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Short communication

Prevention of peak overlap in the capillary electrophoretic separation of inorganic cations by use of a positively charged coated capillary

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Abstract

The use of a quaternary amine-coated capillary in the separation of alkali and alkaline earth cations yielded a decisively better resolution than is possible with a conventional fused-silica capillary. Improved resolution is required in particular when sample solutions contain small amounts of cations in the presence of others with much higher concentrations. The determination of, for example, Mg^{2+} in seawater is not possible with standard conditions, as the large Na^{+} peak overlaps strongly with the peak due to Mg^{2+}. With the coated capillary, baseline resolved separations without peak overlapping could readily be obtained for seawater, albeit at a somewhat longer analysis time. The approach might also be useful in the analysis of other cations and types of samples. © 1997 Elsevier Science B.V.

Keywords: Water analysis; Inorganic cations

1. Introduction

Capillary electrophoresis has become an interesting alternative for the determination of inorganic cations [1–3] in the last few years. Since many metal cations have quite similar electrophoretic mobilities in aqueous solutions [4], published methods often use one or more complexing agents, such as α-hydroxyisobutyric acid, in the background electrolyte to modify the mobilities of the cations [5,6].

However, in the determination of small amounts of cations in the presence of cations with high concentrations, peak overlap caused by the high concentration cation occurs for methods that work fine with solution where all analytes are present at similar concentrations. This makes the analysis of some minor constituents unreliable, or even impossible, in samples such as seawater. For the analysis of this kind of sample matrices, it has been suggested that the electrophoretic flow should be reduced, by increasing the levels of complexing agents, to such an extent that separation is possible [7]. This method is problematic as the resulting increase in current (due to increased conductivity) may require a reduction in the applied separation voltage to keep column heating under control. The addition of an organic solvent such as methanol to the buffer has also been suggested [8,9]. Magnesium and calcium alone, in seawater, have been determined as their ethylenediaminetetraacetic acid (EDTA) complexes [10].

A more generally applicable, simple method that yields higher resolution than usually achievable

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would be of interest. For cation separations in bare fused-silica capillaries, the electroosmotic flow is codirectional with species migration. If the electroosmotic flow is decreased or reversed in direction so that it moves in the opposite direction to the electrophoretic flow of the cations, their residence time in the electric field is prolonged, which is consistent with an increase in the effective length of the separation path. It has been demonstrated recently that by either using neutral [11] or positively charged [12] coated capillaries to alter the electroosmotic flow, the separation of cations can indeed be enhanced.

In this work, a quaternary amine-coated, positively charged capillary was used for the separation of inorganic metal cation solutions. We demonstrate for solutions of alkali and alkaline-earth cations that this approach allows easy separation of minor constituents from major ones. Emphasis was placed on the separation of seawater samples, a matrix where the separation of Ca^{2+} and Mg^{2+} from Na^{+} can otherwise only be achieved with difficulty.

2. Experimental

2.1. Apparatus

A Spectraphoresis 100 capillary electrophoresis system, equipped with a Spectra 100 variable-wavelength UV–Vis CE detector from Thermo Separation Products (Fremont, CA, USA) was used in all analyses. The uncoated fused-silica capillary (70 cm length from the injection point to the detector (effective length) and with an inner diameter of 75 μm) was obtained from Thermo Separation Products. The quaternary amine-coated capillary (CElect-Amine), with an effective capillary length of 55 cm and an inner diameter of 75 μm, was obtained from Supelco (Buchs, Switzerland). Electropherograms were recorded with a MacLab/4s (AD Instruments Pty, Ltd., Castle Hill, Australia) data acquisition system and a PowerMac 7200/90 (Apple Computer, Cupertino, CA, USA) and were processed with MacLab Chart 4.3.s software (AD Instruments) and Igor Pro Version 3 software (WaveMetrics, Lake Oswego, OR, USA).

2.2. Materials

Imidazole, α-hydroxyisobutyric acid and 18-crown-6 were obtained from Fluka (Buchs, Switzerland) and glacial acetic acid was obtained from Merck (Darmstadt, Germany). Standard solutions were prepared from metal chlorides (Fluka). All chemicals were of the highest purity available. Water, used for the preparation of all solutions and rinsing of the capillaries, was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). The seawater sample originated from Auckland, New Zealand.

2.3. Methods

The carrier electrolyte for separations in the uncoated fused-silica capillary contained 5 m*M*

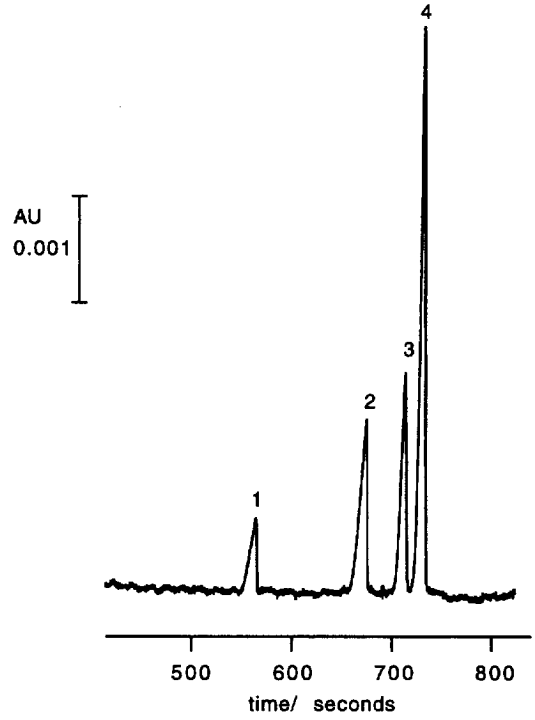

Fig. 1. Separation of a standard solution containing K^{+}, Na^{+}, Ca^{2+} and Mg^{2+} (10 ppm each). Capillary: fused-silica, uncoated, 70 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 5 m*M* imidazole, 6.5 m*M* α-hydroxyisobutyric acid, 2 m*M* 18-crown-6, adjusted to pH 4.1 with glacial acetic acid; 20 kV, detector cathodic; injection: vacuum, 1 s; detection: indirect UV, 220 nm; Peaks: 1=K^{+}, 2=Ca^{2+}, 3=Na^{+} and 4=Mg^{2+}.

imidazole, 6.5 m*M* α-hydroxyisobutyric acid and 2 m*M* 18-crown-6. The pH was adjusted to 4.1 with glacial acetic acid. The carrier electrolyte for the coated capillary contained 10 m*M* imidazole, 6 m*M* α-hydroxyisobutyric acid and 5 m*M* 18-crown-6, and the pH was adjusted to 3.91 with glacial acetic acid. Samples were introduced by vacuum, with the times specified in the figures. Positive voltages of 20 kV (uncoated fused-silica capillary) and 30 kV (coated capillary) were applied at the injector end. The detector time constant was set to 0.3 s. Indirect UV detection at 220 nm was used, with negative signal polarity giving positive peaks. The uncoated capillary was washed with 0.1 *M* NaOH for 5 min, then with water for 5 min and finally with carrier electrolyte for 10 min prior to separations. The coated capillary was rinsed with water for 5 min and then with carrier electrolyte for 10 min before analysis. The capillaries were purged for 2 min with electrolyte before and after every run to remove later-eluting peaks. The seawater sample was filtered through a membrane filter (0.22 μm pore size, Semadeni, Ostermundingen, Switzerland) as soon as possible after collection.

3. Results and discussion

The electropherogram for a standard solution containing 10 ppm of K^+ ($2.56 \cdot 10^{-4}$ mol/l), Na^+ ($4.28 \cdot 10^{-4}$ mol/l), Ca^{2+} ($2.45 \cdot 10^{-4}$ mol/l) and Mg^{2+} ($4.12 \cdot 10^{-4}$ mol/l) is shown in Fig. 1. The separation was carried out in an uncoated fused-silica capillary according to a method that was recommended for the routine analysis of water samples [13]. In this concentration range, baseline resolution was just obtained for all four cations and the peak

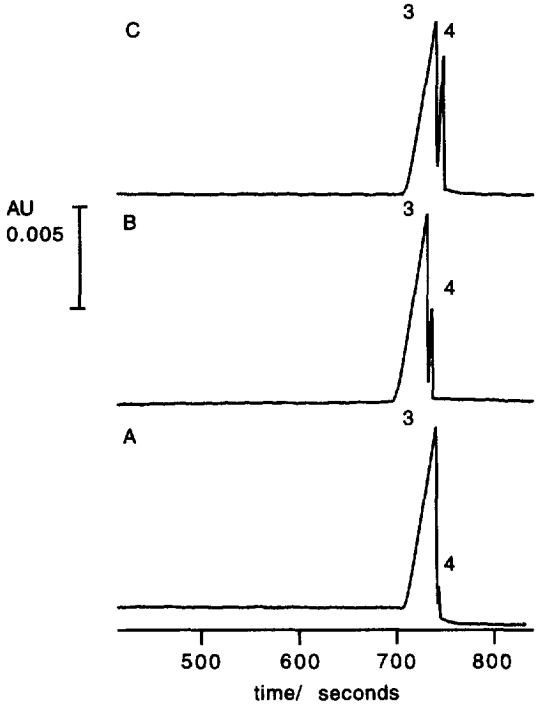

Fig. 2. Separation of 1 ppm (A), 5 ppm (B) and 10 ppm (C) magnesium from 100 ppm sodium. Capillary: fused-silica, uncoated, 70 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 5 m*M* imidazole, 6.5 m*M* α-hydroxyisobutyric acid, 2 m*M* 18-crown-6, adjusted to pH 4.1 with glacial acetic acid; 20 kV, detector cathodic; injection: vacuum, 1 s; detection: indirect UV, 220 nm; Peaks: 3=Na^+ and 4=Mg^{2+}.

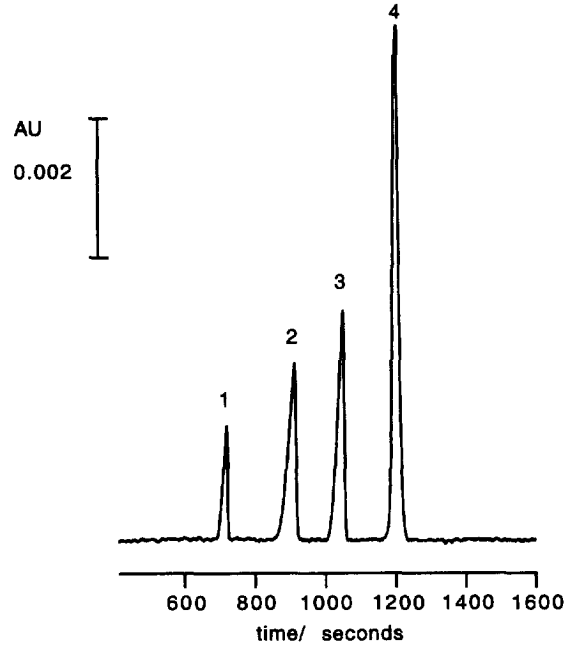

Fig. 3. Separation of a standard solution containing K^+, Na^+, Ca^{2+} and Mg^{2+} (10 ppm each). Capillary: fused-silica, quaternary amine-coated, 55 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 10 m*M* imidazole, 6 m*M* α-hydroxyisobutyric acid, 5 m*M* 18-crown-6, adjusted to pH 3.91 with glacial acetic acid; 30 kV, detector cathodic; injection: vacuum, 2 s; detection: indirect UV, 220 nm; Peaks: 1=K^+, 2=Ca^{2+}, 3=Na^+ and 4=Mg^{2+}.

shapes were symmetrical. When the sodium concentration is increased ten-fold to 100 ppm ($4.28 \cdot 10^{-3}$ mol/l), however, it is then no longer possible to resolve the Mg^{2+} peak from the now broadened Na^+ peak. This situation is demonstrated for three levels of Mg^{2+} [1 ppm ($4.12 \cdot 10^{-5}$ mol/l), 5 ppm ($2.06 \cdot 10^{-4}$ mol/l) and 10 ppm] in Fig. 2. Note that it was found feasible to alter the relative position of the Ca^{2+}, Na^+ and Mg^{2+} peaks by slight modifications of the buffer composition. However, the complete separation of all three peaks could not be achieved when Na^+ was present at 100 ppm.

The electropherogram of the standard mixture of the four ions at 10 ppm separated in the positively charged coated capillary is shown in Fig. 3. Clearly, the four inorganic cations are now separated with considerably better resolution than in the uncoated capillary. The overall migration times increased by a factor of approximately 1.6. These separations with the coated capillary were done by using essentially the same conditions (other than the capillary coating) as above. However, the electrolyte composition was adapted somewhat with respect to reagent concentrations and pH. This yielded optimal peak shapes and reasonable analysis times. The conditions used in this separation (e.g. capillary length, buffer composition) represent a compromise between resolution and analysis time. The use of a longer capillary and changes in the electrolyte system can further increase separation, but they also prolong the analysis time.

As illustrated by the electropherogram shown in Fig. 4, it is possible to completely remove the overlap of the Na^+ and Mg^{2+} peaks at the higher concentration of Na^+ by using the coated column.

The analysis of a seawater sample using the standard uncoated capillary is shown in Fig. 5. Peaks of the four inorganic cations, K^+, Na^+, Ca^{2+} and Mg^{2+} are obtained with a sodium concentration of

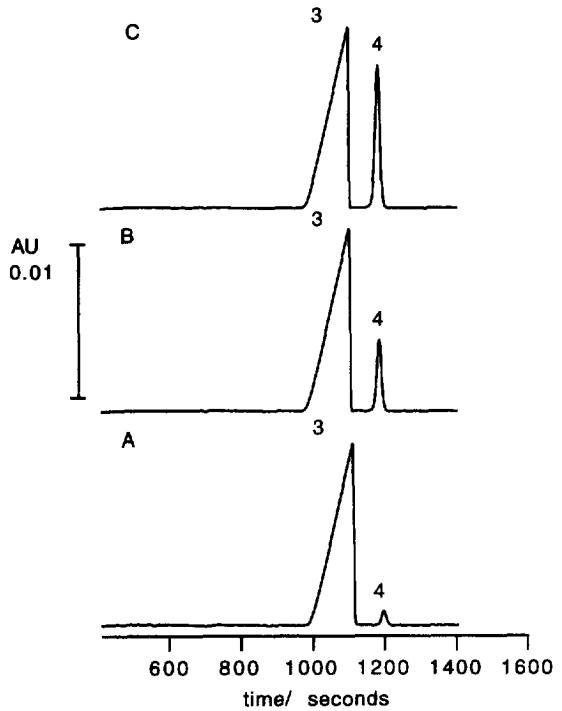

Fig. 4. Separation of 1 ppm (A), 5 ppm (B) and 10 ppm (C) magnesium from 100 ppm sodium. Capillary: fused-silica, quaternary amine coated, 55 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 10 m*M* imidazole, 6 m*M* α-hydroxyisobutyric acid, 5 m*M* 18-crown-6, adjusted to pH 3.91 with glacial acetic acid; 30 kV, detector cathodic; injection: vacuum, 2 s; detection: indirect UV, 220 nm; Peaks: 3=Na^+ and 4=Mg^{2+}.

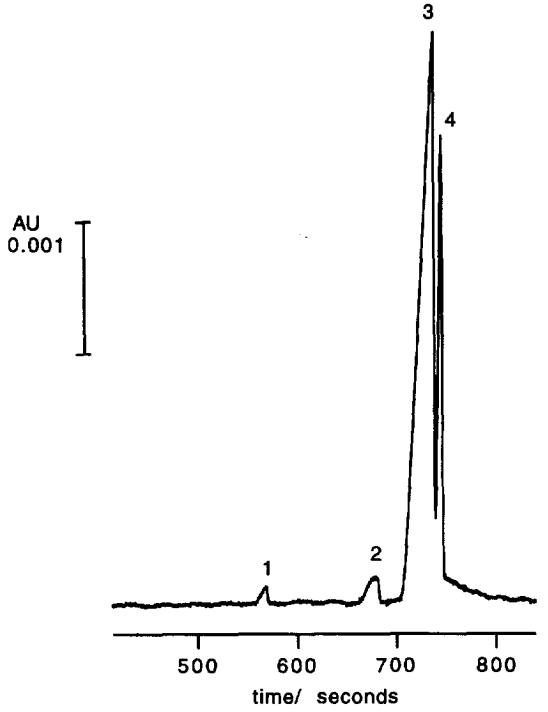

Fig. 5. Separation of seawater (dilution 1:100, v/v). Capillary: fused-silica, uncoated, 70 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 5 m*M* imidazole, 6.5 m*M* α-hydroxyisobutyric acid, 2 m*M* 18-crown-6, adjusted to pH 4.1 with glacial acetic acid; 20 kV, detector cathodic; injection: vacuum, 1 s; detection: indirect UV, 220 nm; Peaks: 1=K^+, 2=Ca^{2+}, 3=Na^+ and 4=Mg^{2+}.

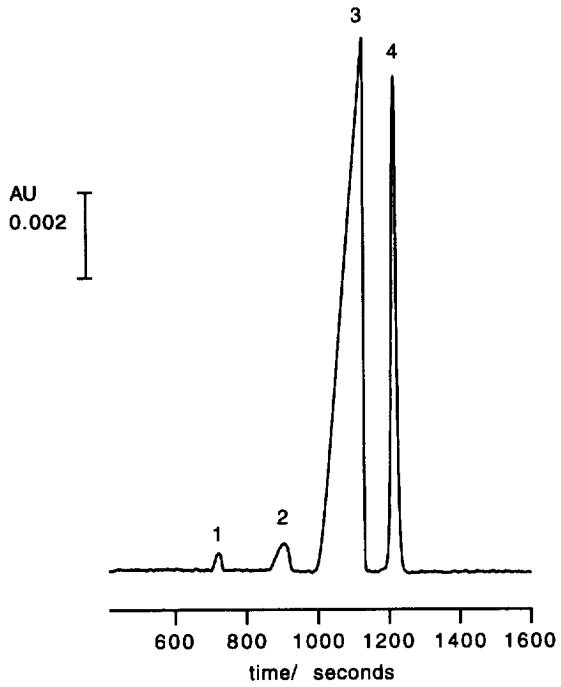

Fig. 6. Separation of seawater (dilution 1:100, v/v). Capillary: fused-silica, quaternary amine-coated, 55 cm effective length×375 μm O.D.×75 μm I.D.; carrier electrolyte: 10 m*M* imidazole, 6 m*M* α-hydroxyisobutyric acid, 5 m*M* 18-crown-6, adjusted to pH 3.91 with glacial acetic acid; 30 kV, detector cathodic; injection: vacuum, 2 s; detection: indirect UV, 220 nm; Peaks: 1=K^+, 2=Ca^{2+}, 3=Na^+ and 4=Mg^{2+}.

around 100 ppm. For the determination of magnesium and sodium as well, the resolution is insufficient, because these two peaks do overlap. Clearly, the standard conditions are not adequate for this sample matrix.

The separation of the same seawater sample in the coated capillary can be seen in Fig. 6. Now baseline-separated peaks of the four inorganic cations, K^+, Na^+, Ca^{2+} and Mg^{2+}, are obtained, allowing the simultaneous determination of these cations in the seawater sample.

4. Conclusions

It has been demonstrated, using seawater as an example, that the use of positively charged capillaries can resolve the peak overlap that occurs when one species with a high concentration dominates. This was readily achieved simply by changing the type of capillary and with only minor adaptations to other reported conditions, such as buffer composition. It is hoped that the approach should represent an easy solution to similar problems with other types of samples. The somewhat prolonged analysis times should not be a hindrance in practise. As positively charged coated capillaries for electrophoresis have recently become available commercially, the technique is easily accessible.

Acknowledgments

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