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Separation and direct UV detection of lanthanides complexed with pyridine-2-carboxylic acid by capillary electrophoresis

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Abstract

Separation and detection of lanthanides by capillary zone electrophoresis in the presence of pyridine-2-carboxylic acid (picolinic acid) as UV-absorbing complexing agent were investigated. The resolution of partially complexed positively charged complexes is improved by using two buffer ligands competing with picolinic acid for metal ions. When hydroxyisobutyric acid (HIBA) and formic acid are used together as competing ligands, this provides complete separation of all 14 lanthanides with good peak shapes. An on-column separation of 14 lanthanides was achieved in only 9 min using 0.8 mmol/l picolinic acid, 10 mmol/l HIBA and 25 mmol/l formic acid at pH 4.7. Determination of lanthanide complexes was performed by direct detection at 210 nm. Detection limits (signal-to-noise ratio=3) are ca. 0.53–0.96 µg/ml. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Complexation; Pyridinecarboxylic acid; Picolinic acid; Lanthanides; Metal cations

1. Introduction

In recent years, capillary electrophoresis (CE) has been used as a fast and effective method of separation for metal ions [1–3]. Complex formation is used to differentiate the electrophoretic mobilities of hydrolyzed metal ions with equal charge. Thanks to the superior separation efficiency of the method, small differences created by the degrees of complex formation are sufficient to achieve separation. However, although a large number of ligands that complex with metal ions are known, it is generally seen that only a limited number of ligands have been used to date in CE separations and that only a limited number of metal ions are separated in a given run, in

spite of the powerful separation capability of the method.

Of special challenge is the separation of lanthanide metal group ions, due to their equal charge and almost similar ionic radii. Furthermore, as a results of the very similar characteristics of the group elements, the stability constants of these metal ions with a given ligand show only slight differences. In particular, in many works, Eu and Gd occur as overlapped peaks or one of these two metal ions are not included in the standard solution. This group of metal ions was first separated by Foret et al., using the weak-complexing hydroxyisobutyric acid (HIBA), thus yielding indirect detection [4]. It is seen that subsequent works, in general also using HIBA or a limited number of other weak-complexing ligands, are based on similar on-column complexation and indirect analysis methods [5–8]. Verma et al. have studied the effect of temperature on sepa-

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ration using HIBA and have reported that in particular the resolution of Eu and Gd increases at low temperature [9]. It is seen in the literature that only a few UV-active ligands have been used for CE lanthanide separation. Timerbaev and co-workers have separated (i) a limited number of lanthanide ions using arsenazo III and 2,6-diacetylpyridine bis(*N*-methylenepyridinohydrazone) [10,11] (ii) 13 lanthanide ions using cyclohexane-1,2-diaminetetraacetic acid (CDTA) [12]. Macka et al. have separated 10 lanthanide ions with arsenazo III [13]. Since lanthanide ions have very similar stability constants for complexation, differentiation in complex formation could be exploited at partial complexation. In cases where a single ligand does not provide enough mobility differentiation, the competitive effect of a second ligand can be used to differentiate the levels of complexation and consequent electrophoretic mobilities. Vogt and Conradi have shown that separation with HIBA is improved by the addition of acetic acid [8]. Macka et al., using citrate as an auxiliary ligand to arsenazo III, have resolved 10 lanthanides [13]. Timerbaev et al., in their work using CDTA, have indicated that separation occurs only in a very basic medium, with the formation of mixed hydroxy complexes [12].

It may be possible to achieve wide use of CE separation by creating different complexation equilibria, especially with ligands of chromophoric structure. In our first study in this direction, we have reported the separation by direct detection of the lanthanide ions with the previously unused ligand cupferron [14]. In that study, the levels of cupferron complexation were differentiated by using HIBA as a competitive ligand. Since cupferron is a UV-active ligand, the complete CE separation of all 14 lanthanide metal ions, direct detection, and quantitative determination were accomplished. Furthermore, it was shown that this separation can also be performed in a matrix with high concentration of uranium and thorium ions.

Further studies are necessary, using the large number of known complexing agents, in order to develop such separations and determinations. In the current work, pyridine-2-carboxylic acid (picolinic acid) was used for the direct detection of lanthanide metal ions. The differentiation between the degrees of complexation was achieved by the addition of two auxiliary ligands, in addition to picolinic acid.

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 (80 mbar, 0.1 min) at the anodic side. A 71 cm×50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used. The distance from the detection window was 59 cm. 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

Ce(NO₃)₃·6H₂O, was purchased from Merck (Darmstadt, Germany). La₂O₃, Nd₂O₃, Sm₂O₃, Eu₂O₃, Gd₂O₃, Dy₂O₃, Ho₂O₃, Er₂O₃, Yb₂O₃, HIBA and pyridine-2-carboxylic acid were from Fluka (Buchs, Switzerland); Tb₂(CO₃)₃, Tm₂(CO₃)₃ from Aldrich (Milwaukee, WI, USA); Pr₆O₁₁ and Lu₂O₃ from Sigma (St. Louis, MO, USA). 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

It is known that picolinic acid forms UV-active complexes with lanthanides. Preliminary experiments showing no peaks from injections with the addition of picolinic acid to the lanthanide sample are to be expected in view of the complexation constants (Table 1). The pre-column complexation method

Table 1
Stability constants of La and Lu with picolinic acid, HIBA and formic acid [15]

	Picolinic acid	HIBA	Formic acid
La	Log K_1 = 3.51	Log K_1 = 2.62	Log K_1 = 1.10
	Log K_2 = 2.95	Log K_2 = 1.80	Log K_2 = 0.99
	Log K_3 = 2.28	Log K_3 = 1.11	
Lu	Log K_1 = 4.41	Log K_1 = 3.67	Log K_1 = 0.99
	Log K_2 = 3.86	Log K_2 = 2.80	Log K_2 = 1.10
	Log K_3 = 2.98	Log K_3 = 2.35	

requires strong and complete complexation. However, complex formation between picolinic acid and lanthanide metal ions occurs stepwise and the formed complexes are weak. Employing the on-column method, separation of metal ions can be performed in the picolinic acid concentration area of partial complexation. Fig. 1 shows the complexation curves of lanthanide metal ions with picolinic acid. As seen in the figure, while in the partial complexation field differences are apparent between the degrees of complexation, towards completion the differentiation subsides. By choosing the optimal picolinic acid concentration from these curves, experiments were conducted. Nevertheless as seen from the electropherogram in Fig. 2, the small differences in complexation were not sufficient to procure the mobilities for the separation of all 14 lanthanides. The clear differences in complexation degrees in the first three lanthanides, seen in Fig. 1, are also reflected in the electropherogram of Fig. 2.

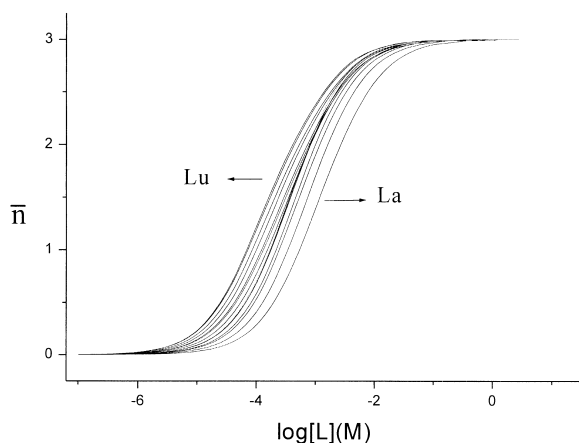


Fig. 1. Dependence of the average degree of complexation \bar{n} on the picolinic acid concentration.

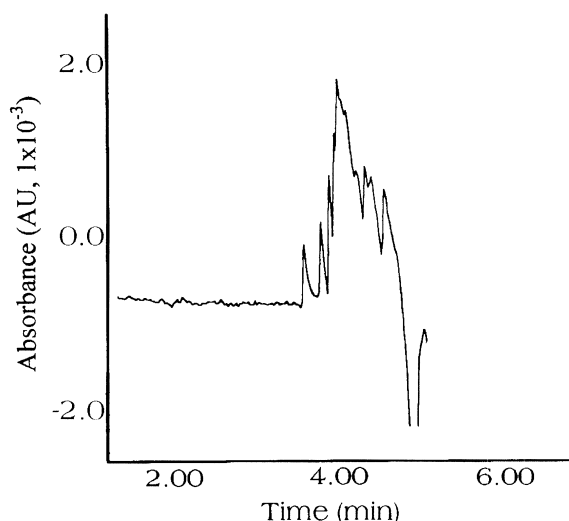


Fig. 2. Electropherogram of lanthanides in picolinic acid containing buffer.

Differentiation in the complexation, and thereby electrophoretic mobilities, of the latter lanthanides can be obtained by the addition of a second complexing ligand. In our previous work, we had achieved the separation and direct detection of the 14 lanthanides using HIBA as a complementary ligand to cupferron. Thus, in the present case, we again chose HIBA as the complementary ligand (in addition to being used as buffer) to picolinic acid.

3.1. Effect of HIBA concentration

Fig. 3 shows the changes in the electrophoretic mobilities as a function of HIBA concentration at constant picolinic acid concentration. With the increase of HIBA, a clear decrease is seen in the electrophoretic mobilities of the lanthanide metal ions. This decrease is largest in La and smallest in Lu. With the increase of HIBA, the resolution increases in the first and middle lanthanides, especially in Eu and Gd, but the last lanthanide peaks approach each other and the negative electroosmotic mobility (EOM) peak.

When isobutyric acid and butyric acid were tried as competitive ligands in lieu of HIBA, the separation of all 14 peaks did not occur. However, the addition of a third ligand, namely formic acid, to the picolinic acid–HIBA system, yielded the separation of the latter peaks.

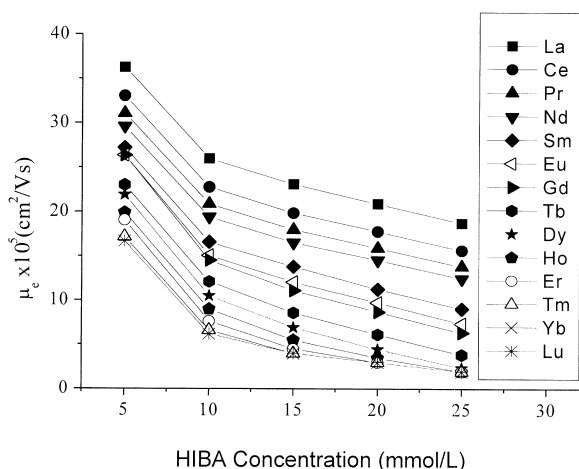


Fig. 3. Plot of effective electrophoretic mobilities of lanthanides vs. HIBA concentrations in a buffer of 0.8 mmol/l picolinic acid at pH 4.7.

3.2. The effect of formic acid

The use of formic acid alone (i.e., with no HIBA) with picolinic acid did not yield separation. Fig. 4 shows the electropherogram of lanthanide metal ions in a medium containing formic acid and picolinic acid. When formic acid was added as complementary ligand to a medium containing HIBA and picolinic acid, a noticeable decrease in the mobility of the last peaks and, consequently, an increase in the resolution was found. In Fig. 5, with the addition of 15 mmol/l formic acid to a medium containing 0.8

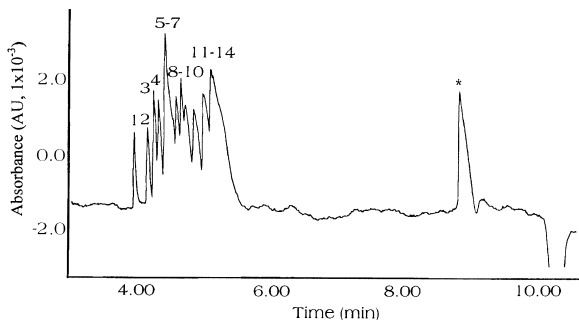


Fig. 4. Electropherogram of lanthanides in 0.8 mmol/l picolinic acid and 30 mmol/l formic acid containing buffer. 1=La, 2=Ce, 3=Pr, 4=Nd, 5=Sm, 6=Eu, 7=Gd, 8=Tb, 9=Dy, 10=Ho, 11=Er, 12=Tm, 13=Yb, 14=Lu. The asterisk indicates the peak due to the perchloric acid in the sample.

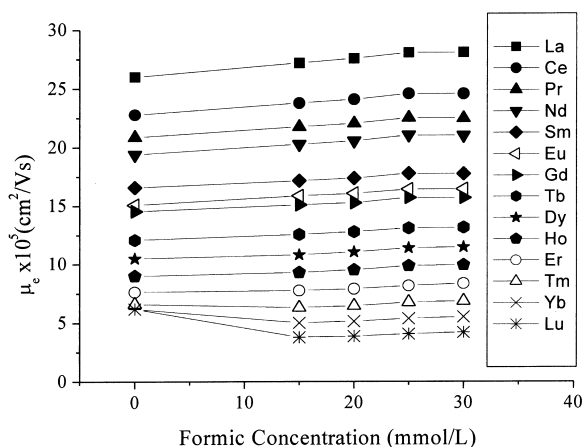


Fig. 5. Plot of effective electrophoretic mobilities of lanthanides vs. formic acid concentrations in a buffer of 0.8 mmol/l picolinic acid, 10 mmol/l HIBA at pH 4.7.

mmol/l picolinic acid and 10 mmol/l HIBA, the change of the electrophoretic mobilities of the last peaks is clearly seen. Further addition of formic acid does not further influence these mobilities. However, the separation between the last lanthanide peak and the peak which we identified as due to the perchloric acid in the sample is optimal with the usage of 25 mmol/l formic acid. This positive peak is marked with an asterisk (*) in the electropherogram.

The pH range from 3.9 to 5.1 was examined. In

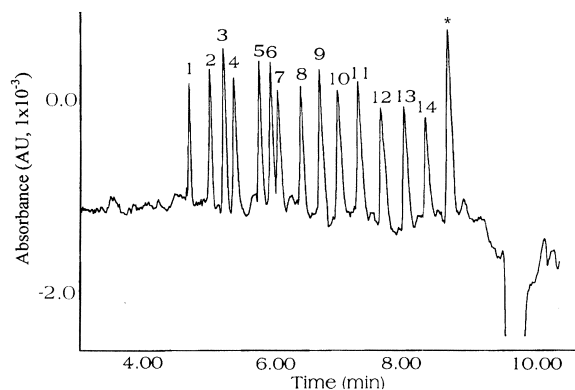


Fig. 6. Electropherogram of the optimized separation of 14 lanthanides. Buffer: 0.8 mmol/l picolinic acid, 10 mmol/l HIBA, 25 mmol/l formic acid, 33 mmol/l Tris. pH 4.7. Injection 80 mbar, 0.1 min. Run voltage: 28 kV. Peak identifications as in Fig. 4.

the low pH values, the baseline is not smooth and because of insufficient protonation of picolinic acid the heights of the peaks are small. At the higher pH values, increased electroosmotic flow, which is in the same direction as the electrophoretic mobilities, causes resolution loss because of fast migration. pH 4.7 was selected as optimal. pH calibration was made with Tris instead of NaOH to have a lower separation current. This process improved peak shapes.

3.3. Optimized separation

Complete separation of 14 lanthanides was achieved under the optimized conditions as seen in Fig. 6. Theoretical plate numbers of the peaks are between 52 000 and 148 000. Minimum detection limits of the lanthanides (three times the background noise) are between 0.53 and 0.96 $\mu\text{g}/\text{ml}$. Relative standard deviations (RSDs) of migration times and relative areas (A/t) are given in Fig. 7.

4. Conclusion

The electrophoretic mobility of metal ions, differentiated by the degree of complexation with a given ligand, can be further differentiated by the addition of a second, competitive ligand. In this work, the separation of lanthanide ions was achieved for the first time with the UV-active ligand picolinic acid, thereby enabling direct detection, with the aid of the competitive effect of two ligands. As seen in Table 1, the equilibrium constants of the competitive ligands with the lanthanide metals are weaker than those of picolinic acid. This difference yields the separations, and the participation of picolinic acid in complexing is large enough to give direct UV detection.

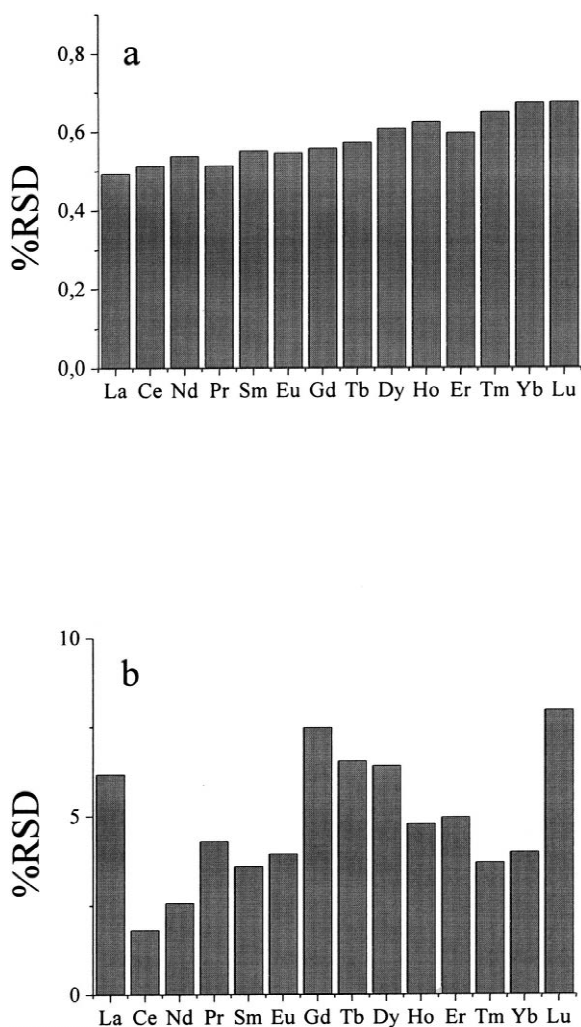


Fig. 7. Reproducibility of (a) migration times and (b) peak area (A/t). Concentrations, $2 \cdot 10^{-4}$ mol/l for Yb and Lu; $3 \cdot 10^{-4}$ mol/l for Eu, Gd and Tm; $4 \cdot 10^{-4}$ mol/l for others.

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