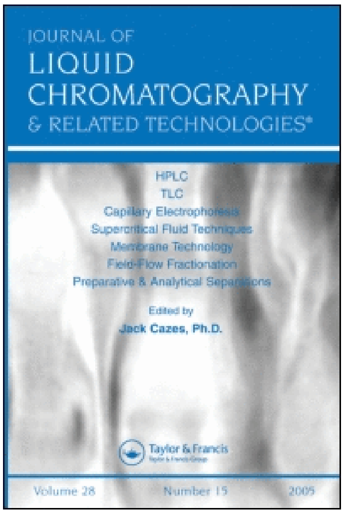

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THE USE OF ALKYLMETHYLMORPHOLINE-BASED BACKGROUND ELECTROLYTES IN CAPILLARY ELECTROPHORESIS

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ABSTRACT

A homologous series of alkylmethylmorpholinium 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 co-ions vary over the $25*10^{-5}$ to $45*10^{-5}$ cm^2/Vs range in 25 mM mobility-matching background electrolytes, prepared from phosphoric acid and the respective alkylmethylmorpholinium hydroxides. It was demonstrated experimentally that electromigration dispersion-related extra peak broadening could be minimized and difficult electrophoretic separations could be realized when these alkylmethylmorpholinium cations were used as co-ions.

1 INTRODUCTION

Ideally, in capillary electrophoresis (CE), separation efficiency is high because the sole cause of peak dispersion is longitudinal diffusion [1]. In well designed instruments this is indeed the case as long as severe solute — wall interactions are absent and electromigration dispersion is [2,3] negligible. Electromigration dispersion (ED) is caused by the local distortion of the electric field strength in the sample zone (*vis-a-vis* to the electric field strength in the surrounding pure background electrolyte) that occurs when the conductivities are different in the sample zone and in the pure background electrolyte (BGE). Generally, ED-induced band broadening can be minimized by keeping the transference number of the sample low [2,3]. Unfortunately, this simple approach is not always feasible due to the limited detection sensitivity of the currently used UV detectors. Mikkers et al. [2] suggested that ED is absent when the mobilities of the analyte and the BGE co-ion are equal. Such equality can be established, for example, by dynamically controlling the mobility of a zwitterionic BGE co-ion via multiple secondary chemical equilibria [4], a feasible, though difficult approach. An alternative, much easier approach was suggested by our laboratory when it was discovered that by assigning the buffering functions of the BGE solely to the counter-ion and the mobility matching functions solely to the co-ion, the mobilities of the analyte and the co-ion could be matched without altering the pH of the BGE [5]. For example, for weak base analytes, the BGE can be formed by adjusting the pH of a weak acid solution to the desired value with a strong base, the cation of which has a mobility equal to the effective mobility of the analyte at the given pH and ionic strength. Such BGEs were termed mobility-matching BGEs and the validity of the principle has been demonstrated with tetralkylammonium ions as co-ions [6]. The drawback of the tetralkylammonium cations is that the choice of available mobilities is limited. Therefore, a research program was initiated to synthesize and characterize a homologous series of cationic co-ions which cover the useful mobility range more evenly than the tetralkylammonium ions do. The power of the suggested mobility-matching BGEs is illustrated in Figure 1 by showing the electropherograms of a sample (the reaction mixture of stryrene epoxide and N,N,N,N-

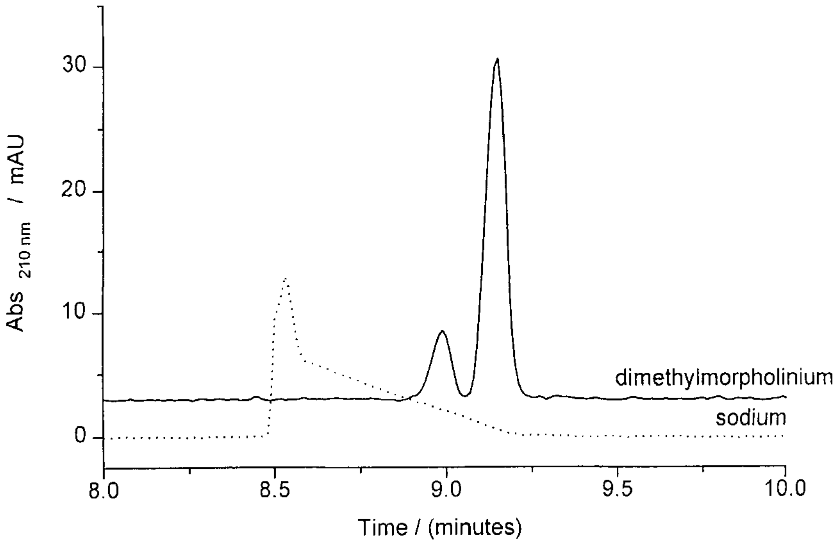

Figure 1.

Electropherograms of the major components (a and b) of the reaction mixture shown in Figure 2 in 0.2% hydroxyethyl cellulose, 35 mM phosphate, pH 1.9 BGEs, (*i*) co-ion: 35 mM sodium (dotted line), (*ii*) co-ion: 35 mM N,N-dimethylmorpholinium (solid line). Field strength: 169 V/cm. Other conditions as in Experimental.

tetramethylethylenediamine) in 0.2% hydroxyethylcellulose, 35 mM phosphoric acid, pH 1.9 BGEs, containing 35 mM NaCl and N,N-dimethylmorpholinium chloride, respectively. Except for the two co-ions, all other conditions of the separations were identical (injected amounts, BGE concentrations of all the components, field strengths, etc.). Clearly, the electropherogram obtained with the Na^+-containing BGE is useless, while symmetrical peaks and complete separation is obtained for the N,N-dimethylmorpholinium-containing BGE. Note, that the tail-end of the skewed second peak (representing infinite dilution) in the Na^+-containing BGE appears at the same time as the maximum of the symmetrical second peak in the N,N-dimethylmorpholinium-containing BGE, indicating that the infinite dilution electrophoretic mobilities of the analyte are identical in both BGEs and no selectivity change has taken place.

Naturally, even the alkylmethylmorpholinium-based BGEs are useful only for the analytes whose mobility is within the range covered by the homologous series. The synthesis and characterization of these co-ions, as well as the results of their use will be discussed in this paper.

2 EXPERIMENTAL

A P/ACE 5510 system (Beckman Instruments, Fullerton, CA) was used for the mobility measurements. The injection-side electrode was kept at the high positive potential. 47 cm long (40 cm from injector to detector) uncoated, 25 μm I.D. fused silica capillaries (Polymicro Technologies, Phoenix, AZ), thermostatted at 37 °C, were used for the mobility determinations. 50 μm I.D. eCAP Neutral capillaries (Part Number 477441, Beckman) with a neutral internal coating was used for the analysis of the samples shown in Figure 6. Unless otherwise noted, power dissipation was kept between 170 to 300 mW/m, corresponding to field strengths of 80 to 320 V/cm. All samples were injected electrokinetically. The electroosmotic flow velocity was measured by electrokinetically injecting a benzylalcohol solution. The reported mobilities are corrected for the effects of the linear potential ramp at the beginning of the separation [7].

All chemicals used for the synthesis of the mobility markers and the mobility-matching co-ions were reagent grade (phosphoric acid, sodium hydroxide, trimethylamine, triethylamine, tripropylamine and tributylamine, N,N,N,N-tetramethylethylenediamine, N-methyl-morpholine, styrene oxide, and 1,2-epoxy-3-

phenoxy-propane as well as methyl-, ethyl-, propyl-, butyl-, hexyl- and dodecyliodide, octyl- and nonylbromide), and were obtained from Aldrich (Milwaukee, WI). The 250 MHR PA hydroxyethylcellulose sample was obtained from Aqualone (Wilmington, DE). Deionized water from a Millipore Q unit (Millipore, Milford, MA) was used to prepare the BGEs.

The quaternary ammonium test solutes were synthesized according to a modified version of the procedure described in [8] and shown by the reaction scheme in Figure 2. The co-ions were synthesized according to a modified version of the procedure described in [9,10] and shown by the reaction scheme in Figure 3. Briefly, 0.1 moles of the starting amine and the respective alkyl halide were added to 200 mL of toluene. The solution was refluxed overnight. (The reaction with N-methylmorpholine and methyliodide was very exothermic and required cooling in an ice water bath.) The off-white precipitate formed in the reaction was separated from the reaction mixture, washed with toluene, dissolved in deionized water, and extracted with dichloromethane. The aqueous phase was then passed over an anion exchange column in hydroxide form (Dowex 1X8, Fluka, Ronkonkoma, NY). The collected effluent was reduced to 100 mL and assayed by titrating it with a standardized solution of HCl. Overall product yields varied between 10 and 60%. The structures of the newly synthesized compounds were confirmed by ^{1}H NMR spectroscopy.

3 RESULTS

The mobilities of the newly synthesized alkylmethylmorpholinium BGE co-ions at a constant ionic strength, were determined according to the simple electrophoretic method described in [6]. UV active quaternary ammonium analytes, whose electrophoretic mobilities varied in the $(10\text{-}55)*10^{-5}$ cm^2/Vs range were analyzed in pH 2.2 BGEs, which were prepared from a 50 mM phosphoric acid solution. The pH of the

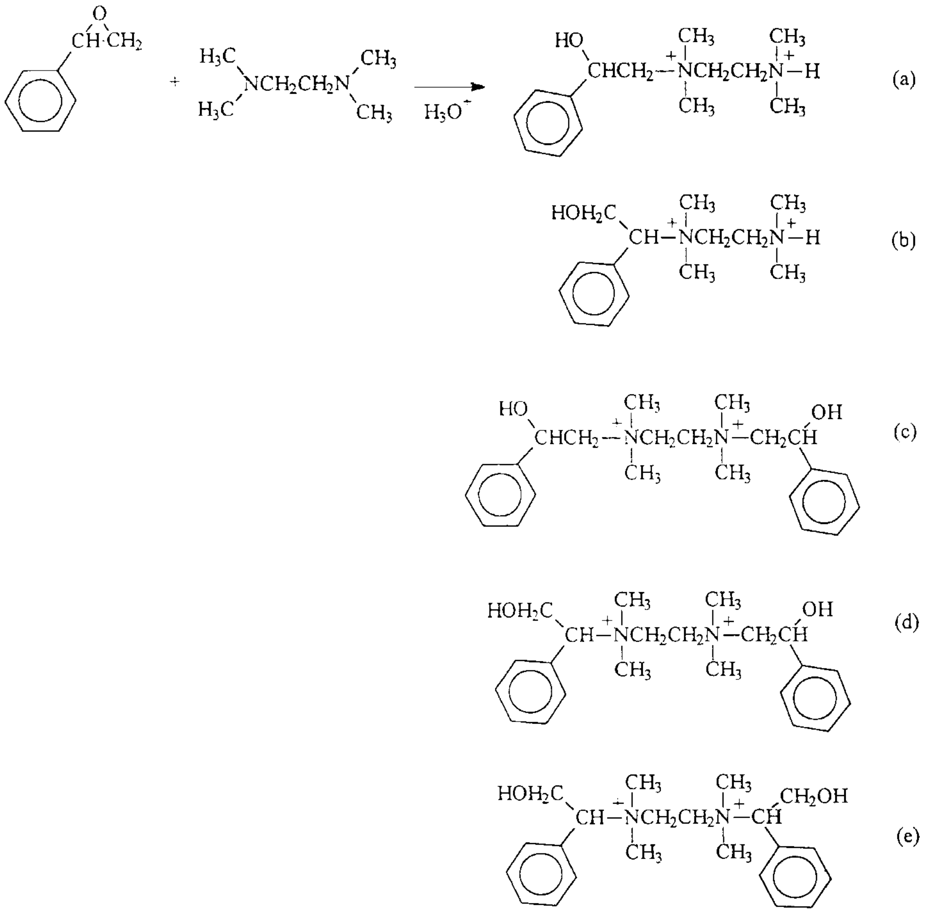

Figure 2.

Reaction scheme used for the synthesis of the cationic mobility markers.

BGEs was adjusted with the respective alkylmethylmorpholinium hydroxide solutions. Since the analyte concentrations were high, fronting (or tailing) analyte peaks were observed when their mobilities were larger (or smaller) than those of the BGE co-ion. By measuring the peak asymmetries (at 10% peak height) and plotting them as a function of the electroosmotic flow corrected effective mobility of the particular peak,

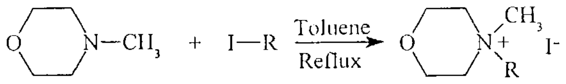

Figure 3.

Reaction scheme used for the synthesis of the N-alkyl-N-methylmorpholinium hydroxides.

plots similar to that shown in Figure 4 for N-methyl-N-propylmorpholinium, were obtained. The mobility where the log(peak asymmetry) *vs.* effective mobility curve crosses the zero line is equal to the effective mobility of the BGE co-ion studied at the given ionic strength. The closer the mobilities of the test solutes are to those of the BGE co-ions, the more accurate is the determined value of the mobility of the BGE co-ion.

By repeating these measurements (Figure 4) for all the newly synthesized co-ions, the effective electrophoretic mobilities of the alkylmethylmorpholinium cations could be determined as shown in Figure 5. The effective electrophoretic mobilities of the alkylmethylmorpholinium cations decrease almost linearly with the logarithm of their molecular weight. However, since the solubility of the nonyl and higher derivatives is more limited than desirable for a buffer component, the relationship could not be tested beyond the N-methyl-N-octylmorpholinium co-ion.

Compared to the lower tetraalkylammonium co-ions [6], the effective mobilities of the alkylmethylmorpholinium cations cover the (25-45)*10^{-5} cm^2/Vs range more evenly and permit closer mobility matching for the analytes. Together, the two cation series offer 11 different mobilities in the (20-50)*10^{-5} cm^2/Vs range.

To demonstrate the value of co-ion-based mobility-matching in difficult CE separations, the electropherograms of a sample that contains the disubstituted geometric isomers (c and d in Figure 2) are shown in Figure 4. To facilitate

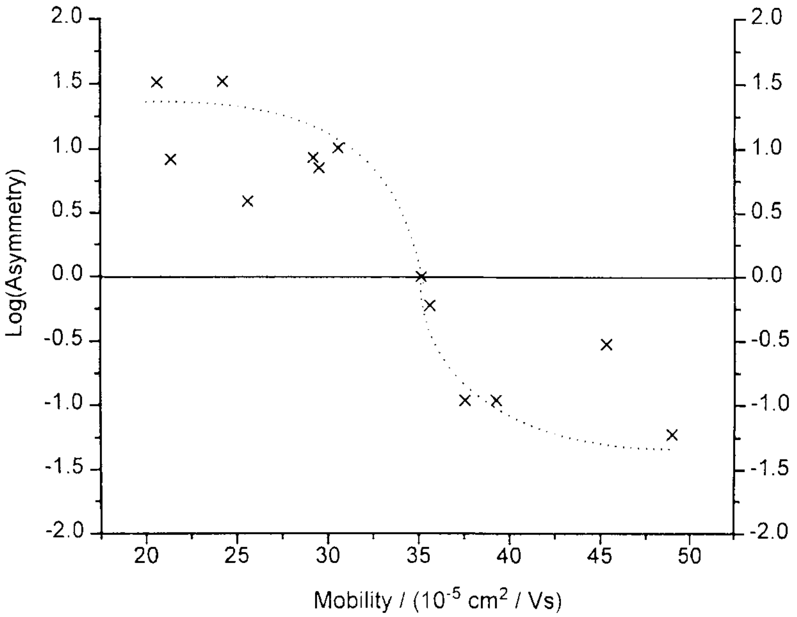

Figure 4.

Determination of the mobility of the N-methyl-N-propyl-morpholinium BGE co-ion. BGE: 50 mM phosphoric acid, pH 2.2, adjusted with N-methyl-N-propyl-morpholinium hydroxide. Field strength: 320 V/cm. Other conditions as in Experimental.

comparison, the electropherograms are plotted in terms of mobilities, rather than migration times. The electropherograms were obtained with 50 mM phosphoric acid BGEs whose pH was adjusted to 2.2 with N-ethyl-N-methyl-morpholinium hydroxide, N-methyl-N-propyl-morpholinium hydroxide and N-hexyl-N-methylmorpholinium hydroxide, respectively. The counter-ion (dihydrogenphosphate), hydronium, and N-methyl-N-alkylmorpholinium ion concentrations are the same in all three BGEs. It can be seen that the mobilities of the tail-ends and the front-ends of the skewed peaks (mobilities at infinite dilutions) and at the peak maxima of the symmetric peaks are

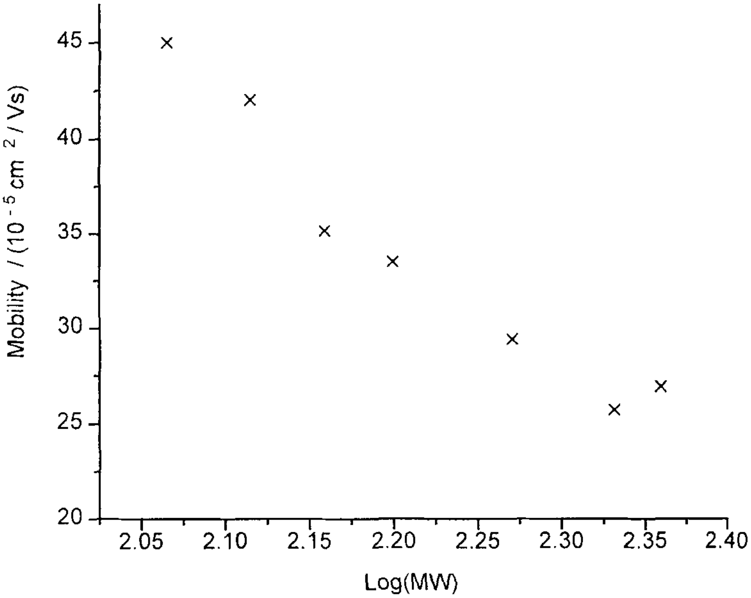

Figure 5.

Effective mobility *vs.* logarithm of molecular weight plot for the N-alkyl-N-methylmorpholinium cations in 50 mM phosphoric acid BGEs, pH 2.2 adjusted with the respective N-alkyl-N-methylmorpholinium hydroxide. Other conditions as in Figure 4.

identical, consequently the separation selectivities, which are the ratios of the effective mobilities, are also identical. However, the extent of peak distortion is very different and leads to peak resolutions which range from inadequate to almost baseline-baseline. The N-methyl-N-propyl-morpholinium ion ($\mu^{eff} = 35*10^{-5}$ cm^2/Vs) results in symmetric solute peaks ($\mu_1^{eff} = 35.3*10^{-5}$ cm^2/Vs and $\mu_2^{eff} = 34.6*10^{-5}$ cm^2/Vs), the N-ethyl-N-methyl-morpholinium ion ($\mu^{eff} = 42*10^{-5}$ cm^2/Vs), the faster co-ion results in tailing solute peaks, while the N-hexyl-N-methylmorpholinium ion ($\mu^{eff} = 28*10^{-5}$ cm^2/Vs), the slower co-ion, results in fronting solute peaks.

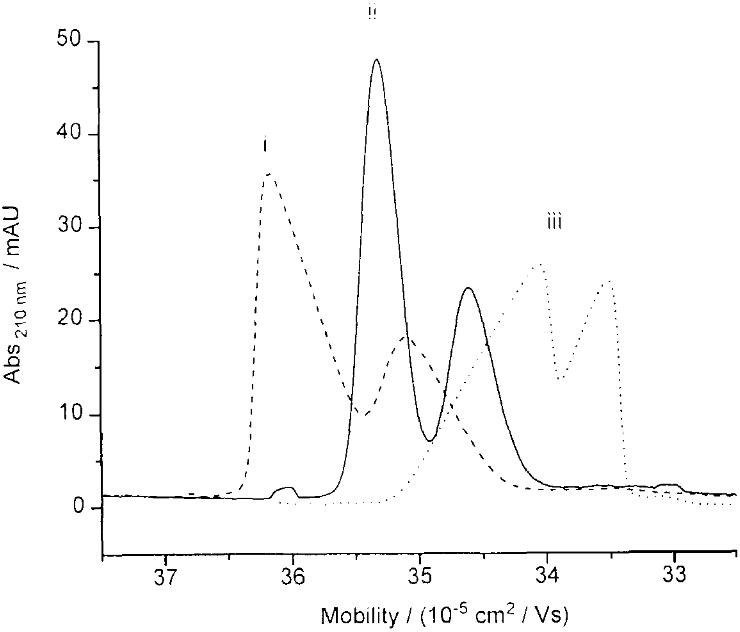

Figure 6.

Electropherograms of the minor components (c and d) of the reaction mixture shown in Figure 2 in 50 mM phosphate, pH 2.2 BGEs, (*i*) co-ion: 25 mM N-ethyl-N-methyl-morpholinium (dashed line), (*ii*) co-ion: 25 mM N-methyl-N-propyl-morpholinium (solid line), (*iii*) co-ion 25 mM N-hexyl-N-methyl-morpholinium (dotted line). Field strength: 80 V/cm. Other conditions as in Experimental, except the eCap Neutral capillary was used.

4. CONCLUSIONS

A set of new N-alkyl-N-methyl-morpholinium hydroxides were synthesized. The mobilities of the cations were determined in pH 2.2, 50 mM phosphate buffer BGEs and found to span the (25-45)*10^{-5} cm^2/Vs range, permitting their use in weak acid-derived mobility matching background electrolytes. Together with the tetraalkylammonium cations, these new reagents offer 11 distinct co-ions which can be used in the (20-50)*10^{-5} cm^2/Vs) effective mobility range to eliminate much of the electromigration dispersion that plagues most CE separations. By utilizing the concept of mobility matching BGEs, co-ion mobility-independent pH-control (as required by separation selectivity) can be achieved by the counter-ion, while pH-independent mobility matching can be achieved by the appropriate co-ion. These BGEs facilitate difficult separations that could not be achieved otherwise, even if the concentration of the analytes was kept very low. The utility of the approach is limited only by the lack of strong electrolytes with appropriately low mobilities. Therefore, further work is under way in our laboratory to synthesize cations which have mobilities below 20*10^{-5} cm^2/Vs.

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