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Separation of metal ions by capillary electrophoresis with a complexing electrolyte

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ABSTRACT

Excellent separations of metal ions can be obtained very quickly by capillary electrophoresis provided a weak complexing reagent is incorporated into the electrolyte to alter the effective mobilities of the sample ions. Indirect photometric detection is possible by also adding a UV-sensitive ion to the electrolyte. Separations are described using phthalate, tartrate, lactate or hydroxyisobutyrate as the complexing reagent. A separation of twenty-seven metal ions was achieved in only 6 min using a lactate system. A mechanism for the separation of lanthanides is proposed for the hydroxyisobutyrate system.

INTRODUCTION

Although ion chromatography continues to be the major method used to determine anions and many inorganic cations, capillary zone electrophoresis (CZE) holds the promise of even better separations. It is becoming more and more common to use the term capillary electrophoresis (CE) instead CZE. Some excellent separations of ions have been achieved by CE [1–8]. A complete separation of all thirteen lanthanides has also been obtained [2,8].

Separations by CE are based on differences in the electrophoretic mobilities of the sample ions. In cases where the mobilities of the free cations are very similar, a reagent is often added to partially complex the sample cations and thereby increase the differences in effective mobilities. The separations are affected by the type and concentration of complexing reagent and by other factors such as pH, ionic strength, and viscosity. When indirect photometric detection is used, the separation may also be influenced by the chemical nature and concentration of the UV-active reagent.

In the present work, some new reagents are evaluated for the separation of a number of inorganic metal ions. In the CE of the lanthanides, the distribution of free metal ion and metal–complex species is calculated and a mechanism is proposed for the excellent separation that is obtained.

EXPERIMENTAL

CE was performed with Waters Quanta 4000 capillary electrophoresis system (Millipore waters, Milford, MA, USA), equipped with a positive power supply. Polyamide-coated, fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA), 60 cm in length with an I.D. of 75 μm, were used. A window for on-column detection was created 7.3 cm from the end of the capillary. Indirect UV detection was employed at 214 nm. The separation voltage applied was 15 or 30 kV.

At the beginning of each experimental day, it was sufficient to rinse the capillary with deionized water for about 15 min. However, in order to keep the capillary clean, the capillary was flushed with 0.5 *M* KOH solution after being used three or four days.

Hydrostatic sample mode was selected for injection and sample time was set at 30 s. Before each run, a 2-min purge of capillary with electrolyte was programmed. A Curken 250-1B plotter (Curken,

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Danbury, CT, USA) was used for recording electropherograms.

All standards and electrolytes were prepared using 18 MΩ deionized waters by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All reagents for preparing electrolytes were of analytical-reagent grade. Hydroxyisobutyric acid (HIBA) and UV-Cat1 were obtained from Waters (Milford, MA, USA). Phthalic acid, *p*-toluidine and phenylethylamine were purchased from Aldrich (Milwaukee, WI, USA). Tartaric acid and 4-methylbenzylamine were supplied by Fisher Scientific (Fair Lawn, NJ, USA) and Fluka (Ronkonkoma, NY, USA), respectively.

RESULTS AND DISCUSSION

General conditions for separation

CE separation of metal ions is based on differences in the mobility of the ions in an electric field. Unfortunately, in some cation groups the individual cations have almost the same mobility. The divalent transition metal cations and the lanthanide cations are examples. In such cases addition of a water-soluble complexing reagent to the capillary electrolyte is used to obtain larger differences in effective mobility. This occurs by complexing the metal ions to differing extents. Ions that are complexed to a greater degree move more slowly through the capillary than those that have a lower fraction of the element in the complexed form. The effects of pH and concentration of complexing reagent have been previously pointed out [2].

The problem of detecting the separated metal ions was solved by indirect photometric detection [3,4]. An organic cation is added to the electrolyte which absorbs in the UV spectral region. A proprietary reagent developed at Waters (UV-Cat1) was found to work well. We also tried a number of aryl derivatives of aliphatic amines, which form cations when protonated. Of those tried, phenylethylamine, benzylamine, *p*-toluidine and 4-methylbenzylamine were the most satisfactory. In order to optimize separation and detection efficiencies, it is important to choose a suitable combination of complexing reagent and UV-sensitive reagent. Some adjustment in the concentration of a UV-sensitive reagent is often required to obtain optimal separation conditions.

Separations using phthalate

Several complexing reagents were tried in connection with the attempted separation of several mono- and divalent metal cations. Only partial complexing of the metal cations is desired because complete complexing is apt to result in species that move at the same rate as the electroosmotic flow. The following complexing reagents were tried initially: HIBA, phthalic acid, malonic acid and succinic acid. Of these, HIBA and phthalic acid were the most promising. HIBA had been used previously for the separation of several mono- and divalent metal ions [3]. However, we obtained even better separations with phthalic acid. A good separation of eight metal ions in about 6 min is shown in Fig. 1.

Separations with phthalic acid were usually better when some methanol was added to the electrolyte. Methanol caused the moving times to be longer and resolution to be better. Elution times of the ions studied increased with added methanol up to about 20% methanol (by volume). With further increases in the proportion of methanol there was a plateau with no further changes in elution times.

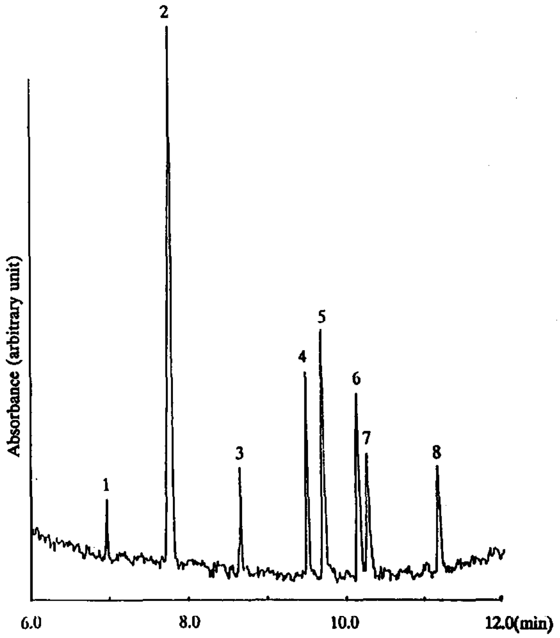

Fig. 1. Separation of metal ions using phthalate. Electrolyte, 2 m*M* phthalic acid, UV-Cat1, 20% methanol, pH 3.3; applied voltage, 15 kV. Peaks: 1 = K^+; 2 = Na^+; 3 = Pb^{2+}; 4 = Mn^{2+}; 5 = Co^{2+}; 6 = Ni^{2+}; 7 = Zn^{2+}; 8 = Cd^{2+}.

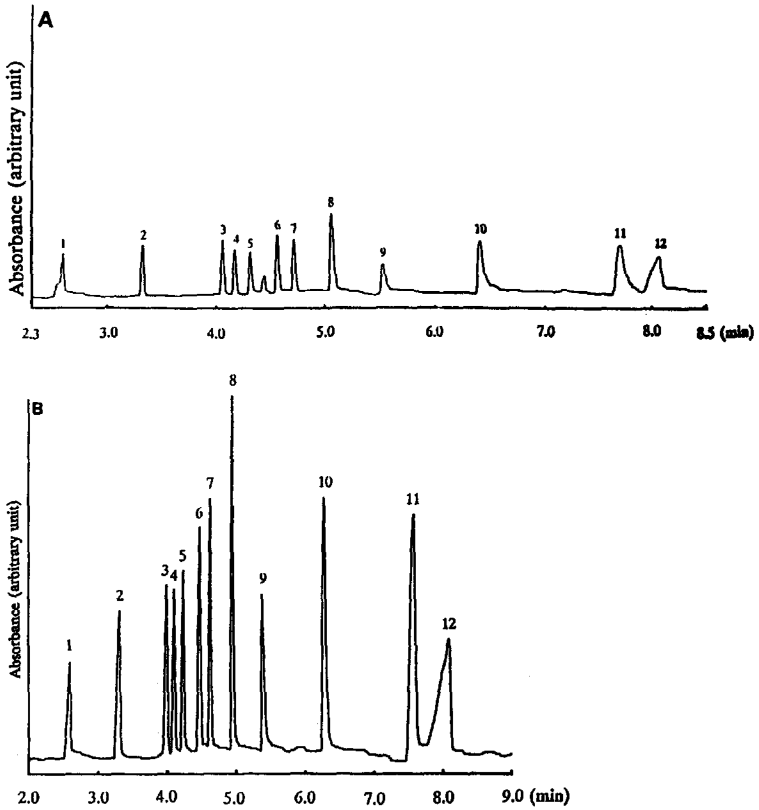

Fig. 2. (A) Separation of 12 alkali, alkaline earth and transition metal ions using tartrate. Electrolyte, 2.5 m*M* tartaric acid, 6 m*M* *p*-toluidine, 20% methanol, pH 4.8; applied voltage, 30 kV. Peaks: 1 = K^+ (1.3 μg/ml); 2 = Na^+ (0.8 μg/ml); 3 = Li^+ (0.2 μg/ml); 4 = Mg^{2+} (0.3 μg/ml); 5 = Ba^{2+} (1.3 μg/ml); 6 = Sr^{2+} (1.3 μg/ml); 7 = Mn^{2+} (0.8 μg/ml); 8 = Ca^{2+} (0.8 μg/ml); 9 = Cd^{2+} (1.3 μg/ml); 10 = Co^{2+} (1.0 μg/ml); 11 = Ni^{2+} (1.0 μg/ml); 12 = Zn^{2+} (1.0 μg/ml). (B) Separation of 12 alkali, alkaline earth and transition metal ions using tartrate. Four times higher concentration of each analyte as in (A). Electrolyte conditions and applied voltage same as described in (A).

Separations using tartrate

After optimizing conditions for CE with a phthalate system, it was found that even better separations of metal cations could be obtained with tartrate as the complexing reagent. Tartrate forms comparatively weak complexes with a number of metal ions and is therefore suitable for capillary ion electrophoresis. However, copper(II) is more strongly complexed and elutes very late or not at all.

An excellent separation of twelve metal ions in a total time of only 8 min is shown in Fig. 2. Resolution is excellent with a steady, flat baseline, as shown in Fig. 2A. At the higher concentration in Fig. 2B, the peaks are very narrow and well shaped.

Some optimization of conditions was required to obtain separations of the quality shown in Fig. 2. The concentration of tartrate and *p*-toluidine used, pH and applied voltage were the most important variables. Methanol (20%) was added to the electrolyte to improve resolution of the divalent metal ions.

Much of the literature on CE does not adequately address the question of the quantitative aspects. We therefore prepared calibration curves for the metal ions studied in the tartrate system. Good linear calibration curves were obtained for the ions studied in the 0.4–10 μg/ml concentration range. The only exception was zinc(II) which gave a relatively poor correlation coefficient for linear regression.

Separations using lactate

Lactate has the same complexing groups as tar-

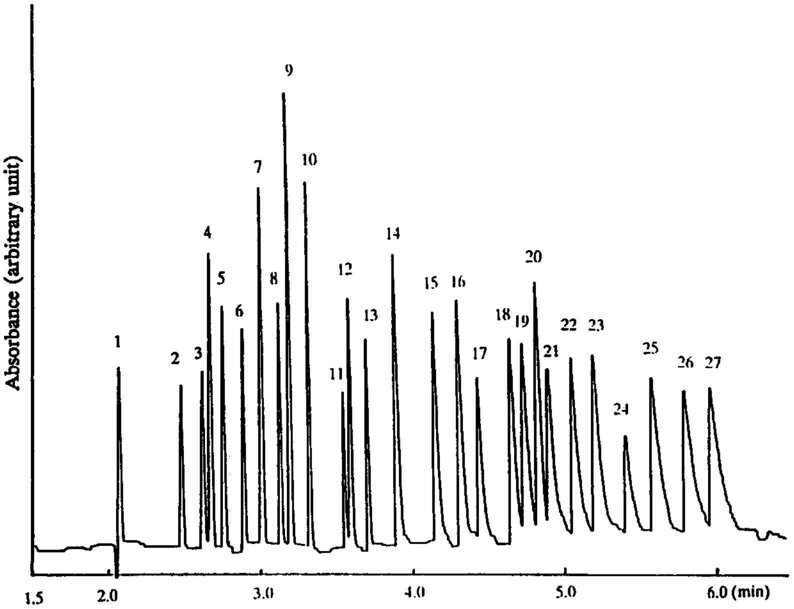

Fig. 3. Separation of 27 alkali, alkaline earth, transition and rare earth metal ions in a single run using lactate. Electrolyte, 15 mM lactic acid, 8 mM 4-methylbenzylamine, 5% methanol, pH 4.25; applied voltage, 30 kV. Peaks: 1 = K^+; 2 = Ba^{2+}; 3 = Sr^{2+}; 4 = Na^+; 5 = Ca^{2+}; 6 = Mg^{2+}; 7 = Mn^{2+}; 8 = Cd^{2+}; 9 = Li^+; 10 = Co^{2+}; 11 = Pb^{2+}; 12 = Ni^{2+}; 13 = Zn^{2+}; 14 = La^{3+}; 15 = Ce^{3+}; 16 = Pr^{3+}; 17 = Nd^{3+}; 18 = Sm^{3+}; 19 = Gd^{3+}; 20 = Cu^{2+}; 21 = Tb^{3+}; 22 = Dy^{3+}; 23 = Ho^{3+}; 24 = Er^{3+}; 25 = Tm^{3+}; 26 = Yb^{3+}; 27 = Lu^{3+}. The concentration of each lanthanide, Ba^{2+}, Sr^{2+}, and Pb^{2+} was 5 μg/ml; the concentration of transition metals, K^+, Na^+, and Ca^{2+} was 3–4 μg/ml, Mg^{2+} was 2 μg/ml and Li^+ was 1 μg/ml.

trate (carboxyl and hydroxyl) but is a smaller molecule and forms somewhat weaker complexes with most metal ions. Preliminary experiments indicated that a lactate system might give good separations both for divalent metal ions and for trivalent lanthanides.

A brief optimization of major conditions was first carried out for separation of the lanthanides. These conditions included the concentrations of lactate and UV visualization reagent, and pH. A very good separation of the thirteen lanthanides was obtained. It was also found that excellent separations could be obtained under the same conditions for alkali metal ions, magnesium and the alkaline earths, and several divalent transition metal ions. All of these except copper(II) eluted before the lanthanides. An excellent separation of 27 metal ions was obtained in a single run that required only 6 min (Fig. 3).

The detection limit for these metal ions was in the range 0.05–0.5 μg/ml. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentrations used.

Separations using HIBA

HIBA has been used extensively for CE. Very good separations of magnesium, the alkaline earths and several of the divalent transition metal ions have been reported [3]. Excellent separation of all of the lanthanides have also been obtained [1,3]. In one instance, thirteen lanthanides plus six other metal ions were separated in a single run, although the shapes of some of the earlier peaks were rather poor [1,3].

We experimented with separation of the lanthanides using HIBA as the complexing reagent. A brief optimization was employed to establish pH and suitable concentrations of HIBA and the UV-sensitive reagent. The concentration of HIBA needed was found to be considerably lower than lactic acid in the previous system. A truly excellent separation of the lanthanides was obtained (Fig. 4). The conditions we established are quite similar to those previously reported.

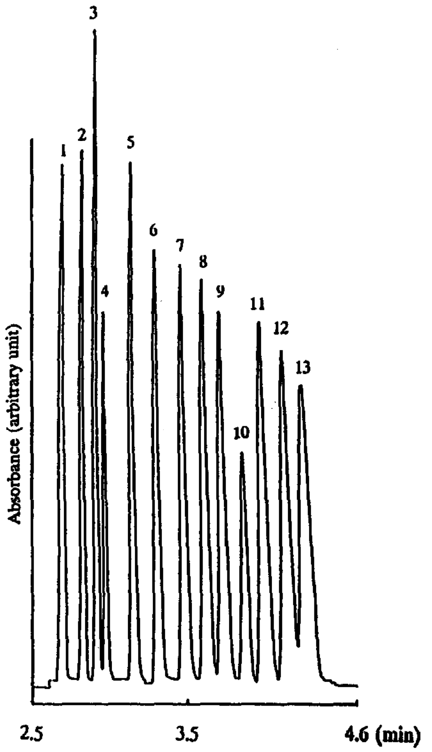

Fig. 4. Separation of 13 lanthanides using HIBA. Electrolyte, 4 m*M* HIBA, 5 m*M* UV Cat1, pH 4.3; applied voltage, 30 kV. Peaks: 1 = La^{3+}; 2 = Ce^{3+}; 3 = Pr^{3+}; 4 = Nd^{3+}; 5 = Sm^{3+}; 6 = Gd^{3+}; 7 = Tb^{3+}; 8 = Dy^{3+}; 9 = Ho^{3+}; 10 = Er^{3+}; 11 = Tm^{3+}; 12 = Yb^{3+}; 13 = Lu^{3+}.

The separation mechanism

In CE it is now common to use a reagent to partially complex metal ions and improve the quality of their separation by altering their net flow characteristics. For example, addition of tartrate to a solution containing metal cations (M_1^{2+}, M_2^{2+}, etc.) may convert some fraction of the metal ions to tartrate complexes (M_1–tart, M_2–tart, etc.). The free cations move along the capillary at rates proportional to their ionic mobilities while the *complexed* metal ions move at a slower speed. Continuous equilibration between free and complexed metal ions causes each metal to move through the capillary as a tight zone. Separation occurs because of the different overall rates at which the zones move. The situation is again somewhat analogous to HPLC except that in this case the "stationary phase" (metal tartrate complexes) is not a real phase and is not stationary, but moves at a slower speed than the free metal cations.

The more completely a metal ion is complexed, the slower will be its rate of movement. If the fraction of metal in the free cation is too small, it may take a long time for the metal to emerge from the capillary and no good separation will be obtained. However, if the various metal ions are not sufficiently complexed (too large a fraction of free metal ion), ions of similar ionic mobility may be poorly

TABLE I

FRACTIONS OF FREE (α_M) AND COMPLEXED (α_{ML_n}) RARE EARTH METAL IONS AND AVERAGE NUMBER OF LIGANDS ($\bar{n}$) IN 4 m*M* HIBA ELECTROLYTE SOLUTION AT pH 4.3

Metal	α_M	α_{ML}	α_{ML2}	α_{ML3}	α_{ML4}	$\bar{n}$
La	0.578	0.360	0.612			0.482
Ce	0.448	0.496	0.052	0.004		0.612
Pr	0.407	0.496	0.093	0.005	0.000	0.697
Nd	0.333	0.572	0.085	0.010	0.000	0.772
Sm	0.296	0.520	0.170	0.013	0.001	0.903
Gd	0.250	0.481	0.244	0.024	0.001	1.045
Tb	0.181	0.470	0.307	0.040	0.002	1.212
Dy	0.141	0.384	0.387	0.084	0.004	1.426
Ho	0.122	0.365	0.413	0.093	0.006	1.494
Er	0.097	0.309	0.472	0.112	0.010	1.629
Tm	0.079	0.309	0.473	0.123	0.016	1.686
Yb	0.070	0.296	0.431	0.169	0.033	1.797
Lu	0.047	0.222	0.514	0.172	0.045	1.946

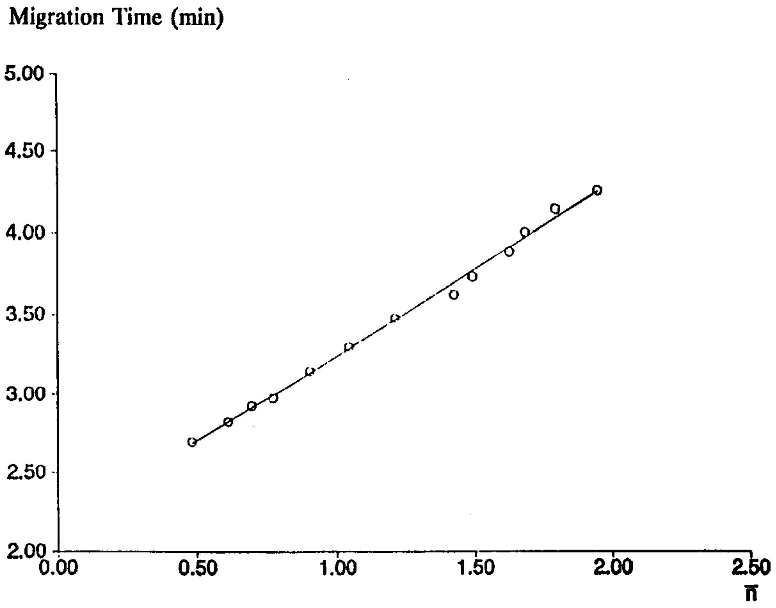

Fig. 5. Plot of moving time *vs.* average number of ligands for 13 lanthanides. Electrolyte conditions and applied voltage same as described for Fig. 4.

separated. Almost nothing has appeared in the literature concerning how strongly metal ions should be complexed for optimal separation by CE. However, Hirokawa *et al.* [9] computed the absolute mobilities of some lanthanide complexes used in isotachophoresis.

We considered the separation of rare earths using 4.0 m*M* HIBA at pH 4.3 as the complexing reagent (see Fig. 4). Using published formation constants [10], the fraction of rare earths present in various chemical forms was calculated by a well-known method [11,12] under the same conditions of pH and HIBA concentration used for the CE separation in Fig. 4. The calculated distribution of chemical species for each rare earth is shown in Table I.

Some interesting conclusions can be drawn from the information in Table I. The predominating species are the free metal ion (M^{3+}), the 1:1 metal–ligand complex (probably ML^{2+}), the 2:1 complex (probably ML_2^+) and the 3:1 complex (probably ML_3). A small fraction of the higher rare earths is also present as the 4:1 complex. Another striking feature is that the average number of ligands associated with a rare earth ($\bar{n}$ increases rapidly with increasing atomic number. This occurs in a fairly regular manner as demonstrated by a plot of $\bar{n}$ against atomic number which has a linear regression correlation coefficient of 0.9958.

In CE the positively charged complexes, as well as the free metal cation, would be expected to move through the capillary by electrophoretic flow as well as by the electroosmotic flow that affects all species. However, the electrophoretic mobility should be slower for ML^{2+} than for M^{3+} and still slower for the larger ML_2^+. Even if the different species move at different rates, rapid equilibrium shifts should keep a tight zone for each of the rare earths. For example, as the faster moving M^{3+} starts to move ahead of the other species, it reequilibrates with the ligand (L) to form a larger fraction of the slower-moving species. At the back edge of the zone, the slower-moving complexes reequilibrate to give a greater fraction of M^{3+}. The average rate of movement should depend on te weighted average of the mobilities of the different species.

This last speculation was tested by plotting the moving time of the various rare earths (from Fig. 4) against the $\bar{n}$ values in Table I. This plot is shown in Fig. 5. It is essentially linear with a correlation coefficient of 0.9978.

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