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Separation and direct UV detection of lanthanides complexed with cupferron by capillary electrophoresis

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Abstract

Separation and detection of lanthanides by capillary zone electrophoresis in the presence of cupferron (*N*-nitroso-*N*-phenylhydroxylamine) as UV absorbing complexing agent were investigated. The resolution of partially complexed positively charged cupferron complexes is improved by using a buffer ligand competing with cupferron for metal ions. When hydroxyisobutyric acid (HIBA) is used as buffer and competing ligand, it provides complete separation of all 14 lanthanides with good peak shapes. An on-column separation of 14 lanthanides was achieved in only 7 min using 0.1 mmol/l cupferron, 15 mmol/l HIBA at pH 4.9. The separation efficiencies for the optimum separation condition are between 77 000 and 208 000 theoretical plates. Determination of lanthanide complexes was performed by direct UV detection at 210 nm. Detection limits (signal-to-noise ratio=3) are ca. 0.24–0.47 µg/ml for lanthanides. Under optimum conditions, the complete separation of thorium and uranium from mixed lanthanides was achieved. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Recently capillary electrophoresis (CE) has been successfully applied to the separation of metal ions [1,2]. The separation of lanthanide group metal ions is especially challenging because of their equal charge and almost similar ionic radii. As a result of the very similar characteristics of the group elements, the stability constants of metal ions with a particular ligand show only slight differences. Thus, in order to achieve a good separation of lanthanides by CE, it is necessary to choose a proper complexing agent and adjust to an optimum ligand concentration range at which all complexation degrees and electro-

phoretic mobilities differ appreciably from each other. This condition is fulfilled in the area of partial complexation. Foret et al. first reported the separation of lanthanides by a weak complexing agent, hydroxyisobutyric acid (HIBA), with indirect UV detection [3]. Vogt and Conradi demonstrated a prediction method for optimum ligand concentration during the capillary electrophoretic separation using complex forming constants of lanthanides with HIBA [4]. Most applications used the same on-column complexation and indirect analysis method but with only limited number of weak ligands possessing differences in stability constants [5–7]. Only a number of UV absorbing reagents have been employed to-date for lanthanide separation. Timerbaev and co-workers used arsenazo III and 2,6-diacetylpyridinebis (*N*-methylene-pyridino-hydrazone) for the separation of only a limited number of

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lanthanide metal ions [8,9]. The same researchers separated 13 lanthanides employing pre-column complexation with cyclohexane-1,2-diaminetetraacetic acid (CDTA) [10]. With CDTA, complete complexation of lanthanides occurs. The mobility differences between strong complexes were obtained by forming mixed hydroxo complexes using a buffer with high pH. Macka et al. separated 10 of the 14 lanthanides using on-column arsenazo III complexation and utilizing secondary equilibria with an auxiliary complexing agent [11].

In the present study, we have used the UV active cupferron as complexing agent for lanthanides. By adding cupferron to the separation buffer which was chosen as HIBA, a good separation of all 14 lanthanides was achieved.

2. Experimental

2.1. Instrumentation

A commercial CE injection system (Prince Technologies, Emmen, The Netherlands) in combination with an on-column variable-wavelength UV–visible detector (Lambda 1000, Bishoff, Leonberg, Germany) was used. The wavelength was set at 210 nm. The analysis voltage was 28 kV. Sample injection was carried out with pressure (40 mbar, 0.06 min) at the anodic side. A 65 cm (effective length 54 cm) × 50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used. The capillary was flushed with 1 mol/l NaOH and water and running buffer each for 10 min at the beginning of each day. A washing step of 2 min with buffer between runs was applied. Automated capillary rinsing, sample injection and execution of the electrophoretic runs were controlled by a personal computer. Data processing was carried out with a commercial CE software (Caesar 1995, Prince Technologies).

2.2. Chemicals

Cupferron (in the form of ammonium salt), $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Th}(\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$ were purchased from Merck. La_2O_3 , Nd_2O_3 , Sm_2O_3 , Eu_2O_3 , Gd_2O_3 , Dy_2O_3 , Ho_2O_3 , Er_2O_3 ,

Yb_2O_3 , HIBA were from Fluka, $\text{Tb}_2(\text{CO}_3)_3$, $\text{Tm}_2(\text{CO}_3)_3$ were from Aldrich and Pr_6O_{11} and Lu_2O_3 from Sigma. Stock solutions of lanthanides were prepared by dissolving their oxides or carbonates in an excess of ultrapure perchloric acid (Merck), and nitrates in water. Other chemicals were analytical-reagent grade. Deionized water obtained from an Elgacan C114 filtration system was used for the preparation of solutions.

3. Results and discussion

Cupferron gives UV active complexes with lanthanides. Pre-column complexation of UV active complexes is mostly preferred in the capillary electrophoretic separation of metal ions. However, for the pre-column complexation method, metal ions should give strong and complete complexation. Consequently, the resulting complexes are expected to be inert.

When cupferron was added to the lanthanide sample and injected into the buffer in the capillary, no peak appeared, which can be understood from the complexation properties. Complex equilibria data between cupferron and lanthanides was only reported for La and Sm [12]. As seen from Table 1, complex formation occurs stepwise and the formed complexes are moderately stable. Employing the on-column method, separation of metal ions can be performed in the cupferron concentration area of partial complexation. The use of cupferron as buffer alone in high concentrations is not suitable because of more noise produced and because of the occurrence of complete complexation, rather than partial complexation. Therefore, several carboxylic acids were tried as buffer and cupferron was added in small concentrations to the buffer.

Table 1
Stability constants of La and Sm with cupferron and HIBA

	Cupferron	HIBA
La	$\log K_1=5.30$	$\log K_1=2.62$
	$\log K_2=4.30$	$\log K_2=1.80$
	$\log K_3=3.30$	$\log K_3=1.11$
Sm	$\log K_1=5.75$	$\log K_1=2.99$
	$\log K_2=4.75$	$\log K_2=2.40$
	$\log K_3=3.75$	$\log K_3=1.38$

3.1. Choice of buffer and pH

Three carboxylic acids were tried as buffer, namely HIBA, acetic and lactic acids. Since all these acids complex with lanthanides, an improvement of the separation can be expected by the combination of two ligands to achieve higher differences in the degree of complexation and of the electrophoretic mobilities. The peak shapes and separation windows are much better for HIBA than for the others. This buffer was used in the experiments. As seen in Table 1, stabilities of lanthanide complexes of HIBA are weaker than that of cupferron [13]. As shown by Vogt and Conradi [4], the difference in stabilities for the complexation can be expected to lead to a better separation, and the participation of cupferron in the complexing is large enough to give direct UV detection.

The pH range from 3.5 to 5.3 was examined. In the low-pH region, the baseline is not smooth and because of insufficient dissociation of cupferron the heights of peaks are small. At the higher pH values, increasing electroosmotic flow, which is in the same direction as the electrophoretic mobilities, causes resolution loss because of fast migration. As in Ref. [11], pH calibration is made with Tris instead of NaOH to obtain a lower separation current. This process improved peak shapes.

3.2. Effect of cupferron concentration

Fig. 1 shows the change in electrophoretic mobilities of lanthanides with cupferron concentration. A threshold amount of $5 \cdot 10^{-2}$ mmol/l cupferron gives satisfactory peak heights. With increasing cupferron concentration, the positive charge of the complexes decreases because of the increasing cupferron portion in the complexes. This decrease is much more for La compared to Lu, which shows almost no change in electrophoretic mobility and, consequently, in complexation degree. This is because Lu reaches maximum complexation much sooner than La. The stability constants of subsequent complexations are weaker than those of first complexation. La binds more readily to cupferron, as compared with Lu. As the electrophoretic mobilities of the first lanthanides become more negative, their peaks are slowed down, but the Lu peak does not

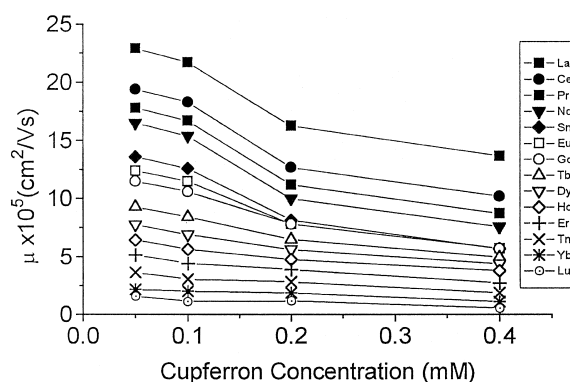


Fig. 1. Plot of electrophoretic mobilities of lanthanides vs. cupferron concentrations in a buffer of 15 mmol/l HIBA, 13 mmol/l Tris at pH 4.9.

move. The separation window becomes narrower and the separation selectivity of the middle lanthanides is lost.

3.3. Effect of HIBA concentration

Fig. 2 shows the separation of the lanthanides at constant cupferron concentration and increasing HIBA concentrations. More HIBA, compared to cupferron, is needed to change electrophoretic mobilities. As the HIBA concentration increases, the mobilities of the lanthanides decrease. This effect is seen more in the last lanthanides. HIBA captures the metal ions from the cupferron complexes and, since the cupferron-to-metal ion ratio increases in the complexes, the negative charge density increases and the mobilities decrease. After 20 mmol/l of HIBA, the last lanthanide peaks disappeared in the electropherogram, probably because complete complexation is reached and the solubilities of neutral complexes are very slight.

3.4. Optimized separation

Complete separation of all 14 lanthanides was achieved under the optimized conditions as seen in Fig. 3. Theoretical plate numbers of the peaks are between 77 000 and 208 000. Minimum detection limits of the lanthanides are between 0.24 and 0.47 $\mu\text{g/ml}$. Relative standard deviations (RSDs) of peak

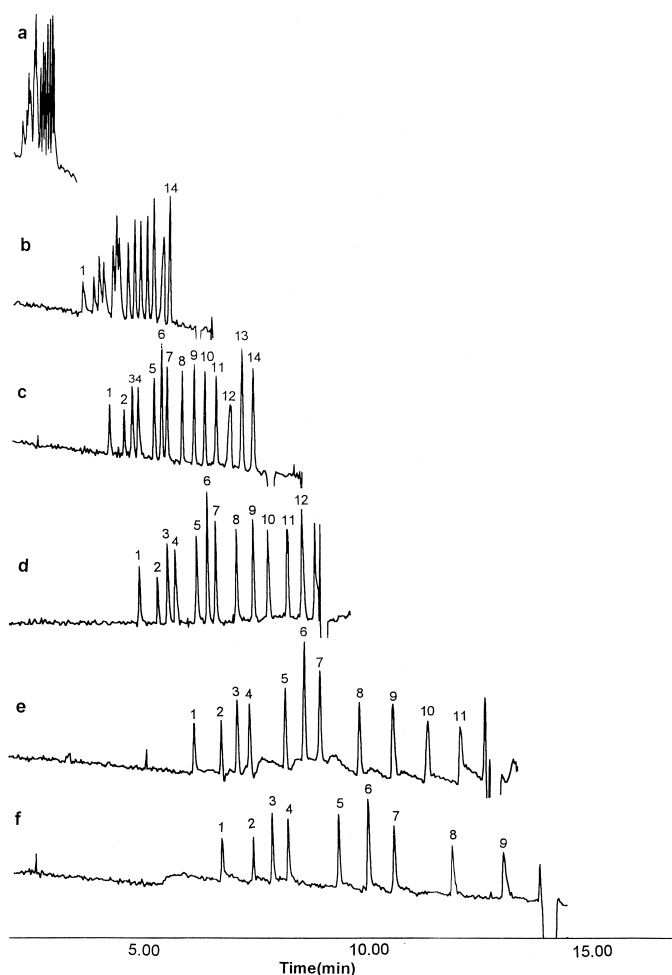


Fig. 2. Separation of lanthanides with increasing HIBA concentrations. (a) 5 mmol/l, (b) 10 mmol/l, (c) 15 mmol/l, (d) 20 mmol/l, (e) 25 mmol/l, (f) 35 mmol/l. All buffers contain 0.05 mmol/l cupferron at pH 4.9. Injection 40 mbar, 0.06 min. Run voltage 28 kV. 1=La, 2=Ce, 3=Pr, 4=Nd, 5=Sm, 6=Eu, 7=Gd, 8=Tb, 9=Dy, 10=Ho, 11=Er, 12=Tm, 13=Yb, 14=Lu.

migration times and relative areas (A/t) are given in Fig. 4.

3.5. Separation of lanthanides in uranium- and thorium-containing solutions

Since lanthanides often are together with U or Th matrix, the separation of lanthanides in this matrix is important. When UO_2^{2+} and Th^{4+} ions are injected into the separation buffer, two broad peaks appear just after the negative peak. It is not possible to quantify these peaks, but the separation of the lanthanides is seen to be possible in these matrix.

Fig. 5 shows the separation of lanthanides from 10- and 100-times more concentrated UO_2^{2+} - and Th^{4+} -containing matrices.

4. Conclusion

It is rather difficult to induce different electrophoretic mobilities in CE separation, among the lanthanide group elements which, in general, have similar characteristics: the majority of the chromophore ligands used in analytical separations are large molecules that form, in one step, chelates with the

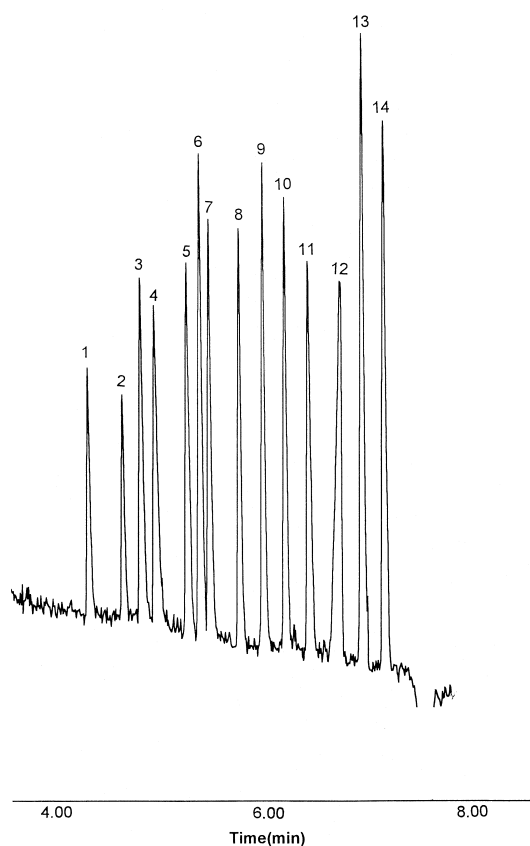


Fig. 3. Electropherogram of the optimized separation of 14 lanthanides. Buffer: 15 mmol/l HIBA, 13 mmol/l Tris, 0.1 mmol/l cupferron, pH 4.9. Peak identifications and other conditions as in Fig. 2.

metal ions. The stability constants of the formed complexes being very similar, it is difficult to induce differences between these stable and large-sized chelate of the lanthanide metal ions. Accordingly, it can be seen from the literature that the lanthanide separations have been performed indirectly with a few partially complexing, UV inactive ligands [3–7]. Full lanthanide separation with direct detection has been reported in only two works [10,11].

The cupferron used in this study, being a UV-active small-molecule ligand, gives partial complexation with the lanthanides with medium stability, and provide the electrophoretic mobility differences that a sufficient for a satisfactory direct detection of all 14 lanthanides. Although cupferron is a small molecule, the detection limits of lanthanide ions are

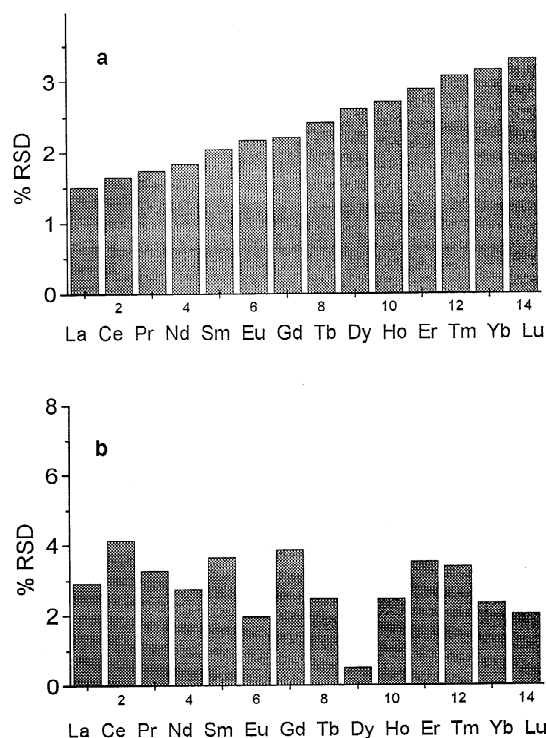


Fig. 4. Reproducibility of (a) migration times and (b) peak area (A/t). Concentrations, $1 \cdot 10^{-4}$ mol/l of each cation. Other conditions as in Fig. 2.

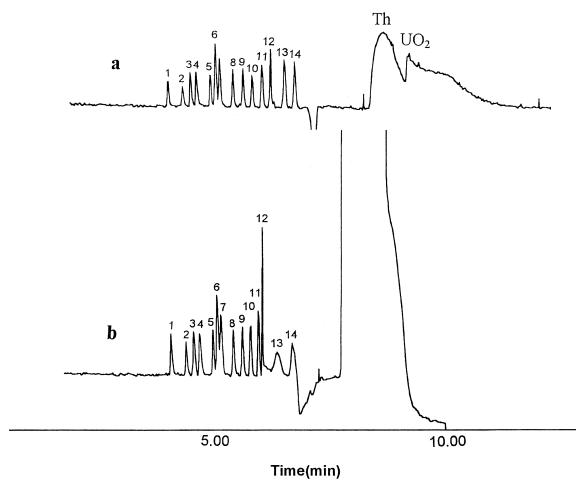


Fig. 5. Separation of lanthanides from 10 (a) and 100 (b) times more concentrated UO_2^{2+} and Th^{4+} matrix. Conditions as in Fig. 3.

comparable to or better than that in the CE of lanthanides with indirect UV detection.

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