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Capillary electrochromatography with monolithic stationary phases III. Evaluation of the electrochromatographic retention of neutral and charged solutes on cationic stearyl-acrylate monoliths and the separation of water-soluble proteins and membrane proteins ☆

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Abstract

This article, which is closely related to part II, is concerned with the evaluation of the retentive properties of cationic stearyl-acrylate monoliths (i.e. cationic C_{17} monoliths) over a wide range of elution conditions with various uncharged and charged solutes including proteins. The retention parameters for charged solutes including the retention factor k^* observed under capillary electrochromatography conditions and the velocity factor k^*_{ep}, which reflects the electrophoretic process, were measured for weak, moderate and strong basic compounds. These retention parameters allowed the assessment of the respective contributions from electrophoretic and partitioning separation mechanisms. The cationic C_{17} monoliths exhibited sufficient hydrophobic interactions with relatively weak basic solutes. Moderate and strong bases showed migration behaviors dominated by their relatively strong electrophoretic mobility with marginal chromatographic partitioning. At low pH, the cationic C_{17} monoliths allowed the separation of proteins with minimum electrostatic interactions between proteins and the cationic sites on the surface of the stationary phase. The utility of the cationic C_{17} monoliths was demonstrated in the rapid and efficient separation of two crude extracts of membrane proteins, namely galactosyl transferase and cytochrome *c* reductase. Short capillary columns (8.5 cm effective length) of the cationic C_{17} monoliths allowed rapid and efficient separations of neutral and charged pesticides and metabolites, phenylthiohydrantoin amino acids and proteins at the time scale of seconds at relatively high flow velocity.

Keywords: Monolithic columns; Stationary phases, electrochromatography; Stearyl-acrylate monoliths; Electrochromatography; Proteins; Amino acids; Anilines; Pesticides

1. Introduction

Despite the fact that capillary electrochromatography (CEC) is a powerful separation technique that combines differential chromatographic partitioning and differential electromigration for charged solutes, CEC has been predominantly applied to the separation of small neutral molecules (e.g. polyaromatic hydrocarbons, alkylbenzenes, alkyl phenyl ketones, etc.) where electrophoretic mobility is not a factor. For a recent review article on CEC applications, see Ref. [1]. The major difficulty in performing CEC

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separations of charged solutes is that the charged molecules exhibit electrophoretic velocity, which can be sometimes higher in magnitude and opposite in direction to the mobile phase flow velocity, a condition that may prohibit the elution and separation of the analytes. This has been recently addressed by the design of stationary phases of relatively high electroosmotic flow (EOF) velocity including both particulate-based [2,3] and monolithic-based columns [4–6]. These stationary phases, which possess on their surface non-polar ligands to ensure the chromatographic retention and selectivity needed for separation and fixed charges to support the EOF, are referred to as amphiphilic stationary phases. However, the presence of charged functionalities on the stationary phase surface may establish electrostatic interactions, which for large bio-molecules such as proteins could lead to irreversible binding under elution conditions normally used in reversed-phase CEC. Of course, electrostatic interactions can be greatly reduced by using relatively high electrolyte concentration in the mobile phase. Unfortunately, reversed-phase CEC is limited to low electrolyte concentration due to the presence of organic solvent in the mobile phase and also to keeping the Joule heating as low as possible. Irreversible proteins binding to CEC columns occurred when anionic reversed-phase columns containing carboxylic or sulfonic acid moieties were used [5,7]. Usually, anionic reversed-phase sorbents should be more suitable for the analysis of negatively charged species while cationic reversed-phase columns eliminate electrostatic interactions with positively charged species including proteins (specially at pH< isoelectric point, p*I*). Although not numerous, some representative contributions to CEC of charged solutes include small and large nucleic acids [8,9], sialo-glycosphingolipids [10], amino acids [6,11–13], peptides [7,11,12,14–19], and proteins [5,18,19]. While most of these separations were carried out on anionic reversed-phase columns, the CEC of proteins necessitated the exclusive use of cationic reversed-phase sorbents to eliminate electrostatic interactions.

Although significant progress has been already made in the CEC of charged species, there is still a need for a better understanding of the retention mechanisms for charged analytes. Thus far, only a few attempts have been made in this regard to assess the respective contributions from electrophoretic and partitioning separation mechanisms to only a limited number of charged solutes [6,13,20].

This report is concerned with the evaluation of the electrochromatographic retention of some representative charged species on the cationic stearyl acrylate monoliths, i.e. cationic C_{17} monoliths, described in the preceding article. In addition, the utility of the cationic stearyl acrylate is demonstrated in the separation of proteins including membrane proteins and in the ultra-fast separation of various species using short columns at relatively high flow velocity.

2. Theory: migration of charged solutes

The migration of charged solutes in CEC was described in our previous contributions [6,13]. Briefly, the retention factor k^* of a charged solute in CEC is given by [21]:

$$k^* = \frac{t_m(1 + k^*_{ep}) - t_0}{t_0} \quad (1)$$

where t_m is the migration time of the analyte, t_0 is the migration time of the EOF marker in the CEC column, and k^*_{ep} is the velocity factor given by [21]:

$$k^*_{ep} = \frac{u_{ep}}{u_{eo}} \quad (2)$$

where u_{eo} is the velocity of the mobile phase and u_{ep} is the electrophoretic velocity of the analyte. k^* and k^*_{ep} are measured under conditions used in the CEC experiments. The electrophoretic velocity u_{ep} is obtained from separate capillary zone electrophoresis (CZE) measurements (i.e. open tube) using the same mobile phase and other conditions as in the CEC experiment. The mobile phase linear velocity u_{eo}, is the actual ''interstitial'' EOF velocity in the monolithic column, which is equal to the apparent EOF velocity u^*_{eo} within the CEC column multiplied by the ratio of current in open tube to that in the monolithic or packed column [22,23]. For neutral solutes, $k^*_{ep} = 0$, and consequently $k^* = k'$, the retention factor normally encountered in chromatography.

The velocity factor, k^*_{ep}, takes a positive or a

negative value when the electrophoretic and electroosmotic mobilities are co- or counter-directional, respectively. Usually, k^* is a positive number except when solutes are eluted before the neutral marker [13], a situation that occurs when charged solutes undergo little or no interactions with the stationary phase. With reversed-phase stationary phases, this is usually the case when the value of k^*_{ep} is relatively high. A high k^*_{ep} reflects strongly charged solute (high charge-to-mass ratio) and consequently weak interactions with non-polar stationary phases.

Because of the presence of k^*_{ep} in Eq. (1), which reflects the contribution of the electrophoretic process to solute's retention, the k^* does not serve as a useful peak locator as its counterpart k' in chromatography. To facilitate the description of the elution order of charged solutes in CEC, a peak locator, k^*_{cc}, based on chromatographic formalism, has been suggested [21]:

$$k^*_{cc} = \frac{t_m - t_0}{t_0} \quad (3)$$

where t_m and t_0 denote the migration time of the analyte and that of an inert and neutral tracer in the CEC column, respectively. Unlike k^* and k^*_{ep}, k^*_{cc} is devoid of any mechanistic insight, and so has limited utility. However, k^*_{cc} is useful for calculating resolution and increases with the migration time as k' in chromatography [21]. k^*_{cc} is negative for components migrating faster than the EOF tracer.

3. Experimental

3.1. Instrumentation

The instrument used was an HP^{3D}CE system from Hewlett-Packard (Waldbornn, Germany) equipped with a photodiode array detector. Electrochromatograms were recorded with a personal computer running an HP^{3D}CE Chemstation. A pressure of 10 bar was applied to both ends of the capillary during the experimental runs to minimize bubble formation. The temperature was held constant at 25 °C. All samples were injected electrokinetically at various times and applied voltages, which are stated in figure captions.

3.2. Reagents and materials

Pentaerythritol diacrylate monostearate (PEDAS), [2-(acryloyloxy)ethyl]trimethylammonium methyl sulfate (AETA), 2,2′-azobisisobutyronitrile (AIBN), *N*-[3-(trimethoxysilyl)propyl]-*N*′-(4-vinylbenzyl)-ethylenediamine hydrochloride, 2,4-dichlorophenol, 3,4,5-trichlorophenol, Troger's base, anilines, (*R*)-(+)-1,1′binaphthyl-2,2′diamine and analytical grade acetone were purchased from Aldrich (Milwaukee, WI, USA). Cyclohexanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium phosphate monobasic and dibasic and sodium acetate were from Mallinckrodt (Paris, KY, USA). Ethylene glycol, hydrochloric acid, acetic acid, phosphoric acid and HPLC-grade methanol and acetonitrile were from Fisher Scientific (Fair Lawn, NJ, USA). Phenylthiohydantoin (PTH) amino acids, bupivacaine, ephedrine, and chlorotetracycline were purchased from Sigma (St. Louis, MO, USA). Concanavalin A (Con A) from *Canavalia ensiformis*, bovine pancreas α-chymotrypsinogen A, bovine erythrocytes carbonic anhydrase, bovine milk β-lactoglobulin B, turkey egg white lysozyme and horse skeletal muscle myoglobin were purchased from Sigma. Crude extract of porcine heart cytochrome *c* reductase was obtained from Sigma (catalog number C3381) and crude extract of bovine milk galactosyl transferase was purchased from Fluka/BioChemika (Ronkonkoma, NY, USA; catalog number 48279). Pesticides were obtained from ChemService (West Chester, PA, USA). Fused-silica capillaries with an internal diameter of 100 μm and an outer diameter of 360 μm were from Polymicro Technology (Phoenix, AZ, USA).

3.3. Column pretreatment

The inner wall of the fused-silica capillary was treated with 1.0 *M* sodium hydroxide for 30 min, flushed with 0.10 *M* hydrochloric acid for 30 min, and then rinsed with water for 30 min. The capillary inner wall was then allowed to react with a 50% (v/v) solution of *N*-[3-(trimethoxysilyl)propyl]-*N*′-(4-vinylbenzyl)ethylenediamine hydrochloride in methanol for 12 h to vinylize the inner wall of the capillary. Finally, the capillary was rinsed with

methanol and water and dried with a stream of nitrogen.

3.4. In situ polymerization

Polymerization solutions weighing 1.6 g each were prepared from monomers, e.g. PEDAS and AETA monomers, and a porogenic solvent in ratios of monomers–solvent (30:70, w/w). The mixtures of monomers were dissolved in a ternary porogenic solvent consisting of 75.4% (w/w) cyclohexanol, 21% (w/w) ethylene glycol (EG) and at a 3.6% (w/w) water [6]. AIBN (1.0%, w/w, with respect to monomers) was added to the solution as initiator. The solution was then sonicated to obtain a clear solution, and to free the solution from dissolved air. The monolithic columns were prepared from polymerization solutions containing 3.2% (w/w) AETA (column D), 1.6% (w/w) AETA (column F) or 0.6% (w/w) AETA (column G), see part II [23].

A 40-cm section of the pretreated capillary (see Section 3.3) was filled with the polymerization solution up to 30 cm (for a column of 25 cm effective length) or 12 cm (for a column of 8.5 cm effective length) by immersing the inlet of the capillary into the solution vial and applying a negative pressure (i.e. sucking with a syringe) to the outlet. The capillary ends were then sealed in an oxygen flame, and the capillary submerged in a 60 °C water bath for 18 h. The resulting monolithic column was washed with acetonitrile–water (80:20, v/v) mixture using an HPLC pump. A detection window was created at 1–2 mm after the end of the polymer bed using thermal wire stripper. Finally, the column was cut to a total length of 33.5 cm with an effective length of either 8.5 cm or 25 cm.

4. Results and discussion

4.1. Evaluation of the chromatographic retention

Various charged and uncharged species were electrochromatographed on the cationic stearyl acrylate monoliths under investigation (see part II [23] for the characteristics of the monoliths) over a wide range of elution conditions to assess the retentive properties of these novel stationary phases, which are considered as amphiphilic stationary phases with nonpolar moieties as well as fixed charges on their surface. Weak bases such as anilines are useful test solutes as both positively charged species at low pH ($pH < pK_a$) and neutral at high pH (e.g. > pH 5.0). To probe in the reversed-phase properties, we have selected PTH amino acids, which in majority are neutral solutes with varying degree of polarity. Also, we have selected some water-soluble standard proteins as well as two crude extracts of membrane proteins. To further assess the retentive properties of these phases we have selected several charged and neutral pesticides and metabolites and devised ultra-fast separations on short columns of 8.5 cm × 100 μm I.D.

4.1.1. Ionizable species

The retention behavior of three kinds of basic compounds was studied on the monolithic stationary phases under evaluation. Anilines, 7-chlorotetracycline, and binaphthyldiamine were used as models of relatively weak bases, bupivacaine and Troger's base as typical models of moderate bases and ephedrine as a representative of strong basic compounds.

Fig. 1 shows an electrochromatogram of anilines obtained on column G at pH 3.0 at a flow velocity of

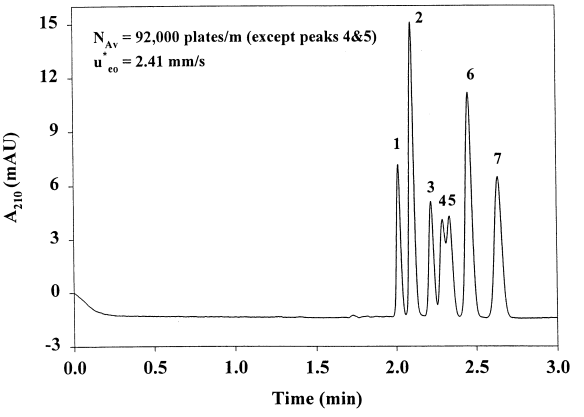

Fig. 1. Electrochromatogram of anilines obtained on column G. Column, 33.5 cm (25 cm effective length) × 100 μm I.D.; mobile phase, 1 m*M* sodium phosphate, pH 3.0, at 80% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s. Solutes: 1, aniline; 2, 3-methylaniline; 3, 4-chloroaniline; 4, 4-isopropylaniline; 5, 4-bromoaniline; 6, 3-chloro-4-methylaniline; 7, 3,4-dichloroaniline.

2.41 mm/s. Under these conditions, seven anilines were separated in ~2.7 min with an average separation efficiency of 92 000 plates/m excluding peaks 4 and 5 which are not well separated. Column G has 0.6% (w/w) of the AETA in the monomers mixture [24]. Table 1 gives the retention parameters obtained at pH 2.5 and 3.0 where anilines are positively charged, and in addition the k' values at pH 7.0 where anilines are neutral. Their electrophoretic mobility were estimated from CZE runs under the same conditions for capillary and electrolyte as in CEC. As can be seen in Table 1, in all cases k^*_{ep} are negative numbers simply because the electrophoretic mobility and electroosmotic mobility are opposite in direction. The absolute value of k^*_{ep} is commensurate with the pK_a of the aniline solutes. The value of k^* is highest for those solutes with the lowest absolute value of k^*_{ep} (Table 1). Increasing the mobile phase ionic strength at pH 2.5 (see Table 1) decreased the absolute value of k^*_{ep} and that resulted in increasing k^* in proportion to the strength of the base (i.e. magnitude of pK_a) such as in the case of 3-methylaniline and aniline. Anilines are uncharged at pH 7.0, and are consequently separated due to differences in their chromatographic partitioning at this pH, see Table 1 for k' values.

Table 2
Values of retention parameters of some basic compounds obtained on column G using a mobile phase consisting of 4.0 m*M* sodium phosphate, pH 2.5, at 40% (v/v) acetonitrile; $u^*_{eo}=2.66$ mm/s

Solutes	k^*_{cc}	k^*_{ep}	k^*
Chlorotetracycline pK_a 3.3[a]	0.48	−0.25	0.12
Bupivacaine pK_a 8.21[b]	0.94	−0.38	0.21
Ephedrine pK_a 10.25[c]	1.59	−0.51	0.28
Troger's base pK_a 3.21[d]	3.06	−0.16	2.40
Binaphthyldiamine	14.00	−0.065	13.03

[a] pK_a values were taken from Ref. [25].
[b] pK_a values were taken from Ref. [28].
[c] pK_a values were taken from Ref. [27].
[d] pK_a values were taken from Ref. [29].

The electrochromatographic behavior of the other amines is shown in Table 2. Bupivacaine, Troger's

Table 1
Values of retention parameters of anilines obtained on column G using four different mobile phases: 4.0 or 8.0 m*M* sodium phosphate, pH 2.5, at 80% (v/v) acetonitrile; 4.0 m*M* sodium phosphate, pH 3.0, at 80% (v/v) acetonitrile; 4.0 m*M* Bis–Tris propane, pH 7.0, at 80% (v/v) acetonitrile

Solutes	pH 2.5 4.0 m*M* $u^*_{eo}=2.37$ mm/s			8.0 m*M* $u^*_{eo}=2.00$ mm/s			pH 3.0 4.0 m*M* $u^*_{eo}=1.74$ mm/s			pH 7.0 4.0 m*M* $u^*_{eo}=0.78$ mm/s
	k^*_{cc}	k^*_{ep}	k^*	k^*_{cc}	k^*_{ep}	k^*	k^*_{cc}	k^*_{ep}	k^*	k'
Aniline pK_a 4.66[a]	0.55	−0.13	0.35	0.70	−0.10	0.53	0.22	−0.13	0.070	0.18
3-Methylamine pK_a 4.91[a]	0.70	−0.22	0.32	0.89	−0.17	0.57	0.27	−0.10	0.15	0.22
4-Isopropylaniline pK_a 4.85[b]	1.12	−0.12	0.87	1.52	−0.28	0.81	0.43	−0.18	0.17	0.32
4-Chloroaniline pK_a 4.06[a]	0.55	−0.066	0.45	0.63	−0.016	0.60	0.31	−0.040	0.26	0.35
4-Bromoaniline pK_a 3.58[a]	0.61	−0.012	0.59	0.70	−0.012	0.68	0.35	−0.075	0.25	0.37
3,4-Dichloroaniline pK_a 3.33[a]	0.79	−0.017	0.76	0.89	−0.010	0.87	0.56	−0.082	0.43	0.45
3-Chloro-4-methylamine pK_a 4.05[c]	0.70	−0.045	0.62	0.80	−0.017	0.77	0.44	−0.065	0.34	0.53

[a] pK_a values are taken from Ref. [26].
[b] pK_a values are taken from Ref. [27].
[c] pK_a values are taken from Ref. [25].

base, chlortetracycline, binaphthyldiamine and ephedrine were electrochromatographed on column G, using 4.0 m*M* sodium phosphate pH 2.5 at 60% (v/v) acetonitrile as the mobile phase. In all cases, since the electrophoretic and electroosmotic mobilities are counter-directional, the values of k^*_{ep} are negatives (same as for anilines). Binaphthyldiamine is the most hydrophobic solute among this group of amino compounds as manifested by its smallest absolute k^*_{ep} value (i.e. weak base) and its highest k^* value. Troger's base, which is the second hydrophobic compound, yielded a small k^*_{ep} (in absolute value) and a high k^*. On the other hand, ephedrine, which is the strongest base, yielded the highest absolute k^*_{ep} value while bupivacaine, which is the next in base strength's yielded the second highest k^*_{ep} (in absolute value). Chlorotetracycline, which yielded a relatively low $|k^*_{ep}|$ (weak base) had the smallest k^* thus indicating its strong hydrophilic character.

In summary, the above retention data have provided a quantitative evaluation of the retention of positively charged solutes on the amphiphilic stationary phases with fixed positive charges and C_{17} ligands. Very recently, we have provided a quantitative evaluation of the retention of (i) negatively charged solutes (dansyl amino acids) in one previous contribution involving similar amphiphilic stationary phases with fixed negative charges and C_{17} ligands [6] and (ii) of both positively charged solutes (anilines) and negatively charged species (dinitrophenyl amino acids) in another contribution involving a C_{18}-silica based monoliths [13].

4.1.2. Examples of separations

4.1.2.1. PTH amino acids

Fig. 2 shows the separation of 10 PTH amino acids obtained on column G using a mobile phase consisting of 30 m*M* sodium phosphate buffer, pH 2.5, at 30% (v/v) acetonitrile. The 10 PTH amino acids were separated in ~4.5 min at a flow velocity of 3.45 mm/s with an average separation efficiency of 54 800 plates/m. PTH amino acids are neutral derivatives except those with unprotected side chains. Among the 10 analytes used in this study, the only PTH amino acids that has an unprotected side chain is that of aspartic acid, which at the pH of the experiment (pH 2.5) may be slightly dissociated, a fact that explains its early elution in Fig. 1. The remaining PTH amino acids are neutral and eluted according to the decreasing polarity of the molecule.

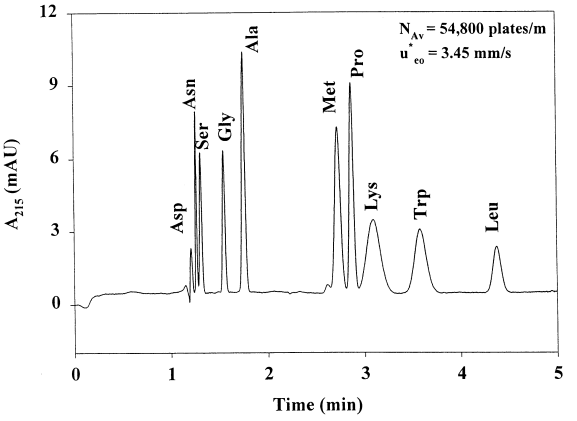

Fig. 2. Electrochromatogram of 10 PTH amino acids obtained on column G. Column, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, 30 m*M* sodium phosphate, pH 2.5, at 30% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s.

4.1.2.2. Water-soluble proteins

Fig. 3 shows the separation of 6 proteins obtained on column D in less than 5 min at a flow velocity of

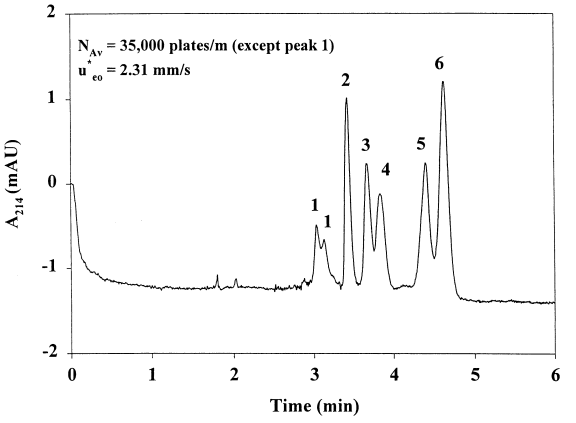

Fig. 3. Electrochromatogram of protein mixture obtained on column D. Column, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 2.5, at 45% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s. Solutes: 1, concanavalin A; 2, α-chymotrypsinogen A; 3, carbonic anhydrase; 4, β-lactoglobulin B; 5, myoglobin; 6, lysozyme.

2.31 mm/s, using a mobile phase consisting of 10 m*M* sodium phosphate, pH 2.5, at 45% (v/v) acetonitrile. The average separation efficiency (excluding peak 1) was 35 000 plates/m. The migration times of the proteins under investigation were examined at different % of acetonitrile in the mobile phase, which consisted of 10 m*M* sodium phosphate buffer, pH 2.5 (Fig. 4). As can be seen in Fig. 4, the protein migration time increased as the % acetonitrile in the mobile phase was increased in the range studied. This may be due to the varying effects of organic solvent on hydrophobic interaction of proteins with the stationary phase, the electrophoretic mobility of the proteins and the electroosmotic mobility of the mobile phase. In other words, increasing the organic content of the mobile phase decreases (i) the nonpolar adsorption of the protein to the stationary phase, (ii) the protein electrophoretic mobility due to decreasing the dielectric constant of the mobile phase and (iii) the electroosmotic mobility of the mobile phase due the same reason as in (ii). The net result of these effects is an overall increase in protein migration time. The ionic strength of mobile phase also affected the migration time of the protein polyelectrolytes. Varying the ionic strength of the mobile phase from 5 to 20 m*M* sodium phosphate (see Fig. 5) resulted in modulating solute migration time with an overall decrease in protein retention at sodium phosphate concentration in the range 10 to 20 m*M*. This may indicate the attenuation of some electrostatic interactions between the weakly dissociated carboxylic groups of proteins and the fixed positive charges of the cationic monolith.

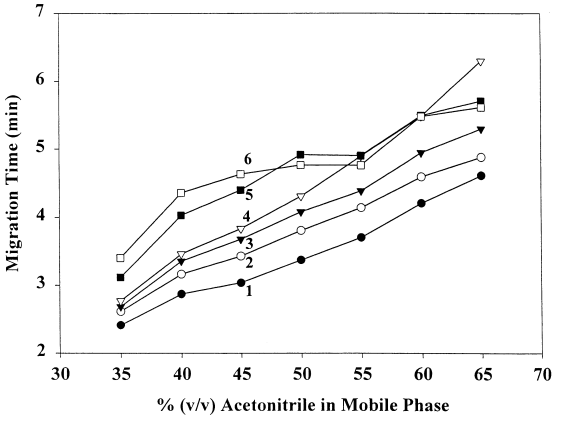

Fig. 4. Plots of the migration time of six proteins as a function of percentage acetonitrile in the mobile phase. Monolithic column D, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 2.5, at various % (v/v) of acetonitrile; running voltage, −25 kV. Solutes: 1, concanavalin A; 2, α-chymotrypsinogen; 3, carbonic anhydrase; 4, β-lactoglobulin B; 5, myoglobin; 6, lysozyme.

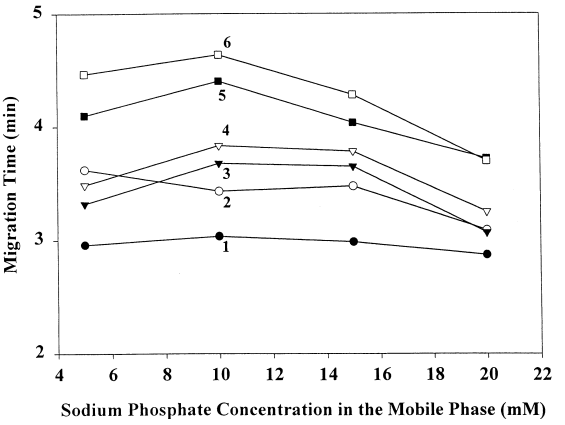

Fig. 5. Plots of the migration time of 6 proteins as a function of ionic strength of the mobile phase. Monolithic column D, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, sodium phosphate, pH 2.5, at 45% (v/v) of acetonitrile; running voltage, −25 kV. Solutes: 1, concanavalin A; 2, α-chymotrypsinogen A; 3, carbonic anhydrase; 4, β-lactoglobulin B; 5, myoglobin; 6, lysozyme.

4.1.2.3. Membrane proteins

To demonstrate the usefulness of the monolithic columns under investigation in the separation of difficult to separate proteins, two crude extracts of membrane proteins, namely galactosyl transferase (M_r = 86 000) and cytochrome *c* reductase (M_r = 80 000) were electrochromatographed with a mobile phase of 10 m*M* sodium phosphate, pH 2.5, at 45% (v/v) acetonitrile. As shown in Figs 6 and 7, the samples were resolved into their components within relatively short analysis time. The asterisks indicate the peaks derived from the sample components. The arrows signal the peaks on which average separation efficiency was estimated (Fig. 7). The observed separation efficiencies for galactosyl transferase and cytochrome *c* reductase samples were 53 500 and 114 800 plates/m, respectively, at a flow velocity of ~2.6 mm/s.

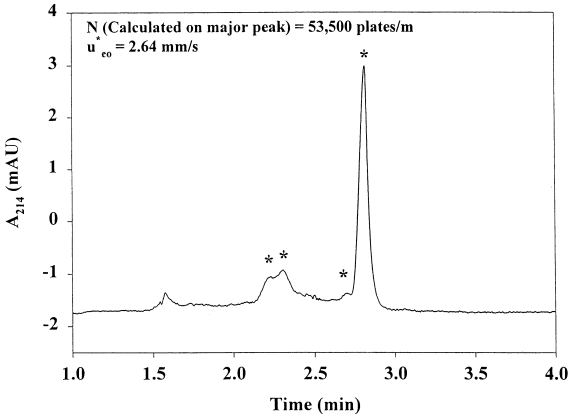

Fig. 6. Electrochromatogram of a crude extract of galactosyl transferase obtained on column F. Column, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 2.5, at 45% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s.

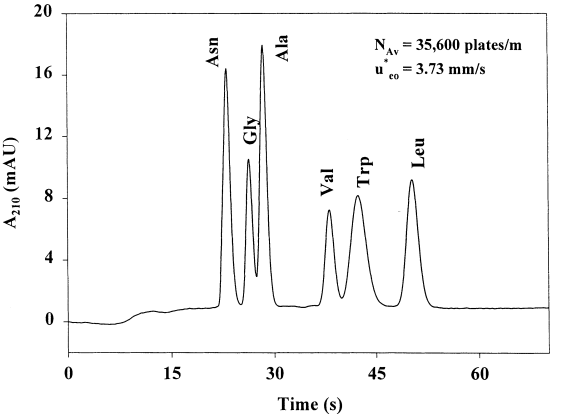

Fig. 8. Ultra fast separation of six PTH amino acids obtained on monolithic column G. Column, 33.5 cm (8.5 cm effective length)×100 μm I.D.; mobile phase, 30 m*M* sodium phosphate, pH 2.5 at 30% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s.

4.2. Ultra-fast separation of neutral and ionizable species

Using a short capillary column of 8.5 cm×100 μm I.D. of type G, six PTH amino acids (Asn, Gly, Ala, Val, Trp and Leu) were separated in almost 50 s at a flow velocity of 3.73 mm/s, using a mobile phase of 30 m*M* sodium phosphate buffer, pH 2.5, at 30% (v/v) acetonitrile as shown in Fig. 8. The average separation efficiency was 35 600 plates/m which corresponds to ~3030 plates per column and about 60 plates/s for the separation of nearly 50 s. Also, three proteins, namely concanavalin A (Con A), β-lactoglobulin B and lysozyme, were separated in about 55 s as shown in Fig. 9 at a flow velocity of

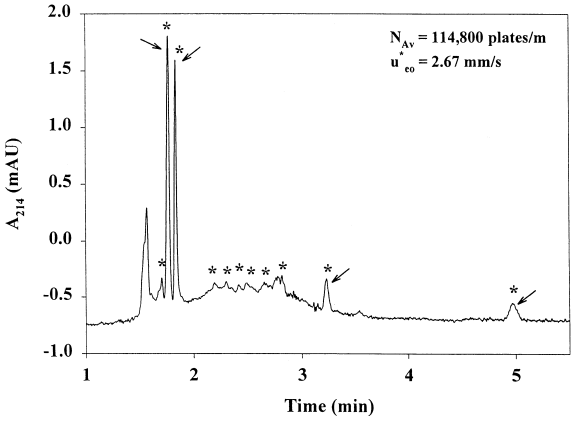

Fig. 7. Electrochromatogram of a crude extract of cytochrome *c* reductase obtained on column F. Column, 33.5 cm (25 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 2.5, at 45% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s.

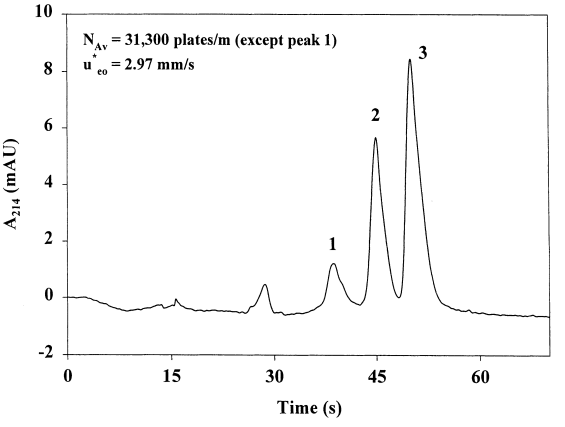

Fig. 9. Ultra fast separation of three proteins obtained on monolithic column G. Column, 33.5 cm (8.5 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 2.5 at 45% (v/v) acetonitrile; running voltage, −25 kV; electrokinetic injection, −10 kV for 2 s. Solutes: 1, concanavalin A; 2, β-lactoglobulin B; 3, lysozyme.

2.97 mm/s. The separation efficiency is about 2700 plates per column which comes to ~50 plates/s

Fourteen pesticides and metabolites were separated in less than 2 min at a flow velocity of 3.2 mm/s on column G (see Fig. 10) using a mobile phase consisting of 10 m*M* sodium phosphate, pH 3.0, at 55% (v/v) acetonitrile. These 14 solutes comprised three carbamate insecticides (oxamyl, aldicarb and carbaryl), three phenylurea herbicides (monuron, diuron, and neburon), three triazines (propazine, prometon and prometryne of pK_a of 1.7, 4.2 and 4.1, respectively [25]), three phenoxy acid herbicides esters (2,4-D isopropyl ester, 2,4-D butyl ester, 2,4,5-T isopropyl ester) two chlorophenols (2,4-dichlorophenol and 3,4,5-trichlorophenol of pK_a of 7.85 and 7.84, respectively [26]). It should be noted that 2,4-dichlorophenol is the metabolite of 2,4-D butyl ester or 2,4-D isopropyl ester. At the pH of the experiment (pH 3.0), with the exception of the two positively charged triazines, namely prometon and prometryne, the other 12 solutes are neutral and eluted in the order of increasing hydrophobicity. Despite the relatively high flow velocity (3.18 mm/s) and short separation path, the separation efficiency is ~53 200 plates/m (excluding peaks 13 and 14) or about 4500 plates per column (8.5 cm) which is about 56 plates/s for a separation of ~80 s for the first 12 peaks, see Fig. 10.

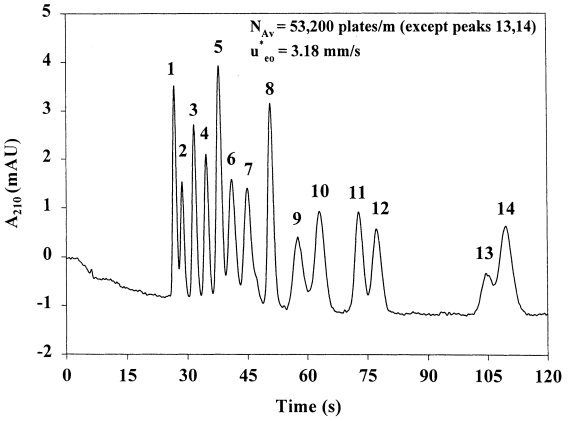

Fig. 10. Ultra-fast separation of 14 pesticides and metabolites obtained on monolithic column H. Column, 33.5 cm (8.5 cm effective length)×100 μm I.D.; mobile phase, 10 m*M* sodium phosphate, pH 3.0 at 55% (v/v) acetonitrile; running voltage, −30 kV; electrokinetic injection, −10 kV for 2 s. Solutes: 1, oxamyl; 2, aldicarb; 3, monuron; 4, carbaryl; 5, diuron; 6, prometon; 7, propazine; 8, 2,4-dichlorophenol; 9, prometryne; 10, neburon; 11, 2,4-D isopropyl ester; 12, 3,4,5-trichlorophenol; 13, 2,4-D butyl ester; 14, 2,4,5-T isopropyl ester.

In summary, 50–60 plates/s can be obtained on short columns at high flow velocity for separations on the time scales of seconds (e.g. 50–80 s) for small solutes as well as for large protein molecules. The achievement of rapid separation is very important for monitoring rapid processes and for multidimensional separation schemes where the second dimension must be very rapid in order to process the individual fractions as they are produced by the first dimensions.

5. Conclusions

We have shown that cationic stearyl acrylate monolithic capillaries are useful in the separation of small, neutral and charged solutes as well as proteins including membrane proteins. Also, we have provided quantitative and qualitative evaluation of the retention of neutral and charged solutes on the cationic stearyl-acrylate monoliths. The amphiphilic stationary phases exhibited retention toward the various charged solutes dominated primarily by hydrophobic interactions and to a lesser extent by electrostatic attraction/repulsion, and the migration of these solutes was also affected by their electrophoretic mobilities. Short monolithic columns allowed rapid separations of multicomponent mixtures at the time scale of seconds with high plate counts due to the favorable mass transfer at high flow velocity.

6. Nomenclature

AETA	[2-(acryloyloxy)ethyl]trimethyl ammonium methyl sulfate
AIBN	2,2′-azobisisobutyronitrile
Con A	concanavalin A
PEDAS	pentaerythritol diacrylate monostearate

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