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N-(Polyethyleneglycol monomethyl ether)-N-methylmorpholinium-based background electrolytes in capillary electrophoresis

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Abstract

A homologous series of N-(polyethyleneglycol monomethyl ether)-N-methylmorpholinium hydroxides has been synthesized, characterized and used as the source of cationic co-ions in mobility-matching background electrolytes designed to eliminate the efficiency-degrading effects of electromigration dispersion. The mobilities of the new cationic co-ions vary in the $6 \cdot 10^{-5}$ to $35 \cdot 10^{-5}$ $cm^2/V\,s$ range in 25 m*M* mobility-matching background electrolytes, prepared from phosphoric acid and the respective N-(polyethyleneglycol monomethyl ether)-N-methylmorpholinium hydroxide. It is demonstrated experimentally that electromigration dispersion-related peak broadening can be minimized and difficult electrophoretic separations can be realized without significant alteration of the separation selectivity when these N-(polyethyleneglycol monomethyl ether)-N-methylmorpholinium cations are used as co-ions in mobility-matching background electrolytes.

Keywords: Background electrolyte composition; Electromigration dispersion; (Polyethylene glycol monomethyl ether)-N-methylmorpholinium hydroxides; Aryltrialkylammonium chlorides; Homatropin; Aminogluthetimide

1. Introduction

In recent years, capillary electrophoresis (CE) has evolved into a widely used separation technique. Due to the absence of slow adsorption–desorption processes, separation of the band centers is much more rapid than the longitudinal broadening of the bands. When dilute samples are used in concentrated background electrolytes (BGEs), and when the instrument is adequately thermostatted, extremely high separation efficiencies may be observed in CE [1]. However, when the conductivity of the sample is sufficiently different from that of the BGE to locally disrupt the homogeneity of the electric field in the capillary, severe peak distortion is observed. This peak distortion phenomenon has been quantitatively described by Mikkers et al. [2] and is referred to as electromigration dispersion (ED). Mikkers [2], and later others [3], showed that ED may be minimized when the extent of the local electric field disruption is small, i.e. when the concentration of the sample relative to that of the BGE is low, or when the mobility of the analyte is equal to that of the BGE co-ion. Unfortunately, the utility of the first approach is hampered by the limited concentration sensitivity of most CE detectors, while that of the second one by the lack of an easy way to match the mobilities of the analyte and the BGE co-ion. Furthermore, if the BGE co-ion is derived from a weak electrolyte (a weak acid or base), any fortuitous mobility matching is usually lost when the pH of the system is changed to alter the selectivity of the separation. Previously, we demonstrated that ED can be controlled dynamically through the use of a zwitterionic BGE co-ion that is involved in multiple secondary chemical equilibria which alter the mobility of the co-ion

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[4]. The dynamic approach, however, is only successful when separation selectivity for the analytes is not altered by the additional secondary equilibria invoked to modify the mobility of the co-ion. Even then, optimization of the composition of such BGEs is difficult because it requires a knowledge of the equilibrium constants involved. As an alternative to the dynamic approach, we proposed a static approach to minimize ED. We suggested that the two main roles of the BGE, pH control and mobility matching, be provided exclusively by the co-ion and the counter-ion of the BGE, respectively. The BGE counter-ion would be a weak acid (or base) to provide buffering for the BGE, while the BGE co-ion would be a strong base (or acid), with a known mobility, to provide mobility-matching for the analyte of interest. Such a buffer system, referred to as a mobility-matching BGE, will minimize ED, independently of the pH of the BGE. We first demonstrated the mobility-matching BGE concept by separating cationic analytes using commercially available tetraalkylammonium hydroxides as co-ion sources [5,6]. The mobility range encompassed by the tetraalkylammonium co-ions was not sufficiently broad to cover the desired range of co-ion mobilities. Moreover, the spacing between the tetraalkylammonium homologues was wide and the solubility of the larger homologues was limited [6]. In an attempt to extend the range of cationic co-ions, we synthesized a series of alkylmethylmorpholinium hydroxides with mobilities more evenly spaced through the $(20–55)\cdot 10^{-5}$ cm^2/V s range [7]. In this paper, we describe the synthesis, characterization and use of the first homologous series of polyethyleneglycol-based cationic mobility-matching reagents designed to reduce the extent of electromigration dispersion of cationic analytes and extend the mobility range of the available cationic co-ions to below $20\cdot 10^{-5}$ cm^2/V s.

The utility of the suggested cationic mobility-matching BGEs is illustrated in Fig. 1 by the electropherograms of a sample that contained (α-hydroxymethylbenzyl)trimethyl ammonium ion, (α-hydroxymethylbenzyl)triethyl ammonium ion and (α-hydroxymethylbenzyl)tributyl ammonium ion as analytes. The BGE is made of 50 m*M* phosphoric acid, the pH of which is adjusted to 2.2 with N-(polyethyleneglycol MW150 monomethyl ether)-N-methylmorpholinium hydroxide (150-PEGMME MEMOR). The time axis in the electropherograms has been converted to mobility axis (shown in 10^{-5} cm^2/V s units) to permit direct comparisons. In agreement with theoretical predictions, the first ana-

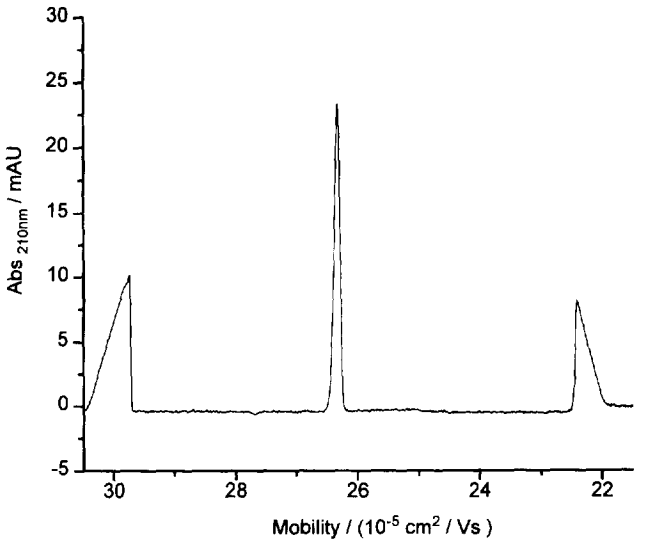

Fig. 1. Electropherogram of a sample containing 2.5 m*M* each of (α-hydroxymethylbenzyl)trimethylammonium chloride, (α-hydroxymethylbenzyl)triethylammonium chloride and (α-hydroxymethylbenzyl)tributylammonium chloride. Conditions: 1 s, 1.5 p.s.i. pressure injection, capillary: 25 μm fused-silica capillary. Field strength: 299 V/cm. Background electrolyte: 50 m*M* phosphoric acid, pH adjusted to 2.2 with 150-PEGMME MEMOR. Other conditions as in Section 2.

lyte peak, (α-hydroxymethylbenzyl)trimethyl ammonium ion, which has a mobility faster than the BGE co-ion, exhibits severe fronting, while the last analyte peak, (α-hydroxymethylbenzyl)tributyl ammonium ion, which has a mobility slower than the BGE co-ion, exhibits severe tailing. The middle analyte peak, which has a mobility close to that of the BGE co-ion, is symmetrical. Separation efficiency improves extraordinarily as the mobility of the BGE co-ion matches that of the middle analyte.

2. Experimental

Both a P/ACE 2100 and a P/ACE 5510 system (Beckman Instruments, Fullerton, CA, USA) were used for the electrophoretic experiments. The detector-side electrode was kept at the high positive potential. 57 cm long (50 cm from injector to detector)×50 μm I.D. eCAP Neutral capillaries (Part No. 477441, Beckman) with a neutral internal coating, 47 cm long (40 cm from injector to detector)× 50 μm I.D. eCAP Amine capillaries (Part No. 477431, Beckman), and 47 cm long (40 cm from injector to detector)×25 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), thermostatted at 37°C, were used for the mobility determinations. Unless otherwise noted, the field strength was 214 V/cm, resulting in power dissipation between 80 to 275 mW/m. All samples were injected by 1.5 p.s.i. nitrogen pressure for 1 s (1 p.s.i.=6894.76 Pa). The electroosmotic flow velocity was measured with benzylalcohol, immediately after each run, using the pressure-assisted capillary electrophoretic method developed in our laboratory [8]. The reported mobilities are corrected for the effects of the linear potential ramp at the beginning of the separation [9].

All chemicals used for the synthesis of the mobility-matching co-ions (methyl morpholine, anhydrous pyridine, toluenesulfonic chloride, toluene, dichloromethane (DCM), 2-methoxyethanol, 2-(2-methoxyethoxy)ethanol, triethylene glycol monomethyl ether, 350-, 550-, 750- and 2000-polyethylene glycol monomethyl ether) and some of the mobility markers (benzyl alcohol, benzyltrimethylammonium bromide, benzyltriethylammonium chloride and benzyltributylammonium bromide, phenyl alanine, tripelennamine hydrochloride, benzyl piperazine, dibenzyl amine, N,N-dimethylbenzyl amine, homatropine and aminoglutethimide) were reagent grade chemicals and, along with the BGE components [phosphoric acid, morpholinoethane sulfonic acid (MES) and lithium hydroxide], were obtained from Aldrich (Milwaukee, WI, USA). Deionized water from a Milli-Q unit (Millipore, Milford, MA, USA) was used to prepare the BGEs. β-Cyclodextrin was a generous gift from American Maize Products Corporation (Hammond, IN, USA).

Test analytes (α-hydroxymethylbenzyl)trimethylammonium chloride, (α-hydroxymethylbenzyl) triethylammonium chloride, (α-hydroxymethylbenzyl)tripropylammonium chloride, (α-hydroxymethylbenzyl)tributylammonium chloride, (α-hydroxymethylphenoxy)trimethylammonium chloride, (α-hydroxymethylphenoxy)triethylammonium chloride, (α-hydroxymethylphenoxy)tripropylammonium chloride and (α-hydroxymethylphenoxy)tributylammonium chloride were synthesized as described in Ref. [10]. The (polyethyleneglycol monomethyl ether) tosylates were prepared according to Tipson [11] as shown in Fig. 2. Briefly, equimolar amounts of *p*-toluenesulfonyl

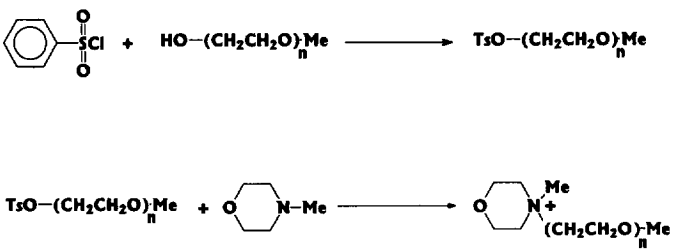

Fig. 2. Reaction scheme for the synthesis of the PEGMME MEMOR hydroxides.

chloride and PEGMME were mixed in a 50% solution of anhydrous pyridine and DCM. The product was obtained by adding 5 *M* sulfuric acid to the reaction mixture and extracting it with DCM. DCM was removed by vacuum evaporation yielding the tosylated PEGMME. The tosylated PEGMME was then reacted with N-methylmorpholine by adding the tosylated PEGMME to an excess of N-methylmorpholine and stirring the reaction mixture at 60°C overnight. 100–200 ml of deionized water was then added and the excess N-methylmorpholine was stripped in vacuum. The aqueous reaction mixture was then passed through a hydroxide-form strong anion-exchange resin column. The basic solution was extracted with DCM to remove residual traces of N-methylmorpholine. The 400–600 m*M* aqueous base solutions were assayed by titration using a standardized HCl solution, as were the residual N-methylmorpholinium concentrations (generally ≤5 mol%). Overall product yields varied between 40 and 60%.

3. Results

The synthesized ammonium probe molecules have effective mobilities in the 25 to $35 \cdot 10^{-5}$ $cm^2/V\,s$ range. They were used, together with the commercially available UV-absorbing quaternary ammonium compounds and amine analytes (with effective mobilities varying in the 5 to $55 \cdot 10^{-5}$ $cm^2/V\,s$ range) to determine the effective mobilities (at constant ionic strength) of the newly synthesized PEGMME MEMOR co-ions according to the electrophoretic method described in [6]. The analytes were electrophoresed in BGEs prepared from 25 m*M* solutions of the respective PEGMME MEMOR, adjusted to $2.2 \leq pH \leq 2.8$ with phosphoric acid. In order to minimize the effects of localized pH disruptions [12], the amine 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 MEMOR BGE co-ion 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. 3 for 150-PEGMME MEMOR, the unknown effective mobility of the co-ion studied (at the given ionic strength) could be read off from the point where the log(peak asymmetry) vs. mobility curve intersected the zero line. The closer the mobilities of the test

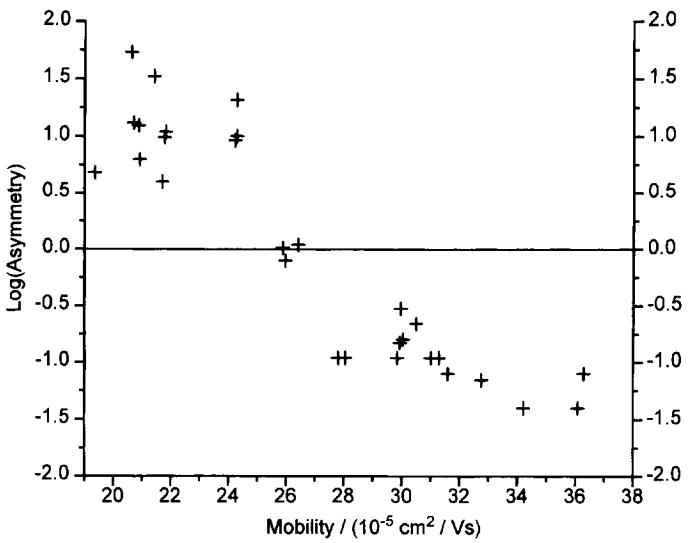

Fig. 3. Determination of the mobility of the 150-PEGMME MEMOR BGE co-ion. Field strength: 299 V/cm, capillary: 25 μm fused-silica. Other conditions as in Section 2.

solutes bracket the mobility of the BGE co-ion, the greater the accuracy of this mobility determination method.

By repeating these measurements for all the newly synthesized PEGMME MEMOR co-ions, their effective electrophoretic mobilities could be obtained and plotted against the logarithm of the ratio of their molecular mass and charge as shown in Fig. 4. These data indicate that with 2000-PEGMME MEMOR, analyte mobilities as low as $6 \cdot 10^{-5}$ cm^2/V s can be matched.

To demonstrate the utility of cationic co-ion-based mobility-matching in CE separations, the electropherograms of a chiral analyte, homatropine were obtained at the high, 2.5 m*M* analyte concentration level as shown in Fig. 5. In this figure, the time axis is once again replaced by the mobility axis. The electropherograms were obtained with 5 m*M* β-cyclodextrin, 50 m*M* MES BGEs whose pH was adjusted to 6.0 with lithium hydroxide (dashed line of Fig. 5) and 350-PEGMME MEMOR (solid line of Fig. 5), respectively. The β-cyclodextrin, counter-ion and co-ion concentrations are the same in both BGEs. Clearly, most of the electromigration dispersion is eliminated in the 350-PEGMME MEMOR-containing BGE. The utility of the PEGMME MEMOR cationic mobility-matching reagents is further demonstrated in Fig. 6, which shows the electropherograms of the much less mobile aminoglutethimide enantiomers. The separations were obtained with 50 m*M* phosphoric acid BGEs, whose pH was adjusted to 2.2 with lithium hydroxide (dotted line in Fig. 6) and the much slower 550-PEGMME MEMOR hydroxide (solid line in Fig. 6), respectively. Both BGEs contained 15 m*M* β-CD. The peaks tail badly even with the slowest inorganic co-ion, Li^+, but they are symmetrical, devoid of any electromigration dispersion when 550-PEGMME $MEMOR^+$, with a matching mobility is used as co-ion.

Though the efficiency improvements afforded by the use of mobility-matching BGEs are obvious from Figs. 5 and 6, one has to make certain that the replacement of lithium-based BGEs with PEGMME MEMOR-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. 7 as a function of the β-cyclodextrin concentration of the BGEs for the chiral test analytes homatropine and aminoglutethimide. The two selectivity curves are identical within experimental error and indicate the absence of untoward selectivity-modifying secondary effects.

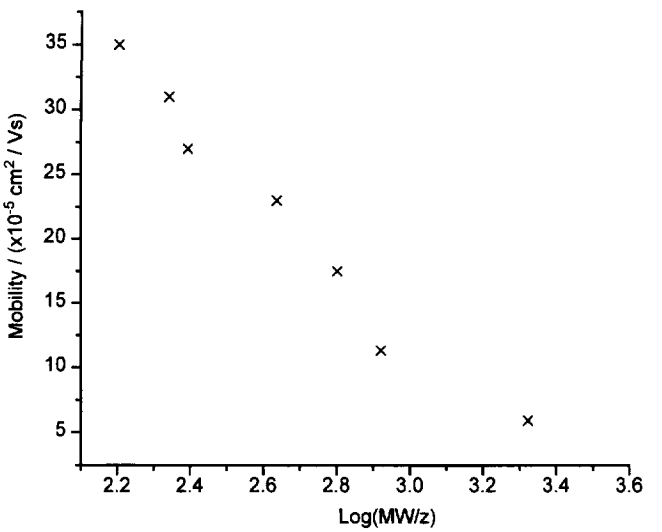

Fig. 4. Effective mobilities of the PEGMME MEMOR cations as a function of the logarithm of the ratio of their molecular mass and charge.

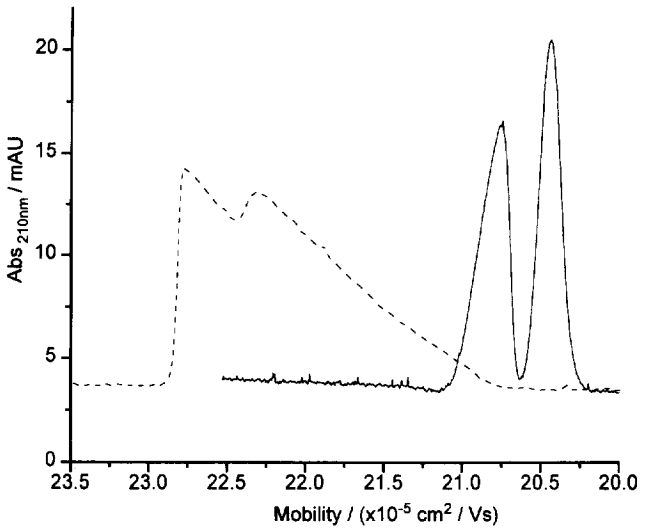

Fig. 5. Separation of the enantiomers of homatropine using 50 m*M* MES, 25 m*M* co-ion, pH 6.1, 5 m*M* β-CD BGEs. The pH was adjusted with lithium hydroxide (dashed line) and 350-PEGMME MEMOR hydroxide (solid line). Sample concentration 2.5 m*M*. Capillary: 50 μm eCAP Amine capillary. Field strength: 214 V/cm. Other conditions as in Section 2.

4. Conclusions

A series of new N-(polyethyleneglycol monomethylether)-N-methylmorpholinium hydroxides were synthesized and the effective electrophoretic mobilities of their cations were determined in pH 2.2–pH 2.8 phosphoric acid BGEs, where the co-ion concentration was kept constant at 25 m*M*. The effective mobilities of these PEGMME MEMOR co-ions span the $(6–35)\cdot10^{-5}$ cm^2/V s range. When combined with the previously reported tetraalkylammonium and N-alkyl-N-methylmorpholinium co-ions

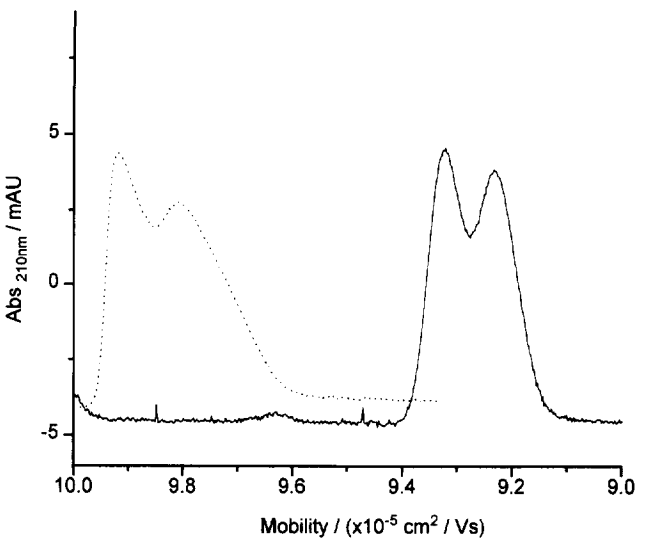

Fig. 6. Separation of the enantiomers of aminoglutethimide. 50 m*M* phosphoric acid, 25 m*M* co-ion, pH 2.2, 15 m*M* β-CD BGEs. The pH was adjusted with lithium hydroxide (dotted line) and 550-PEGMME MEMOR hydroxide (solid line). Sample concentration 1.0 m*M*. Capillary: 50 μm eCAP Neutral capillary. Field strength: 176 V/cm. Other conditions as in Section 2.

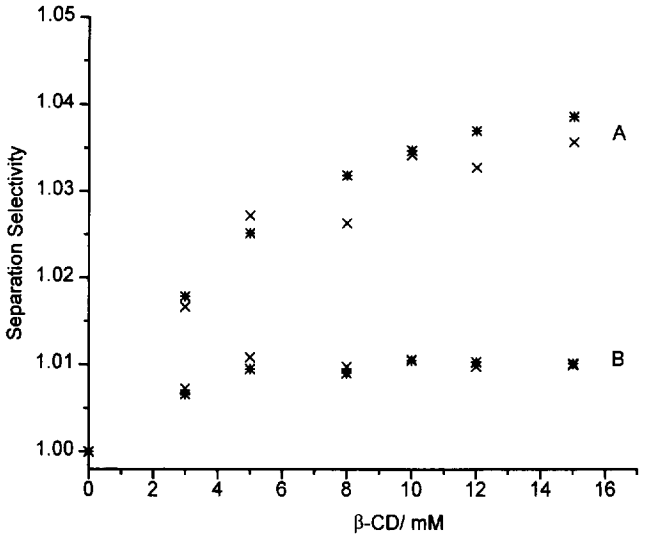

Fig. 7. Separation selectivity for homatropin (A) and aminogluthetimide (B) as a function of the β-cyclodextrin concentration in 50 m*M* phosphoric acid, pH 2.2 BGEs. Co-ions: 550-PEGMME MEMOR (*), lithium (×). Capillary: 50 μm eCAP Neutral capillary. Field strength: 176 V/cm. Other conditions as in Section 2.

(see Refs. [6,7]), they can cover the entire electrophoretically useful mobility range, $(6–50)\cdot10^{-5}$ cm^2/V s, and allow the preparation of mobility-matching background electrolytes which eliminate much of the electromigration dispersion that plagues the CE separations of cationic analytes. In the use of the mobility-matching BGEs, the pH of the BGE, required for optimum separation selectivity, can be set by the weak acid counter-ion, while the co-ion mobility, required for the elimination of electromigration dispersion and the realization of maximum separation efficiency, can be set by selecting the appropriate strong base whose mobility is independent of the pH of the BGE.

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