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Increased selectivity for electrochromatography by dynamic ion exchange

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ABSTRACT

The separation of compounds with similar mobilities is expected to be difficult with capillary zone electrophoresis. Increased selectivity is shown for compounds of this type when other modes of separation are added to the system. A capillary coated with a hydrophobic stationary phase is shown to be a dynamic ion exchanger when a quaternary ammonium compound is added to the running buffer. Compounds are shown to have a decrease in retention when the concentration of the buffer ion is increased. The effect of adding an organic modifier and the influence of the concentration of the surface active reagent are also studied.

INTRODUCTION

Capillary zone electrophoresis (CZE) has been demonstrated to provide highly efficient separations of many charged species including small organic molecules, amino acids, peptides and nucleic acids [1–5]. Because CZE separates compounds based on their differential migration rates, compounds with similar mobilities pose resolution problems, however [6,7]. The most common optimization techniques for CZE are changing the pH of the running buffer, coating the column with a hydrophobic stationary phase, and using additives to decrease the electroosmotic flow.

The addition of hydrophobic ion-interaction reagents (IIR) to the mobile phase to enhance retention and resolution has been widely used in the liquid chromatographic (LC) separation of charged species on reversed-phase columns [8–13]. The ion-pair model presumes that an ion pair between the IIR and the sample ion is formed in the mobile phase. The ion-piar then partitions into the stationary phase. Alternatively, retention can be explained by a dynamic ion-exchanged model [14]. This model presumes that the IIR is adsorptively bound to the column where it behaves as an ion-exchanger. The retention and separation in this mode are very similar to those in ion-exchange chromatography. There is, however, added flexibility in dynamic ion-exchange due to the many parameters that affect the retention of a species. Another variation is the ion-interaction model where the IIR is dynamically adsorbed to the column forming a charged double layer. Sample ion are then retained due to their coulombic attraction to the double layer. The actual mechanism of ion-pair chromatography is probably a mixture of these models depending upon the specific conditions.

In this paper, separation of compounds with similar electrophoretic mobilities is performed using a reversed-phase coated capillary with dynamic ion-exchange sites. An IIR, cetyltrimethylammonium bromide (CTAB), is added to the running buffer and is sorptively bound to the hydrophobic stationary phase to allow ion-exchange. The results of these experiments support the dynamic ion-exchange model for retention and separation.

EXPERIMENTAL

The buffers used in these experiments were prepared in deionized water (Millipore, Bedford, MA,

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USA; Milli-Q system). CTAB solutions were prepared by diluting with buffer solution and adjusting to pH 7 with a 0.1 *M* solution of NaOH. The anions, 4-amino-1-naphthalenesulphonic acid (4A1N) and 5-amino-2-naphthalenesulphonic acid (5A2N) (both from Aldrich, Milwaukee, WI, USA), were prepared in running buffer prior to injection.

The fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 50 cm long and nominally 10 μm in I.D. These capillaries were used as supplied or coated, and the coating later cross-linked with 10.0% (w/w) OV-17v (Alltech Assoc., Deerfield, IL, USA) as described in ref. 15. The polyimide coating was removed with hot sulphuric acid 40 cm from the injection end of the capillary to facilitate on-column detection. The CZE–electrochromatographic system is similar to the one described previously [16]. The electric field is supplied by either a positive or a negative high-voltage supply (0–30 kV, Glassman High Voltage, Whitehouse Station, NJ, USA; Model MJ30P0400-11 or MJ30N0400-11).

Sample introduction was accomplished by using electromigration in all cases. Detection was accomplished by UV absorption (240 nm, ISCO Model 3850, Omaha, NE, USA) or by using a laser-based fluorescence detector similar to one described previously [16] with the exception being that the laser line used was the 325-nm line of a He–Cd laser (Liconix, Sunnyvale, CA, USA; Model 4240NB).

All data were either collected on a strip chart recorder or subjected to analog-to-digital conversion (Data Translation, Marlborough, MA, USA; Model DT 2827, 5–10 Hz) and later stored on a personal computer (IBM PC-AT).

DISCUSSION

The resolution of compounds separated by CZE is given by

$$R = \frac{1}{4\sqrt{2}} (\mu_{e,1} - \mu_{e,2}) \sqrt{\frac{V}{D(\bar{\mu}_e + \mu_{eo})}} \quad (1)$$

where $\mu_{e,1}$ and $\mu_{e,2}$ are the electrophoretic mobilities of the solutes, $\bar{\mu}_e$ is the average electrophoretic mobility, μ_{eo} is the electroosmotic flow coefficient, V is the applied potential and D is the diffusion coefficient. As can be seen from this equation, resolution is directly proportional to the difference in mobilities of the two species of interest.

The separation of compounds with similar mobilities is therefore expected to be difficult with CZE [7]. This can be demonstrated by the attempted separation of positional isomers, which would be expected to have similar mobilities. When 4A1N and 5A2N are injected into the commercial capillary electrophoresis system at pH 7 (10 m*M* sodium phosphate buffer), no separation is achieved. One way to enhance the selectivity of this separation would be to change the pH of the buffer which may change the charge, and therefore the mobility, of each species to different degrees. This, however, would not be expected to work in all cases due to a small range of pK values for the components in the mixture. Compounds with very similar but distinct mobilities will not separate unless $\mu_{eo} \approx -\mu_e$, which can be achieved through the addition of modifiers to the buffer. This condition leads to analysis times that approach infinity, however.

Selectivity can also be enhanced by adding another mode of separation to the system. One way to do this for the compounds we have selected is to add anion-exchange sites to the column. Compounds with larger affinities for the ion-exchange sites would be impeded and would have longer migration times. This can be accomplished by first coating the column with polymeric stationary phase [17]. This will have two effects on the column. First, the charged silanol groups on the surface of the column would be shielded, thus drastically reducing electroosmotic flow. Second, the surface of the column would be changed from a polar to a relatively non-polar, hydrophobic surface. Compounds with a hydrophobic character can therefore partition into the stationary phase and can be separated based on retention [18].

For the separation of anions, the dynamic ion-exchange sites are added to the column by the addition of a surface-active quaternary ammonium compound to the buffer. The hydrophobic end of this molecule is expected to adsorb to the hydrophobic surface of the coated capillary, allowing the charged end of the molecule to be exposed to the polar (aqueous) mobile phase. The surface of the capillary would therefore be expected to carry a positive charge with a corresponding association of anions in the double-layer region that extends to the diffuse layer. This is in contrast to a bare fused-silica column, where under normal (aqueous) conditions

the solid surface has an excess anionic charge resulting from ionization of surface silanol groups. The counter-ions in the diffuse layer in that case would therefore be positive. The migration of hydrated counter-ions in the diffuse layer is the propulsive force behind electroosmotic flow [19]. In the case of a bare fused-silica capillary we would expect the flow to be towards the cathode while in the case of the dynamic exchange mode the flow would be in the direction of the anode.

The fact that the electroosmotic flow is reversed when a surface-active quaternary ammonium compound is added to the buffer supports the dynamic ion-exchange model for ion-pair chromatography. This is the process where the surface-active agent is first adsorbed to the surface of the column, and the sample ion is then retained by an ion-exchange mechanism. The mechanism is more complex than ion-exchange, however, because the ion-exchange site is sorptively bound, while in an ion chromatography column the ion-exchange site is covalently bound.

The C_{18} chain of the CTAB molecule is expected to be readily adsorbed onto the surface of a reversed-phase column by hydrophobic attraction and would tend to behave as an ion-exchange site. Since conductance data indicate that R_4N^+ salts are highly dissociated under similar conditions it can be assumed that ion-pair formation is negligible [12]. The adsorption of the CTAB to the column was confirmed by washing the column with acetonitrile and the observation that the electroosmotic flow changes back to its original direction, *i.e.*, towards the cathode.

The retention of a compound by dynamic ion-exchange, as discussed above, is complex. Some of the parameters that can influence the dynamic ion-exchange retention of compounds in HPLC are the size of the surface-active agent, pH, type and concentration of the organic modifier and the stationary phase characteristics [13,14]. These parameters would be expected to have similar effects in electrochromatography.

In Fig. 1, the two compounds, 4A1N and 5A2N, are separated on a column coated with 2% OV-17v. The buffer consists of potassium phthalate, a common eluent used in ion-exchange chromatography, and 400 μM CTAB. The column was equilibrated with the buffer for 2 h to increase the stability of the

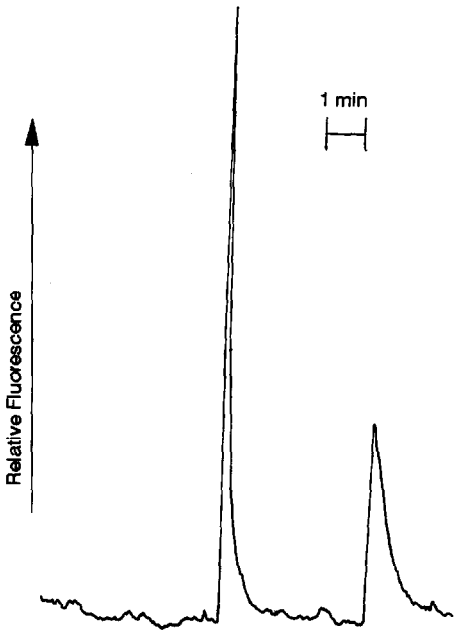

Fig. 1. Elution profile for (left) 4A1N and (right) 5A2N. Buffer conditions: 32 mM potassium phthalate, 400 μM CTAB, pH 7.0.

migration times. There is a reduction in efficiency compared to CZE in this system based on the observed number of theoretical plates in each peak. This would be expected due to the increased contribution of mass transfer in the stationary phase to the plate height.

The equilibrium describing the exchange of an anion A and the buffer ion E is

$$A_{aq} + nE_s \rightleftarrows A_s + nE_{aq} \quad (2)$$

where the subscripts s and aq refer to the stationary and mobile phases, respectively.

Models for dynamic ion-exchange based on HPLC experiments predict that increased buffer ion concentration will have two effects on the retention of sample ions. The increased concentration of the counter ion increases the amount of the surface-active agent adsorbed to the surface of the capillary [11]. This is greatly offset, however, by the increased competition for the exchange sites as can be seen from eqn. 2. Pfeffer and Yeung [18] and Tsuda [20] have shown that the addition of a surface-active reagent, such as CTAB, to the hydrophobic surface of a coated capillary column has the effect of

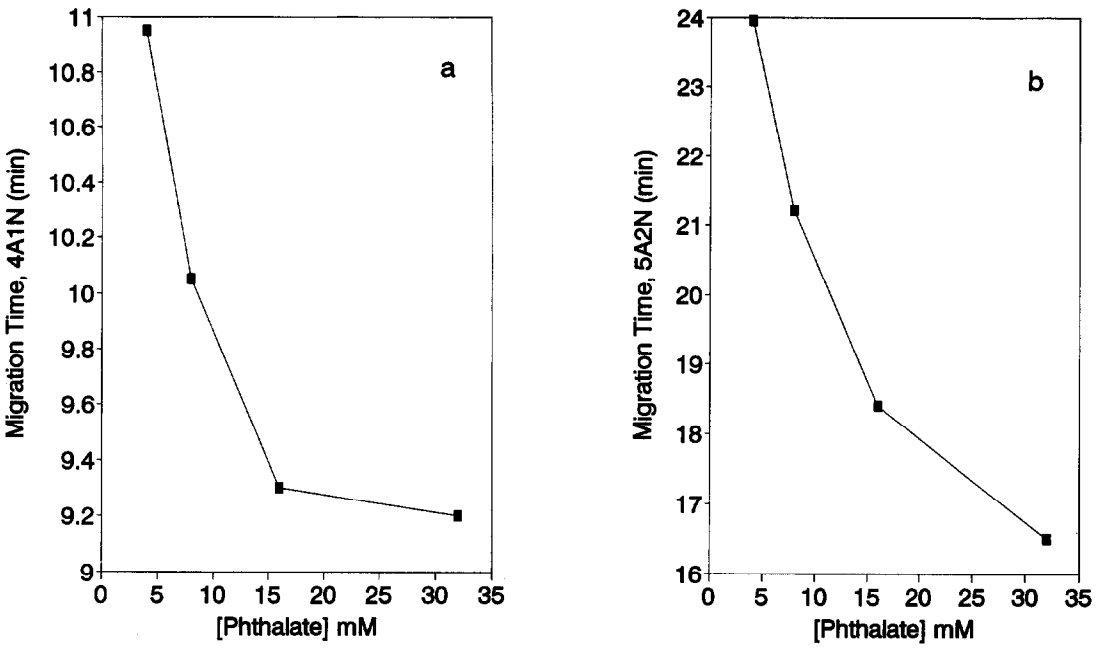

Fig. 2. Dependence of migration times on buffer ion concentration for (a) 4A1N and (b) 5A2N. Buffer conditions are the same as in Fig. 1.

increasing electroosmotic flow. A maximum in flow-rate is reached when charge repulsion prevents additional CTAB adsorption and the hydrophobic phase is essentially saturated. With a 10 m*M* phosphate and 10% acetonitrile buffer this maximum was reached in the range of 20 μM CTAB. With no organic solvent in the mobile phase the saturation point would be expected to be even lower.

In our system we used a CTAB concentration of 400 μM to ensure that the walls of the capillary were saturated. The net effect, therefore, of an increase in buffer ion concentration should be a decrease in migration time of the sample ions. This was confirmed in our experiments as shown in Fig. 2. Another interesting result is that 4A1N, the least retained compound, showed very little change in retention at the highest concentration of buffer ion. This is because this compound is only slightly retained at the high buffer ion concentrations. Therefore, its migration time depends almost entirely upon its mobility as in zone electrophoresis. 5A2N, on the other hand, showed a decrease in retention at all buffer ion concentrations.

In addition to the cation-exchange sites, there will also be reversed-phase character to this column due to the hydrophobic nature of the polymeric-film coating. This reversed-phase character can be examined by adding an organic component, acetonitrile, to the mobile phase and observing its effect on the migration times in the absence of CTAB.

Fujiwara and Honda [21] have shown that the addition of acetonitrile to a CZE buffer can increase the velocity of electroosmotic flow by almost 30%. We expect that the addition of acetonitrile will decrease the migration times of non-retained compounds by the increase in flow-rate, and decrease the migration times of retained compounds by an even larger amount, due to the decrease in retention. As seen from Fig. 3, the addition of acetonitrile to our system did have the predicted effect on the migration times of these species. In our experiment the retention time of 4A1N decreased about 40%, indicating that there is some reversed-phase retention of this compound. The 5A2N migration time decreased about 200%, indicating that this compound has a strong reversed-phase component.

Because the amount of organic modifier added to the system also affects the adsorption of CTAB to

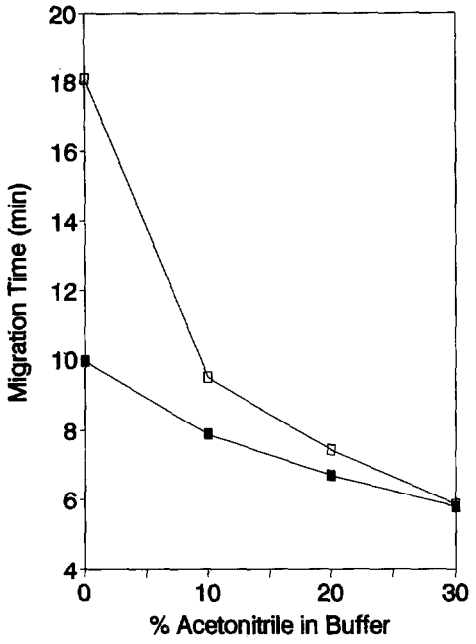

Fig. 3. Dependence of migration times on the concentration of organic modifier. Buffer conditions: 32 m*M* potassium phthalate, pH 7.0, no CTAB. (□) 4A1N; (■) 5A2N.

the surface of the capillary, this could be an additional way to adjust retention in this system. The contribution of these two retention mechanisms to the separation can be estimated by noting the dependence of migration times on the concentration of the ion-exchange sites on the surface of the column. As noted before, as the CTAB concentration increases the velocity of electroosmotic flow also increases, up to the point of saturation of the column wall with the CTAB molecules. The addition of CTAB would, therefore, shield reversed-phase sites on the column up to the point of saturation. A compound with a stronger reversed-phase component would have a decreased migration time with an increase in CTAB concentration, while a compound with a stronger ion-exchange component would be expected to have an increased migration time as the number of ion-exchange sites increased.

Fig. 4 shows the effect of CTAB concentration on the migration times of 4A1N and 5A2N. The compound with the strongest reversed-phase component, 5A2N, did show a decrease in retention as the CTAB was increased. The 4A1N migration time, however, shows a sharp increase between 50 and 100 μM CTAB, and then slowly increases as the CTAB concentration is increased further. The diminished effect of further addition of CTAB can be explained by the saturation of CTAB on the walls of the column. 4A1N, therefore, has a stronger ion-exchange component, while 5A2N has a stronger reversed-phase component.

In summary, we have shown that manipulation of the surface of a capillary electrophoresis column can add selectivity to separations. The retention of the sample ions can be varied in this system by changing the concentration of the buffer ion, the addition of an organic modifier, and by changing the concentration of the IIR. The mechanism described here is complementary to that of ion pairing [7] in our

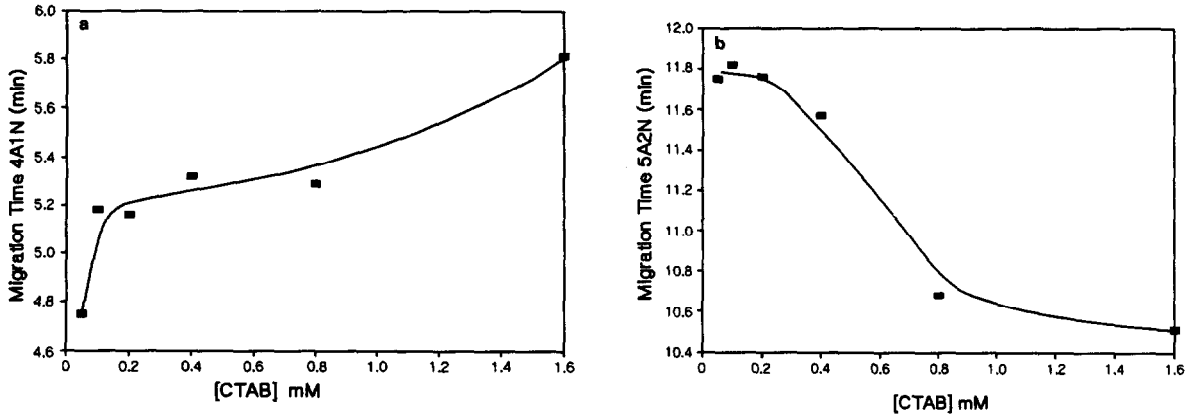

Fig. 4. Dependence of migration times on the CTAB concentration for (a) 4A1N and (b) 5A2N. Buffer conditions: 8 m*M* potassium phthalate, CTAB as noted, pH 7.0.

earlier work for the same analytes. The addition of the dynamic ion-exchange mode to electrochromatography extends it use while adding flexibility. The system can be converted back to the reversed-phase mode by simply washing the column with a less polar mobile phase, which removes the IIR adsorbed to the hydrobic surface.

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