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Maximization of separation efficiency in capillary electrophoretic chiral separations by means of mobility-matching background electrolytes

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Abstract

It has been predicted, both theoretically and by computer simulation, that in capillary electrophoresis the electromigration–dispersion-induced peak broadening can be eliminated by matching the mobilities of the analyte and the background electrolyte co-ion. Though mobility matching can be achieved by invoking multiple secondary chemical equilibria in the background electrolyte – such as protonation or complexation – to change the mobility of the co-ion, this approach is not feasible when the composition of the background electrolyte is dictated by the need to achieve a certain separation selectivity. In this paper, a background electrolyte preparation principle is outlined which decouples the dual roles of the background electrolyte, namely the buffering function and the mobility matching function, by ascribing the buffering function solely to the counter-ion (a conjugate acid or conjugate base) and the mobility matching function solely to the co-ion (a strong electrolyte). The power of this approach is demonstrated by solving difficult enantiomer separations.

Keywords: Capillary electrophoresis; Enantiomer separation; Optimization, efficiency; Buffer composition; Alkyl ammonium ions

1. Introduction

According to Giddings [1], "Separation is the art and science of maximizing separative transport relative to dispersive transport". In capillary electrophoresis (CE), the first objective has been tackled by the introduction of various resolution equations [2–5] which specify that resolution, R_s, depends on the mobility difference, $\Delta\mu$ and average mobility, μ_{ave}, of the solute pair [2–4], or the mobility ratio, α, and the effective charges, z_{eff}, of the analyte pair [5,6]. When secondary chemical equilibria are invoked to bring about the desired α values, such as protonation to separate weak electrolytes [5,7] or complexation with cyclodextrins to separate enantiomers [6,8–15], analytical expressions can be obtained which relate $\Delta\mu$ and μ_{ave} [8–10] or α and z_{eff} [6,11–15] to the composition of the background electrolyte. These analytical expressions can then be used to rationally select the operating conditions which yield thermodynamically optimized peak resolution values.

However, in CE, diffusional band broadening is often accompanied by electromigration dispersion [16]. Electromigration dispersion (ED) lowers peak resolution below the theoretical value. Both theoretical discussions [16,17] and computer simulations [18–20] indicate that ED-induced band broadening is

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at a minimum when (i) the concentration of the BGE is much higher than that of the analyte or (ii) the mobilities of the analyte and the background electrolyte (BGE) co-ion are identical. Often, the ratio of the analyte ion concentration and the co-ion concentration cannot be minimized because the concentration detection limits of the commonly used UV detectors are poor. Therefore, a more viable solution is to try to match the mobilities of the analyte and the BGE co-ion. Using fenoprofen as an example, we have demonstrated that the mobility of a properly selected zwitterionic BGE co-ion can be adjusted dynamically in cyclodextrin-containing BGEs by fixing any two of the following three analytical BGE concentrations: (i) the cyclodextrin concentration, (ii) the zwitterionic buffer concentration or (iii) the hydronium ion concentration, and varying the third [21]. Though the dynamically matched mobilities resulted in symmetric enantiomer peaks and high separation efficiencies, the method is not as simple and fast as might be desired.

In this paper we describe an alternative approach to efficiency maximization [22]. This approach is based on the recognition that BGEs play a dual role: (i) they provide the chemical environment that dictates separation selectivity, and (ii) they, together with the sample, set the electric field strength profile that controls the extent of electromigration dispersion. If one could decouple the two roles and ascribe them respectively to the counter-ion and the co-ion, one should be able to independently vary the pH of the BGE (to achieve the desired selectivity) and the mobility of the co-ion (to eliminate the local distortion of the electric field). For example, the BGE for weak base analytes can be made from a weak acid titrated to the desired pH with a strong base. The desired pH dictates the selection of the buffering weak acid (pK_a), while the average mobility of the cationic analyte pair to be separated dictates the selection of the strong base. As long as the sample contained the same counter-ion as the BGE, one could independently vary the pH and the mobility of the co-ion: the first by varying the ratio of the amounts of the weak acid and the strong base, the second by varying the chemical identity of the strong base (e.g. KOH vs. LiOH). Naturally, this mobility matching approach works only for analytes with closely similar mobilities, but then again, these are the critical separations where ED-induced peak broadening may obviate the separation. The feasibility and power of the proposed mobility matching buffers is demonstrated in Fig. 1 showing the electropherograms of a mixture of benzyltrimethylammonium and benzyldimethylammonium ions in a series of 50 m*M* phosphoric acid, pH 2.2 BGEs. The injected amounts and the BGE concentrations of all components are identical, except that the first electropherogram is obtained with 25 m*M* Na^+ as co-ion, the second one is obtained with 25 m*M* Li^+ as co-ion, the third is obtained with 25 m*M* tetraethylammonium (TEA) as co-ion and the fourth is obtained with 25 m*M* tetrapropylammonium (TPrA). In agreement with the theoretical predictions [16], only the TEA BGE yields symmetrical peaks, even though the analyte mobilities are identical in all four BGEs.

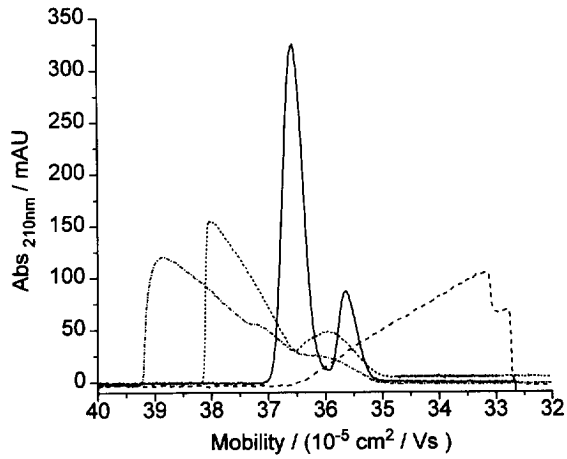

Fig. 1. Electropherograms of a mixture of benzyltrimethylammonium and benzyldimethylammmonium ions in 50 m*M* phosphate, pH 2.2. BGEs co-ions: (a) 25 m*M* Na^+ (dash-dot-dash line), (b) 25 m*M* Li^+ (short dash line), (c) 25 m*M* tetraethylammonium (solid line), (d) tetrapropylammonium (long dash line). For the sake of easy comparison, the horizontal axis is scaled in mobility units. Field strength: 135 V/cm. Other conditions as in Experimental.

2. Experimental

All CE separations were carried out with either a

P/ACE 2100 or a P/ACE 5510 system (Beckman Instruments, Fullerton, CA, USA), using 210 nm as the detection wavelength. The electrode at the injection end of the capillary was kept at positive potential. Uncoated, 25 μm I.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) (40 cm from injector to detector, 47 cm total length), and 50 μm I.D. neutral coated capillaries (Beckman Instruments, Part no. 477441) (30 cm from injector to detector, 37 cm total length), thermostatted at 37°C were used. The field strength was kept at 130–170 V/cm during the co-ion mobility determinations and at 320 V/cm during the separation of the enantiomers (Fig. 4–6). The samples were injected both electrokinetically and hydraulically. The electroosmotic flow velocity was determined by injecting a dilute solution of benzylalcohol and taking into account the linear potential ramp at the beginning of the separation [23].

β-Cyclodextrin was a generous gift from American Maize Products Corporation (Hammond, IN, USA). Reagent grade phosphoric acid, tetramethyl-, tetraethyl-, tetrapropyl and tetrabutylammonium hydroxide, sodium hydroxide, lithium hydroxide, trimethylamine, triethylamine, tripropylamine and tributylamine, and styrene oxide were obtained from Aldrich (Milwaukee, WI, USA). All BGEs were prepared using deionized water from a Milli-Q unit (Millipore, Milford, MA, USA).

The quaternary ammonium test solutes were synthesized according to [24] and their structure was confirmed by ^{1}H NMR spectroscopy.

3. Results

3.1. CE method for the determination of the mobility of the BGE co-ion

First, a simple electrophoretic method was developed to determine the actual mobilities of the BGE co-ions. A series of permanently cationic, UV-active analytes, either commercially available or synthesized in our laboratory from styrene oxide and trialkylamines [24], were assembled with ionic mobilities spanning the relevant $(15–50)\cdot10^{-5}$ cm^2/Vs mobility range. The analytes were organized into several test suites such that the components did not co-migrate, even at concentrations high enough to cause severe ED-induced peak distortion. The test mixtures were separated in pH 2.2 BGEs prepared from a 50 m*M* phosphoric acid solution. The pH of the test mixture was adjusted with the tetraalkylammonium hydroxide under study. The fast migrating peaks of the test mixtures were fronting, the slow ones were tailing. The test solute mobilities were calculated from the peak start times for fronting peaks and the peak end times for tailing peaks representing the best approximations of the "infinite dilution" mobilities. Next, the peak asymmetries at 10% peak height were determined (calculated as $A = b/a$, where a is the time difference between the peak front at 10% peak height and the peak maximum, and b is the time difference between the peak tail at 10% peak height and the peak maximum), and their logarithm was plotted as a function of the respective "infinite dilution" mobilities as shown in Fig. 2 for the tetrabutylammonium co-ion. The point where peak asymmetry becomes unity corresponds to the effective mobility of BGE co-ion. This method, which requires only CE measurements, is very simple and sensitive because when the test solute concentrations are high, even small differences between the mobilities of the BGE co-ion and the test solute lead to large peak asymmetry values. Obviously, test solutes with only slightly higher and slightly lower mobilities than that of the BGE co-ion are needed if accurate co-ion mobility values are to be obtained.

3.2. Effective mobilities of the tetraalkylammonium ions

Using the method described in section 3.1, the effective mobilities of the lower members of the symmetrical tetraalkylammonium ions series were determined and plotted in Fig. 3 as a function of the logarithm of their molecular mass. The determined effective mobilities agree reasonably well with the literature values of 44.9 32.7 23.4 and $19.5\cdot10^{-5}$ cm^2/Vs reported for TMA, TEA, TPrA and TBA, respectively [25]. Thus, using tetraalkylammonium

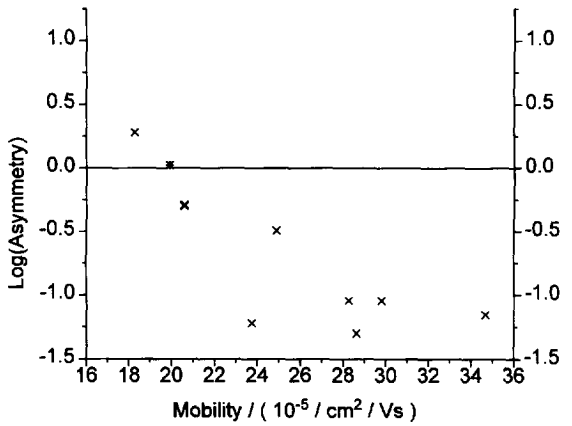

Fig. 2. BGE co-ion mobility determination method. BGE: 50 m*M* phosphoric acid, pH 2.2, adjusted with tetrabutylammonium hydroxide. Other conditions as in Experimental.

hydroxides, one can access BGE co-ion mobilities that are significantly lower than that offered by LiOH, the slowest member of the alkali hydroxide family.

3.3. Effect of co-ion mobility matching on peak resolution in the separation of enantiomers

To indicate the dramatic influence of co-ion mobility matching on peak resolution, a test mixture

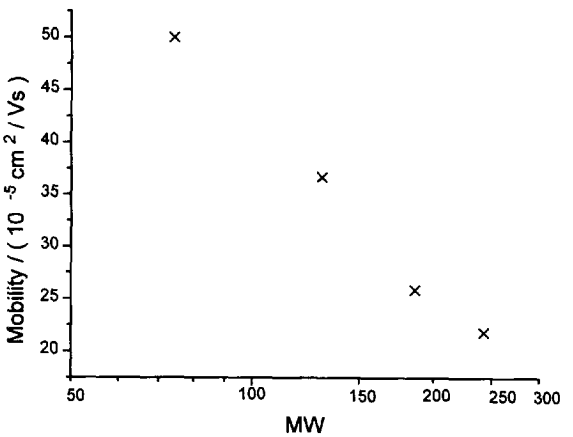

Fig. 3. Effective mobilities vs. molecular mass plots for the lower tetraalkylammonium cations in 50 m*M* phosphoric acid BGE, pH 2.2, adjusted with the respective tetraalkylammonium hydroxide.

containing five permanently cationic racemic solute pairs was assembled and the enantiomers were separated using 15 m*M* β-cyclodextrin, 50 m*M* phosphoric acid BGEs whose pH was adjusted to 2.2 with tetraethylammonium hydroxide, tetrapropylammonium hydroxide and tetrabutylammonium hydroxide, respectively. Since the dihydrogenphosphate, hydronium, and tetraalkylammonium ion concentrations are the same in all three BGEs, and since the tetraalkylammonium ions have been shown not to influence the selective binding of chiral analytes on β-cyclodextrin significantly [14], the separation selectivities and analyte charges are identical in all three BGEs. Therefore, according to the generalized resolution equation [5,6], peak resolution should be identical in all three BGEs. In order to demonstrate the extraordinary power of proper co-ion mobility matching, solute concentrations were kept as low as possible (peak heights for the strongly UV absorbing aromatic test solutes were only 2 mAU). This, alone, minimizes ED-induced peak distortion and enhances the chance of peak resolution. Yet, as shown in Fig. 4–6, none of the enantiomeric pairs can be resolved, even under the most favorable analytical conditions without the use of proper mobility matching.

The electropherograms, obtained with these three BGEs are shown in Figs. 4–6, for the tetraethyl-, tetrapropyl- and tetrabutylammonium co-ions, respectively. For the sake of easy comparison, the horizontal axis of each electropherogram is scaled in mobility units, rather than migration time. It can be seen that for the fastest co-ion, the tetraethylammonium ion (effective mobility=$37 \cdot 10^{-5}$ cm^2/Vs), all peaks, even the fastest enantiomeric pair (effective mobility ~$27.5 \cdot 10^{-5}$ cm^2/Vs), tail. Partial separation can be seen for the first, third and fourth peak pairs, but there is no hint of separation for the second and fifth pairs. For the slower BGE co-ion, the tetrapropylammonium ion (effective mobility=$27 \cdot 10^{-5}$ cm^2/Vs), the first peak pair fronts and the peaks are almost completely separated. The second peak pair tails slightly and the peaks become partially separated. The third to fifth peak pairs tail increasingly and there is yet no separation on the last, the slowest peak pair. For the slowest BGE co-ion, the tetrabutylammonium ion (effective mobility=$20 \cdot 10^{-5}$ cm^2/Vs), the first and second

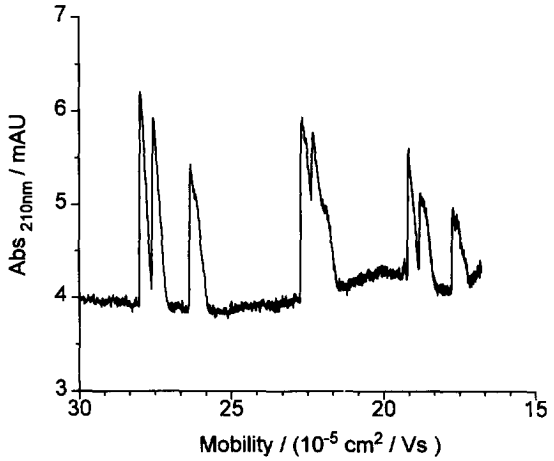

Fig. 4. Electropherogram of a test mixture containing five racemic solutes: (α-hydroxymethylbenzyl)trimethyl ammonium ion, (α-hydroxymethylbenzyl)triethyl ammonium ion, (α-hydroxymethylbenzyl)tributyl ammonium ion, N-(2-phenyl-1-hydroxyethyl)-N,N,N-tributyl ammonium ion (in decreasing order of mobility) in 15 m*M* β-cyclodextrin, 50 m*M* phosphoric acid BGE, pH 2.2 adjusted with tetraethylammonium hydroxide. Other conditions as in Experimental.

peak pairs front badly and peak resolution is lost. Complete resolution is achieved for the very slightly fronting and very slightly tailing third and fourth peak pairs indicating that the effective mobility of

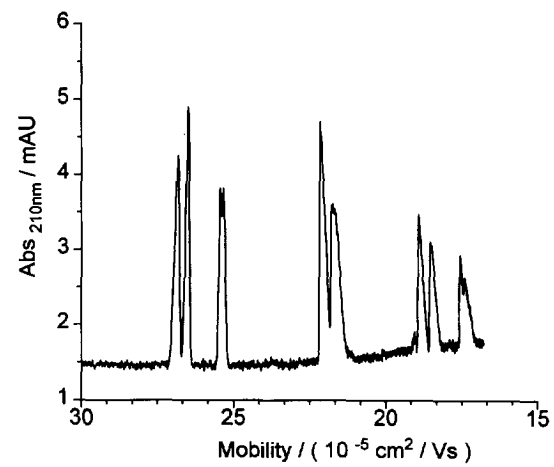

Fig. 5. Electropherogram of a test mixture shown in Fig. 4. Conditions as in Fig. 4, except pH adjusted with tetrapropylammonium hydroxide.

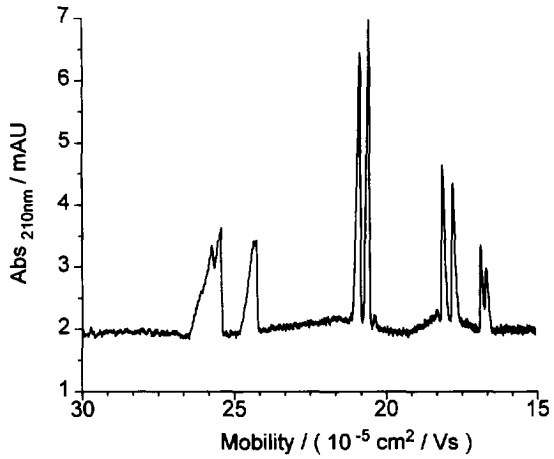

Fig. 6. Electropherogram of a test mixture shown in Fig. 4. Conditions as in Fig. 4, except pH adjusted with tetrabutylammonium hydroxide.

the tetrabutylammonium co-ion lies in between their mobilities. Partial separation is achieved, for the first time, for the fifth, the slowest peak pair (effective mobility = $17 \cdot 10^{-5}$ cm^2/Vs).

4. Conclusion

The concept of mobility matching BGEs has been introduced here. In these mobility matching BGEs, the role of pH control is ascribed to the counter-ion, while the role of mobility matching is assigned solely to the co-ion. When the counter-ion is a weak electrolyte and the co-ion is a strong electrolyte, the roles of pH control and mobility matching are decoupled from each other and both pH (separation selectivity) and peak shape (electromigration dispersion) can be adjusted independently. The power of this approach has been demonstrated via the separation of enantiomers. Without proper mobility matching, these separations could not be achieved, even when the concentration of the analytes was very low.

Though this paper used enantiomers to demonstrate the power of mobility matching buffers, such buffers can be used advantageously whenever the

sample contains a peak pair whose migration behavior is very similar. Practical utility of the method depends on the availability of a sufficiently large number of strong electrolytes with mobilities covering the useful 0 to $50 \cdot 10^{-5}$ cm^2/Vs range. Further work is under way in our laboratory to synthesize such agents.

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