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Polyethylene glycol monomethyl ether sulfate-based background electrolytes in capillary electrophoresis

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Abstract

A homologous series of (polyethylene glycol monomethyl ether) hydrogensulfates has been synthesized, characterized and used as the source of anionic co-ions in mobility-matching background electrolytes designed to eliminate the efficiency-degrading effects of electromigration dispersion. The mobilities of the new anionic co-ions vary in the $5 \cdot 10^{-5}$ to $45 \cdot 10^{-5}$ cm^2/Vs range in 25 m*M* mobility-matching background electrolytes, prepared from ϵ-aminocaproic acid and the respective (polyethylene glycol monomethyl ether) hydrogensulfate. It is demonstrated experimentally that the electromigration dispersion-related peak broadening can be minimized and difficult electrophoretic separations can be realized without significant alteration of the separation selectivity when these (polyethylene glycol monomethyl ether) sulfate anions are used as co-ions in mobility-matching background electrolytes.

Keywords: Background electrolyte composition; (Polyethylene glycol monomethyl ether) hydrogensulfates; Aminocaproic acid; Dinitrobenzamidophenylalanine; Nitrobenzoic acids

1. Introduction

Capillary electrophoresis (CE) has proved to be a very powerful separation technique because under ideal conditions (well designed instrument, dilute samples in sufficiently concentrated background electrolytes (BGE), absence of slow sorption processes, etc.), separation of the band centers is much more rapid than longitudinal broadening of the bands [1]. However, when the conductivity of the sample band is significantly different from that of the BGE, the local electric field strength in the sample band becomes significantly different from that of the BGE and band distortion occurs. This phenomenon, known as electromigration dispersion (ED), has been quantitatively described by Mikkers et al. [2]. It was suggested [2,3] that ED can be minimized by minimizing the transference number of the sample or by matching the mobilities of the analyte and the BGE co-ion. In general, the mobilities of the analyte and the BGE co-ion are equal only in fortuitous cases. If the BGE co-ion doubles as the conjugate acid or conjugate base component of the buffer, even fortuitous mobility matching is lost when the pH of the BGE is changed. In an attempt to reduce the extent of ED, we demonstrated that the mobility of a zwitterionic BGE co-ion can be controlled dynamically by invoking multiple secondary chemical equilibria [4]. Unfortunately, this approach only works when separation selectivity is not altered by the additional secondary chemical equilibria invoked, and optimization of such BGEs can become quite involved. In a second ED-reducing approach, we suggested that the BGE co-ions be derived from strong electrolytes while the counter-ions be derived from weak electrolytes. This way, the pH of the BGE

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can be altered at will, fulfilling the buffering function of the BGE, without influencing the mobility of the co-ion. Simultaneously, the chemical identity of the co-ions can be selected freely to match their mobility to that of the analyte, fulfilling the mobility-matching function of the BGE [5]. Accordingly, the BGE for the separation of a weak base analyte is prepared by adjusting the pH of a weak acid solution to the desired value with a strong base, the cation of which has an effective mobility equal to the effective mobility of the analyte at the desired pH and ionic strength. The BGE for the separation of a weak acid analyte is prepared by adjusting the pH of a weak base solution to the desired value with a strong acid, the anion of which has an effective mobility equal to the effective mobility of the analyte at the desired pH and ionic strength. We call these BGEs mobility-matching BGEs. The mobility-matching BGE concept was first demonstrated using tetralkylammonium hydroxides as co-ion sources [5,6]. Unfortunately, the choice of commercially available, sufficiently water-soluble tetralkylammonium ions is very limited [6]. Therefore, in order to obtain cationic co-ions with more closely spaced mobilities we synthesized a series of alkylmethylmorpholinium ions [7] and later, to lower the range of accessible mobilities, we synthesized a series of N-(polyethylene glycol monomethyl ether)-N-methylmorpholinium ions as cationic co-ions [8]. In this paper, we describe the synthesis, characterization and use of the first homologous series of anionic mobility-matching reagents designed to reduce the extent of electromigration dispersion of anionic analytes.

The utility of the suggested anionic mobility-matching BGEs is illustrated in Fig. 1 by showing the electropherograms of a sample that contained 4-nitrobenzoic acid and 3-nitrobenzoic acid as analytes in two BGEs. Both BGEs were prepared by adding 25 mmol of the respective strong acid, hydrochloric acid, and (polyethylene glycol monomethyl ether) hydrogensulfate (550-PEGMME hydrogensulfate) to a 0.1 l volumetric flask and adjusting the pH of the solution to 3.2 with ϵ-aminocaproic acid. In order to facilitate direct comparison, the time axis in the electropherograms has been converted to mobility axis (shown in 10^{-5} cm^2/Vs units). Except for the two co-ions (chloride vs. 550-PEGMME sulfate), all other conditions (injected amounts, BGE

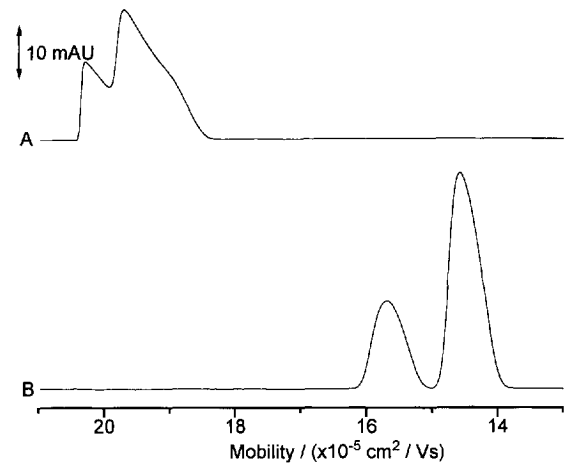

Fig. 1. Electropherograms of a sample containing 2 m*M* each of 4-nitrobenzoic acid (4-NBA) and 3-nitrobenzoic acid (3-NBA). Conditions: 1 s, 1.5 p.s.i. pressure injection; capillary, 50 μm eCAP Amine capillary; field strength, 105 V/cm; background electrolytes, 25 m*M* co-ion, pH adjusted to 3.2 with ϵ-aminocaproic acid. Electropherograms: BGEs, 25 m*M* chloride co-ion (electropherogram A); 25 m*M* 550-PEGMME sulfate co-ion (electropherogram B). The more mobile peak corresponds to 4-NBA. Other conditions as in Section 2.

concentrations of all components, field strengths, etc.) were identical in both separations. Clearly, peak distortion with the Cl^--containing BGE (electropherogram A in the top part of Fig. 1) is so excessive that the separation is useless for analytical purposes. On the other hand, symmetrical peaks and complete separation is obtained with the 550-PEGMME sulfate-containing BGE. Since the viscosity of the 550-PEGMME sulfate-containing BGE is higher than that of the Cl^--containing BGE, it is not surprising that the tail-end mobilities of the second peak (representing infinite dilution conditions) in the two BGEs are slightly different.

2. Experimental

A P/ACE 5510 system (Beckman Instruments, Fullerton, CA, USA) was used for all electrophoretic experiments. The detection-side electrode was kept at the high positive potential. Capillaries, 57 cm long (50 cm from injector to detector) 50 μm I.D. (eCAP Neutral capillary, Part Number 477441, Beckman) with a neutral internal coating, and 47 cm long (40

cm from injector to detector) 50 μm I.D. eCAP Amine capillary (Part Number 477431, Beckman), thermostated at 37°C, were used for the mobility determinations. Unless otherwise noted, the field strength was 176 V/cm, resulting in power dissipation between 93 to 200 mW/m. All samples were injected by 1.5 p.s.i. (1 p.s.i.=6894.76 Pa) nitrogen pressure for 1 s. The electroosmotic flow velocity was measured with benzyl alcohol immediately after each run using the pressure-assisted capillary electrophoretic method developed in our laboratory [9]. The reported mobilities are corrected for the effects of the linear potential ramp at the beginning of the separation [10].

All chemicals used for the synthesis of the mobility-matching co-ions (chlorosulfonic acid, anhydrous ethyl ether, 2-methoxyethanol, 2-(2-methoxyethoxy) ethanol, triethylene glycol mono methyl ether, 350-, 550-, 750- and 2000-polyethylene glycol monomethyl ether) and some of the mobility markers (benzyl alcohol, 2-phenyl ethanol, 3-phenyl propanol, 4-phenyl butanol, 5-phenyl pentanol) were reagent grade chemicals and were obtained from Aldrich (Milwaukee, WI, USA), along with the other mobility markers (benzyl sulfonic acid, *p*-toluenesulfonic acid, 4-ethyl benzyl sulfonic acid, naphthalenesulfonic acid, naphthalenedisulfonic acid, naphthalenetrisulfonic acid, benzoic acid, 2-chlorobenzoic acid, 3-chlorobenzoic acid, 4-chlorobenzoic acid, 2-nitrobenzoic acid, 3-nitrobenzoic acid, 4-nitrobenzoic acid) and BGE components (hydrochloric acid and ϵ-aminocaproic acid). Deionized water from a Millipore Q unit (Millipore, Milford, MA, USA) was used to prepare the BGEs. β-cyclodextrin was a generous gift from American Maize Products (Hammond, ID, USA).

Test analyte 3,5-dinitrobenzoyl phenylalanine was synthesized from 3,5-dinitrobenzoyl chloride and phenylalanine as described in [11]. The (polyethylene glycol monomethyl ether) sulfate co-ions (PEGMME sulfates) were prepared according to the general reaction scheme published by Sandler and Karo [12], as shown in Fig. 2. Briefly, equimolar amounts of chlorosulfonic acid and PEGMME were mixed in cold anhydrous ethyl ether and the evolving hydrochloric acid was removed by a nitrogen stream. Next, 250 to 500 ml of deionized water was added to the reaction mixture and ethyl ether was removed by

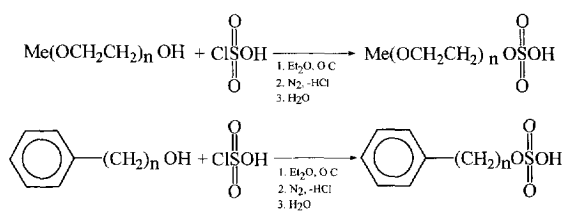

Fig. 2. Reaction scheme for the synthesis of the PEGMME hydrogensulfates.

vacuum. The 400–600 m*M* aqueous acid solutions were assayed by titration using a standardized LiOH solution. The residual hydrochloric acid and sulfuric acid concentrations (generally <5 mole%) in the products were determined by CE using indirect UV detection (25 m*M* *p*-toluenesulfonic acid, 50 m*M* morpholinoethanesulfonic acid BGE at pH 6.1 [13,14]). Overall product yields varied between 60 and 80%. Test analytes, phenyl methyl hydrogensulfate, 2-phenyl ethyl hydrogensulfate, 3-phenyl propyl hydrogensulfate, 4-phenyl butyl hydrogensulfate and 5-phenyl pentyl hydrogensulfate were synthesized in a similar manner.

3. Results

The newly synthesized phenyl alkyl hydrogensulfate probe molecules have effective mobilities in the $25 \cdot 10^{-5}$ to $35 \cdot 10^{-5}$ cm^2/Vs range, as shown in Fig. 3. They were used, together with the commercially available UV active sulfonic acid and carboxylic acid analytes (with effective mobilities varying in the $5 \cdot 10^{-5}$ to $55 \cdot 10^{-5}$ cm^2/Vs range) to determine the effective mobilities (at constant ionic strength) of the newly synthesized PEGMME sulfate co-ions according to the electrophoretic method described in [6]. Briefly, the analytes were electrophoresed in BGEs prepared from 25 m*M* solutions of the respective PEGMME hydrogensulfates. The pH of the BGEs was adjusted to 3.2, 3.4, 4.0 and 4.4 with ϵ-aminocaproic acid. In order to minimize the effects of localized pH disruptions [15], the carboxylic acid analytes were used at 1 m*M* concentrations. Fronting, symmetrical or tailing analyte peaks were observed depending on whether their mobilities were larger than, equal to or smaller than the mobility of the PEGMME sulfate BGE co-ion

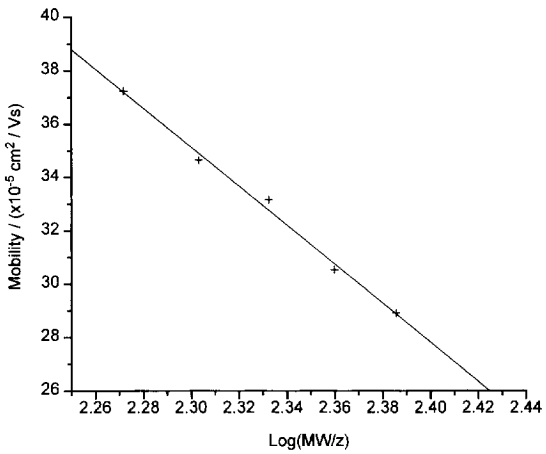

Fig. 3. Effective mobilities for members of the homologous series of phenyl alkyl hydrogensulfates. Conditions: 50 m*M* ϵ-amino-caproic acid, pH 4.4 adjusted with HCl; field strength, 315 V/cm.

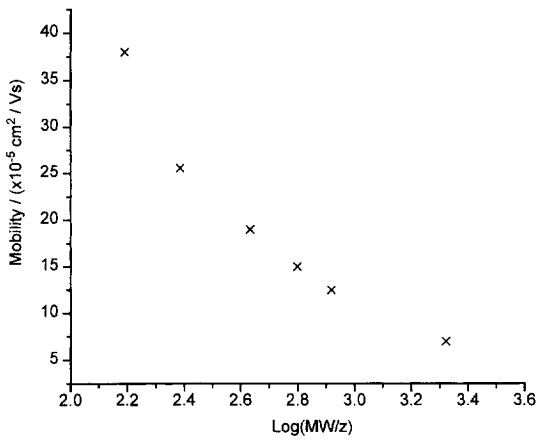

Fig. 5. Effective mobilities of the PEGMME sulfates as a function of the logarithm of their molecular mass.

studied. By plotting the asymmetries of the analyte peaks (measured at 10% peak height) as a function of their effective mobilities, as shown in Fig. 4 for 550-PEGMME sulfate, the unknown effective mobility of the co-ion studied (at the given ionic strength) could be read off at the intersection of the log(peak asymmetry) curve and the zero line. The closer the mobilities of the test solutes bracket the mobility of the BGE co-ion, the more accurate the determination.

By repeating these measurements for all the newly synthesized PEGMME sulfate co-ions, their effective electrophoretic mobilities could be obtained and plotted against the logarithm of their molecular mass as shown in Fig. 5. These data indicate that with 2000-PEGMME sulfate, analyte mobilities as low as $7 \cdot 10^{-5}$ cm^2/Vs can be matched.

To demonstrate the utility of anionic co-ion-based mobility-matching in CE separations, the electropherograms of a 2.5 m*M* sample of a chiral analyte, 3,5-dinitrobenzamidophenylalanine were obtained as shown in Fig. 6. Once again, to facilitate

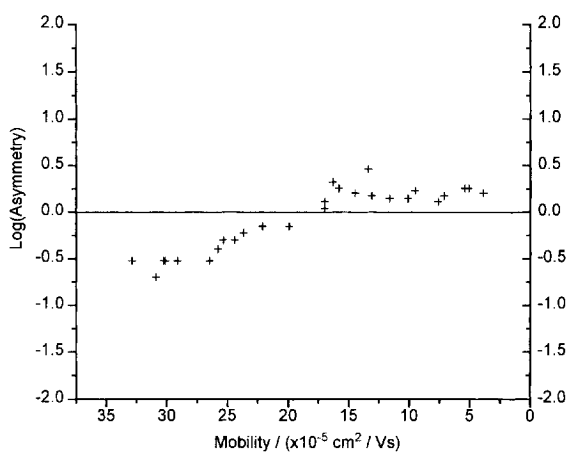

Fig. 4. Determination of the mobility of the 550-PEGMME sulfate BGE co-ion. Field strength: 176 V/cm. Other conditions as in Section 2.

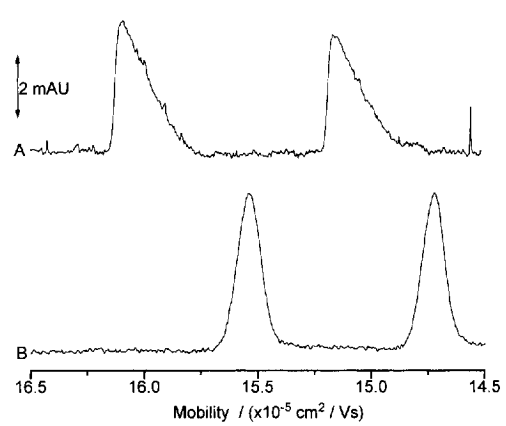

Fig. 6. Separation of the enantiomers of 3,5-dinitrobenzamidophenylalanine. BGEs: 25 m*M* chloride co-ion (electropherogram A); 25 m*M* 550-PEGMME sulfate co-ion (electropherogram B). Field strength: 176 V/cm. Other conditions as in Section 2.

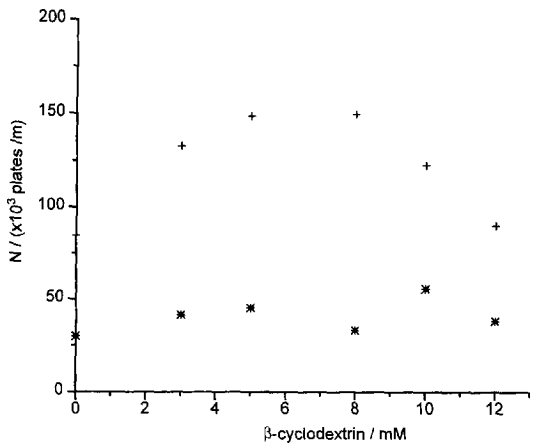

Fig. 7. Plate numbers calculated for the less mobile enantiomer of 3,5-dinitrobenzamidophenylalanine as a function of the β-cyclodextrin concentration of the BGE. Co-ion: 550-PEGMME sulfate (+), chloride (*). Other conditions as in Section 2.

direct comparison, the time axis is replaced by the mobility axis. The electropherograms were obtained with 8 m*M* β-cyclodextrin, 50 m*M* ε-aminocaproic acid BGEs whose pH was adjusted to 4.4 with hydrochloric acid (electropherogram A in the top part of Fig. 6) and 550-PEGMME hydrogensulfate (electropherogram B in the bottom part of Fig. 6). The β-cyclodextrin, counter-ion and co-ion concentrations are the same in both BGEs. Clearly, most of ED is eliminated in the 550-PEGMME sulfate-containing BGE. The same conclusion is supported by the plate number comparisons shown in Fig. 7.

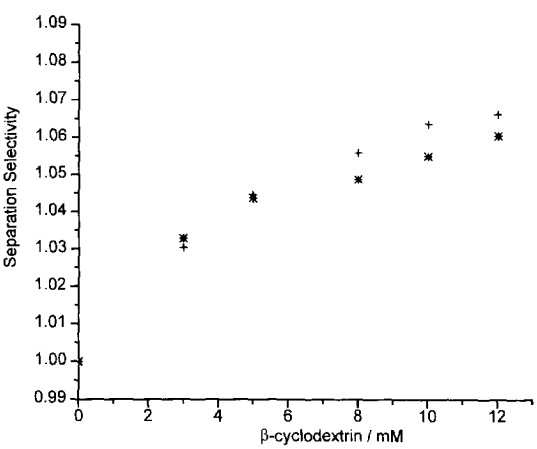

Fig. 8. Separation selectivity as a function of the β-cyclodextrin concentration of the BGE. Co-ion: 550-PEGMME sulfate (+), chloride (*). Other conditions as in Section 2.

Though the efficiency improvements afforded by the use of mobility-matching BGEs are obvious from Fig. 6 and Fig. 7, one has to make certain that the replacement of chloride-based BGEs with PEGMME sulfate-based BGEs does not alter the selectivity of the separation significantly. Therefore, the separation selectivities (the ratios of the effective mobilities of the two enantiomers) were determined and plotted in Fig. 8 as a function of the β-cyclodextrin concentration of the BGEs. The two selectivity curves are identical, within experimental error, indicating the absence of undesired secondary effects.

4. Conclusions

A set of new (polyethylene glycol monomethyl ether) hydrogensulfates were synthesized and their effective electrophoretic mobilities were determined in pH 3.2, 3.4, 4.0, and 4.4 ε-aminocaproic acid BGEs where the co-ion concentration was constant at 25 m*M*. The effective mobilities of these PEGMME sulfate co-ions span the $(5–45)\cdot10^{-5}$ cm^2/Vs range, permitting their effective use in mobility-matching BGEs to eliminate much of the electromigration dispersion that plagues the CE separations of anionic analytes. By utilizing the concept of mobility-matching BGEs, co-ion mobility-independent pH-control (required to insure optimum separation selectivity) can be realized by selecting the appropriate counter-ion, and pH-independent mobility matching (required to insure adequate separation efficiency) can be realized by selecting the appropriate co-ion.

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