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Separation of metal cations by electrophoresis in a positively charged coated capillary

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Abstract

In the expanding field of capillary electrophoresis, the use of coated capillaries is becoming more widespread. Application of a thin polymer coating on the surface of the silica capillary wall makes it possible to generate a capillary wall of positive or no charge when using a typical buffer of a pH of 5.0 to ca. 6.0, thereby altering the electroosmotic flow. Indeed, when a coating is used which carries a positive charge under appropriate buffer conditions, the electroosmotic flow is reversed from the normal direction. This reversal increases the migration times of metal cations and also improves separation efficiency. In this work, a positively charged coated capillary was used with a 2-aminopyridine buffer system for the separation of metal cations. Separations using this system compared favorably with other published results. The positively charged coated column yielded cation separations superior to those obtained with uncoated columns. This system proved effective for the separation of many cations, including lanthanides.

1. Introduction

Fused-silica capillaries are the most common columns used in capillary electrophoresis (CE) separations. Resolution is the function of the column efficiency, N, and relative migration velocity, $\Delta U/U_A$, expressed as $R_s = 1/4n^{1/2}(\Delta U/U_A)$ [1]. For biological samples, such as proteins or amino acids, there are two means by which higher resolution can be achieved. First, since the species of interest are often negatively charged, the electroosmotic flow (EOF) moves in a direction opposite that of the analytes, causing a large relative velocity difference. Second, biochemical analytes have large molecular masses and correspondingly small diffusion coefficients which contribute to a high theoretical plate number. By contrast, in metal cation separations, the EOF is codirectional with species migration, and band broadening results from the relatively high axial diffusion of the low-molecular-mass cations. If the EOF can be effectively decreased or reversed in direction so that it moves opposite the cations, then resolution can be increased due to the increase in $\Delta U/U_A$. Coating of the inner capillary wall is a technique commonly used to change the surface characteristic of such columns in order to bring out the desired change of EOF.

Capillary column technology for chromatography advanced significantly in the early 1980s. The chemistry involved in the production of

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state-of-the art capillary columns for gas chromatography has been studied and reported in detail [2,3]. The fundamental physical and chemical properties of the capillary surface, the techniques of chemical modification of the capillary surface, and the production of uniform and stable stationary phase films have been described. Coated columns are commonly used in CE analysis of biological samples to increase separation efficiency and resolution [4,5]. Up to now, few cation separations by CE have adopted these column alteration techniques. C_1- and C_{18}-saturated hydrocarbon coatings were applied by Chen and Cassidy [6]. In their work with 75 μm I.D. C_{18}-coated capillaries, comparison of the heights equivalent to a theoretical plate (HETP values) suggested that the overall column efficiencies were smaller than those for an uncoated capillary. The interactions between the silica surface and the positively charged ions were reduced due to the hydrophobic coating. In their experiments, adsorption of the larger hydrophobic ions and associated peak tailing were observed. In this work we describe the use of coated capillaries in the separation of metal cations, and compare our results with other published metal cation separations by capillary electrophoresis.

2. Experimental

2.1. Apparatus

The following equipment was used in this research: Metrohm 654 pH Meter (Brinkmann, Metrohm, Switzerland); fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA); CE system CES-1 (Dionex, Sunnyvale, CA, USA); Milli-Q water system (Millipore, Milford, MA, USA).

Metal cations were indirectly detected on-column by UV absorbance at 214 nm. Data were collected with Dionex AI400 and AI450 software together with a Model SP4270 integrator (Spectra-Physics, San Jose, CA, USA).

2.2. Materials

3-Aminopropyltrimethoxysilane (Aldrich) was used for the capillary coating The commercial 2-aminopyridine used to make the buffer was pale yellow. Therefore it was recrystallized in cyclohexane to yield white, flaky crystals. 2-Aminopyridine (0.941 g) was dissolved in water to produce 100 ml of buffer solution. To make the pH adjustment solution, acetic acid (99%) was diluted 10-fold. Buffer solutions of 15 m*M* 2-aminopyridine/acetate were prepared by diluting 2-aminopyridine stock solution and adjusting pH to 5.0 using the diluted acetic acid solution. In the separations of lanthanide samples, 2-hydroxyisobutyric acid (HIBA) (3.5 m*M*) was included in the buffer. The composition and pH of 2-aminopyridine/acetate buffer have been optimized for cation separations [7].

The 1.00 mg/ml metal cation standard solution was made by dissolving the appropriate weights of the nitrates of K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+} and lanthanides in 2% nitric acid.

All above solutions were prepared with deionized water (18 mΩ cm). Before use, all solutions were filtered through a 0.45-μm cellulose acetate membrane and degassed in an ultrasonic bath.

2.3. Preparation of coated columns

Several coated capillaries with different surface charges were prepared as previously described [8]. The coatings are listed in Table 1. In brief, the positively charged capillaries were prepared by the following steps: the capillaries were treated with deionized water (18 MΩ cm) before coating in order to ensure uniform distribution of silanol groups on the silica surface. The capillaries were filled with deionized water, then drained and sealed under nitrogen. They were then heated at 250°C for 2 h to increase the population of silanol groups on the surface. The capillaries were opened and purged with nitrogen for 1 h. The coating solutions consisted of varying amounts of 3-aminopropyltrimethoxysilane in methylene chloride. The capillaries

Table 1
Capillary coatings

Name	Coating material	Description
Superox 4	Polyethylene glycol	Neutral
	Acryloyl-amido-2-2-methylpropanesulfonic acid	Negative charge
	3-Aminopropyltrimethoxysilane	Positive charge

were filled with coating solution and statically coated [9] at 40°C. The coated capillaries were purged with nitrogen for 1 h at 40°C and heated to 120°C under nitrogen pressure for 2 h to cross-link and bond the 3-aminopropyltrimethoxysilane on the surface. Before installing a coated capillary in the CE system, it underwent a rinsing process using 5-ml quantities each of methylene chloride, methanol and deionized water, in that order.

3. Results and Discussion

3.1. Selection of capillary coating

One method to modify the EOF in the capillary involves application of different coating materials to alter the surface charge on the capillary wall. A negatively charged capillary generates an EOF toward the cathode, while a positively charged capillary generates an electroosmotic flow toward the anode. The EOF, in turn, affects the separation. Fig. 1 shows electropherograms of the same metal cation standard using capillaries with coatings of different charge. The capillaries with charged coatings did not achieve good separations for biological samples [10]. However, we found that the column with the positive coating retained the cationic species much longer than the either the uncoated or neutral coated column. The uncoated column, on the other hand, unexpectedly retained the cations longer than the neutral coated column. No reasonable explanation for this anomaly has been found, and it will be the subject of future investigation.

The variation of separation efficiency determined from different cation peaks using different capillary coatings was measured in terms of theoretical plate numbers (Fig. 2). The positively charged coating (3-aminopropyltrimethoxysilane) gave higher efficiency than the uncoated column for most of the metal cations tested. The increase in plate number resulted from the repulsive force between the respective positive charges of the cations and of the capillary surface. The neutral polymer coating increased the plate number for the later peaks in the electro-

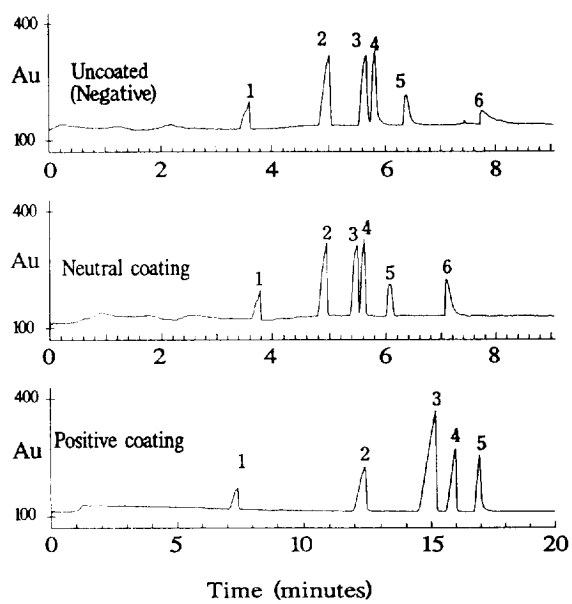

Fig. 1. Electropherograms of the separation of K^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+} (which are labeled peaks 1, 2, 3, 4, 5 and 6, respectively) with different coated capillaries: neutral coating is polyethylene glycol; positive coating is 3-aminopropyltrimethoxysilane. Conditions: 15 m*M* 2-aminopyridine acetate buffer, pH 5.0, 25 000 V, 80 cm × 75 μm I.D. fused-silica capillary, 100 mm gravity injection for 30 s and indirect detection at 214 nm.

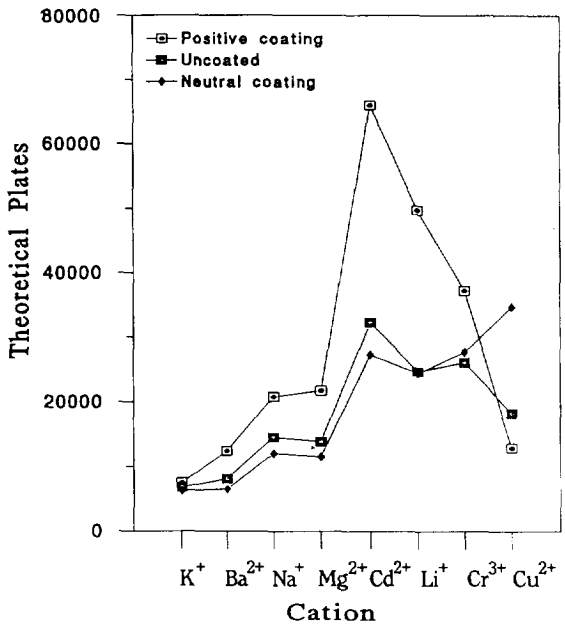

Fig. 2. Variation of separation efficiencies with different charged capillaries (the average of four determinations, standard deviation <3%). Neutral coating is polyethylene glycol; positive coating is 3-aminopropyltrimethoxysilane. Conditions as in Fig. 1.

pherogram because the interaction between the column wall and the cations was less than for a negatively charged wall surface. A negatively charged coating (sulfonic acid) was also evaluated. It yielded results similar to those of the uncoated capillary, as we expected. However, the later peaks in the electropherogram were more deformed than those using the uncoated capillary, so the corresponding results were not included in Fig. 2. Poor peak shapes were probably due to greater negative charge density which may have been present on the surface of the coated capillary. The positively charged capillary was selected for further experimentation, in part because of its superior separation efficiency.

Compared to uncoated capillaries, Chen and Cassidy's [6] C_{18}-coated capillaries yielded better peak resolution, although adsorption and peak tailing were observed for larger hydrophobic ions. In that work, the preparation of the C_{18}-coated capillary required two to three days. In contrast, the positively charged coating in our research is relatively simple and quick to prepare. Only 3 or 4 h were needed to apply a coating of 3-aminopropyltrimethoxysilane on the capillary wall. The resulting positively charged capillary provides improved separation efficiency and resolution in metal cation analysis over uncoated or C_{18}-coated columns. In contrast to the C_{18}-coated capillary, no adsorption of ions was observed in our experiments with the neutral coating, as indicated by the reproducibility of the cation migration times and by the peak shapes. In similar fashion, the statically coated capillaries did not display significant adsorption in protein separations reported in previous work [11]. Furthermore, using the 2-aminopyridine/acetate buffer and the positively charged capillary, we obtained a system which provided reproducible separation and high performance during hundreds of metal cation separation experiments.

3.2. The selection of coating solution concentration

A comparison was made of coated capillaries for which different 3-aminopropyltrimethoxysilane concentrations were used to prepare the columns. Fig. 3 shows that except in the case of Zn^{2+}, the separation efficiencies were similar for a given cation between different coating concentrations. The migration times of the cations changed with different capillary coating concentrations, but no variation was observed between 0.5 and 3.0 mg/ml (Fig. 4), which presents a reasonable working range. The variation of cation migration times is due to differences in EOF, as discussed in the next section. The cation bands broadened with increasing migration time, so the resolution of the peaks did not change significantly with coating concentration over the range 0.5–3.0 mg/ml.

The capillaries coated with the solution ranging from 0.5 to 3.0 mg/ml displayed similar metal cation separation behavior in terms of migration times and resolution. This behavior is due to similar EOFs, as will be discussed in more detail in the following section. Of these capillaries, the 2.0 mg/ml coated capillary was chosen

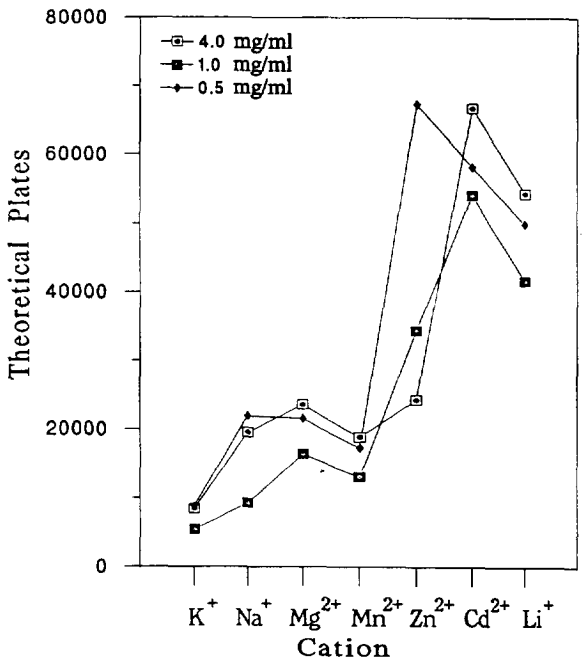

Fig. 3. Variation of separation efficiencies with capillaries coated by different concentrations of 3-aminopropyltrimethoxysilane (the average of four determinations, standard deviation <14%). Conditions as in Fig. 1.

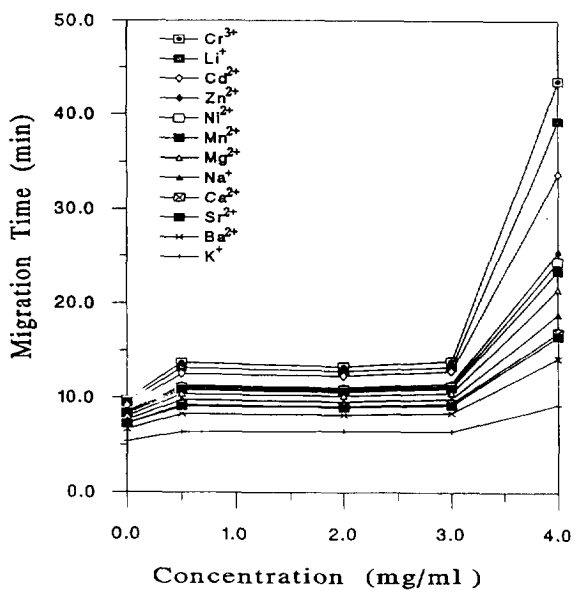

Fig. 4. Variation of cation migration times with capillaries coated with different concentrations of 3-aminopropyltrimethoxysilane. The standard deviation was <2.0%. Conditions as in Fig. 1.

for further investigation since it lies at the center of the acceptable working range.

3.3. Structure of the coating layer

The separation behavior of the coated column should be related to the properties of the coating surface. Generally in chromatography, coated columns are evaluated in terms of thickness, d_f, of the stationary phase, given by $d_f = cr/2000\rho$, where c is the concentration of the coating reagent, r is the column inside radius, and ρ is the density of the coating solution. However, this approach is not useful in CE because the capillary coating does not serve as a stationary phase. Specifically, only a portion of the coating solute was bound to the surface of the capillary as the solvent was evaporated because the bound portion is dependent on the reaction equilibrium. The remainder may have been washed away with the rinse. In CE, the separation behavior should be related to the percent of the capillary wall covered rather than to the coating thickness.

3.4. Electroosmotic flow

The concentration of coating solution should be related to the charge density on the column surface up to the point where the surface is completely covered. The charge density, in turn, can affect the direction and strength of the EOF. Using dimethylsulfoxide (DMSO) as the neutral marker, the EOF was observed to change direction when the capillary was coated with 3-aminopropyltrimethoxysilane. The rate of flow increased with the concentration of 3-aminopropyltrimethoxysilane coating solution, but not in a linear fashion (Fig. 5). This result accounts for the non-linearity in the change of cation migration times with coating (Fig. 4). The variation in cation migration times and in EOF could be caused by dynamic factors in the coating reaction or by technological factors in the column coating process. The dynamic factors could include the degree of activation of the original column surface, the evaporation rate of the solvent, and the temperature and flow-rate of the

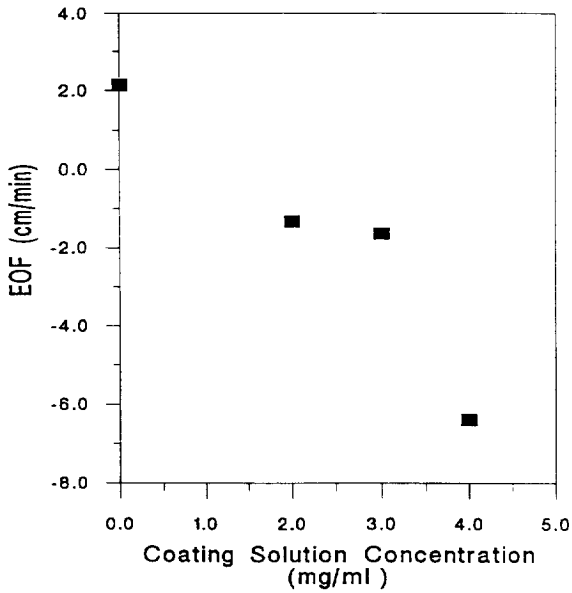

Fig. 5. Variation of electroosmotic flow (EOF) with capillaries coated using different concentrations of 3-aminopropyltrimethoxysilane (the average of six determinations, standard deviation <3%). Conditions: 15 m*M* 2-aminopyridine–acetate buffer, pH 5.0, 25 000 V, 80 cm × 75 μm I.D. fused-silica capillary, 100 mm gravity injection and indirect detection at 214 nm.

inert gas in the cross-linking reaction. The technological factors involve our ability to exactly reproduce the preparation of coating each time. Similar phenomena were observed in Chen and Cassidy's research [6], wherein the electroosmotic flow in a C_{18}-saturated hydrocarbon coated capillary varied with a relative standard deviation of 13%. This variation in coating efficiency has been noted in the reports of coated capillaries in gas chromatography [9] as well.

3.5. The selection of working voltage

The working voltage directly affects the migration of analytes and background electrolyte in the buffer, as well as the electroosmotic flow. Fig. 6 shows the effect of working voltage on the migration times of the metal cations using the 3-aminopropyltrimethoxysilane coated capillary. At working voltages of 18 000 and 22 000 V, a longer analysis time was needed than at higher

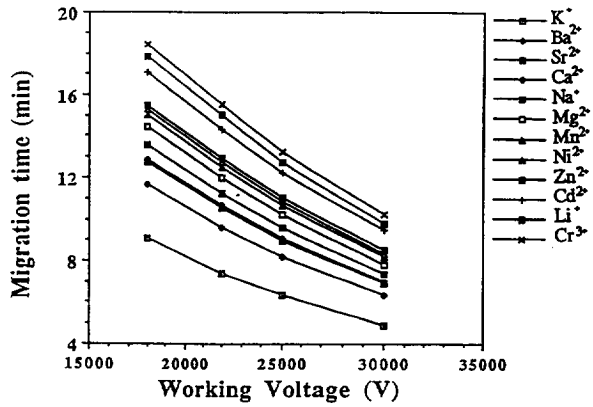

Fig. 6. Effect of working voltage on the migration times of the metal cations. Conditions as in Fig. 1.

voltage, with little variation in resolution between peaks. At 30 000 V working voltage, the resolution decreased between Sr^{2+} and Ca^{2+}, even though the analysis time shortened (Fig. 7). A similar situation was observed by Beck and Engelhardt [12]. A working voltage of 25 000 V was selected as optimal for our experiments to maintain good resolution and short analysis time.

In a CE system, the working voltage is one parameter to be optimized. However, it was observed that the signal-to-noise ratio was affected primarily by the working *current*. In contrast to voltage, the resolution between peaks was not altered by the working current. When the working current was lower than 15 μA, the electropherograms had broad peaks and longer migration times. When the current was between 18 and ca. 20 μA, the electropherogram had normal peak shapes and shorter migration times. If the current increased over 22 μA, the analysis time was further shortened, but more noise appeared because of Joule heating effects [13]. A typical working current of between 18 and ca. 20 μA was observed with the 2-aminopyridine/acetate buffer under our experimental conditions.

3.6. Metal cation separations

Twelve metal cations, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and

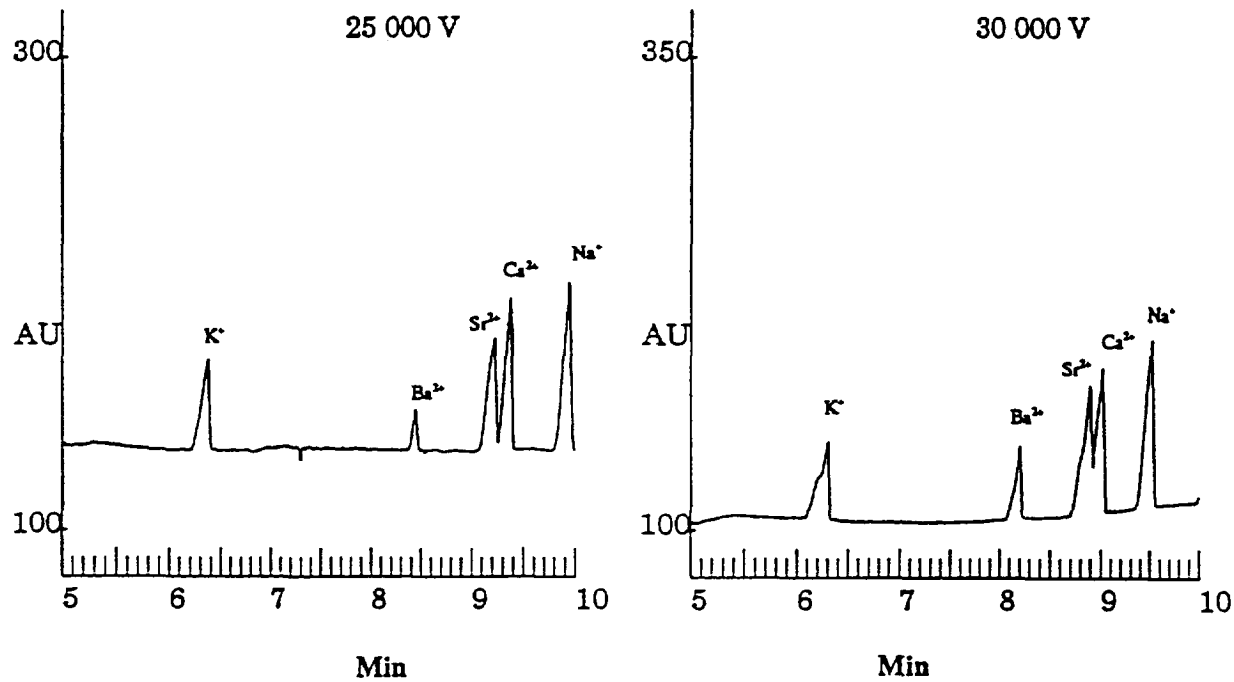

Fig. 7. Comparison of the resolution between the Sr^{2+} and Ca^{2+} peaks with different working voltages. Conditions as in Fig. 1.

Cd^{2+} were separated with baseline resolution using the 15 m*M* 2-aminopyridine/acetate buffer and the 2.0 mg/ml 3-aminopropyltrimethoxysilane coated capillary (Fig. 8, bottom). The concentrations of these cations were 5 μg/ml Na^+, K^+, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}; 8.5 μg/ml Ba^{2+}; 5.5 μg/ml Sr^{2+}; 2.5 μg/ml Ca^{2+}; 1.5 μg/ml Mg^{2+}; 1.0 μg/ml Li^+. This separation was achieved based on the effect of the positively charged coating on the EOF and on the mobility differences between the metal cations. Compared to uncoated capillaries, the positively charged capillary provided better resolution, although overall migration times increased. Fig. 8 compares the separation of a sample using an uncoated capillary (top) to that using a 2.0 mg/ml 3-aminopropyltrimethoxysilane coated capillary (bottom). A complete separation of twelve metal cations was achieved using the coated capillary with a longer total analysis time. Complete separation was not achieved by the uncoated capillary.

When we attempted to separate additional cations using the coated capillary, K^+ and NH_4^+ coeluted; however, when the concentrations of K^+ and NH_4^+ were diluted to lower than 0.1 μg/ml (close to the detection limit), K^+ and NH_4^+ were separated into two peaks. In the latter case, the difference in migration times was only 0.05 min (3 s). Other pairs of coeluted cations were Mn^{2+} and Fe^{2+}, Ni^{2+} and Co^{2+}, and Pb^{2+} and Cd^{2+}. Although a signal was obtained for Cu^{2+}, its migration time was so long (ca. 35–40 min) that axial diffusion produced a flat, large tailing peak.

Cation separations by the 3-aminopropyltrimethoxysilane coated capillary with the 2-aminopyridine/acetate buffer produced higher resolution but somewhat greater band broadening than with the uncoated capillary. As shown in Fig. 8, the twelve metal cations, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, were separated in 14 mins. This separation has good resolution for all peaks, even though it requires a relatively longer analysis time due to the direction of the electroosmotic flow. This separation compares favorably to those reported elsewhere [14–17].

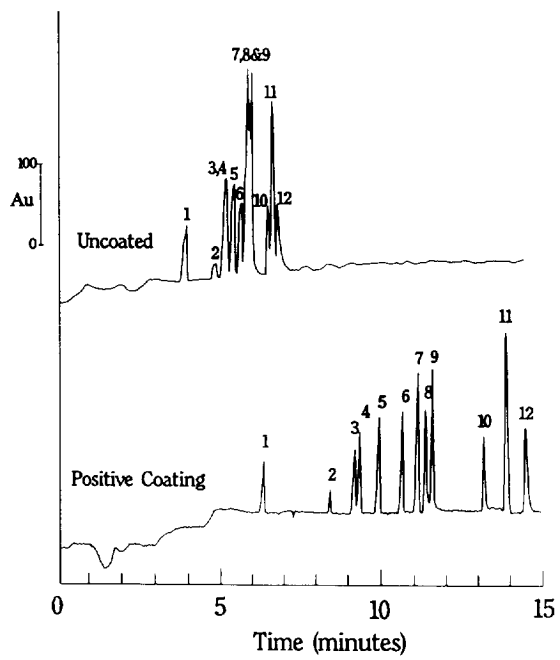

Fig. 8. Electropherogram comparing separations using an uncoated capillary (top) and a 2.0 mg/ml 3-aminopropyltrimethoxysilane coated capillary (80 cm × 75 μm I.D.) (bottom). Conditions as in Fig. 1. The peaks are K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Li^+ and Cr^{3+} from 1 to 12 in order.

3.7. Quantitative analysis

Simultaneous, quantitative analysis of the twelve metal cations listed above was achieved by our method. The equations of the calibration curves, correlation coefficients, average standard deviations, and detection limits for these cations are listed in Table 2. Chen and Cassidy [6] observed that the signal-to-noise ratio obtained with a neutrally coated capillary was similar to that obtained with the uncoated capillary at pH 4.5 to ca. 5.0. In our experiments, the linear range of quantitative analysis and the detection limits of the positively charged capillary were superior to those of the uncoated capillary.

The sensitivity of our system was superior to that reported by others [12,15,16,18]. The principal reason for this result is that stacking efficiency, which focuses the sample zone into a narrow band, was more effective in the positively charged column than in the uncoated columns because the EOF was in the direction opposite to that of cation migration. Furthermore, baseline noise with the positively charged column was lower than with the uncoated column, resulting in a better signal-to-noise ratio. For the metal cations studied, the detection limits with the

Table 2
Parameters for quantitative analysis using 2 mg/ml 3-aminopropyltrimethoxysilane coated capillary

Ions	EC	r^2	R.S.D. (%)	DL (μg/ml)
Li^+	$y = 11.971 + 429.74x$	1.000	2.8	0.02
Na^+	$y = -10.971 + 52.054x$	1.000	1.4	0.08
K^+	$y = 13.069 + 27.265x$	1.000	2.0	0.03
Mg^{2+}	$y = -13.018 + 170.20x$	1.000	1.4	0.02
Ca^{2+}	$y = 7.8364 + 72.381x$	0.999	1.0	0.02
Sr^{2+}	$y = -9.3126 + 46.665x$	1.000	2.1	0.15
Ba^{2+}	$y = 16.400 + 4.1449x$	0.999	6.6	0.34
Cr^{3+}	$y = -17.566 + 61.992x$	1.000	2.4	0.15
Mn^{2+}	$y = -59.695 + 87.821x$	0.998	1.8	0.08
Ni^{2+}	$y = -51.144 + 67.331x$	0.999	1.2	0.35
Zn^{2+}	$y = 36.546 + 63.778x$	0.998	3.2	0.20
Cd^{2+}	$y = 5.2129 + 36.206x$	0.998	5.7	0.35

EC = Equations of calibration curves; y is absorbance and x is concentration (μg/ml); r^2 = correlation coefficient of the calibration curves; R.S.D. = relative standard deviation; DL = detection limit, signal-to-noise ratio 3.

coated capillary approached 0.1 to ca. 0.01 μg/ml. The linear ranges were from 0.5 to ca. 50 μg/ml for Na^+, K^+, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, from 0.15 to ca. 15 μg/ml for Mg^{2+}, from 0.5 to ca. 5 μg/ml for Ca^{2+}, from 0.5 to ca. 55 μg/ml for Sr^{2+}, from 0.8 to ca. 20 μg/ml for Ba^{2+}, and from 0.1 to ca. 10 μg/ml for Li^+. In this simultaneous determination, the linear range of the calibration curves for alkaline earth metals were not as good as with the uncoated capillary. The linear range of Ca^{2+} was also smaller, because the peak of Sr^{2+} was superimposed on that of Ca^{2+} at higher concentrations. Also, a relatively large standard deviation of determination appeared at high concentrations of Sr^{2+}. For all others, the linear calibration range and the detection limits with the coated capillary were superior.

3.8. Use of a complexing agent to separate lanthanides

Two different buffer characteristics were varied to improve cation separations by our CE methods. First, by adjusting the pH of the buffer, we were able to change the EOF [9]. The second characteristic involved the addition of complexing agents to the buffer. The complexation equilibria enhanced the mobility differences between sample ions.

In the separation of metal cations, HIBA is commonly used as a complexing agent. In 1981, Nukatsuka *et al.* [19] described the use of HIBA as a complexing agent in the separation of lanthanides. In 1990 Foret *et al.* [16] reported its use in the separation of rare earth cations, alkali metal cations and Mg^{2+}. Researchers at Waters/Millipore have published comprehensive papers [14,18] describing the use of HIBA as a complexing agent. Differences in the mobilities of lanthanide ions were enhanced by the addition of HIBA to the buffer, but the elution order was not changed. For transition metals, on the other hand, the enhancement of mobility differences was accompanied by changes in elution order, for example, between Co^{2+} and Pb^{2+}.

In our experiments, 3.5 m*M* HIBA was added to the buffer. Efficient separation (Fig. 9) was achieved for almost all cations under the conditions: 60 cm × 75 μm I.D. capillary coated with 2.0 mg/ml 3-aminopropyltrimethoxysilane, 15 m*M* 2-aminopyridine/3.5 m*M* HIBA/acetate buffer (pH 5.0), 30 kV working voltage. The 24 cations were separated in 20 min. With HIBA added to the buffer, baseline noise was significantly increased.

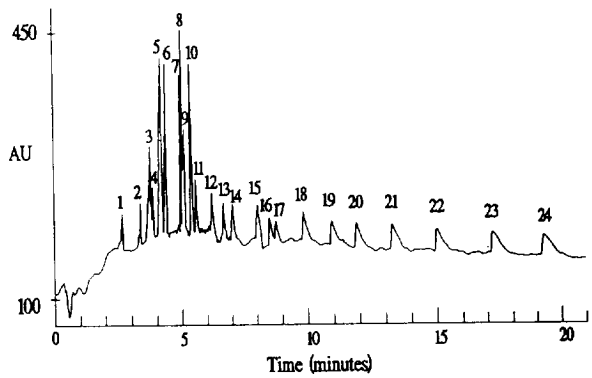

Fig. 9. Electropherogram showing the separation of 24 cations: K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Li^+, Cr^{3+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+} which are labeled from 1 to 24 in order. Concentrations: Li^+ was 1 μg/ml and others were 5 μg/ml. Conditions: 3-aminopropyltrimethoxysilane coated capillary (60 cm × 75 μm I.D.), 15 m*M* 2-aminopyridine–3.5 m*M* HIBA–acetate buffer, pH 5.0, 30 000 V, 100 mm gravity injection for 30 s, and detection at 214 nm.

4. Conclusions

Coated capillaries, commonly employing neutral coating materials, have been used in CE to separate biological samples [4,5,11]. The 3-aminopropyltrimethoxysilane coated capillary has been successfully used to separate 12 transition metal cations (Fig. 8). Both separation efficiency and resolution were superior to those obtained with uncoated capillaries. The lower limits of the linear range and the detection limits were improved ten fold over uncoated capillaries using the "UV-Cat 1" buffer [20] or the creatinine buffer [15]. Our method of capillary coating is easier and faster than the C_{18}-coated capillary [6]. No adsorption of analytes on the

bonded coating was observed in our experiments. The electroosmotic flow was stable for a long period of time, even after 50–ca. 60 runs. With the addition of complex-forming HIBA to the buffer, 24 metal cations were separated with the positively charged capillary in 20 min. With this capillary, the column efficiency and resolution achieved were higher than those obtained with the C_{18}-coated capillary.

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