Use of quasi-equimolar, closely-spaced, poly-co-ion background electrolytes in capillary electrophoresis for the reduction of electromigration dispersion over a wide mobility range

Robert L. Williams[a], Bart Childs[b], Eric V. Dose[c], Georges Guiochon[d], Gyula Vigh[a],*

[a]*Chemistry Department, Texas A&M University, P.O. Box 300012, College Station, TX 77842-3012, USA*
[b]*Computer Science Department, Texas A&M University, College Station, TX 77843-3255, USA*
[c]*470 Clarendon Ave. Winter Park, FL 32789, USA*
[d]*Chemistry Department, University of Tennessee, Knoxville, TN 37996-1600 USA*

Abstract

The dependence of analyte peak shape and separation efficiency as a function of the mobility spacing of the co-ions was investigated by computer simulation of the electrophoretic process in poly-co-ion background electrolytes. The simulation results indicated that when the mobilities of the strong electrolyte co-ions differed from each other by less than $1 \cdot 10^{-5}$ $cm^2 V^{-1} s^{-1}$, much of the electromigration dispersion could be eliminated for the analytes that migrated in the mobility window of the co-ion pair. Accordingly, a poly-component, quasi-equimolar mixture of polyethyleneglycol monomethyl ether hydrogensulfates was synthesized in which the effective mobilities of the strong electrolyte anions ranged from about $10 \cdot 10^{-5}$ to $23 \cdot 10^{-5}$ $cm^2 V^{-1} s^{-1}$, and the effective mobilities of these anions differed from each other by about $0.5 \cdot 10^{-5}$ to $1.5 \cdot 10^{-5}$ $cm^2 V^{-1} s^{-1}$. A comparison of the electropherograms of the same seven-component sample obtained with a single co-ion background electrolyte and the poly-co-ion background electrolyte (at identical ion strength) verified that the quasi-equimolar, poly-co-ion background electrolyte eliminated much of the peak distortion that was caused by electromigration dispersion and resulted in good separation efficiencies over the entire, broad mobility range covered by the new poly-co-ion background electrolyte.   © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In capillary electrophoresis (CE), conductivity differences between the analyte zones and the pure background electrolyte (BGE) zones lead to local electric field strength differences which, in turn, distort the shape of the analyte bands and result in

*Corresponding author.

reduced separation efficiencies. This phenomenon is known as electromigration dispersion (EMD) [1]. For BGEs which contain only a single co-ion and a single counter-ion, Mikkers et al. [1] derived an analytical expression which relates the local electric field strengths in the sample zone, E^{Sample}, and in the pure BGE zone, E^{BGE} to the concentration of the analyte, $C^{analyte}$, the concentration of the BGE co-ion, C^{BGE}, the mobility ratio of the counter-ion and

the co-ion, $r^{counter-ion}$, and the mobility ratio of the analyte and the co-ion, $r^{analyte}$ as:

$$\frac{E^{Sample}}{E^{BGE}} =$$

$$\frac{1}{1 - \dfrac{C^{analyte}}{C^{co-ion}} \dfrac{(1 - r^{analyte})(r^{counter-ion} + r^{analyte})}{(1 + r^{counter-ion})r^{analyte}}} \qquad (1)$$

In previous papers, we described BGE systems which were designed to minimize the extent of EMD, and the subsequent loss of separation efficiency, by matching the mobility of the BGE co-ion to that of the critical analyte, either by introducing additional secondary chemical equilibria (complexation) [2] or by selecting a single co-ion from a family of strong electrolyte ions which have regularly spaced mobilities (MobiMatch BGEs) [3–6]. Though EMD on the critical analyte, for which mobility matching was achieved, was eliminated, the practical utility of these BGE systems is limited when the sample contains several critical analytes with widely different mobilities, because only the mobility of one analyte can be matched at any given time by the single BGE co-ion.

Since no analytical expression analogous to Eq. (1) is available for multiple-co-ion BGEs, computer simulations of the electrophoretic process are required to study the analyte peak profiles that develop under nonlinear conditions [7–21]. Some of these programs were used to simulate the analyte peak profiles in two-co-ion BGEs [19–21] and in three-co-ion BGEs [16] where the co-ions had widely different mobilities. It was observed that EMD was reduced for the analytes whose mobilities were close to those of the respective co-ions. Unfortunately, it was also observed [19–21] that in these BGEs, noncomigrating system peaks were generated which propagated with mobilities intermediate to the mobilities of the two neighboring co-ions. These non-comigrating system peaks were associated with severe local electric field strength perturbations [20,21]. The large local electric field strength disturbances grossly distorted the analytes peaks whose mobilities were close to them. Occasionally, these peak distortions were so extreme that the analyte peak could not be distinguished from the baseline and indicated that the objective, i.e. uniform elimina-

tion of EMD over a broad mobility range, could not be achieved by using BGEs which contained a few, widely spaced co-ions [21]

These observations prompted us to try to create a BGE that contains a large number of very closely spaced co-ions, each present in similar concentration, so that (i) evenly-distributed mobility matching could be achieved over a broad range of analyte mobilities, and (ii) large local electric field strength disturbances (associated with the noncomigrating system peaks) — which distort analyte peak shapes and lower separation efficiencies — could be avoided. In order to ease the synthetic burden, computer simulations were first carried out with binary co-ion systems to develop a feel for the mobility spacing required to fill the above objectives.

2. Experimental

The test analytes (2-nitrobenzoic acid, 2NBA, 3-nitrobenzoic acid, 3NBA, 4-nitrobenzoic acid, 4NBA, 2-chlorobenzoic acid, 2CBA, 3-chlorobenzoic acid, 3CBA, 4-chlorobenzoic acid, 4CBA, benzoic acid, BA, and benzyl alcohol), the chemicals used for the BGEs (ϵ-aminocaproic acid, hydrochloric acid, p-toluene sulfonic acid, PTSA, tris(hydroxymethyl)aminomethane, Tris) and the chemicals used for the synthesis of polyethyleneglycol monomethyl ether hydrogensulfates (chlorosulfonic acid, polyethyleneglycol monomethyl ethers with average molecular masses of 350, 550 and 750 and oligomer numbers ranging approximately from 4 to 24) were reagent grade chemicals (Aldrich, Milwaukee, WI, USA) and were used as obtained.

A P/ACE 2100 and a P/ACE 5200 CE unit (Beckman Instruments, Fullerton, CA, USA) were used for the electrophoretic experiments. The high positive potential was applied to the detector side of the 47 cm long (40 cm from injector to detector)×25 μm I.D. 150 μm O.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ). A 57 cm long (50 cm from injector to detector), 50 μm I.D. eCAP neutral capillary (Part No. 477441, Beckman Instruments) was used for the indirect UV detection CE measurements. The electric field strength was kept at 319 V/cm, resulting in power dissipation of 230–590 mW/m. The capillary cartridge coolant was thermo-

stated at 37°C. The BGEs contained 140 mM ϵ-aminocaproic acid, their pH was adjusted to 4.0 (resulting in a nominal ionic strength of 100 mM) either with hydrochloric acid for the single co-ion BGEs, or with the quasi-equimolar mixture of poly-ethyleneglycol monomethyl ether hydrogensulfates synthesized in our laboratory as described in [5]. All analytes were injected at 6 mM concentration, dissolved in the respective BGEs, by 0.5 p.s.i. nitrogen for 1 s (1 p.s.i.$=$6894.76 Pa). Immediately after each analysis, the electroosmotic flow-rate was measured using the PreMCE method [22]. The reported mobility values are corrected for the effects of the linear voltage ramp at the beginning of the separation [23].

The CE simulation program first described by Dose and Guiochon [12] was modified to accommodate up to 10 ions of the same charge. Unless otherwise noted, the following input parameters were used for the calculations: total length of capillary: $L_T=4.0$ cm (equivalent to 26 667 cells); length of capillary from inlet to detector: $L_D=3.6$ cm (equivalent to 24 000 cells); length of the injection band: $L_i=0.045$ cm (equivalent to 300 cells); applied voltage: 708 V; initial time increment: 0.05 s; electroosmotic flow velocity: 0 cm/s; counter-ion mobility: $+14\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. The simulations were performed on a Gateway2000 P5-120 computer (Gateway, Sioux City, SD, USA) which was equipped with a 120 MHz pentium CPU and 32 Mbytes of EDO RAM.

3. Results

Previous work indicated that EMD could only be reduced in the vicinity of a matching co-ion and, also, that the analyte peak shape distortions were governed by the local electric field strength perturbances [19–21]. This led to our hypothesis that EMD could be reduced over a broad range of analyte mobilities by adding to the BGE a large number of co-ions whose mobilities are regularly spaced, provided that the extent of local electric field strength disturbances between each neighboring co-ion pair could be kept at a minimum. Therefore, computer simulations were carried out for several two co-ions/single counter-ion/single analyte systems to study the extent of EMD on the analyte peak as a function

of the mobility difference ('mobility spacing') of the co-ions. The objective of these simulations was to determine how close the co-ion mobilities had to be in order to limit the EMD-related separation efficiency-loss (with respect to the separation efficiencies observed with a perfectly matched, single co-ion BGE) to about 20%. The mobility of the analyte, around which the mobilities of the co-ions were centered, was kept at $-39.0\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. Unlike in any of the previous simulation work [7–21], the mobility spacing of the co-ions was varied from very narrow to moderately broad: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 2.0, 3.0, 4.0 and $5.0\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. The total co-ion concentration was fixed at 25 mM and divided equally between the two co-ions (equimolar multiple co-ion BGEs). For reference, separations in a single co-ion BGE with a total co-ion concentration of 25 mM and mobility of $-39.0\cdot10^{-5}$ cm^2 V^{-1} s^{-1} (matched to the mobility of the analyte), were also simulated.

It is known from our previous work, that non-comigrating system peaks arise which propagate with a mobility intermediate to that of the co-ion pair [21,22]. The noncomigrating system peaks are accompanied by local electric field strength perturbations which distort the peak shape of any analyte that migrates in the vicinity. Some of the simulated local electric field strength curves are shown in Fig. 1. The corresponding simulated analyte peak profiles are shown in Fig. 2. To facilitate direct comparison of the simulations, the time axis has been replaced with mobility axis. Where the local electric field strength is higher (the higher mobility side), the peaks have a sharper front, where the local electric field strength is lower (the lower mobility side), the peaks have a more diffuse front. The extent of local electric field strength distortion decreases as the co-ion spacing is decreased (Fig. 1). Consequently, the extent of peak shape distortion also decreases as the co-ion spacing is decreased (Fig. 2).

To further illustrate the effect of co-ion mobility spacing on separation efficiency, the number of theoretical plates was calculated for each two-co-ion system and divided by the number of theoretical plates calculated for the single co-ion, mobility matched system, yielding a quantity that can be thought of as relative separation efficiency. The results, plotted in Fig. 3, indicate that in order to
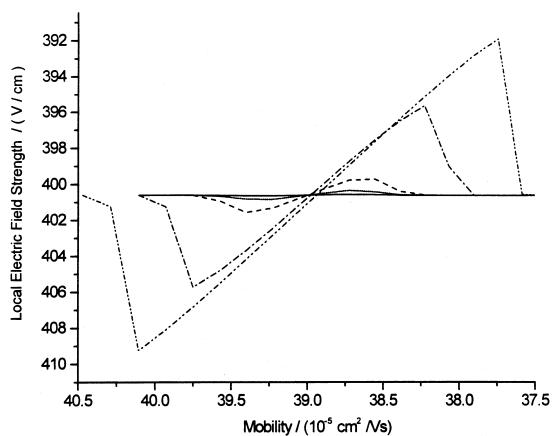
Fig. 1. Simulated local electric field strength profiles corresponding to the noncomigrating system peak region in two-co-ion BGEs with co-ion spacings of $0.1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (solid line), $0.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dotted line), $1.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dashed line), $3.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dash dot line) and $5.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dash double dot line). Other conditions as in Section 2.

keep the relative separation efficiency loss within 20%, the co-ion mobility spacing must be kept equal to, or smaller than, $1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}.

These results, combined with the conclusions in [20,21], suggest that using a BGE which contains a large number of co-ions whose mobilities are closer than $1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, one would be able to limit

Fig. 3. Effect of co-ion mobility spacing on the relative separation efficiency in two-co-ion BGEs. Other conditions as in Fig. 1.

EMD-related peak distortion over a practically useful, broad enough mobility range. To demonstrate the feasibility of this idea, a quasi-equimolar mixture of polyethyleneglycol monomethyl ether hydrogensulfates were synthesized according to the procedure described in [5] using chlorosulfonic acid and a mixture of polyethyleneglycol monomethyl ethers. The indirect UV detection electropherogram [5] of this quasi-equimolar polyethyleneglycol monomethyl ether hydrogensulfate mixture is shown in Fig. 4. The electropherogram was obtained using a 25 m*M*
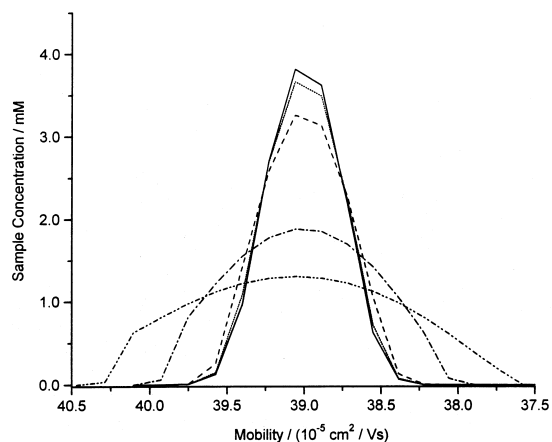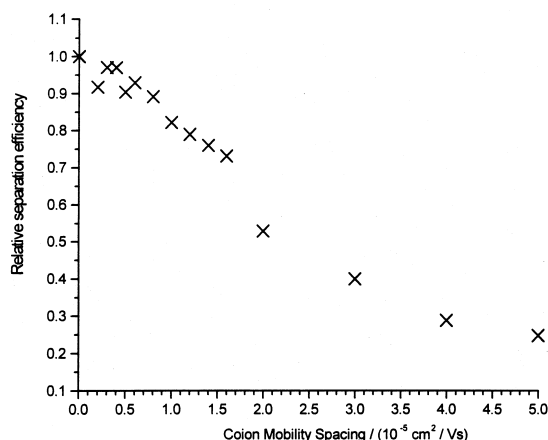

Fig. 2. Simulated analyte peak profiles for two-co-ion BGEs with co-ion spacings of $0.1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (solid line), $0.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dotted line), $1.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dashed line), $3.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dash dot line) and $5.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (dash double dot line). Other conditions as in Fig. 1.
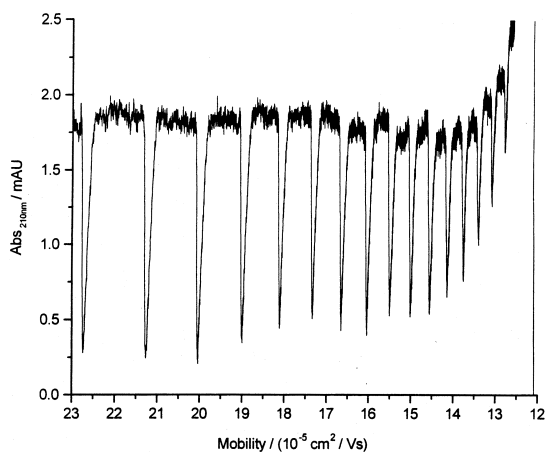

Fig. 4. Indirect UV detection electropherogram of the quasi-equimolar multiple-component polyethyleneglycol monomethyl ether hydrogensulfate sample. Electroosmotic flow mobility: $9.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. Other conditions as in Section 2.

PTSA BGE whose pH was adjusted to 8.0 with Tris. This co-ion mixture covers a mobility range of $12 \cdot 10^{-5}$ to $23 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (at 25 mM ionic strength), while the mobility spacing of the co-ions varies from about $0.5 \cdot 10^{-5}$ to about $1.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. The mixture offers a sufficiently uniform concentration distribution for its constituents (quasi-equimolar mixture), to permit a meaningful testing of the utility of equimolar, closely spaced, poly-co-ion BGEs suggested by the simulations.

Therefore, a quasi-equimolar, poly-co-ion BGE was prepared by titrating a 140 mM ε-aminocaproic acid to pH 4.0 (nominal ionic strength = 100 mM) with the polyethyleneglycol monomethyl ether hydrogensulfate mixture shown in Fig. 4. For comparison purposes, a single co-ion BGE was also prepared in a similar manner, except that hydrochloric acid (infinite dilution ionic mobility $-79.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} [24]), the strong acid most frequently used today to adjust the pH of ε-aminocaproic acid to the acidic side, was used. The same seven-component test mixture was then analyzed with both BGEs. The electropherograms obtained are shown in Fig. 5; the time axis in the figure has once again been replaced with the electrophoretic mobility axis. As expected, in the single co-ion BGE, all analytes tail, the separation efficiencies are poor and analytes

4NBA and 3NBA cannot be completely resolved (electropherogram A in Fig. 5). With the quasi-equimolar poly-co-ion BGE, almost completely symmetrical peak shapes and good separation efficiencies were obtained over the entire analyte mobility range. Also, the 4NBA and 3NBA analyte peaks became baseline resolved. Only the analyte peak corresponding to 2NBA showed the effects of strong EMD: the peak was broad and dome-shaped. This indicates that 2NBA, which has a mobility of $-23.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, lies outside the upper mobility boundary of the quasi-equimolar poly-ethyleneglycol monomethyl ether hydrogensulfate mixture used. It is noteworthy, that the analyte mobilities in the quasi-equimolar poly-co-ion BGE are somewhat lower than in the chloride-containing BGE. This is so, because the viscosity of the polyethyleneglycol monomethyl ether hydrogensulfate-containing BGE is higher than that of the chloride-containing BGE.

4. Conclusions

Computer simulations of the electrophoretic process indicate that much of the electromigration dispersion can be eliminated, and a significant portion of the nominal separation efficiency can be retained, when a BGE that contains a large number of closely spaced co-ions (mobility spacing $1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} or less), is used. Such a quasi-equimolar mixture of co-ions was successfully synthesized by reacting a carefully blended polyethyleneglycol monomethyl ether mixture with chlorosulfonic acid. Experimental results proved that over the $10 \cdot 10^{-5}$ to $22 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} mobility range covered by this polyethyleneglycol monomethyl ether hydrogensulfate mixture, the quasi-equimolar poly-co-ion mixture performed as predicted. Compared to the single co-ion BGE, it lead to improved peak shape, even when the concentration of the injected analytes was as high as 6 mM.
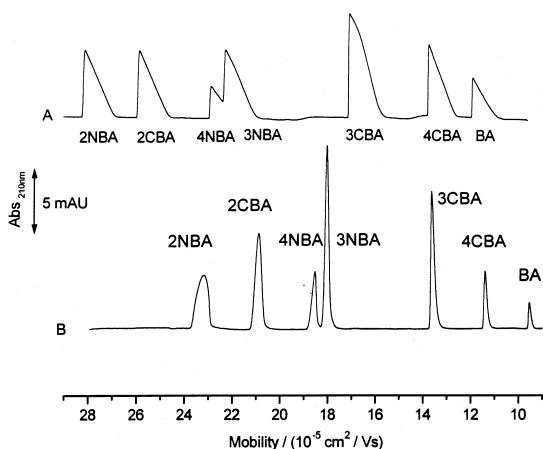

Fig. 5. Comparison of the electropherograms of a seven-component sample obtained with a single co-ion BGE (chloride co-ion, trace A on top) and the quasi-equimolar poly-co-ion BGE prepared with the polyethyleneglycol monomethyl ether sulfates shown in Fig. 4 (trace B on bottom). Other conditions as in Section 2.

Acknowledgements

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