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Comparison of silanization/hydrosilation and organosilanization modification procedures on etched capillaries for electrokinetic chromatography

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Abstract

Etched capillaries for use in open tubular electrochromatography are modified by silanization/hydrosilation and organosilanization. The migration behavior of both types of capillaries is evaluated with small basic molecules, peptides and proteins. Comparisons of peak symmetry and efficiency are used to measure the effectiveness of the two methods for modifying the etched surface. From this information, the suitability of each method for use with etched capillaries can be determined.

Keywords: Capillary electrochromatography; Stationary phases; Basic solutes

1. Introduction

Capillary electrochromatography (CEC) is an emerging separation technology that combines features of both electrochrophoresis and liquid chromatography [1,2]. The stationary phase is fabricated within the capillary in three basic formats: on a particle packed in the column; as a monolith; or attached to the wall of the capillary [3–5]. The latter approach is the one utilized in this investigation and is generally referred to as open tubular capillary electrochromatography (OTCEC). The open tubular configuration is the simplest mode for CEC since it does not require any frits as in packed columns or polymerization processes such as those developed for monoliths (except for stationary phases based on molecularly imprinted polymers or polymeric surfactants). A significant drawback in most OTCEC formats is the low phase ratio of the bonded material because of the small surface area on the inner wall of the capillary where the stationary phase is attached. This OTCEC column feature results in both low loadability of the sample and very often insufficient chromatographic effects because of the small amount of stationary phase and the relatively long distance the solute must travel in order to interact with the bonded or adsorbed material.

A new capillary configuration has been developed for improving performance to overcome the fundamental disadvantages of open tubular columns. This separation medium is fabricated through etching the inner wall of a fused silica capillary by heating it at a high temperature (300 or 400 °C) in the presence of ammonium bifluoride (NH_4HF_2) for three to four hours. The etching process results in an increase in the surface area of the inner wall by a factor of 1000 or more and in radial extensions of up to 5 μm in length being created [6,7]. The larger surface area can partially alleviate the low capacity of the bare capillary and the protrusions can shorten the distance solutes must travel to interact with a stationary phase attached to the etched inner wall. Another important feature of etched capillaries is that elements from the etching reagent, nitrogen and fluoride, are also incorporated into the new surface matrix [8]. The presence of these components significantly decreases the strong adsorptive properties of the silanols thus making the new surface more amenable to the analysis of

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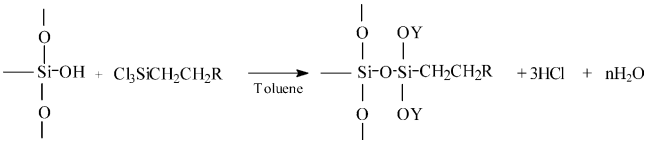
a broader range of compounds, especially basic species. The new surface matrix composed of silicon and oxygen as well as fluoride and nitrogen has a significant effect on the electrophoretic behavior of the capillary. At low pH the nitrogens near the surface become protonated resulting in an overall positive charge on the inner wall of the capillary and a reverse electroosmotic flow (EOF). As the pH is raised to less acidic conditions, the EOF passes through zero and then becomes cathodic due to the deprotonation of the nitrogens, ionization of the remaining silanols, and the presence of the negatively charged fluoride species [8,9].

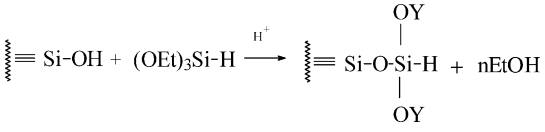
The final step in producing etched capillaries for open tubular CEC is to chemically modify the surface by bonding or adsorbing a stationary phase. For chemically bonded stationary phases, there are two possible approaches for attaching an organic moiety to the etched surface. One possibility is organosilanization, the method used for virtually all silica-based commercial phases for HPLC, and the second choice is silanization/hydrosilation, the process used in the fabrication of all such capillaries to date [7]. In organosilanization, a trifunctional organosilane would react with surface silanols and then crosslink to form the bonded phase. This reaction has already been used on ordinary (unetched) bare silica surfaces and can produce significant suppression of EOF and a substantial reduction in basic solute interactions with silanols [9].



where $n = 1–3$ and Y = H or $Si(OSi)_2R$ depending on the extent of crosslinking.

In the second approach, a silanization reaction is used to create a surface composed primarily of hydride moieties [10,11].

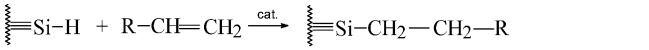
1.1. Silanization



where $n = 1–3$ and Y = H or $Si(OSi)_2R$ depending on the extent of crosslinking.

Under optimized reaction conditions the extent of crosslinking has been determined to be quite high (>95%) so that few residual silanols will remain on the surface to affect basic solutes. The high degree of crosslinking means that the Si–H moiety is firmly anchored to the wall. Attachment of an organic group to the hydride determines some of the selectivity properties of the capillary and is accomplished through a hydrosilation reaction [8,12].

1.2. Hydrosilation



cat = catalyst, metal complex such as hexachloroplatinic acid or free radical initiator such as *t*-butyl peroxide

This process results in a stable silicon–carbon bond between the capillary wall and the organic moiety.

In this investigation, the two reaction protocols, organosilanization and silanization/hydrosilation, are tested as modification methods for etched capillaries. By evaluating electrochromatograms for the same samples on the two capillary formats, it should be possible to determine effects of the hydride surface as well as steric considerations that might results from bonding the same moiety (C18) by two different reaction sequences.

2. Experimental

2.1. Materials

The fused silica capillaries used had a 380 μm o.d. with a 50 μm i.d. inner channel and were obtained from Polymicro Technologies, Phoenix, AZ, USA. Capillary dimensions for the etched modified capillaries were total length (L) = 58.5 cm and distance to the detection window (l) = 50.0 cm.

Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ammonium bifluoride, the etching agent, was purchased from Sigma–Aldrich (St. Louis, MO/Milwaukee, WI, USA), triethoxysilane (Gelest, Morrisville, PA, USA), 1-octadecene and hexachloroplatinic acid (Sigma–Aldrich) were used for the modification of the inner walls of the capillary by silanization/hydrosilation. The octadecyltricholorosilane and pyridine catalyst used for the organosilanization procedure were obtained from Sigma–Aldrich. The buffer materials were as follows: TRIS [*tris*[hydroxymethyl] amino methane] and phosphoric acid (Sigma–Aldrich). The following were used as test solutes and purchased from Sigma–Aldrich: cytochrome c; lysozyme; bovine serum albumin; tryptamine; 3-hydroxytyramine; *R*-epinephrine; DL-propranolol; DL-DOPA; minocycline; tetracycline; chlorotetracycline; oxytetracycline; doxycycline; methacycline; and meclocycline.

2.2. Instrumentation

The HPCE instrument used was an Agilent (Waldbronn, Germany) 3D Capillary electrophoresis instrument having a UV detector. The oven used for etching of capillaries was part of a Hewlett-Packard Model 5890 gas chromatograph. The GC oven was used for the control of the etching temperature and was modified so that multiple capillaries could be accommodated.

2.3. CEC experiments

The etched capillaries modified by silanization/hydrosilation were prepared as described previously [7,8]. Briefly the fused silica surface was etched with a 5% ammonium bifluoride/methanol solution for a total of 4 h at elevated temperature (300–400 °C) in a gas chromatographic oven. A silica hydride layer was then covalently attached by reacting the etched surface with ~1 M triethoxysilane solution in dioxane (total volume = 7.5 mL) with a hydrochloric acid catalyst (115 μL of 2.3 M). The C18 (1-octadecene) was attached to the hydride via hydrosiliation using hexachloroplatinic acid as a catalyst. 1-Octadecene was added to 2.0 mL of toluene and 100 μL of catalyst and heated to 70 °C for 1 h. The solution was then passed through the capillary which was heated at 100 °C for 24 h. Additional solution was passed through the capillary each day for 4 days with the column being kept in the GC oven at 100 °C. At the end of the process the capillary was rinsed with toluene and methanol.

The etched capillaries modified by organosilanization were prepared as follows: All glassware was dried at 110 °C for 24 h. First, 30 mL of toluene was placed in the dried flask. The solution was covered with an argon blanket. Then, 10 mL of octadecyltrichlorosilane was added to the toluene (under argon). Finally, 0.5 mL of pyridine was added. The mixture was transferred to the capillaries. This capillary with the octadecyltricholorsilane/pyridine was kept in the oven at 100 °C for 24 h.

The buffer composition (diluted 1:10 from a stock solution) and pH value used in this study were: pH 2.14, 0.03 mol/L H_3PO_4 and 0.019 mol/L TRIS. Injection was done either electrokinetically or by pressure.

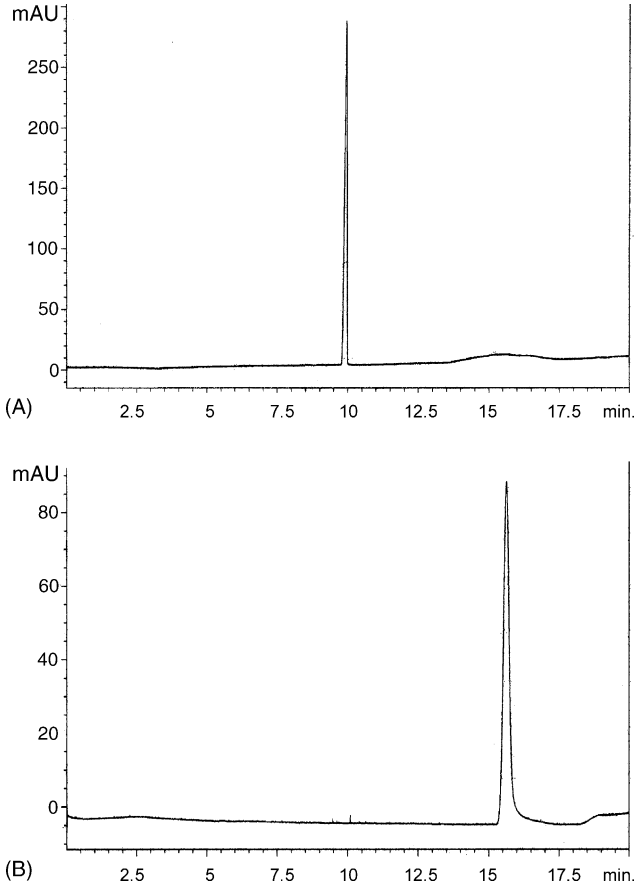


Fig. 1. Comparison of electromigration profiles of tryptamine on etched capillaries modified by two methods. (A) C18 by silanization/hydrosilation and (B) C18 by organosilanization. Both capillaries: i.d. = 50 μm, 58.5/50 cm. Buffer pH 2.14 + 10% ethanol, 25 kV, injection 3 s @ 50 mbar, detection @ 220 nm.

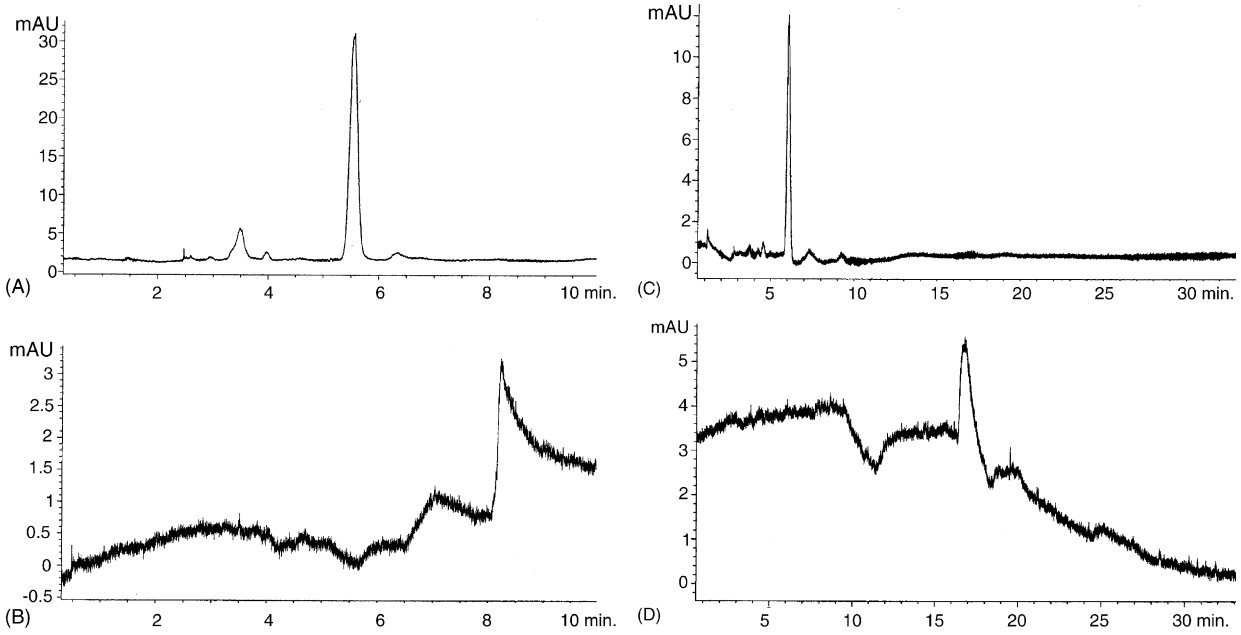


Fig. 2. Electromigration profiles of cytochrome c (A and B) and lysozyme (C and D) on etched C18 capillaries modified by silanization/hydrosilation (A and C) and organosilanization (B and D). Conditions the same as Fig. 1 except 30% ethanol in mobile phase and detection @ 210 nm.

3. Results and discussion

In order to evaluate the two chemical reaction methods for the modification of etched capillaries, a number of solutes were selected to be tested on etched columns having an octadecyl moiety attached by both procedures. In general, molecules with basic functionalities provide the best means for testing the performance of capillaries where the presence of a significant number of silanols is being probed. It has already been demonstrated that the etching process itself creates a surface that is less adsorptive than a typical bare capillary [13].

A simple first test is to observe the peak characteristics of a small basic molecule on the two capillaries. Tryptamine was selected for this comparison. Fig. 1 shows the electrochromatograms for this solute on the two columns under identical experimental conditions with the same sample. The peak obtained on the silanization/hydrosilation column (Fig. 1A) is relatively sharp and symmetric in comparison to the peak observed on the organosilanization column (Fig. 1B). The *N* values for the two tests were determined to be as follows: Fig. 1A = 115,000 plates/m and Fig. 1B = 38,000 plates/m. In addition, the reproducibility of the migration times for the silanization/hydrosilation column was 1.6% RSD while that of the organosilanization column was 9.8% RSD. It is quite clear that for this particular solute, the C18 moiety with the hydride surface underneath provides a more amenable separation medium than the organosilanization structure.

A second single solute comparison under identical experimental conditions was made for the proteins cytochrome c and lysozyme. These basic macromolecules are more complex than trypamine and should be more sensitive to a surface with strongly adsorptive properties. The four electrochromatograms are shown in Fig. 2. The results are similar to those obtained for the tryptamine test, i.e. the peaks obtained on the hydride-based column (Fig. 2A and C) are superior in electrochromatographic performance to those obtained on the organosilane-based capillary (Fig. 2B and D). In fact, the protein electrochromatograms on the organosilane column are even poorer (greater tailing and lower efficiency) than the smaller molecule tryptamine on the same capillary. Some improvement in peak shape and efficiency on both columns is seen for lysozyme in comparison to cytochrome c when ethanol is added to the mobile phase. These results indicate that there is some deficiency in the stationary phase morphology of the column fabricated with the organosilanization reaction protocol, i.e. more silanols on the surface.

While single solutes reveal some of the differences between the two modification methods, a more complete description of the effects of the two surfaces can be seen by comparing the electrochromatograms of mixtures. The first such example is shown in Fig. 3 for a mixture of four basic species. In the case of the hydride based capillary (Fig. 3A) each of the four components is well separated and each peak

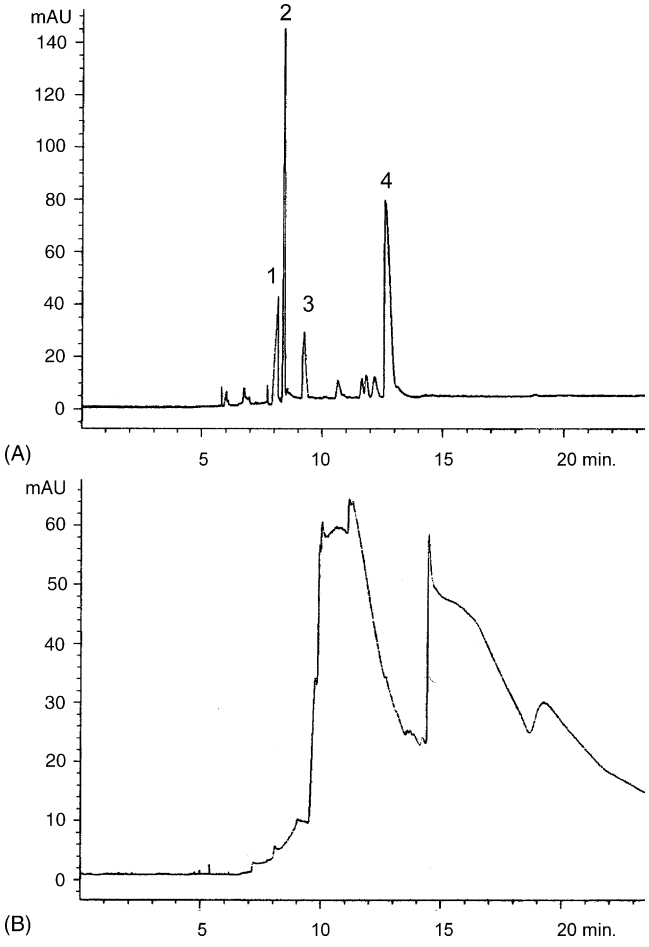


Fig. 3. Comparison of the separation of a mixture of small basic molecules on etched C18 capillaries modified by silanization/hydrosilation (A) and organosilanization (B). Conditions the same as Fig. 1. Solutes: (1) 3-hydroxytyramine; (2) *R*-epinephrine; (3) DL-propranolol; (4) DL-DOPA.

has both high symmetry and good efficiency. In fact, a number of trace impurity compounds are readily resolved by this column. In contrast, the organosilane etched capillary provides no resolution of this mixture since the peak shapes are very broad and appear to tail considerably. No significant improvement was seen in the electrochromatogram by varying the amount or type of organic modifier in the mobile phase.

Electrochromatograms of another multi-component sample are shown in Fig. 4. In this case the analytes are a synthetic peptide and additional species due to the presence of deletion peptides, side chain reaction products and Group I ion adducts produced during the synthesis, purification or upon storage. Under optimum conditions for the capillary produced by silanization/hydrosilation (Fig. 4A), at least nine well-resolved components can be observed in the electrochromatogram. In this case the mobile phase is composed of the running buffer and 30% ethanol. In contrast, under optimum conditions for the organosilane column (Fig. 4B), a number (at least seven) discrete peaks can be obtained using a running buffer containing no organic modifier.

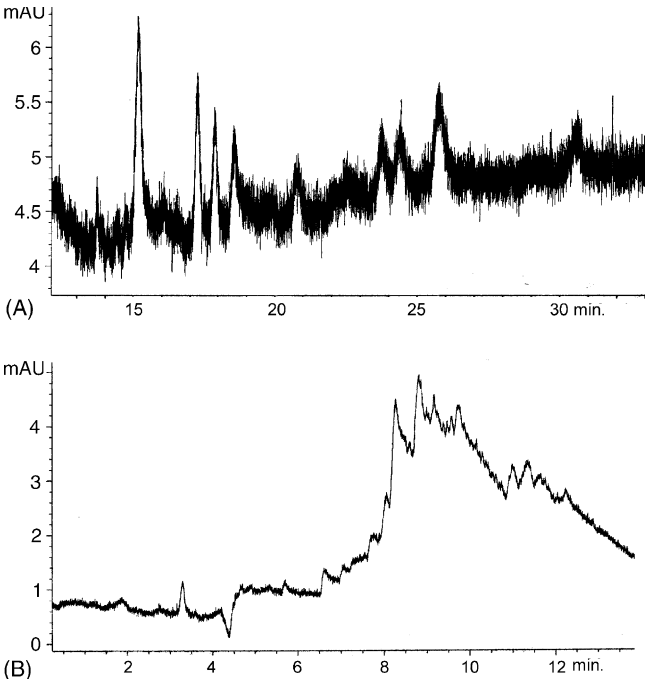


Fig. 4. Comparison of the elution profiles of a synthetic peptide on etched C18 capillaries modified by silanization/hydrosilation (A) and organosilanization (B). Conditions the same as Fig. 1 except A is 30% ethanol and B is 0% ethanol. Solute: JC16 (HTNIHQDQHNHFHR).

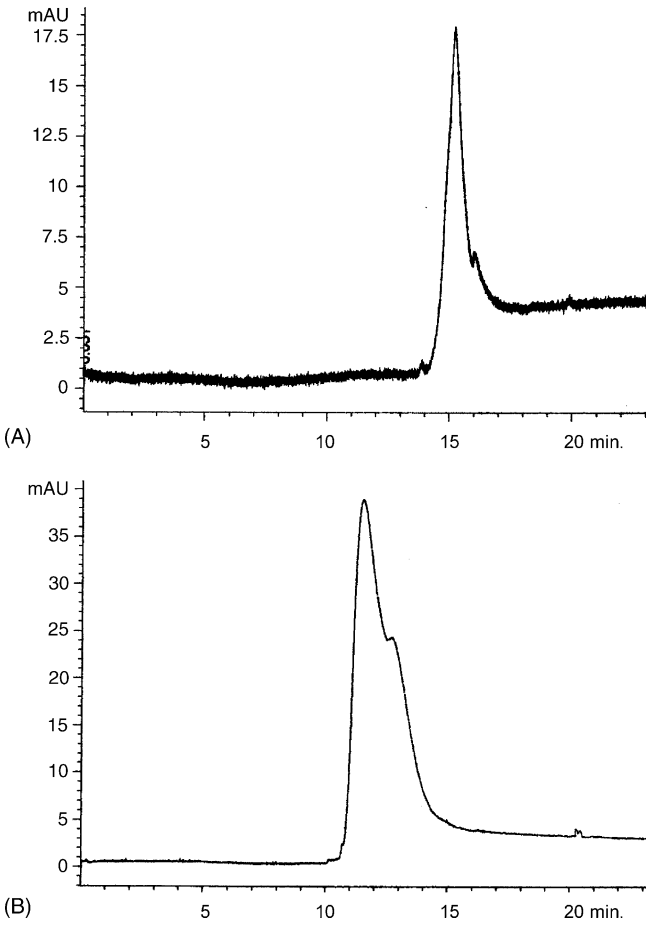


Fig. 5. Comparison of electromigration profiles of bovine serum albumin on etched capillaries modified by two methods. (A) C18 by silanization/hydrosilation and (B) C18 by organosilanization. Conditions the same as Fig. 1.

However, neither a good reproducible migration pattern nor background is obtained for this column. An addition of varying amounts of organic modifiers (ethanol and methanol) to the mobile phase does not improve either the resolution or the background. While the result shown in Fig. 4B is not comparable to the hydride-based column, it is an improvement over the separation of the small basic molecules shown in Fig. 3B.

The examples above focus on those cases where the performance of the etched capillary modified by organosilanization is poorer than that achieved by those columns where the inner wall is subjected to silanization/hydrosilation. However, a few cases have been found where the two types of etched chemically modified capillaries have comparable analytical capabilities. The first example is the electromigration of the protein bovine serum albumin shown in Fig. 5. The two columns display similar peak shapes and partial resolution of the isoforms of this protein. Slightly better efficiency is achieved on the hydride-based column than on the organosilane capillary. But overall the behavior of bovine serum albumin on the organosilane capillary is quite acceptable in comparison to the poor performance in the examples shown in Figs. 1–4.

A final example utilizing a mixture of tetracyclines is shown in Fig. 6. In each case, all seven components in the mixture are resolved as separately identifiable peaks. The only difference is that for one solute, chlorotetracyline, the efficiency is notably poorer for the organosilane capillary (Fig. 6B) in comparison to the electrochromatogram in Fig. 6A. The mobile phase used in Fig. 6 produced the optimum results for both columns so no further improvement for the organosilane column was achieved by varying either the pH, the type of organic modifier or the amount of organic modifier. The results obtained on the hydride-based column for tetracyclines are comparable to those reported in an earlier study [14].

For a variety of the most challenging solutes, small basic compounds, peptides and proteins, the etched capillary modified by the silanization/hydrosilation reaction protocol outperforms a similar capillary modified by organosilanization. Such differences are generally not observed when the capillary is not etched [9]. It is likely that the etched surface provides a geometry (significant roughness and surface extensions) that lead to inefficient crosslinking of the organosilane reagent resulting in a substantial number of residual silanols. In contrast, the triethoxysilane having a hydrogen instead of a large organic moiety (C18) as on the organosilane reagent, leads to much more efficient crosslinking and hence fewer residual silanols on the surface. The presence of a greater number of silanols on the organosilane column

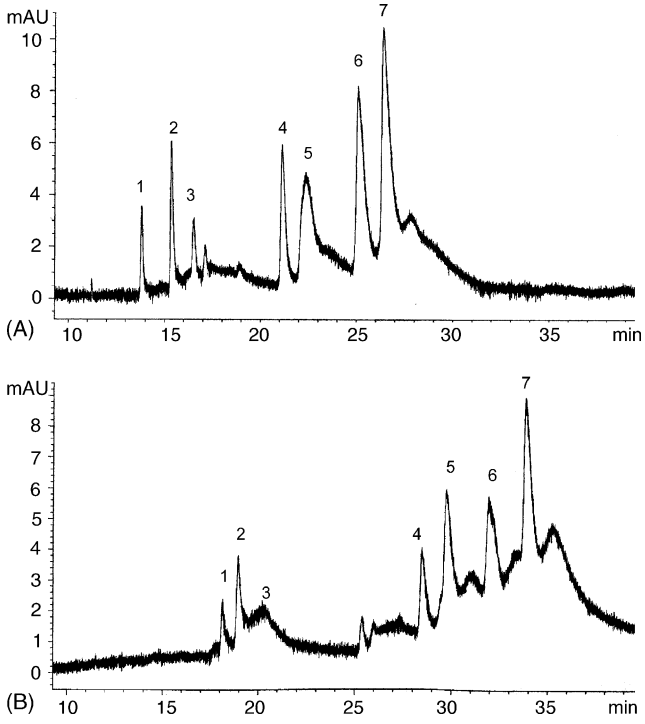


Fig. 6. Comparison of the separation of a mixture of tetracylines on etched C18 capillaries modified by silanization/hydrosilation (A) and organosilanization (B). Conditions the same as Fig. 1 except for 40% ethanol in mobile phase. Solutes: (1) minocycline; (2) tetracycline; (3) chlorotetracycline; (4) oxytetracycline; (5) doxycycline; (6) methacycline; (7) meclocycline.

could account for the differences in electrochromatographic performance between the two methods for chemically modifying the etched capillaries.

4. Conclusions

The silanization/hydrosilation reaction sequence appears to be superior to organosilanization for the modification of etched capillaries for use in open tubular electrochromatography. In many instances, both peak symmetry and efficiency on the organosilane column are significantly poorer than on the hydride-based capillary. These conclusions are drawn from comparisons of electrochromatograms on the two types of columns for the most challenging analytes, those with basic functionalities. It is likely that the superior separation capabilities of capillaries made by the silanization/hydrosilation procedure in contrast to those modified by organosilanization is due to fewer residual silanols.

Acknowledgments

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