

1       **Selective Ultratrace Detection of Al(III) and Ga(III) Complexed with a Calcein**  
2       **Isomer by Capillary Zone Electrophoresis with Laser-induced Fluorescent**  
3       **Detection**

4  
5       **Comparison of Emissive Polyaminocarboxylates as Derivatizing Ligands**

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1 **Abstract**

2 A highly sensitive and selective capillary zone electrophoresis with laser-induced fluorescent detection  
3 (CZE-LIF) for Al<sup>3+</sup> and Ga<sup>3+</sup> was developed using a calcein 4',5'-isomer as a pre-capillary complexing  
4 agent. Direct fluorescent detection without an agent added to the carrier buffer provided the notable  
5 detection limits of  $3.9 \times 10^{-10}$  and  $4.6 \times 10^{-10}$  mol dm<sup>-3</sup> for Al and Ga, respectively. By applying a river  
6 water sample successfully to this system, its potential for practical applications was ascertained. A  
7 comparison of three aromatic polyaminocarboxylate ligands related to the coordination structure and  
8 the distance between the fluorophore and the chelating moiety, revealed some interesting factors  
9 regarding their selectivity.

10 **Keywords.** Capillary zone electrophoresis, Laser-induced fluorescent detection, Calcein, Al, Ga,  
11 kinetics

12

## 1 **1. Introduction**

2 When capillary zone electrophoresis (CZE) is employed, it is difficult to obtain high sensitivity  
3 because of its short optical path length (typically 50-100  $\mu\text{m}$ ). For organic analytes, a laser-induced  
4 fluorescent (LIF) detection technique is frequently employed with detection levels of  $10^{-10}$ - $10^{-12}$  mol  
5  $\text{dm}^{-3}$  easily achievable with pre-capillary derivatization due to the covalent bonds of analytes with  
6 emissive labeling agents. For metal ions, however, such trace levels of detection are not able to be  
7 obtained if even when LIF is employed [1-7]. This is due to the customary addition of fluorescent  
8 agents for detection in the carrier buffer solution, which also plays a role as a complexing agent for  
9 separation. The emissive agents in the carrier give a substantially large baseline noise, and the signals  
10 of the metal complexes are invariably superimposed on the large background. Although the complexing  
11 agent can be omitted from the carrier buffer solution with pre-capillary complexing techniques, it  
12 presents a problem in that kinetic stability on the dissociation reaction is essential for the detection of  
13 metal ions [8-14]. The driving force to dissociate metal complexes arises when the bands of a metal  
14 complex and a ligand are completely separated from each other in the capillary, which corresponds to  
15 the concentration jump of the agent. At this point, the on-capillary complex equilibrium is no longer  
16 maintained.

17 While several detection systems using CE and employing kinetically stable metal complexes with  
18 pre-capillary complexation have been developed, fluorescent detection has been used sparingly; i.e.  
19 UV/Vis detection methods are the most employed. We previously reported on a new aromatic  
20 polyaminocarboxylate system with a fluorescein and a methyl-EDTA portion as a fluorophore and a  
21 chelating moiety, respectively [15]. The CZE-LIF of those metal chelates was demonstrated to be a  
22 highly sensitive, with  $10^{-11}$ - $10^{-10}$  mol  $\text{dm}^{-3}$  level detection limits for  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Pb}^{2+}$   
23 and  $\text{Cd}^{2+}$  ions. Interestingly, however, triply charged metal ions were not detected. On the other hand,  
24 we also reported that a calcein isomer (4',5'-calcein, abbreviated as calcein or L or **1** in this paper) was  
25 useful for the selective detection of  $\text{Al}^{3+}$  in reversed-phase HPLC with fluorescent detection, in which a  
26 detection limit of  $7.6 \times 10^{-11}$  mol  $\text{dm}^{-3}$  ( $2.1 \text{ ng l}^{-1}$ ) was successfully achieved [16]. Judging from these  
27 results, it is expected that L, which is a kind of aromatic polyaminocarboxylate ligand with two  
28 iminodiacetate and a fluorescein portions, would be suited for detecting triply charged metal ions in  
29 CE-LIF.

30 In this paper, we report on a highly sensitive CZE-LIF system for  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  using L as a pre-  
31 capillary complexing agent and discuss its selectivity, and compare L with the chemical structure of  
32 antenna and the chelating moieties of other aromatic polyaminocarboxylate ligands.

33

## 34 **2. Experimental**

### 35 **2.1. Apparatus**

1 The CE-LIF setup used in this study was assembled as follows. The voltage supply, the HCZE-30P  
2 model, was purchased from Matsusada Precision (Shiga, Japan). An LIF detection system, ZETALIF  
3 (Picometrics, Ramonville, France), equipped with a Model 163D 25 mW argon laser (Spectra-Physics,  
4 CA, USA), was employed. The Bio-Focus 3000 CE system (Bio-Rad Laboratories Japan, Tokyo,  
5 Japan) was employed to obtain electropherograms with UV/Vis absorbance detection. Fused-silica  
6 capillary tubes (inner diameter of 50  $\mu\text{m}$ , outer diameter of 375  $\mu\text{m}$ , a total length of 60.0 cm, and an  
7 effective length to the detection window of 46.5 and 55.7 cm for LIF and UV/vis absorbance detection,  
8 respectively) were obtained from Scientific Glass Engineering (Austin, TX). New capillary tubes were  
9 pretreated by rinsing with a 1 mol  $\text{dm}^{-3}$  solution of NaOH for 60 minutes and deionized water for 60  
10 minutes. All buffer and rinsing solutions in the capillary tubes were exchanged under reduced pressure  
11 by a vacuum pump. To avoid any serious contamination of  $\text{Al}^{3+}$  ions, perfluoroalkoxy (PFA) wares  
12 were exclusively employed.

## 14 2.2. Chemicals

15 The reagents, 4',5'-bis[*N,N'*-bis(carboxymethyl)aminomethyl]fluorecein (calcein, >97% purity)  
16 obtained from Fluka (Buchs, Switzerland) were dissolved in deionized water by the Milli-Q SP. TOC.  
17 system (Millipore Co., Billerica, MA, USA) to give a concentration of  $10^{-2}$  mol  $\text{dm}^{-3}$ . The standard  
18 solutions of metal ions were prepared by dissolving the chloride salts (99.9 % purity, Wako Pure  
19 Chemical Industries, Osaka, Japan) in deionized water with a few drops of concentrated hydrochloric  
20 acid. The *o,o'*-dihydroxyazobenzene (DHAB) was obtained from Dojindo Lab. (Kumamoto, Japan).  
21 The 0.1 mol  $\text{dm}^{-3}$  pH buffer solutions of 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid  
22 (HEPES)-NaOH and *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES)-NaOH (Dojindo Lab.,  
23 Kumamoto, Japan) were prepared for the pH range 7.0-10.

## 25 2.3. CE-LIF procedure

26 The L and pH buffer solutions were added to the sample solution. The concentrations of the mixed  
27 solution were typically  $1 \times 10^{-6}$  mol  $\text{dm}^{-3}$  and  $4 \times 10^{-2}$  mol  $\text{dm}^{-3}$  for the L and pH buffer, respectively.  
28 After 10 minutes, the sample solution was hydrodynamically injected into the capillary from the  
29 cathodic end with  $\Delta 5$  cm in 72 s for LIF detection (an injection volume of 10 nL was calculated using  
30 the Hagen-Poiseuille equation) and 5 psi  $\times$  s for UV/Vis detection (8 nL). Then voltage was applied at  
31 15-20 kV with a constant voltage mode. A carrier buffer of  $10^{-2}$  mol  $\text{dm}^{-3}$  HEPES-NaOH (pH 7.50)  
32 containing  $5 \times 10^{-8}$  mol  $\text{dm}^{-3}$  of EDTA was typically employed. The power output of the laser was set  
33 at 15.5 mW. The voltage of the photomultiplier in the LIF detection system was set at 570 V.

34 To avoid any serious  $\text{Al}^{3+}$  contamination from the reagents used, the chemical suppression technique  
35 reported in our previous work was also examined [16]. The sample was prepared by the following

1 procedure; 50  $\mu\text{L}$  of  $1 \times 10^{-5} \text{ mol dm}^{-3}$  L stock solution containing  $1.5 \times 10^{-5} \text{ mol dm}^{-3}$  of DHAB and  
2  $0.1 \text{ mol dm}^{-3}$  of CHES-NaOH (pH 10.0) was added to the sample solution, and then 20  $\mu\text{L}$  of  $0.1 \text{ mol}$   
3  $\text{dm}^{-3}$  HEPES pH buffer solution containing  $4.0 \times 10^{-6} \text{ mol dm}^{-3}$  of DHAB was added. The mixed  
4 solution was made up to 500  $\mu\text{L}$  with deionized water and injected to the CE-LIF after it had been left  
5 to stand for 15 minutes.

6

### 7 **3. Results and discussion**

#### 8 **3.1. Electrophoretic behavior**

9 The metal complexes with L were injected into the CE with UV/Vis detection at 488 nm after pre-  
10 capillary chelation. Only the distinct peaks of  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  were observed as shown in Figure 1. None  
11 of the other metal complexes ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$ )  
12 showed any trace peaks with simultaneous addition. Regarding  $\text{In}^{3+}$ , an extremely broadened peak was  
13 observed near the peak of the ligand, quantification was impossible due to overlap. The selectivity of  
14  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  over the other metal ions seems to be mainly based on the effect of the kinetic  
15 characteristics. Only the kinetic stable complexes which survived during migration were detected, and  
16 the kinetic active complexes decomposed; i.e. the dissociation kinetics controls the detection selectivity.  
17 The water exchange rate constants,  $k_{\text{H}_2\text{O}}$ , for all undetected metal ions in the CE are more than  $1 \times 10^4$   
18  $\text{s}^{-1}$ , while a metal ion with a large  $k_{\text{H}_2\text{O}}$  value frequently tends to form labile complexes on ligand-  
19 exchange reaction [17]. On the other hand, the values of  $k_{\text{H}_2\text{O}}$  of the triply-charged group IIIA metal  
20 ions are 1.29,  $4.0 \times 10^2$  and  $4.0 \times 10^4 \text{ s}^{-1}$  for  $\text{Al}^{3+}$ ,  $\text{Ga}^{3+}$  and  $\text{In}^{3+}$ , respectively [17]. Judging from these  
21 values, this complexing system seems to be sufficiently inert to be detected in the CE only for metal  
22 ions with  $k_{\text{H}_2\text{O}}$  of less than  $10^4 \text{ s}^{-1}$ . The  $\text{In}^{3+}$  complex on the borderline between inert and labile was  
23 detected as a half-dissociated broad peak. No interference for the detection of  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  was  
24 observed at the concentration range of  $2.0 \times 10^{-5} \text{ mol dm}^{-3}$  of foreign metal ions owing to the kinetic  
25 selectivity.

26 A similar migration behavior to that using UV/Vis detection was observed using the LIF detection  
27 system as shown in Figure 2. Paramagnetic ( $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$ ) and heavy metal ions  
28 ( $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$ ) are generally known to significantly quench the ligand-centered emission and to  
29 be difficult to detect with the fluorescence detection. Meanwhile, the  $\text{Al}^{3+}$ - and  $\text{Ga}^{3+}$ -L complexes were  
30 distinctly detectable with fluorescence detection since these metal ions were closed-shell metal ions,  
31 which did not quench the ligand-centered emission. Other closed-shell metal complexes ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$   
32 and  $\text{Zn}^{2+}$ ) were not detected due to the on-capillary dissociation processes as described above.

33 In Figure 2, the peak of the Al complex is about ten times higher than that of the Ga complex. This is due  
34 to contaminant Al ions ( $4.2 \times 10^{-9} \text{ mol dm}^{-3}$ ). Since the masking reagent, EDTA, was added to the  
35 carrier buffer solution, the contaminant Al in the carrier should have been suppressed. Therefore, the

1 source of the contamination most likely originated from the reagents used for the pre-capillary  
2 complexing process; L and the pH buffer solutions. Accordingly, a contaminant suppression technique  
3 for Al was employed [16]. This simple technique is based on the combination of the kinetics and  
4 thermodynamics of Al complexes; by simply adding of DHAB to used reagents, all contaminant Al in  
5 the reagents used is converted into an inactive DHAB complex on the pre-capillary complexation  
6 process. On the other hand, sample Al ions are derivatized into the L complexes with fast complex  
7 formation kinetics. When this technique was employed, the peak of contaminant Al successfully  
8 decreased by a factor of fifteen. While over 99.9 % of the contaminant Al theoretically was suppressed  
9 by the formation of DHAB complexes, an Al peak of about  $10 \text{ ng l}^{-1}$  ( $3.7 \times 10^{-10} \text{ mol dm}^{-3}$ ) was still  
10 observed. Such behavior was also observed in HPLC experiments, where it was expected that the Al  
11 originated from the instruments. This technique was successfully employed for the determination of Al  
12 but not for Ga. The L complexes with Ga ions seemed to dissociate due to a fast ligand-exchange  
13 reaction in the pre-capillary process ( $\text{Ga-L} + \text{DHAB} \rightarrow \text{Ga-DHAB} + \text{L}$ ; fast). This suggests that the  
14 Ga-L complex is far more labile than the Al-L complex, which is supported by the  $k_{\text{H}_2\text{O}}$  values.

### 16 **3.2. Detection limits and an application to a river water sample**

17 Ultrasensitive detection of metal-L complexes was considered highly likely since the fluorescein  
18 fluorophore with an absorbance maximum at around 490 nm was suitable for the excitation with an  
19 argon laser ( $\lambda_{\text{ex}} = 488 \text{ nm}$ ). The calibration curves for  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  were obtained. Their linear ranges,  
20 correlation factors and detection limits were  $1 \times 10^{-8}$ - $1 \times 10^{-6}$  and  $5 \times 10^{-10}$ - $1 \times 10^{-8} \text{ mol dm}^{-3}$ ,  $R^2 =$   
21  $0.993$  and  $0.998$ , and  $4.2 \times 10^{-9}$  (42 atto mole as amount basis) (based on  $3\sigma$ ) and  $4.6 \times 10^{-10} \text{ mol dm}^{-3}$   
22 (4.6 atto mole as amount basis) (based on  $S/N = 3$ ) for  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$ , respectively. The comparatively  
23 poor detection limit of Al to Ga was due to the fluctuation of the contaminant Al peak. When the  
24 masking technique was employed for Al, the linear ranges, the correlation factor of the calibration  
25 curve and the detection limit based on  $3\sigma$  were  $1 \times 10^{-9}$ - $1 \times 10^{-7} \text{ mol dm}^{-3}$ ,  $R^2 = 0.9938$  and  $3.9 \times 10^{-10}$   
26  $\text{mol dm}^{-3}$  ( $10 \text{ ng l}^{-1}$ , 3.9 atto mole as amount basis). These detection limits are the most sensitive among  
27 the CZE methods for metal ions.

28 The determination of Al in a river water sample (JC0031) was carried out as an example of its  
29 application to real samples (Figure 3). Some unknown peaks appeared with low reliability as shown in  
30 Figure 2 (25-27 min) and Figure 3 (32-36 min). Since these peak heights changed depending on the lot  
31 number of the agent and exposure to extremely low pH solutions, the origin of the peaks seemed to be  
32 from impure substances. However, the reliability of the peaks of  $\text{Al}^{3+}$ ,  $\text{Ga}^{3+}$  and L were sufficiently  
33 high for quantitative determination. The value determined by our method ( $14 \mu\text{g l}^{-1}$ ) was similar to the  
34 certified value ( $13 \mu\text{g l}^{-1}$ ). No Ga peak was observed, which indicated that the contaminant level of Ga  
35 lay at levels lower than  $10^{-10} \text{ mol dm}^{-3}$ .

### 1 3.3. Comparison of three ligands regarding selectivity and chemical structure

2 We have previously reported on some CE and HPLC systems with fluorescence detection using three  
3 kinds of aromatic polyaminocarboxylate ligands as pre-column derivatizing reagents [15,16,18]. A  
4 comparison of these systems with this study is informative and useful with respect to the molecular  
5 design of fluorescent ligands for analytical separation systems (summarized in Table 1).

6 First, regarding emissive characteristics, the distance between the fluorophores and the center metal  
7 ions seems to control the quenching process by paramagnetic and heavy metal effects. The  
8 paramagnetic metal ions ( $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ) quench the ligand-centered emission of **1** and  
9 ABEDTA (**2**) complexes, while other metal ions, including the heavy metal ions ( $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Hg}^{2+}$ ),  
10 retain the emissive characteristics of these complexes. However, the intensity of the ligand-centered  
11 emission of the  $\text{Pb}^{2+}$ -**1** and -**2** complexes was half of that of the free ligands (data not shown).  
12 Furthermore, it was reported that the closed-shell lanthanide ions,  $\text{La}^{3+}$  and  $\text{Lu}^{3+}$ , quench the ligand-  
13 centered emission of **1** [19]. Therefore, a heavy atom effect seems to be at work in certain cases. On the  
14 other hand, the ligand-centered emissions of the FTC-ABEDTA (**3**) complexes with paramagnetic and  
15 heavy metal ions retain their emissive characteristics. An approximation of the structures of the  
16 complexes was made to estimate the distances between the fluorescent moiety and the metal ion as  
17 shown in Figure 4. The MM/MD simulation was made for  $\text{Ca}^{2+}$  complexes since there was no  
18 parameter set for  $\text{Al}^{3+}$  available in the software employed. It is noted that the aim of the calculation is  
19 merely to estimate the distance between a center-metal and a fluorophore for the complexes with  
20 typical metal ions in aqueous solution, and is not to obtain precise chemical structures. Nevertheless,  
21 reasonable calculated values were obtained for the distance between carboxylate O atoms and  $\text{Ca}^{2+}$   
22 (2.22-2.23 Å), and imino N atoms and  $\text{Ca}^{2+}$  (2.48-2.55 Å) in the complexes with **2** and **3**, which values  
23 were comparable to those of EDTA obtained from the crystal structure ( $\text{Ca}\cdots\text{O}$ , 2.37-2.45 Å;  $\text{Ca}\cdots\text{N}$ ,  
24 2.62-2.71 Å) [20]. The distances between the fluorophore and the metal ion for **1**, **2**, and **3** complexes  
25 are approximately 3, 6, 11-12 Å, respectively. This fact suggests that the fluorescent characteristics of  
26 the ligands can be controlled by the distance modulation between the fluorescent and the chelating  
27 moiety. Hence, **1** selectively works as a pre-column derivatizing reagent for closed-shell and non-heavy  
28 metal ions, as described in this study, whereas the simultaneous detection of multiple metal ions is  
29 achieved by **3** since all metal complexes have strongly emissive nature.

30 Secondly, the chemical structure of the chelating moiety, such as the coordination number or cavity  
31 size, has a substantial influence on selectivity based on the decomposition processes in the separation  
32 column. The methyl-EDTA frame in the **2** and **3** ligands provides kinetic stability for metal complexes,  
33 with the exception of the triply charged metal ions. While the same coordination structures of **2** and **3**  
34 provide different selectivity, this is due to the differences in the experimental conditions (at pH 7.5 for  
35 **2** complexes and pH 12 for **3** complexes) and of the charges of the complexes (2- for  $[\text{M}^{2+}\text{-2}]$ ; 3- or 4-  
36 for  $[\text{M}^{2+}\text{-3}]$  complexes). It is well known that the pH value substantially affects the rate of acid  
37 dissociation processes [21], and the charge of the complex frequently affects the rate of ligand-

1 exchange processes with attacking nucleophilic ligands [22]. On the other hand, the **1** ligand provides  
2 kinetic stability selective to only triply charged metal complexes. This may be a result of the cavity size  
3 in the coordination sphere; the ligand, **1**, seems to have a smaller coordination cavity than **2** and **3**  
4 ligands. The coordination structure of the Al-**1** complex remains unknown, yet at least it has been  
5 reported that the composition of the Al-**1** complex is one to one [16]. In the present work, it is  
6 interestingly revealed that a boundary of  $k_{\text{H}_2\text{O}}$  allows an inert complex to form with the **1** ligand at  
7 around  $10^{-4} \text{ s}^{-1}$ . The selectivity in our CE system is probably related with the mechanism of the  
8 dissociation process. It was reported that the solvent exchange reaction of  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$  occurs with a  
9 very similar mechanism (both were dissociation mechanism). However, the association mechanism of  
10  $\text{In}^{3+}$  was quite different [17]. In contrast to **1**, the larger cavity size in **2** and **3** complexes seems to be  
11 suited to doubly charged metal ions. The kinetic stability of the complexes with these ligands seems not  
12 to be interrelated with the  $k_{\text{H}_2\text{O}}$  values; (the order of  $k_{\text{H}_2\text{O}}$ :  $\text{Al}^{3+} < \text{Ga}^{3+} < \text{Ni}^{2+} < \text{Mg}^{2+} < \text{Co}^{2+} < \text{Fe}^{2+} <$   
13  $\text{Mn}^{2+} < \text{Zn}^{2+} < \text{Cd}^{2+} < \text{Hg}^{2+} < \text{Cu}^{2+} < \text{Pb}^{2+}$ ). This suggests that the dissociation kinetics of the **2** and **3**  
14 complexes is rather complicated to be involved in solvolysis, acid-assisted dissociation and ligand-  
15 exchange dissociation processes. It is emphasized that the inertness of the complex is controlled by the  
16 nature of the metal ion for the **1** complex and controlled by the characteristics of the ligand for the **2**  
17 and **3** complexes. Modulating the distance between fluorophores and the chelating moieties, and also  
18 controlling the chelating structure can provide different selectivity for various metal ions.

#### 19 20 **4. Conclusion**

21 It is noteworthy that sub nano molar level detections are successfully achieved using CZE. These  
22 detection limits are comparable to those obtained by instrumental analytical methods. Although CE is  
23 considered a low sensitivity method even when LIF detection is employed [4-7], the researches we  
24 have carried out [15] have overthrown the belief that it is impossible to achieve high sensitivity without  
25 any pre-concentration techniques. The relationship between the coordination structure in  
26 polyaminocarboxylate ligands and the selectivity in the CE is not completely clear at this stage.  
27 However, it is clear that powerful chemical systems for CE will be achieved once we understand the  
28 total design of a ligand based on the combination of emissive and kinetic characteristics.

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## 1 **References**

- 2 [1] A.R. Timerbaev, O.A. Shpigun, *Electrophoresis* 21 (2000) 4179.
- 3 [2] A.R. Timerbaev, *Electrophoresis* 25 (2004) 4008.
- 4 [3] M. Macka, P.R. Haddad, *Electrophoresis* 18 (1997) 2482.
- 5 [4] R.H. Zhu, W.T. Kok, *Anal. Chim. Acta* 371 (1998) 269.
- 6 [5] B.-F. Liu, L.-B. Liu, J.-K. Cheng, *J. Chromatogr. A* 834 (1999) 277.
- 7 [6] M.N. Church, J.D. Spear, R.E. Russo, G.L. Klunder, P.M. Grant, B.D. Andresen, *Anal. Chem.* 70  
8 (1998) 2475.
- 9 [7] L. Ye, J.E. Wong, C.A. Lucy, *Anal. Chem.* 69 (1997) 1837.
- 10 [8] T. Saitoh, H. Hoshino, T. Yotsuyanagi, *J. Chromatogr.* 469 (1989) 175.
- 11 [9] T. Saitoh, H. Hoshino, T. Yotsuyanagi, *Anal. Sci.* 7 (1991) 495.
- 12 [10] N. Iki, H. Hoshino, T. Yotsuyanagi, *Chem. Lett.* (1993) 701.
- 13 [11] N. Iki, H. Hoshino, T. Yotsuyanagi, *J. Chromatogr.* 652 (1993) 539.
- 14 [12] O.V. Krokhnin, W. Xu, H. Hoshino, O.A. Shpigun, T. Yotsuyanagi, *Chem. Lett.* (1996) 1095.
- 15 [13] O.V. Krokhnin, H. Hoshino, O.A. Shpigun, T. Yotsuyanagi, *Chem. Lett.* (1999) 903.
- 16 [14] S. Saito, H. Hoshino, *Anal. Bioanal. Chem.* 378 (2004) 1644.
- 17 [15] S. Saito, R. Suzuki, N. Danzaka, K. Yoshimoto, M. Maeda, M. Aoyama, *Electrophoresis*,  
18 submitted.
- 19 [16] S. Saito, T. Anada, S. Hoshi, K. Akatsuka, *Anal. Chem.* 77 (2005) 5332.
- 20 [17] L. Helm, A. E. Merbach, *Chem. Rev.* 105 (2005) 1923.
- 21 [18] S. Saito, N. Danzaka, S. Hoshi, *J. Chromatogr. A* 1104 (2006) 140.
- 22 [19] I. Berregi, J. S. Durand, J. A. Casado, *Talanta* 48 (1999) 719.
- 23 [20] B.L. Barnett, V.A. Uchitman, *Inorg. Chem.* 18 (1979) 2674.
- 24 [21] D.W. Margerum, G.R. Cayley, D.C. Weatherburn, G.K. Pagenkopf in A.E. Martell (editor),  
25 *Coordination Chemistry Vol. 2*. American Chemical Society, Washington, D.C., 1978, p. 85-105.

- 1 [22] D.W. Margerum, G.R. Cayley, D.C. Weatherburn, G.K. Pagenkopf in A.E. Martell (editor),  
2 Coordination Chemistry Vol. 2. American Chemical Society, Washington, D.C., 1978, p. 163-194.
- 3 [23] G. Chang, W. Guida, W. C. Still, J. Am. Chem. Soc. 111 (1989) 4379.
- 4 [24] W.F. Gunsteren, H.J.C. Berendsen, Mol. Simul. 1 (1988) 173.
- 5 [25] F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T.  
6 Hendrickson, W.C. Still, J. Comp. Chem. 11 (1990) 440.

7

## 1 Legends for Figures

2 **Figure 1.** Typical electropherogram of metal-L complexes with UV/Vis detection. Sample,  $C_L = 5.0 \times$   
3  $10^{-4} \text{ mol dm}^{-3}$ ,  $C_{\text{metal}} = 2.0 \times 10^{-5} \text{ mol dm}^{-3}$  (metal =  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  
4  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ ),  $C_{\text{Al}} = 2.5 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $C_{\text{Ga}} = 7.0 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $C_{\text{HEPES}} = 1.0 \times 10^{-2} \text{ mol dm}^{-3}$ .  
5 Carrier buffer,  $C_{\text{HEPES}} = 2.0 \times 10^{-2} \text{ mol dm}^{-3}$  (pH 7.5),  $C_{\text{EDTA}} = 5.0 \times 10^{-8} \text{ mol dm}^{-3}$ . Applied voltage,  
6 15 kV.

7 **Figure 2.** Typical electropherogram of  $\text{Al}^{3+}$  and  $\text{Ga}^{3+}$ -L complexes with LIF detection. Sample,  $C_L =$   
8  $1.0 \times 10^{-6} \text{ mol dm}^{-3}$ ,  $C_{\text{Al}} = C_{\text{Ga}} = 1.0 \times 10^{-8} \text{ mol dm}^{-3}$ ,  $C_{\text{HEPES}} = 4.0 \times 10^{-3} \text{ mol dm}^{-3}$ . Carrier buffer,  
9  $C_{\text{HEPES}} = 4.0 \times 10^{-3} \text{ mol dm}^{-3}$  (pH 7.5),  $C_{\text{EDTA}} = 5.0 \times 10^{-8} \text{ mol dm}^{-3}$ . Applied voltage, 15 kV.

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11 **Figure 3.** Typical electropherogram of a river water sample (JAC0031). Sample,  $C_L = 1.0 \times 10^{-6} \text{ mol}$   
12  $\text{dm}^{-3}$ ,  $C_{\text{HEPES}} = 2.0 \times 10^{-2} \text{ mol dm}^{-3}$ , river water (JAC0031) was diluted by a factor of 10. Carrier buffer,  
13  $C_{\text{HEPES}} = 4.0 \times 10^{-3} \text{ mol dm}^{-3}$  (pH 7.5),  $C_{\text{EDTA}} = 5.0 \times 10^{-8} \text{ mol dm}^{-3}$ .

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15 **Figure 4.** Possible structures of the  $\text{Ca}^{2+}$  complexes with aromatic moiety-containing  
16 polyaminocarboxylate ligands: (a)  $\text{Ca}^{2+}$ -1, (b)  $\text{Ca}^{2+}$ -2, (c)  $\text{Ca}^{2+}$ -3. The **solid arrows** indicate the  
17 distances between fluorescent moiety and  $\text{Ca}^{2+}$ . These models and distances with standard deviation  
18 were obtained from the Monte Carlo Multiple Minimum (MCMM) procedure [23] and Stochastic  
19 Dynamics simulations [24] (simulation time: 500 ps, time step: 1 fs, 300 K, SHAKE: applied to all  
20 bonds involving hydrogen atoms), respectively. All calculations were made with the OPLS2003 force  
21 field and GB/SA treatment water, as implemented in the Macromodel 9.0 package [25]. In the case of  
22  $\text{Ca}^{2+}$ -3 complex, two stable structures were obtained.