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Journal of Chromatography A, 924 (2001) 211–221

JOURNAL OF CHROMATOGRAPHY A

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Electrochromatographic characterization of etched chemically-modified capillaries with small synthetic peptides

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Abstract

In this investigation, various capillary electrochromatographic (CEC) experiments have been employed to characterize the properties of etched, chemically-modified surfaces of open tubular capillary columns with peptides as solute probes and under conditions of variable voltage, temperature and solvent composition. The separation performance of etched capillaries with either *n*-octadecyl or liquid crystal moieties derived from a cholesterol phase bonded to the surface were compared. With the liquid crystal bonded species, interesting and significantly different variations in retention behavior of peptides are obtained compared to those observed with the corresponding *n*-octadecyl modified surfaces by changes in temperature, solvent composition and field strength. These peptide separations illustrate the usefulness of this CEC approach for practical applications, where both the retention characteristics of the charged analytes as well as the selectivity differences due to the surface properties of the etched chemically-modified surfaces of open tubular capillary columns can be rationally modulated. As in HPLC, appropriate choice of CEC experimental variables, including the chemical properties of the immobilized ligand(s), represents a powerful tool for optimizing resolution.

Keywords: Electrochromatography; Capillary columns; Peptides

1. Introduction

A promising approach for solving various problems associated with frit design, difficulties in packing capillaries with microparticulate chemically-modified sorbents with mean particle diameters of ≤ 3 μm, bubble formation and the delayed elution of basic compounds encountered with packed capillaries is open tubular capillary electrochromatography (OTCEC). OTCEC has been developed in a number of different formats over the last 20 years. An early approach involved the use of 30 μm soda-lime capillaries that were treated with NaOH followed by attachment of an *n*-octadecylsilane coating [1]. These capillaries were shown to be useful for the separation of hydrocarbon samples. Smaller diameter (10–25 μm) capillaries with the same coating have successfully been used for the separation of polycyclic aromatic hydrocarbons (PAHs) [2]. A major problem with the open tubular format is the low phase ratio in these columns and this can lead to limited retention of solutes. Polymer coatings have been proposed as one means of increasing the phase ratio in OTCEC in order to promote solute–bonded phase interactions. Thus, a 25 μm I.D. capillary with a polymethacrylate coating was used for the separation of benzoates with efficiencies greater than 250 000 plates/m [3]. Similarly, an open tubular capillary containing a co-polymer of *N-tert.*-

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00830-5

butylacrylamide and 2-acylamido-2-methyl-1-propanesulfonic acid (AMPS) was able to separate various mixtures of PAHs, parabens and ketones [4]. In this case, AMPS was included in the polymeric coating in order to create a stable electroosmotic flow since AMPS has a negative charge typical of CEC operating conditions. Sol–gel technology offers another avenue to increase the amount of stationary phase in OTCEC, whereby silane reagents with *n*-octadecyl moieties can be used to form a thin film on the inner wall of the capillary. Mixtures of non-polar compounds have been separated with this type of CEC column [5]. The open tubular approach described in this paper utilizes a technology developed previously [6] that involves first etching the inner wall of the fused capillary followed by chemical modification of the new surface via silanization/hydrosilation reactions [7]. This type of etched, chemically-modified capillaries has been found useful for the separation of peptides, proteins and small basic compounds including pharmaceuticals [7–13].

Liquid crystal stationary phases in high-performance liquid chromatography (HPLC) are rapidly gaining interest. An early study of these materials involved a comparison of two terphenyl liquid crystal stationary phases to a standard *n*-octadecyl modified material for the retention of 16 PAHs as well as the separation of C_{60} and C_{70} fullerenes [14]. It was demonstrated that one of the liquid crystal phases behaved in a similar manner to a standard *n*-octadecyl phase for the separation of the fullerenes and had many of the same retention characteristics for the PAHs. However, the other liquid crystal phase displayed both higher retention and greater resolving power (larger α values) than the *n*-octadecyl phase for the two fullerene compounds. A fundamental difference in the retention mechanism between the fullerenes and the PAHs on this liquid crystal phase was demonstrated by variable temperature studies since the enthalpy of transfer (ΔH_{trans}) of these solutes was positive for both C_{60} and C_{70} fullerenes, whilst ΔH_{trans} was negative for the aromatic compounds. It was also shown that these particular terphenyl liquid crystal phases had very good molecular shape recognition capabilities based on the retention of various PAHs. In another study using a cholesteryl-liquid crystal stationary phase [15], it was determined that this material also possessed very high selectivity for more planar molecules. Both the terphenyl phase and the cholesterol phase have α values that are twice as large as the *n*-octadecyl phase when comparing the retention of the same pair of planar and non-planar molecules of the same size. Moreover, a series of benzodiazepines separated on the cholesterol phase had a different elution order from that obtained on an *n*-octadecyl modified phase [16]. This difference in retention properties was attributed to the molecular shape discrimination ability of the cholesterol phase. Several other studies have described work on the synthesis, characterization and applications of cholesterol phases [17–25]. The uniqueness of the bonded liquid crystal has been demonstrated in solid state magnetic resonance studies [20] involving a comparison of the *n*-octadecyl modified stationary phase with two types of bonded liquid crystals. The relaxation time (T_2) for the main peak in the spectrum representing most of the methylene groups in C_{18} decreases regularly with a decrease in temperature indicating an overall restriction in molecular motion. However, some carbon signals on the liquid crystal molecules have a nonuniform change, in some cases an increase, in T_2 with decreasing temperature. A greater degree of association or ordering of the liquid crystal materials in comparison to the C_{18} moiety can explain the difference in T_2 behavior between the phases. This process of association of bonded groups as the temperature is lowered may be similar to a phase transition of the pure material but is not a true change of state since the molecule is immobilized onto the support material at one end. A recent study has shown that liquid crystals used in commercial display devices can be bonded to silica hydride surfaces via hydrosilation of a cyano group [13,26]. This study attempts to contrast and characterize further the separation capabilities with peptides of etched open tubular columns made with cholesterol and *n*-octadecyl modified surfaces in order to exploit some of the advantages identified in the earlier study of liquid crystal CEC separation media [13].

2. Materials and methods

2.1. OTCEC columns

The C_{18} and cholesterol OTCEC columns used in

this study were produced by etching and chemical modification of Agilent Technologies (Waldbronn, Germany) fused-silica capillaries, I.D.=20 μm, with a ''bubble cell'' at the detection window. The process for etching and the synthetic scheme used to produce etched and hydride modified capillaries were based on previously described procedures [6]. The attachment of the *n*-octadecyl [6,27] and the cholesterol [13] moieties also followed established protocols. Each capillary was conditioned overnight with methanol, then washed with Milli-Q water (using a syringe) and finally several volumes of the running buffer (degassed with helium for 30 min) were passed through the capillary. The buffers used in the mobile phases were as follows: buffer A, pH 2.14, 60 m*M* phosphoric acid and 38 m*M* Tris; buffer B, pH 7.3, 50 m*M* Tris and 125 m*M* boric acid. The organic solvent modifiers used in the buffers, methanol, acetonitrile and trifluoroethanol (TFE) were obtained from Merck (Kilsyth, Australia) and were of the highest purity available.

2.2. Peptide samples

The trombin receptor antagonistic peptides (TRAP) used in this study were prepared according to the general principles of solid-phase peptide synthesis based on 9-fluorenylmethylcarbonyl (FMOC) chemistry [28–30]. The exact details of the synthetic procedures for these peptides will be published elsewhere [31]. The generic methods employed for the purification of these synthetic peptides have been described previously [27,32].

2.3. Instrumentation

Experiments for the electrochromatographic characterization of the etched chemically-modified surfaces of open tubular capillary columns with peptides were carried out using a Beckman P/ACE 5510 Series capillary electrophoresis instrument (Beckman, Fullerton, CA, USA) equipped with a UV detector and temperature control. The system was interfaced with a Pentium computer utilizing the System Gold software (version 8.1) for instrument control and data collection.

3. Results and discussion

OTCEC has demonstrated capabilities that are distinctly different from other micro-separation techniques such as μ-HPLC, high-performance capillary electrophoresis (HPCE) and packed column capillary electrochromatography. In order to exploit the potential of this technique in a broad range of separation problems, characterization of the performance and life-time properties of etched chemically-modified capillaries is essential and has proven to be a particularly effective strategy for the development of different OTCEC approaches. Spectroscopic characterization by diffuse reflectance infrared Fourier transform (DRIFT) [12,13], scanning electron microscopy [6] and atomic force microscopy [33] have provided physical as well as topographic information about the inner surfaces of these CEC columns. In this study, the influences of experimental variables such as the field strength, solvent composition and temperatures have been examined with peptide samples to further evaluate the performance of two different types of etched chemically-modified OTCEC capillaries.

3.1. Influence of variable field strength on peptide separation

According to theory, when no surface interactions occur the migration behavior of a typical, charged solute in a capillary electrophoretic or electrochromatographic experiment should be linear with an increase in the electric field strength. However, the nature of the etched chemically modified inner wall of the capillary, particularly when an organic modifier is present in the mobile phase, may produce non-ideal surface effects that lead to a deviation from the expected linear change in migration time with applied electric field strength. In order to understand the effect of etching and subsequent chemical modification of the inner wall of a separation capillary, the migration behavior of two synthetic peptides as a function of field strength was studied. Fig. 1 shows the results for the field strength studies obtained for two etched chemically modified capillaries (cholesterol and C_{18}) at pH 2.14 with the TRAP-related peptide, TRAP-6. The amino acid sequence of the TRAP-related peptides used in this investigation are detailed in Table 1. It can be seen that for TRAP-6

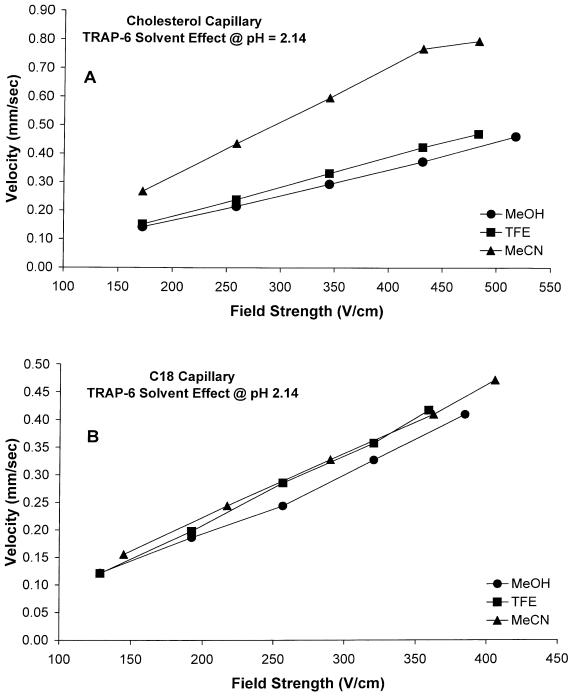

Fig. 1. Plot of the migration velocity of the TRAP-6 peptide as a function of the applied field strength with various organic modifiers (20%) as part of the mobile phase in OTCEC at pH 2.14. (A) Etched cholesterol modified capillary and (B) etched *n*-octadecyl modified capillary.

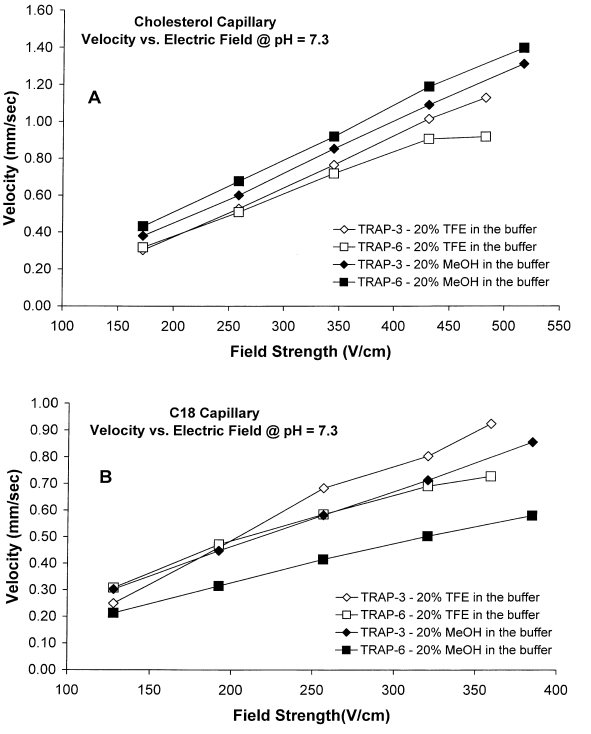

Fig. 2. Plot of the migration velocity of the TRAP-3 and TRAP-6 peptides as a function of the applied field strength with methanol or TFE as part of the mobile phase in OTCEC at pH 7.3. (A) Etched cholesterol modified capillary and (B) etched *n*-octadecyl modified capillary.

the migration velocity was nearly linear as the applied field was varied on both columns. Some minor deviations occurred in the migration velocity of this peptide on the cholesterol column with acetonitrile as the organic modifier and with TFE and methanol on the C_{18} column. A similar comparison is made in Fig. 2 where the migration velocities of two synthetic peptides (TRAP-3 and TRAP-6) are plotted as a function of the applied field strength for the two etched chemically modified capillaries at pH 7.3. With the cholesterol column (Fig. 2A) both peptides exhibit linear migration behavior with methanol as the organic modifier. However, in the presence of 20% (v/v) TFE the migration behavior of TRAP-6 displays a deviation from linearity at higher applied field strengths. On the C_{18} capillary (Fig. 2B) the migration velocity of both peptides as a function of field strength was linear within experimental error with the 20% (v/v) methanol–buffer

Table 1
Peptide code, amino acid sequence and predicted relative hydrophobicity [31,35] of the TRAP peptide analogues

Peptide code	Amino acid sequence	Relative hydrophobicity
TRAP-1	Ser Phe Leu Leu Arg Asn Pro	20.86
TRAP-2	Ser Ala Leu Leu Arg Asn Pro	14.24
TRAP-3	Ser Phe Ala Leu Arg Asn Pro	16.91
TRAP-4	Ser Phe Leu Ala Arg Asn Pro	16.91
TRAP-5	Ser Phe Leu Leu Ala Asn Pro	22.22
TRAP-6	Ser Phe Leu Leu Arg Ala Pro	24.75

mobile phase. When the organic component was changed to 20% (v/v) TFE, then both peptides, particularly TRAP-3, exhibited distinctly non-linear behavior in terms of peptide migration velocity as a function of the field strength. Moreover, the elution order of the two peptides was reversed when changing from low to high applied field strength.

Several conclusions can be drawn from these observations. Since the columns were all made from the same fused-silica capillary tubing etched under the same conditions, then the surface properties of the inner wall of the etched capillaries should, on average, be identical. As the same protocols were employed for the immobilization of the *n*-octadecyl and cholesteryl-ligands, similar ligand densities and coverage were anticipated, consistent with the previous DRIFT [12,13], scanning electron microscopy [6] and atomic force microscopy [33] measurements. Therefore, in general terms, the migration behavior of a particular peptide as a function of applied field should qualitatively if not quantitatively be identical for both cholesterol and C_{18} columns when the same mobile phase is used if it is assumed that the electrophoretic component dominates the CEC migration rather than the surface interaction component and the charge state of the peptide is not significantly affected by the molecular structure and concentration of the solvent [34]. Such a result was observed for several of the peptides, i.e., for TRAP-3 and TRAP-6 in 20% (v/v) methanol at pH 7.3. However, in other cases such as TRAP-6 at pH 2.14 with all organic modifiers or with TRAP-3 in 20% (v/v) TFE at pH 7.3 there were differences in migration behavior of the peptide when the experimental data obtained with the two modified surfaces were compared. These results indicate that the organic ligand bonded to the etched surface was selectively interacting with these peptides, resulting in chromatographic retention of the solutes. One explanation for the non-linear plots in Figs. 1 and 2 is that variations in the applied field may result in changes in the structure (orientation) of the bonded organic ligand on the etched surface. Secondly, as the electrophoretic velocity of a peptide is a function of the dielectric constant of the solvent [34], variations in this parameter will also impact on the overall retention behavior. Structural changes in the orientational arrangement of the bonded phase would result in differences in the extent and mode of interaction with peptide solutes. The combination of these two effects would lead to non-linear changes in migration as the applied voltage varies. The results cannot be attributed to structural (conformational) changes in the peptides themselves, since the same peptide has linear behavior on one column but has a non-linear behavior on the other column under the same mobile phase conditions. Therefore, the use of the applied field can be a useful variable in controlling both retention and resolution of peptides in OTCEC, whilst the shape of the plot of migration velocity versus applied field strength represents a useful diagnostic tool to aid the characterization of the interplay of the electrophoretic and chromatographic components of the retention behavior of different types of OTCEC capillaries.

3.2. Solvent composition studies

The results discussed above suggest that the nature and concentration of an organic modifier in the mobile phase has a significant effect on the retention characteristics of peptides with etched chemically modified capillaries in OTCEC. The variation in solute migration based on the organic solvent composition of the mobile phase is a complex phenomenon in capillary electrochromatography. Recent investigations with packed CEC systems have provided a basis to interpret the influence of organic solvent content in the CEC of peptides [34]. This complex interplay is due to the hybrid nature of CEC, which encompasses aspects of both capillary electrophoresis as well as liquid chromatography. Three factors control the extent of migration of charged solutes, not necessarily in unison, as the organic composition of the mobile phase changes. These variables are: (1) the electroosmotic flow in the capillary; (2) the electrophoretic mobility of the solute; and (3) the chromatographic retention based on the magnitude of the solute–bonded phase interactions, i.e., on the magnitude of the respective k' values. In order to assess the contribution of these three different processes to the overall electromigration of the TRAP-related peptides, several of these charged peptide solutes were used to characterize the etched, chemically modified open tubular columns with variable mobile phase compositions.

Fig. 3 shows the variation in the migration veloci-

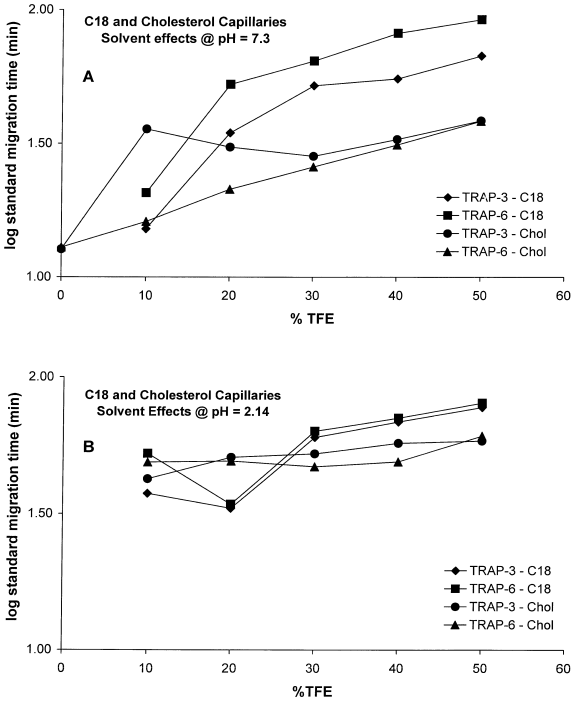

Fig. 3. Plot of the log of the migration time TRAP-3 and TRAP-6 peptides on etched C_{18} modified and etched cholesterol modified capillaries as a function of the percentage of TFE in the mobile phase. (A) Buffer, pH 7.3 and (B) buffer, pH 2.14.

ties of two synthetic peptides on both columns as a function of the amount of TFE in the buffer. A significant difference in the behavior of the two columns was observed at pH 7.3 (Fig. 3A). Both peptides exhibit similar migration characteristics on the etched C_{18} modified capillary. Thus, with the C_{18} modified capillary there was a large increase in the logarithm of the migration time (log t_M) when the organic solvent content was changed from 10 to 20% (v/v) TFE in the buffer, followed by a smaller linear increase of approximately the same slope for both peptides up to 50% (v/v) organic modifier. These results indicate that a lower EOF has occurred with the C_{18} modified capillary as the solvent content was increased counterbalanced by a more significant electrophoretic mobility as a result of the presence of the organic component in the buffer. Under conditions of high organic solvent content, it is likely that the electrophoretic effect dominates the overall migration since this type of C_{18} etched chemically-modified capillary has been shown to have very low electroosmotic flow [12,13]. The decrease in the slope of the plot of migration velocity versus organic solvent content above 20% (v/v) TFE may be due to the consequences of a reduced chromatographic effect where decreased retention is expected as the amount of organic modifier in the mobile phase is increased. The behavior of the two peptides on the etched chemically-modified cholesterol capillary is more complex. TRAP-6 essentially displays a linear increase in log t_M as the percentage of TFE was increased while TRAP-3 shows an initial increase between 0 and 10% (v/v), then a decrease up to 30% (v/v) TFE and finally a progressive near-linear increase between 30 and 50% (v/v) organic modifier. The non-uniform behavior exhibited by the TRAP-3 peptide on the cholesterol column may be due to a physical change of the bonded moiety (degree of association, chain orientation, etc.) on the etched surface as the concentration of the organic solvent modifier in the buffer was varied. This change would make interaction between the cholesterol moiety and TRAP-3 more favorable (higher k' value) leading to an increase in migration time. The decrease in migration velocity of TRAP-3 between 10 and 30% (v/v) TFE can be explained by the expected reduced retention at higher organic composition in the mobile phase. Beyond 30% (v/v) TFE, all the peptides behave in a similar manner. At low pH values, i.e., pH 2.14, the behavior of TRAP-3 and TRAP-6 is somewhat different (Fig. 3B). Analogous to the behavior observed in Fig. 3A, both peptides show a small and approximately linear increase in t_M above 30% (v/v) TFE on the C_{18} column. At lower organic solvent percentages, there is first a decrease in migration time to a minimum at 20% (v/v) TFE, then a sharp increase in t_M before the linear behavior is observed. This behavior could again reflect a change in the surface morphology of the C_{18} bonded moiety where k' interactions are more important at low percentages of organic modifier in the buffer. The TRAP-6 peptide on the cholesterol column has somewhat similar behavior as on the *n*-octadecyl capillary but with smaller changes in t_M. In addition, the minimum point is shifted to between 30 and 40% (v/v) TFE. TRAP-3 on the cholesterol capillary displays a small and non-linear increase in migration over the solvent composition range studied.

The data described above suggest that manipulation of solvent composition represents a useful variable for controlling peptide separations in OTCEC. Analogous observations have been made for the separation of peptides with packed CEC systems [34]. In fact, with etched chemically modified capillaries, changes in the bonded phase morphology might occur in the presence of different organic solvent modifiers, and this effect in combination with changes in dielectric constant of the mobile phase, could provide an additional means of optimizing a particular separation. Some examples from this investigation can be used to illustrate this point with TRAP peptide samples that have been shown to contain several additional components [27] arising from the solid-phase peptide synthesis. The electrochromatrogram of the TRAP-3 peptide on the cholesterol capillary at pH 2.14 at two different percentages of TFE is shown in Fig. 4. It can be clearly seen that resolution of five minor components before the main peak is obtained at 10% (v/v) TFE (Fig. 4A) while only three components are easily seen at 50% (v/v) TFE (Fig. 4B). A similar effect can be seen when the same peptide is run at the same pH on the C_{18} column using methanol as the organic modifier (Fig. 5). At 20% (v/v) methanol (Fig. 5A), five well-resolved minor components are observed before the main peak in the electrochromatogram of TRAP-3 while at 40% (v/v) organic modifier in the buffer (Fig. 5B) only four peaks are readily identified. With 40% (v/v) methanol peaks 2 and 3 are barely resolved. Comparing Figs. 4A and 5A shows another example of the influence of the organic modifier and the type of surface chemistry on the capillary. The minor component that elutes after the main peak is sharp and migrates only slightly slower than the major peak on the C_{18} capillary with methanol as the buffer modifier while on the cholesterol column with TFE as the organic component of the mobile phase it appears considerably later in the electrochromatogram and is noticeably broader.

3.3. Variable temperature studies

Temperature is another variable that can be used to control the retention of and improve the resolution

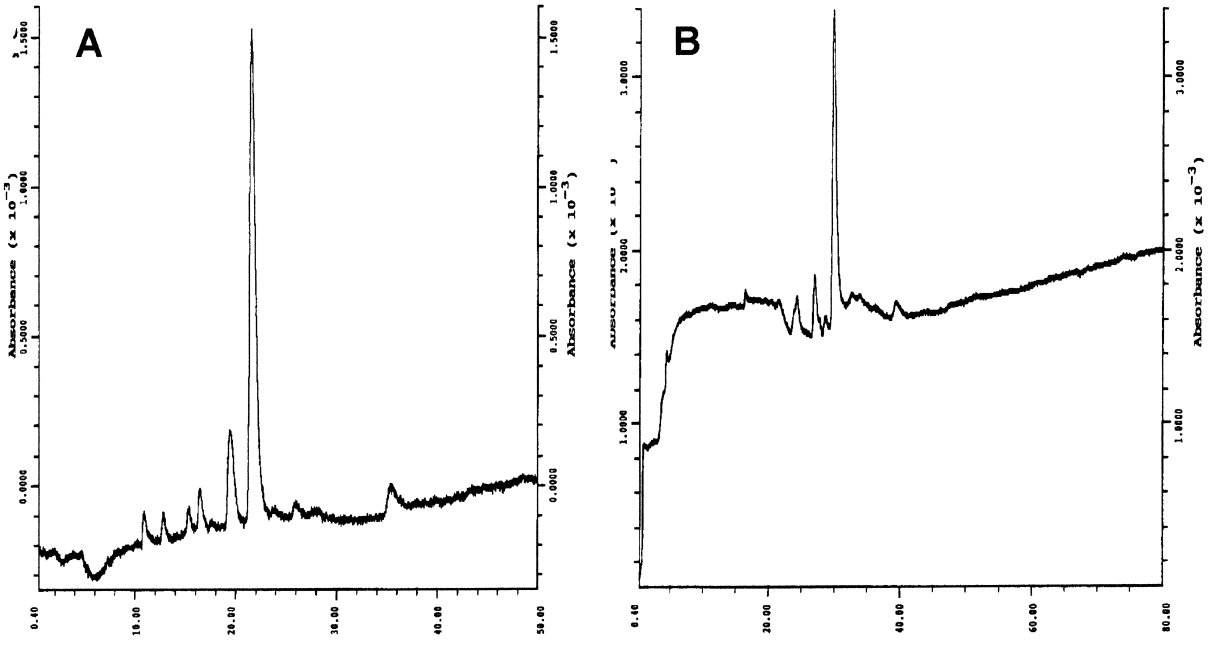

Fig. 4. Electrochromatograms of a TRAP-3 peptide sample on an etched cholesterol modified capillary at pH 2.14 and V=25 kV. (A) Buffer+10% (v/v) TFE and (B) buffer+50% (v/v) TFE. Capillary dimensions: 58 cm (effective length 51 cm)×20 μm I.D. x-Axis: retention time in min.

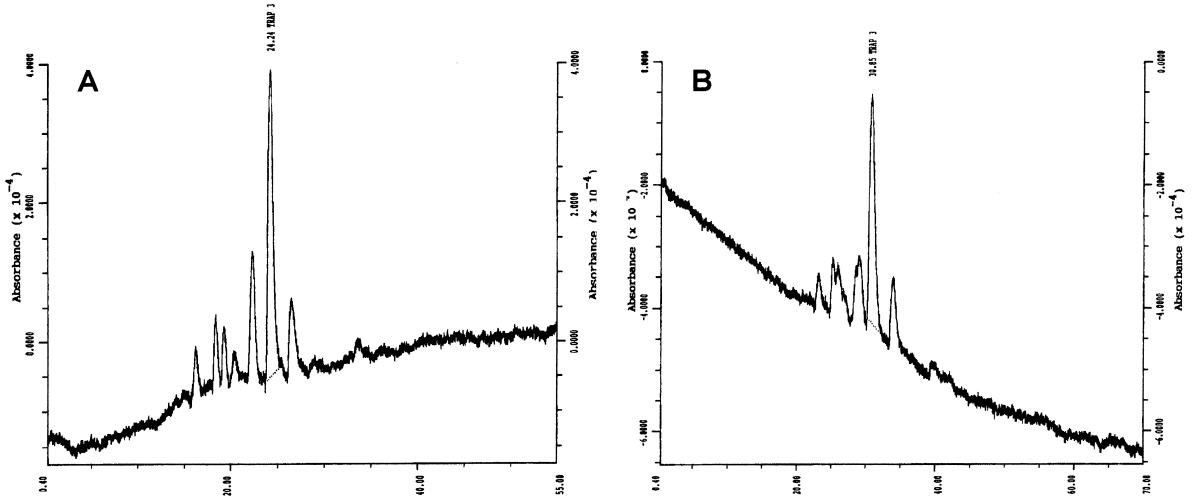

Fig. 5. Electrochromatograms of a TRAP-3 peptide sample on an etched C_{18} modified capillary at pH 2.14 and V=25 kV. (A) Buffer+20% (v/v) methanol and (B) buffer+40% (v/v) methanol. Capillary dimensions: 69 cm (effective length 62 cm)×20 μm I.D. x-Axis: retention time in min.

of peptides in OTCEC separations. Changes of surface morphology as a function of temperature, particularly for the cholesterol moiety used in this study, have been demonstrated by solid state nuclear magnetic resonance (NMR) for organic groups bonded to silica particles [20]. However, temperature also has an effect on the three prime migration factors that control the separation of charge solutes in CEC: electrophoretic mobility; electroosmotic flow; and solute–bonded phase interactions. Therefore, comparisons of general temperature effects on the two columns can be used to learn about the thermodynamics of the migration of solutes in the etched chemically modified columns as well as provide insights into additional means of improving a particular separation.

Fig. 6 is a summary of the migration of the two test peptides (TRAP-3 and TRAP-6) on both columns at two different pH conditions but with the same amount (20%, v/v) and type of organic modifier. In Fig. 6A it can be seen that temperature has very little effect on the migration of the two peptides at low pH for the etched C_{18} modified capillary. This result would suggest that any changes in peptide–bonded phase interactions are being compensated for by changes in electrophoretic mobility and/or the electroosmotic flow. Fig. 6C illustrates the migration of these two peptides at neutral pH on the same capillary and follows the expected behavior for a typical reversed-phase or hydrophobic interaction chromatographic system, i.e., an increase in retention with a decrease in temperature. Therefore, it can be concluded that for these particular peptides separated by CEC on an etched and chemically-modified C_{18} capillary changes in temperature has little or no effect on the separation at low pH while an increase in temperature can be used to shorten the analysis time at neutral pH. These results are in sharp contrast to the data obtained on the etched and cholesterol modified capillary. At low pH it can be seen that the peptide migration time decreases with a decrease in temperature (Fig. 6B). In fact the behavior of the two main peptide peaks is considerably different with a change in elution order occurring twice over the temperature range studied. The results indicate that under the particular conditions of buffer, pH and fixed amount of organic modifier used in these experiments, the bonded cholesterol moiety probably undergoes changes in surface morphology that alter the degree of interaction with the two different solutes. Another example of this effect is seen in Fig. 6D using the cholesterol column at neutral pH. Here the general behavior is similar to that observed for the same two peptides on the C_{18} capillary (Fig. 6C),

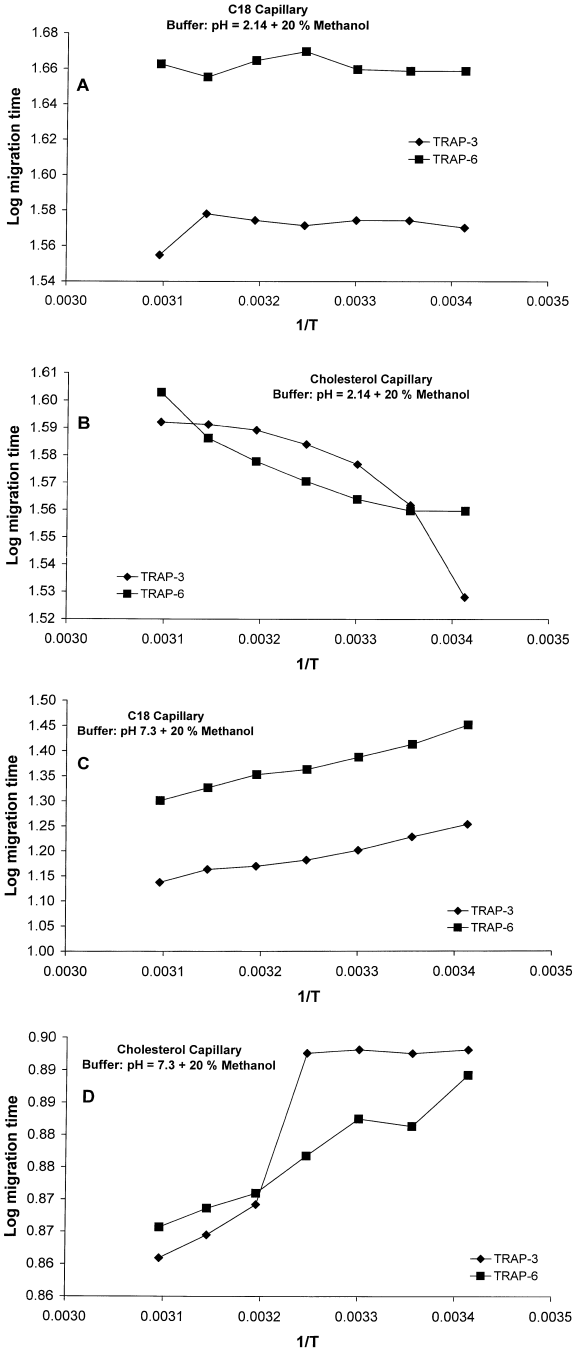

Fig. 6. Plot of the logarithm of the migration time (log t_M) for the TRAP-3 and TRAP-6 peptides versus $1/T$ with 20% (v/v) methanol in the buffer A or buffer B at V=20 kV. (A) Etched C_{18} modified capillary at pH 2.14; (B) etched cholesterol modified capillary at pH 2.14; (C) etched C_{18} modified capillary at pH 7.3; and (D) etched cholesterol modified capillary at pH 7.3. Capillary dimensions as in Fig. 4 for the cholesterol column and as in Fig. 5 for the C_{18} column.

i.e., an increase in t_M as the temperature is decreased. However, this increase is nonlinear in comparison to the data obtained for the *n*-octadecyl capillary. In particular, TRAP-6 show a dramatic increase in migration time over a short temperature range. The migration time of TRAP-3 is linear over an extended range at high temperature but shows a discontinuity (a slight decrease) at low temperature. These results demonstrate that cholesterol and C_{18} phases are interacting with these peptides in a different manner. These variations cannot be ascribed to changes in peptide conformational structure with temperature since their migration behavior on the two columns is distinctly different from each other at both pH values. If conformation changes or other peptide structural differences in temperature were primarily responsible for the migration behavior, then similar data trends would be observed on both columns under the same conditions.

The effect of temperature on the separation characteristics of etched chemically modified capillaries can be seen by examining the electrochromatograms of several of the peptide samples. Fig. 7 shows the migration behavior at two different temperatures of a TRAP-3 sample at low pH. At low temperature (Fig. 7A) there are seven minor components in the sample that elute before the main peak while at higher temperature (Fig. 7B) nine small peaks are observed before the main TRAP-3 component. As shown in Fig. 6B, migration of the TRAP-3 component in the sample is slower (39.0 min) at 50°C than at 20°C (33.2 min). Therefore, for the cholesterol capillary as illustrated by the behavior of the two samples in Fig. 6, significant changes can be made in the migration order of solutes by varying the temperature. These changes can be used to effectively improve resolution as shown in Fig. 7. However, as predicted from the data shown in Fig. 6 for the C_{18} capillary, an increase in temperature will only shorten the analysis time without necessarily causing a change in elution order. Fig. 8 shows the electrochromatograms for a TRAP-6 sample at two temperatures. At low temperature (Fig. 8A) the first peak elutes at 17.0 min and the last peak is at 37.0 min, while at higher temperature (Fig. 8B) the respective migration times are 13.2 and 22.3 min, respectively. As expected, the speed of these CEC analyses is improved at high temperature while resolution is better at low temperature.

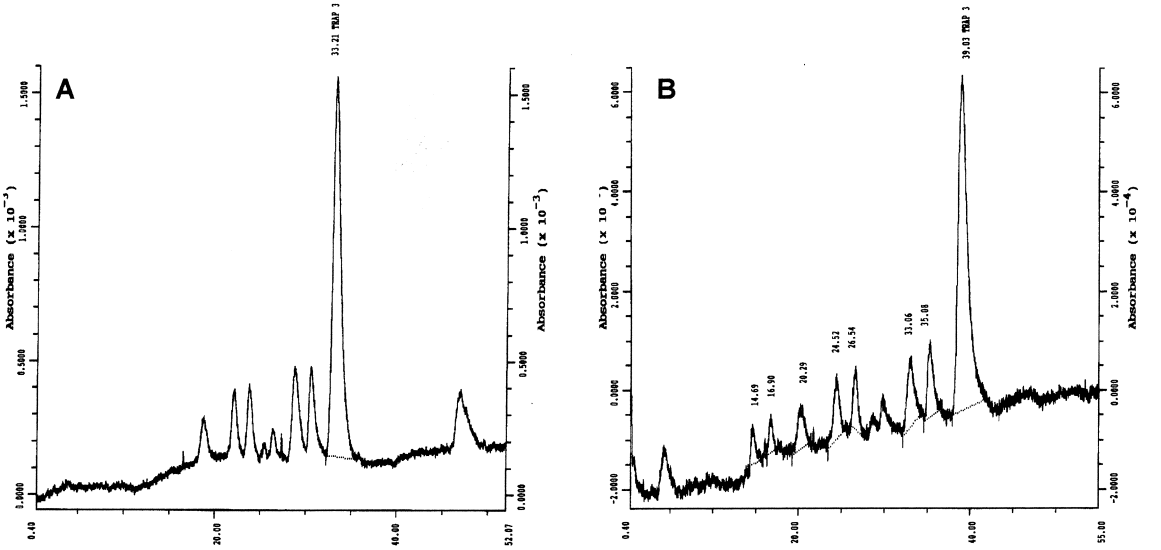

Fig. 7. Electrochromatograms of a TRAP-3 peptide sample on etched cholesterol modified capillary at pH 2.14 with 20% (v/v) methanol in the buffer A and V=25 kV. (A) 20°C and (B) 50°C. Capillary dimensions are as in Fig. 4. x-Axis: retention time in min.

4. Conclusions

The migration behavior illustrated above with this series of TRAP-related peptides is consistent with the dual separation mechanism expected for charged solutes in open tubular CEC, i.e., a combination of electrophoretic mobility and solute–bonded phase interactions. Variables such as temperature and mobile phase composition affect solute migration differently in these two mechanisms. Manipulation of

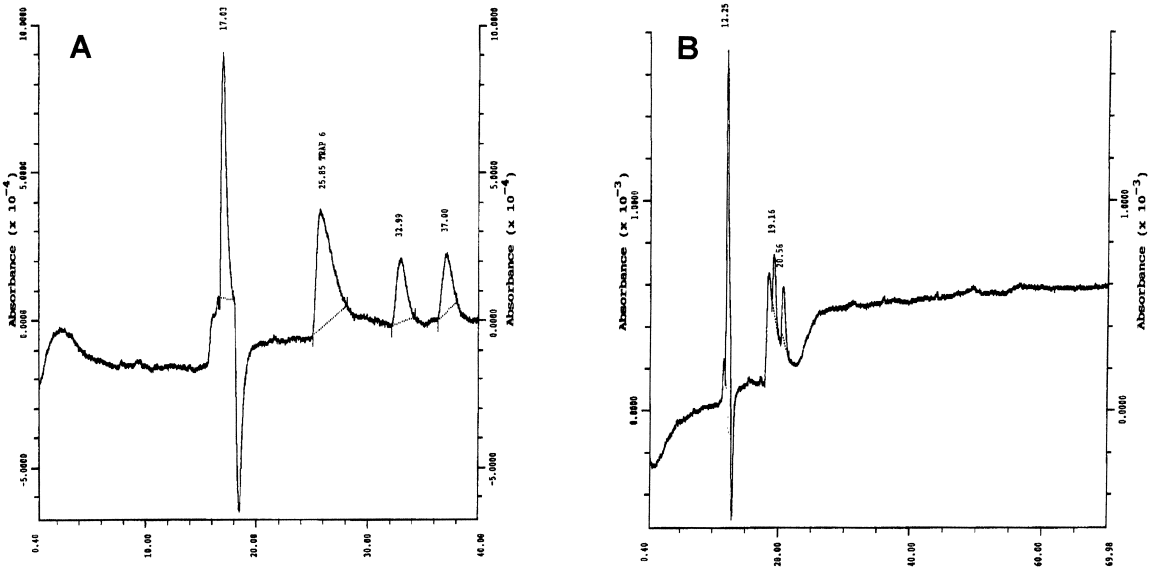

Fig. 8. Electrochromatograms of a TRAP-6 peptide sample on etched C_{18} modified capillary at pH 7.3 with 20% (v/v) methanol in buffer B and V=25 kV. (A) 20° and (B) 50°C. Capillary dimensions as in Fig. 5. x-Axis: retention time in min.

these variables can result in more options for improving separations than if a single mechanism controlled solute migration. In addition, changes in these experimental parameters can have an effect on the bonded phase morphology. Liquid crystal bonded moieties are more likely to demonstrate this behavior since they tend to exhibit stronger intermolecularly bonded chain interactions than other types of organic molecules such as ordinary hydrocarbons (e.g., C_{18}). In some cases, even changes in the magnitude of the applied voltage (field strength) can produce nonuniform changes in peptide migration and this can be exploited to improve separations. Therefore, in addition to the advantages such as ease of experimental operation and compatibility with basic and biological samples demonstrated previously, OTCEC with etched chemically modified capillaries offers a multitude of experimental variables that can be used to optimize difficult separations.

Acknowledgements

The authors wish to thank the National Science Foundation (grant CHE 9625628), the National Institutes of Health (grant R15 GM 49452-01), the Australian Research Council and the National Health and Medical Research Council of Australia for the support of the research presented in this manuscript. The authors gratefully acknowledge the assistance of Dr. G. Rozing from Agilent Technologies for donation of the capillaries with ''bubble cell''.

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