. It was also noted that the mother complexes must be kinetically stable species for detection because of the strong driving force to dissociate the complexes ($\text{Ln-L} \rightleftharpoons \text{Ln}^{3+} + \text{L}$; k_d) which occurs when the complexing equilibrium ($\text{Ln}^{3+} + \text{L} \leftrightarrow \text{Ln-L}$; K_{ML}) breaks down at the point when the band of Ln-L completely separates from that of L without the addition of NBD-ABEDTA in the carrier buffer solution, i.e. when the concentration of L in the Ln-L band falls to very low concentrations. This technique is characterized by both dynamic and inert complexation on one center metal ion. The absence of L in the carrier led to low baseline noise and provided the high sensitivity with a high signal to noise ratio. Although this system is effective, there is still a problem in that the sensitivity is rather low compared with instrumental methods and CE with on-line concentration techniques. However, the collaboration between a pre- and an on-capillary complexing technique, which has been reported in a series of our previous studies,^{7,12-14} indicates a potential gain in the advantages of both modes; high sensitivity and high resolution.

Recently, we developed a complexing system using FTC-ABEDTA (abbreviated as H_6L or L , and shown in Fig. 1) (FTC = fluorescein-thiocarbamyl), in which it was revealed that the quenching process of a ligand-centered emission by paramagnetic and heavy atom effects did not take place at all in the metal-L complexes. In the complexes, the electric charges of the chelating and the antenna moieties and the distance between the moieties was designed to avoid the quenching processes.^{12,15} It is expected that the L complexes with Ln^{3+} ions, which generally quench ligand-centered emission, also possesses emissive characteristics, and can be applied to direct fluorescence detection. The direct fluorescence detection of Ln^{3+} in CE has never been reported, but there are some reports using indirect CZE detection with a laser-induced fluorescence (LIF) method,^{1,3,16} in which detection limits are no lower than in the range of $10^{-7} \text{ mol dm}^{-3}$. Furthermore, with respect to separation, since L has a similar structure to NBD-ABEDTA , which has been successfully applied to separate Ln^{3+} ions, the L is a potential candidate for the combined mode of a pre-capillary derivatizing and an on-capillary dynamic ternary complexing technique. In this report, we demonstrated a highly sensitive method at sub atto mole levels for Ln^{3+} using the mother complex of L by CZE-LIF together with an on-capillary ternary complexing technique.

110 Experimental

Chemicals

The ligand, FTC-ABEDTA , was synthesized as was previously reported.¹² The stock solutions of L and all other reagents were prepared using deionized water (over 18 MW) 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 Ln^{3+} solutions were determined by the EDTA titration method using Xylenol Orange. The following

ternary complex reagents were employed: iminodiacetic acid (IDA), nitrilotriacetic acid (NTA, 99.0 % purity, Tokyo Kasei Kogyo Co., LTD., Japan), trisodium citrate dihydrate (99 % purity), tartaric acid (analytical grade), sodium carbonate (99.7 % purity, Wako Pure Chemical Industries, Japan) and glycine (99.0 % purity, Kanto Kagaku, Tokyo, Japan). Boric acid of high purity (Superpure, 99.8 % purity, Merk, Darmstadt, Germany), used as pH buffer in the carrier solutions, was employed to prevent any serious contamination of Ca ions (*vide infra*). It was dissolved in deionized water and the pH was adjusted to the desirable values of 9-10 with 3 mol dm⁻³ ultra-pure sodium hydroxide solution (Kanto Kagaku, Tokyo, Japan).

Apparatus

The fluorescence spectra were measured using a Shimadzu model RF-1500 spectrofluorometer (Kyoto, Japan). The CE-LIF setup used was made with a voltage supply, a HCZE-30P model purchased from Matsusada Precision Inc. (Shiga, Japan), and a LIF detection system, ZETALIF (Picometrics, Ramonville, France) equipped with a Model 163D 25 mW argon laser (Spectra-Physics, CA, USA). A fused-silica capillary tube (inner diameter, 50 μm; outer diameter, 375 μm; total length, 60.0 cm; effective length to the detection window, 46.5 cm) obtained from Scientific Glass Engineering Inc. (Austin, TX) was employed. New capillary tubes were pretreated by rinsing with 1 mol dm⁻³ solution of NaOH for 60 minutes and deionized water for 60 minutes. All carrier buffer and rinsing solutions in the capillary tubes were exchanged under reduced pressure by a vacuum pump.

Sample preparation and electrophoresis

A solution of L and a pH buffer solution of borate were added to a Ln sample solution. Typical final concentrations in the sample solution were 2.0 × 10⁻⁶ and 1.0 × 10⁻³ mol dm⁻³ for L and borate, respectively, and the pH value was set at 10.0. After one minute standing the prepared sample solution was injected to the capillary tube from the cathodic end (Δ5cm × 72 sec, 5 nL). A high voltage of 20 kV was applied (typical current value was 60-70 μA). The argon laser and the voltage of photomultiplier of detector were set at 8 mA and 570 V, respectively.

Results and discussion

Emissive property of the complexes and separation by CZE without a ternary complex agent

The Ln complexes with L had strong emissive characteristics as **was anticipated**. The fluorescence spectra of all Ln-L complexes were the same shape as that of a free ligand (λ_{ex}/nm 492, λ_{em}/nm 512) (Fig. 2). The ligand design to avoid paramagnetic and heavy atom quenching was described in detail in our previous paper.¹⁵ The excitation maximum wavelength at 492 nm was well suited to LIF detection using an argon laser (λ_{ex}/nm 488). In our system, no fluorescent ligand (L) was added to the carrier buffer. If all the Ln ions **formed** inert complexes on dissociation process, all the Ln ions could be detected using LIF detection.

The typical electropherogram of the Ln-L complexes using a CZE mode without any ternary complex reagents in the carrier solution is shown in Fig. 3. Although the separation among Ln ions resulted in low resolution, at least **each of the fourteen Ln complexes was detected with sharp peak shapes**. This strongly indicates that the Ln-L complexes are kinetically stable species in the separation process. In addition, this was the first time that each of Ln³⁺ ions was detected simultaneously using direct fluorescence detection.

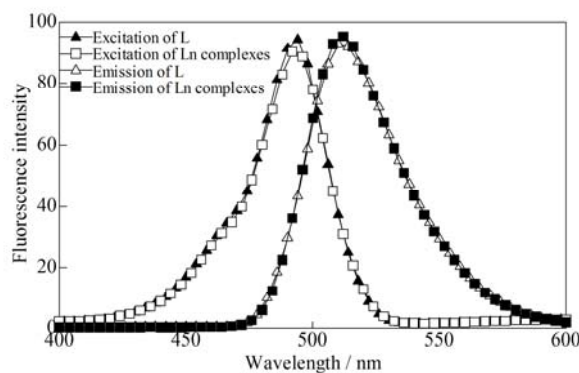


Fig. 2 Fluorescent spectra of FTC-ABEDTA (L) and Ln³⁺-L complexes. C_L = C_{Ln} = 1.0 × 10⁻⁷ mol dm⁻³; C_{borate} = 1.0 × 10⁻³ mol dm⁻³ (pH 9.9). Excitation and emission wavelengths are at 493 nm and 513 nm, respectively. Band pass, 10 nm.

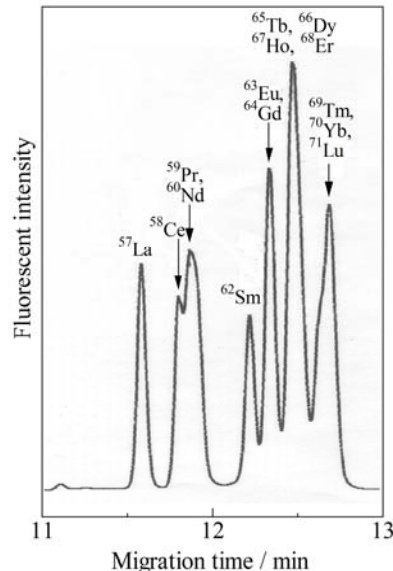


Fig. 3 Typical electropherogram of Ln-L complex with CZE-LIF. Sample, C_{Ln} = 2.0 × 10⁻⁸ mol dm⁻³; C_L = 2.0 × 10⁻⁶ mol dm⁻³; C_{borate} = 1.0 × 10⁻³ mol dm⁻³ (pH 10.0). Carrier buffer, C_{borate} = 1.2 × 10⁻² mol dm⁻³ (pH 10.50).

Separation by CZE with ternary complex agents

Various organic ligands were examined for the on-capillary ternary complexation separation technique in order to improve the resolution; IDA, citrate, nitrilotriacetic acid tartarate carbonate and glycine. In our previous work, the agent, IDA,

provided a desirable effect for the separation of Ln-NBD-ABEDTA system; 13 peaks for 14 Ln ions (only the Pr and Sm peaks overlapped).⁷ However, the single use of IDA resulted in only ten separated peaks for the present system; four Ln peaks overlapped. This, interestingly, indicates that the characteristics of the ternary complex formation changes if the chemical structure of the antenna moiety in the mother complex changes from NBD to FTC even when the chelating portion has the same structure (ABEDTA). After much trial and error, we found that the simultaneous addition of IDA and citrate was highly efficient for separation among Ln ions. The obtained electropherogram is shown in Fig. 4. The ternary complex formation constants of the Gd-EDTA complex with IDA and citrate have been reported; $K_{\text{Gd-EDTA-IDA}} = 10^{4.47, 11}$ $K_{\text{Gd-EDTA-Cit}} = 10^{3.56, 8}$. If the values are similar for the Ln-L system, the population is approximately 80 % and 20 % for Gd-L-IDA and Gd-L-citrate, respectively, under the conditions indicated in Fig. 4. It is emphasized that a fine-tuning of the resolution for large metal complexes is possible by competing reactions of these two kinds of ternary complexations on the center metal ion. The order of migration is as follows; $^{71}\text{Lu} < ^{70}\text{Yb} < ^{69}\text{Tm} < ^{68}\text{Er} < ^{57}\text{La} < ^{67}\text{Ho} < ^{66}\text{Dy} < ^{58}\text{Ce} < ^{65}\text{Tb} < ^{64}\text{Gd} < ^{63}\text{Eu} < ^{59}\text{Pr} = ^{62}\text{Sm} < ^{60}\text{Nd}$. This order is slightly different from that of NBD-ABEDTA system; $^{71}\text{Lu} < ^{70}\text{Yb} < ^{69}\text{Tm} < ^{57}\text{La} < ^{68}\text{Er} < ^{67}\text{Ho} < ^{66}\text{Dy} < ^{65}\text{Tb} < ^{58}\text{Ce} < ^{64}\text{Gd} < ^{63}\text{Eu} < ^{59}\text{Pr} = ^{62}\text{Sm} < ^{60}\text{Nd}$.⁷ This slight difference is likely due to the effect of the ternary complexation with citrate, and suggests that using competing ternary complexation on residual coordination sites is useful for fine tuning the resolution. The inverted migration order with respect to atomic number was explained by the stability of the ternary complex formation on residual coordination sites in the mother complexes; that is, there is a balance between the electrostatic binding energy and the steric hindrance on ternary complexation. The electrostatic binding energy generally increases as the charge density of Ln^{3+} increases along with the atomic number due to the lanthanide contraction, and the steric hindrance increases with the atomic number due to the reduction of the number of the residual coordination sites to form ternary complexes along lanthanide contraction. That is, the electrostatic binding energy and the steric hindrance change with opposite tendencies to each other regarding complexation stability.

Although the resolution was improved among Ln ions, a noticeable saddling phenomenon between the large peak at 19 minutes and the Ce peak at 22.5 minutes was observed (see Fig. 4). The ultratrace detection of Er, La, Ho, Dy, Ce and Tb was difficult due to the interference of the electropherogram. The large peak most probably originated from contaminant calcium ions in the carrier buffer and the stock solution of L. Similar behavior was observed for CE-LIF of transition metal complexes with L.¹² It seems that the saddling behavior takes place as a result of the fast kinetics of the on-capillary formation and dissociation reactions between L and Ca^{2+} . The concentration level of contaminant Ca^{2+} was at the $\sim 10^{-7}$ mol dm^{-3} level. While the contaminant Ca peak was not observed in the case of the NBD-ABEDTA system, this can be explained by the rather low sensitivity (10^{-7} mol dm^{-3} level), which was too low to detect calcium ions in contaminant

level.⁷ In this L system, the electropherogram was influenced by trace level Ca^{2+} due to its substantial high sensitivity. To avoid this effect, a polyaminocarbocylate, CyDTA (*trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid), which forms an inert complex with Ca^{2+} with a half-life of 385 minutes,¹⁷ was added to the carrier buffer solution for blocking contaminant Ca^{2+} . While the peaks of Ln complexes were broadened or diminished in the NBD-ABEDTA system when CyDTA or citrate was added to the carrier buffer (data not shown), interestingly, the electropherogram of L was cleared by the addition of CyDTA. The broadening peaks for the NBD-ABEDTA system is probably because of the rapid progress of a ligand exchange reaction of Ln-NBD-ABEDTA with CyDTA^{3-} or citrate^{3-} ($\text{Ln-L} + \text{L}'^{3-} \rightarrow \text{Ln-L}' + \text{L}$; k_{ex}).

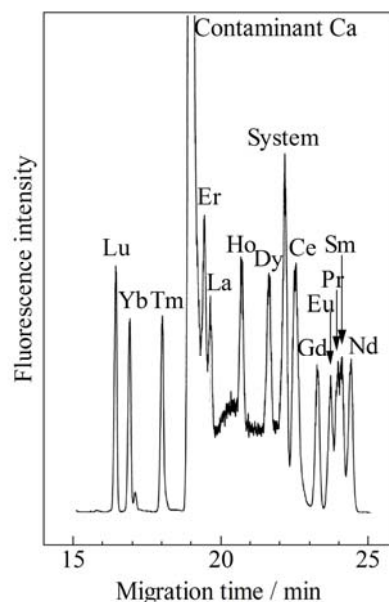


Fig. 4 Typical electropherogram of Ln-L complex with ternary complexing agents in the carrier buffer. Sample, $C_{\text{Ln}} = 2.0 \times 10^{-8}$ mol dm^{-3} ; $C_{\text{L}} = 2.0 \times 10^{-6}$ mol dm^{-3} ; $C_{\text{borate}} = 1.0 \times 10^{-3}$ mol dm^{-3} (pH 10.0). Carrier buffer, $C_{\text{borate}} = 1.2 \times 10^{-2}$ mol dm^{-3} (pH 9.60); $C_{\text{IDA}} = 5.5 \times 10^{-3}$ mol dm^{-3} ; $C_{\text{citrate}} = 9.0 \times 10^{-3}$ mol dm^{-3} .

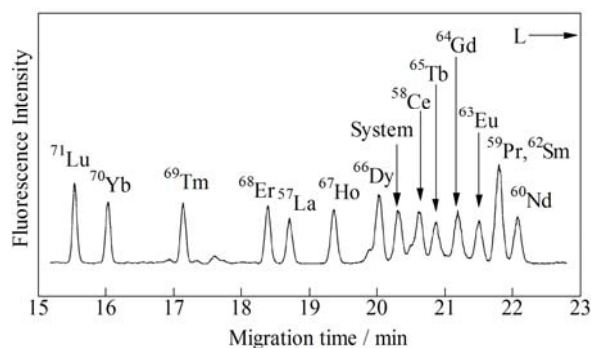


Fig. 5 Typical electropherogram of Ln-L complex under optimal conditions. Sample, $C_{\text{Ln}} = 5.0 \times 10^{-9}$ mol dm^{-3} ; $C_{\text{L}} = 2.0 \times 10^{-6}$ mol dm^{-3} ; $C_{\text{borate}} = 1.0 \times 10^{-3}$ mol dm^{-3} (pH 10.0). Carrier buffer, $C_{\text{borate}} = 1.2 \times 10^{-2}$ mol dm^{-3} (pH 9.60); $C_{\text{IDA}} = 5.5 \times 10^{-3}$ mol dm^{-3} ; $C_{\text{citrate}} = 9.0 \times 10^{-3}$ mol dm^{-3} ; $C_{\text{CyDTA}} = 2.5 \times 10^{-5}$ mol dm^{-3} .

Since Ln-L complexes exist as more negatively charged species (2- or 3-) than Ln-NBD-ABEDTA (1-), and CyDTA and citrate exist as triply charged species in this pH range, it appears to be more difficult in the L system than in the NBD-ABEDTA system to form an outer sphere complex of Ln-L \cdots L $^{3-}$ in the ligand-exchange process,¹⁸ which probably results in the process slowing down. In this manner, the L system is more resistant to the attack of auxiliary ligands by controlling the electric charge. The typical electropherogram of 5.0×10^{-9} mol dm $^{-3}$ Ln $^{3+}$ ions under optimized conditions is depicted in Fig. 5. The disturbance of the electropherogram by contaminant Ca $^{2+}$ ions entirely disappeared, and a high resolution among Ln ions was successfully achieved, except between Sm-Nd.

Determination of Ln $^{3+}$ by CZE-LIF

The linearity of the calibration curve typical for Lu $^{3+}$ was observed in the range of 5.0×10^{-10} – 1.0×10^{-7} mol dm $^{-3}$ with a correlation coefficient, $R^2 = 0.9998$. The relative standard deviations of peak height and migration time were 5.0 % and 0.6 %, respectively. The detection limit was typically 9.1×10^{-11} mol dm $^{-3}$ (15.6 ng dm $^{-3}$) for Lu $^{3+}$ based on S/N = 3. This detectability corresponds to 0.46 atto mole as an amount basis (5nl injection). This detection limit is not surprising, but rather expected with the employment of highly emissive fluorophore and a LIF detection. However, with respect to the detection of metal ions, the obtained detection limit is superior to any other conventional CE methods of more than one magnitude, including LIF detection systems¹⁶ and on-capillary concentration techniques,^{6,16,19,20} to our knowledge.

Conclusion

The field of light-switching chemical systems for sensing metal ions is attracting a considerable amount of attention.^{21,22} Our strategy in this work takes an entirely different approach to the switching system with regard to the molecular design; with not only no switching but no quenching either. Although our strategy doesn't seem so sophisticated at first glance, it integrates some interesting essential chemistry; governing the emissive properties of metal complexes by the distance control between a fluorophore and a chelating moiety, it involves the manipulation of resolution by ternary complex formation depending on residual coordination sites, and the control of the kinetics of metal complexes (spontaneous dissociation, k_d , and ligand-exchange processes, k_{ex}). This work provides a practical method to achieve high sensitivity, selectivity and simplicity by the design of chemical reactions. We have proposed a novel technique combining a pre-capillary complexing technique with an on-capillary complexing technique through a series of our reports.^{7,12-14}

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