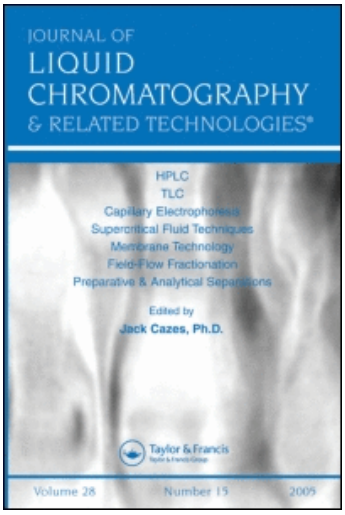

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CHARACTERIZATION OF WALL MODIFIED SILICA CAPILLARIES FOR OTCEC AND CE

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CHARACTERIZATION OF WALL MODIFIED SILICA CAPILLARIES FOR OTCEC AND CE

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ABSTRACT

Fused silica capillaries with an i.d. of 50 and 75 μm are chemically modified by the silanization/hydrosilation method to attach organic moieties for use in electrokinetic chromatography. An etching process was utilized to increase the surface area of the capillary inner wall. A comparison of Si-C bonded capillary columns to those obtained by conventional organosilanization (Si-O-Si-C) bond was performed. The etched capillaries after chemical modification are shown to have an anodic electroosmotic flow (EOF) below pH 4.5 while the bare modified capillaries show a cathodic EOF at all pH's studied.

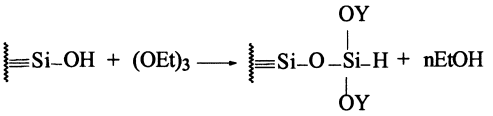
The use of these etched chemically modified capillaries eliminates the need for frits and the difficulties associated with packed capillaries such as bubble formation. This open tubular approach to CEC is also suitable for an analysis of a number of different types of basic compounds.

INTRODUCTION

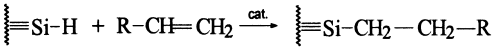
The use of capillary electrochromatography (CEC) as a separation technique in modern analytical laboratories is growing exponentially. Several aspects of CEC have been described in the literature.[1-6] Preparation of packed capillary columns including frit formation, their longevity and reproducibility have been addressed as well.[7-11]

In this manuscript capillary columns for an open tubular approach to CEC are described and characterized.[12] Four types of the capillary columns were investigated: hydride; unetched and etched (increased surface area) diol fused silica capillaries modified by silanization/hydrosilation reaction; and a column modified by conventional organosilanization. The chemistry used in the modification process is based on the reactions shown below:

Reaction A. Silanization/hydrosilation reaction scheme:

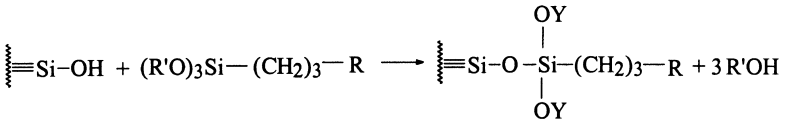


n = 1-3 depending on extent of cross-linking and Y represents -H or ≡Si-



catalyst - hexachloroplatinic acid

Reaction B. Organosilanization:



where R' may be a methyl or ethyl group, R - contains the hydrophilic or hydrophobic moiety and Y represents -H or ≡Si-.

After modification, the electroosmotic flow in the capillary columns is greatly reduced because a significant number of silanols present on the capillary wall are converted to hydrides or they form a linkage with the organic moiety. The mechanism of the separation of the analytes in the etched modified capillaries is based on a combination of differences in electrophoretic mobility and solute/attached moiety interactions.

EXPERIMENTAL

All solvents used were HPLC grade. Distilled water was purified using a Mili-Q water system (DI water). Triethoxysilane (TES), hexachloroplatinic acid and olefins (7-octene-1,2-diol, allyl glycidyl ether) were purchased from Aldrich Co. Inc. (Milwaukee, WI, USA). Proteins and peptides were purchased from Sigma Chemical Co. (St. Louis, Mo, USA) antidepressants from Aldrich and γ-glycidoxypropyltrimethoxysilane (GPTMS) came from Petrarch Systems, Silanes & Siloxanes, Bristol, PA, USA.

The compositions of the buffers used were as follows:

pH 2.14 buffer: 0.3 M phosphate (from 85% H3PO4) + 0.19 M Tris [tris(hydroxymethyl)aminomethane].

pH 3.0 buffer: 0.3 M citric acid + 0.3 M -alanine.

pH 3.7 buffer: 0.3 M lactic acid + 0.3 M -alanine.

pH 4.41 buffer:0.3 M acetic acid + 0.37 M GABA (-aminobutyric acid)

pH 6.0 buffer: 0.3 M MES (4-morpholine ethane sulfonic acid) + 0.2 M histidine

pH 10.5 buffer: 0.3 M CAPS (3-(cyclohexylamino)-1propanesulfonic acid) + 0.52 M ammonia

The fused silica capillary tubing was purchased from Polymicro Technologies Inc. (Phoenix, Az USA). A GC oven was used during the etching and chemical modification reactions. Solvents and reagents were forced through the capillaries by applying nitrogen pressure (40-60 psi). The CE and CEC experimental work was done using an Applied Biosystems 270A-HT Capillary Electrophoresis instrument and a home-made HPCE instrument.

Capillaries Used in This Study

Hydride Modified Capillary

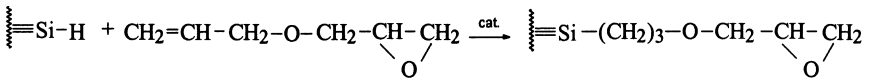
Made by the silanization reaction (first part of reaction A - Introduction). The capillary was first treated with a pH = 10.0 (about 6 mM) ammonia solution for 20 hours with a flow rate of 0.1-0.2 mL/min. at room temperature, then was rinsed with DI water followed by flushing with 0.1 M HCl for 4 hours to remove any ammonia adsorbed on the surface.

After 2 hours rinsing with DI water, the tube was dried with N2 for 20 hours at 100°C and then was rinsed with dioxane and treated with 1M TES (trietoxysilane) solution in dioxane at 90°C for 90 min. Finally the tube was rinsed with dioxane, tetrahydrofuran, and dried under N2 at 100°C for over 24 hours.

Diol from Allyl Glycidyl Ether (Diol-AGE)

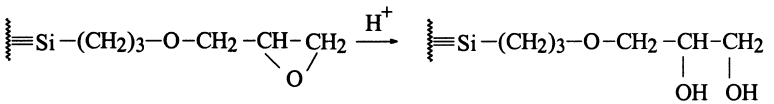
Modified capillary - made from the hydride capillary by the hydrosilation process (second part of reaction A - Introduction) by addition of allyl glycidyl ether (reaction C) followed by hydrolysis of the epoxy groups (reaction D).

Reaction C:



catalyst - hexachloroplatinic acid

Reaction D:



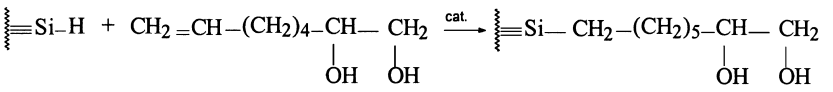
10 mL of 75 mM allyl glycidyl ether solution in toluene + 10 μL of 30 mM Speier's catalyst (hexachloroplatinic acid in 2-propanol) was allowed to equilibrate for 1 hour at 70°C under a dry N_2 blanket. A hydride modified capillary (Point 1 above) was rinsed with toulene and then treated with the equilibrated allyl glycidyl ether /toluene/catalyst solution at 100 1°C under 30 psi of N_2 for 45 hours.

After the reaction was completed, the capillary was washed with toluene, tetrahydrofuran, and dried at 100°C overnight. The epoxy groups were opened by treating the capillary with 0.05 M hydrochloric acid at 65°C for 5 hours. The tube was washed with water and tetrahydrofuran and dried.

Etched Diol from 7-Octene-1,2-Diol (Diol-7OD) Modified Capillary

The inner surface of the capillary was increased by the etching process prior the modification procedure. After the silanization reaction the 7-octene-1,2-diol (7-OD) was attached to the capillary wall (reaction E). The procedure is described elsewhere.[13]

Reaction E:



Conventional Diol from γ-Glycidoxypropyltrimethoxysilane (Diol-GPTMS) Modified Capillary

Made by the organosilanization process (reaction B - Introduction). The epoxy groups were opened by hydrolysis (reaction D - above). A preconditioned capillary (rinsed with pH = 10.0 (6 mM) ammonia solution - 20 hours, then with water, 0.1 M HCl, water and finally dried) was treated with 25 mL of a solution containing 0.8 mL of GPTMS, 15.0 mL of toluene and 9.2 μL of pyridine at 100 1°C under 30 psi of N2 for 25 hours. The capillary was then washed with toluene for 4 hours and tetrahydrofuran for 2 hours. Drying was done with N2 at 100 1°C overnight. Opening of the epoxy groups was done as described in Point 2.

Electroosmotic Mobilty Measurements

μ_{eo} measurements were done using DMSO - dimethyl sulfoxide.[14] First, the marker was injected and the electric field was applied for a specific period of time (about 2-5 min.) to electroosmotically move the DMSO band along with a plug of electrolyte over a short distance of the capillary. A second injection of the marker was then made to mark the end of the electolyte plug. After this injection, the marker peaks sandwiched with the electrolyte plug were moved to the detection window by applying a vacuum. The migration times of the two markers were recorded and the μ_{eo} was calculated using the equation:

$$\mu_{eo} = Ll/Vt_v \times (1-t_1/t_2)$$

where: L = total length of capillary, l = effective length of capillary, V = applied voltage (V). t_v = time for which voltage was applied, t_1 and t_2 = migration times of the two marker peaks.

RESULTS AND DISCUSSION

The main purpose of modification of the capillaries was to reduce the interaction of proteins and peptides with a capillary wall of fused silica. The modification effect on critical analytical parameters for all capillaries studied is presented in Table 1. The solutes (Cytochrome c's) are proteins with molecular weight about 13.4 kDa which only differ by three amino acids in a total

Table 1

Separation Efficiencies and Peak Symmetries for Two Cytochrome C's in Modified Capillaries*

Capillary	Cytochrome C	Plate Count Mean	Plates/m % RSD	Asymmetry Mean	Asymmetry % RSD
Hydride B	Horse	5.26E + 04	29.6	1.17	14.2
	Bovine	4.96E + 04	22.1	1.06	16.2
Diol-AGE	Horse	3.1E + 05	2.7	0.97	9.7
	Bovine	2.93E + 05	5.0	1.02	6.0
Etched Diol-70D	Horse	2.56E + 04	2.2	1.03	3.2
	Bovine	2.42E + 04	2.1	1.01	2.4
Diol-GPTMS	Horse	5.44E + 05	8.0	0.6	30.0
	Bovine	5.13E + 05	22.3	0.71	37.1

* Data based on five replicate measurements.

sequence of 104.[15] Under the same conditions, Diol-AGE and Diol-GPTMS modified capillaries show better efficiency then the other capillary columns investigated. The average plate count for these capillaries are one order of magnitude higher as compared to the remaining two columns. There is a difference in reproducibility of the data. On the hydride capillary surface there are still some silanols remaining which are responsible for protein interaction with the capillary wall leading to lower separation efficiency and poor reproducibility. Diol moieties on the capillary wall in the Diol-AGE column as well as Etched Diol-7OD are anchored to the predominantly hydride surface and additionally they provide shielding of surface silanol groups which results in better precision of the measurements.

The efficiency of the Etched Diol-7OD is lower than Diol-AGE and Diol-GPTMS capillaries due to the interaction of the solute with 7-OD moiety. For the Etched Diol-7OD capillary the inner surface is greater by about 1000-fold than the bare capillary, which results in higher ligand density and better precision of the measurements. In the case of the Diol-GPMS capillary, only silanol species are lying underneath the diol chains, so silanophilic interactions with the basic probes are more likely to occur. The overall precision of this capillary is worse than the other diol columns studied.

For further comparison Diol-AGE and Diol-GPTMS were chosen, because the surface area in both cases was very similar. Table 2 lists the average theoretical plate counts and A/B ratios before and after treating the two Diol

Table 2

Separation Efficiencies and Peak Symmetries for Two Lysozymes in Diol Modified Capillaries Before and After Treatment with High pH Buffer*

Capillary	Lysozyme	Plate Count Mean	Plates/m % RSD	Aymmetry Mean	Asymmetry % RSD
Diol-AGE	Turkey	2.15E + 05	3.1	1.15	9.1
	Chicken	2.12E + 05	4.7	1.32	9.1
Diol-AGE: treated with the buffer pH = 10.5 for 1 hr	Turkey	3.29E + 05	6.7	3.33`	9.9
	Chicken	2.98E + 05	3.9	3.37	9.8
Diol-GPTMS	Turkey	6.72E + 05	13.3	0.73	24.8
	Chicken	5.16E + 05	38.5	0.99	23.8
Diol-GPTM: treated with the buffer pH = 10.5 for 1 hr	Turkey	7.38E + 05	7.1	1.39	56.3
	Chicken	5.84E + 05	44.7	1.83	29.9

* Data based on five replicate measurements.

capillaries with a high pH buffer. From the data it can be concluded that both capillaries are fairly stable under these conditions, however the higher A/B ratio indicates peak tailing that resulted from the slight removal of the bonded moieties from the capillary wall by hydrolysis. The run to run precision of migration time is also better for Diol-AGE than Diol-GPTMS capillary, except that of the first day as shown in Table 3.

Bounding of the hydrophilic moiety on the capillary wall drastically reduces electroosmosis, but it is not eliminated totally due to residual silanols left on the surface after formation of the hydride. Ionization of these species and perhaps formation of new ones become more extensive with high pH buffers. Also unreacted silicon hydride species in Diol-AGE lying underneath the anchored diol-moieties might react as follow with water in the presence of high pH buffer:

$$\equiv Si-H + H_2O \xrightarrow{OH^-} \equiv Si-OH + H_2$$

Table 3

Precision of Migration Times in the Diol-AGE and Diol-GPTMS Modified Capillaries for Cytochrome C (Horse)

Capillary	Day	Migration Time (min.) Mean	% RSD	Buffer pH	Current μA
Diol-AGE	1st	12.99	2.6	3.73	9
	10th	11.88	0.4	3.71	9
	15th	11.59	0.7	3.68	9
	20th	10.86	0.2	3.71	9
	25th	10.12	0.5	3.69	9
Diol-GPTMS	1st	14.40	1.7	3.71	8
	10th	13.72	0.4	3.71	8
	15th	13.23	0.9	3.70	8
	20th	12.86	2.3	3.69	8
	25th	12.44	0.9	3.69	8

* Data based on three replicate measurements.

Table 4

Migration Time of the Marker (Mesityl Oxide) at pH 6 Buffer After Exposure of the Capillary to High pH Buffer (= 10.5) for 1 Hour

Capillary	Migration Time (min.) Before Exposure	Migration Time (min.) After Exposure to High pH Buffer
Diol-AGE	26.05	27.77
	26.01	27.87
	26.02	27.94
	% RSD = 0.1	% RSD = 0.3
Diol-GPTMS	26.88	13.4
	26.79	13.5
	26.7	13.56
	% RSD = 0.3	% RSD = 0.4

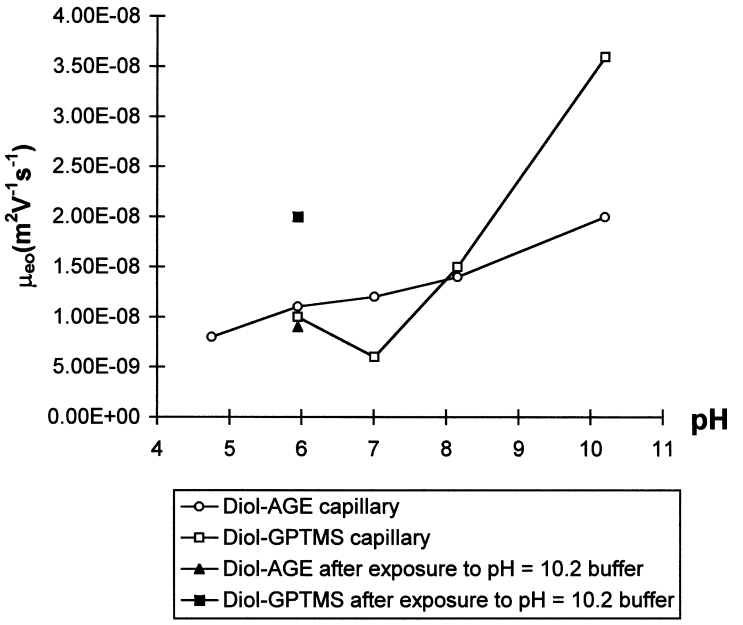


Figure 1. Electroosmotic mobility (μeo) as a function of buffer pH for Diol-AGE and Diol-GPTMS capillaries. Applied voltage: 22 kV. Marker: mesityl oxide at a concentration of 1 mM in the buffers, detection wavelength 254 nm. The solid points represent the μeo measured for each column after prolonged exposure to pH = 10.2 buffer.

In order to test this hypothesis, the migration time of a mesityl oxide marker was measured before and after exposure of the capillaries to high pH buffer. Results from Table 4 indicate, that under the conditions used, the Si-C bond in the Diol-AGE capillary resists hydrolysis under moderately basic conditions. In comparison when the Diol-GPTMS capillary, was tested the migration times for the marker diminished. This may be due to insufficient protection of the siloxane linkages lying under the hydrophilic moiety. As a result silanols can undergo ionization at high pH and siloxane linkages can hydrolyze increasing the anionic charge on the capillary wall. Figure 1 illustrates the electroosmotic mobility (μ_{eo}), as measured with a neutral marker (mesityl oxide) as a function of buffer pH in the Diol-AGE and Diol-GPTMS capillaries. As expected when pH increases the μ_{eo} values increase for both capillaries, but with the Diol-GPTMS column reaching a much higher value for pH = 10.2 buffer. This is a result of increasing ionization of residual silanols and/or the formation of a new ones. From this graph we can conclude that in case of the Diol-GPTMS capillary more residual silanols were accessible.

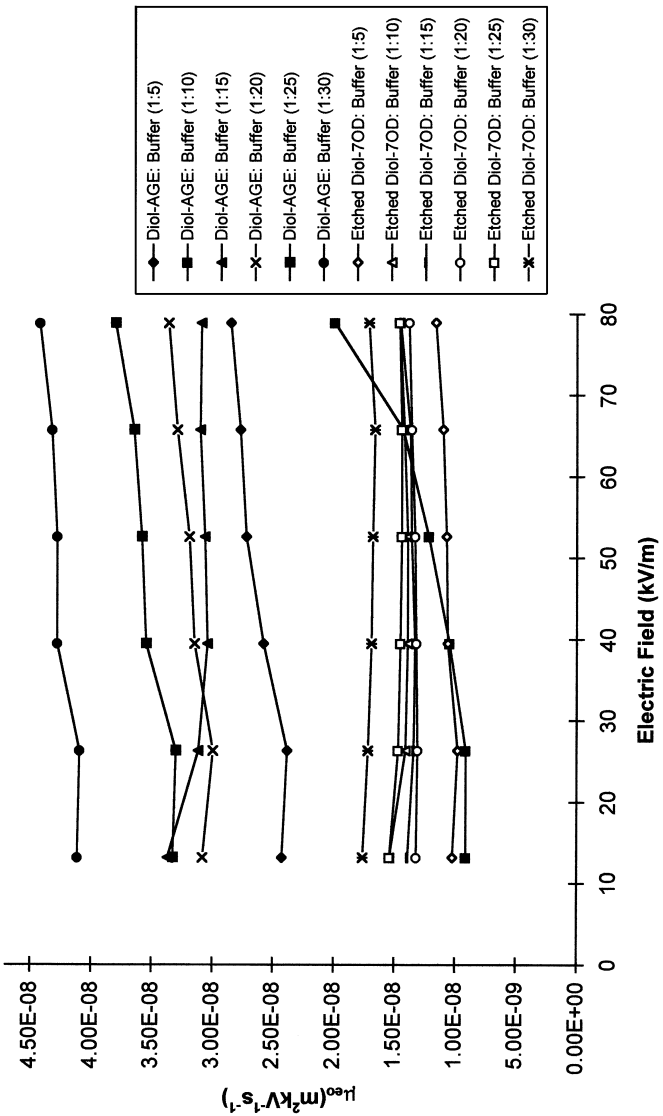


Figure 2. Electroosmotic mobility vs. applied electric field for Diol-AGE and etched Diol-7OD capillaries.

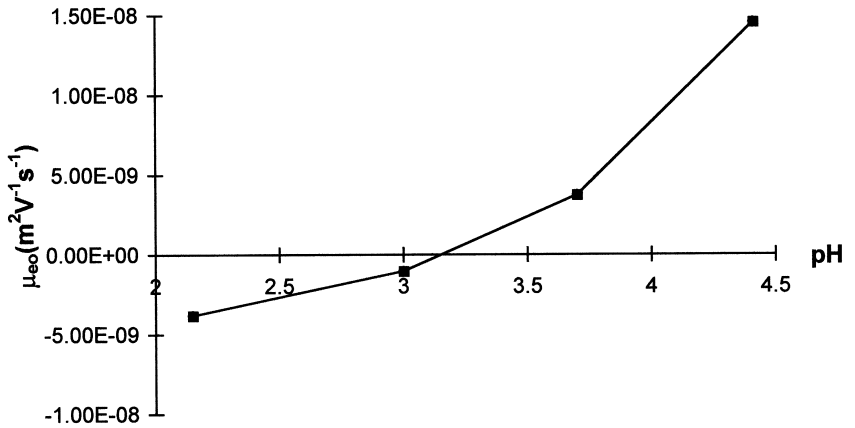


Figure 3. Electroosmotic mobility vs. buffer pH for Etched Diol-7OD capillary. Applied voltage: 30 kV. Marker: DMSO - 0.1% solution in the buffers, detection wavelength 211 nm.

If new silanols are formed upon exposure to high pH buffer, the following measurements at lower pH buffer should give a higher value than original one. After taking the above data, on Figure 1, both capillaries were treated further with pH = 10.2 buffer for 1 hour, then they were again equilibrated with pH = 6.0 buffer. New determinations of electroosmotic mobility were taken (the single solids points on Figure 1) at pH = 6.0. For the Diol-AGE capillary the value was found to be essentially equal to the original one while for the Diol-GPTMS capillary electroosmosis was much higher. This means that under basic conditions, the Si-O-Si-C linkage in the capillary hydrolyzes at least partially to regenerate silanols.

The effect of etching of the capillary on electroosmotic mobility can be observed in Figure 2. Measurements for the Etched Diol-7OD and Diol-AGE capillaries are plotted on one graph. As can be seen from this figure, μ_{eo} measured in the Etched Diol-7OD capillary is much lower and more uniform than measured in Diol-AGE column. This can be explained by greater ligand density on the etched surface and better coverage of the charges on the capillary wall and reduction of the zeta potential. The Etched Diol-7OD capillary itself exhibits unusual properties at low pH. Figure 3 shows the plot of electroosmotic mobility vs. pH over the range from pH = 2.14 to 4.5. At pH = 3.0 and below the electoosmotic flow is reverse - anodic. This is probably due to the etching process, during which ammonium ion from the etching reagent was incorporated into silica surface and at low pH carries positive charge. The electroosmotic mobility for the Etched Diol-7OD capillaries did not change much after one year of use (about 400 injections (see Figure 4)).

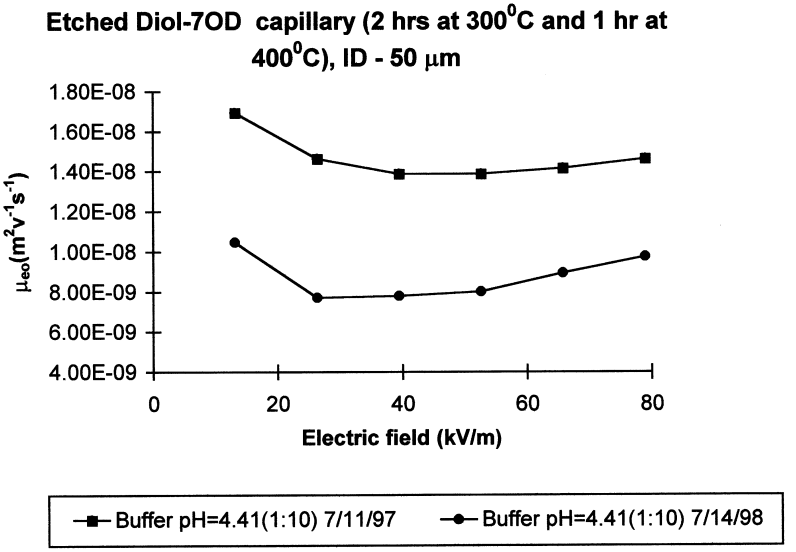


Figure 4. Etched Diol-7Od capillary - electoosmotic mobility measurements vs electric field in one year interval. Marker: DMSO - 0.1 % solution in the buffers; detection wavelength 211 nm.

Figure 5 shows a typical electrochromatogram obtained on the hydride modified capillary for basic proteins. A very good separation was obtained with peaks shape being very symetrical. However the reproducibility of this data was poor as was indicated earlier.

Next five basic proteins were chosen for testing both Diol-AGE and Diol-GPTMS columns, Figures 6 and 7 respectively. A similar separation on Etched Diol-7OD column was reported earlier.[13] The peak shapes for Diol-AGE and Diol-GPTMS capillaries were very symmetrical and excellent separation was obtained. In the case of the Etched Diol-7OD column, peaks were broader due to the mass transfer effects, but the separation was also good. It is worth mentioning, that the migration order for lysozyme and cytochrome C was reversed for the Etched Diol-7OD capillary, when compared with all the other columns tested. This result is significant because it reflects the greater degree of interaction between the protein and the bonded moiety in the etched chemically modified format.

CONCLUSIONS

The Si-C diol modified capillaries prepared by catalytic hydrosilation on a hydride intermediate appear to be well suited for CE and OTCEC separations.

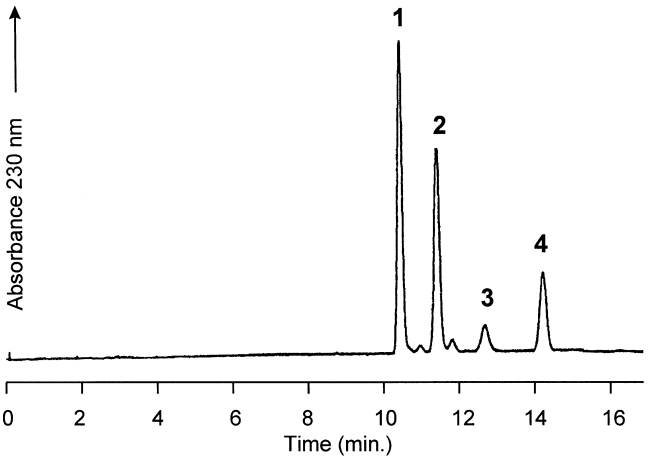


Figure 5. Electrochromatogram of basic proteins obtained on hydride capillary. Total length = 73 cm; effective length = 50 cm; ID = 75 μm; buffer pH = 3.7; applied voltage 20 kV (current 8 μA); detection wavelength 230 nm; protein concentration, 0.25 mg/mL each. Proteins, 1 = lysozyme (turkey), 2 = lysozyme (chicken), 3 = myoglobin, 4 = ribonuclease A.

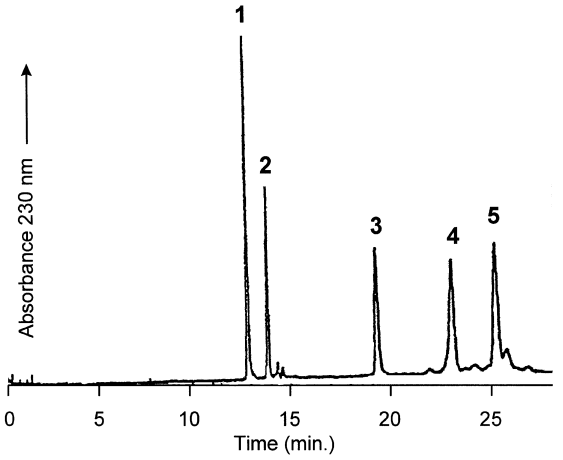


Figure 6. Electrochromatogram of basic proteins obtained on Diol-AGE capillary. Total length = 73 cm; effective length = 50 cm; ID = 75 μm; buffer pH = 3.7; applied voltage 22 kV (current 9 μA); detection wavelength 230 nm; protein concentration, 0.6 mg/mL each. Proteins, 1 = lysozyme (turkey), 2 = cytochrome c(bovine), 3 = ribonuclease A, 4 = trypsinogen, 5 = α-chymotrypsinogen A.

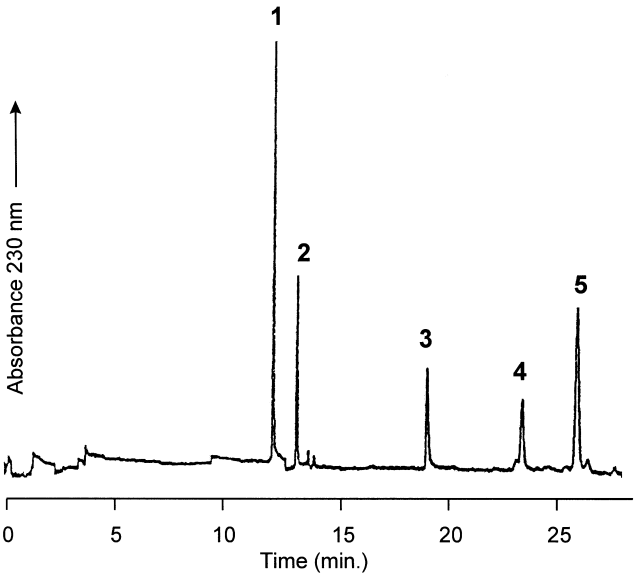


Figure 7. Electrochromatogram of basic proteins obtained on Diol-GPTMS capillary. Total length = 73 cm; effective length = 50 cm; ID = 75 μm; buffer pH = 3.7; applied voltage 22 kV (current 8-9 μA); detection wavelength 230 nm; protein concentration, 0.4 mg/mL each. Proteins, 1 = lysozyme (turkey), 2 = cytochrome c(bovine), 3 = ribonuclease A, 4 = trypsinogen, 5 = α-chymotrypsinogen A.

The overall performance of this type of capillary is superior when compared with capillaries made by the organosilanization process. The etching of the capillary wall in the case of hydride-based columns provided better precision in migration times, by facilitating higher loading of the organic moiety on the surface.

In addition, the etched chemically modified capillaries provided solute/bonded phase interaction that can be exploited in an open tubular capillary electrochromatographic mode.

ACKNOWLEDGMENTS

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REFERENCES

1. M. G. Cikalo, K. D. Bartle, M. M. Robson, P. E. Myers, R. Melvin, Analyst (Cambridge, U.K.), **123(7)**, 87R-102R (1998).

2. J. J. Pesek, M. T. Matyska, Electrophoresis, **18(12-13)**, 2228-2238 (1997).

3. L. A. Colon, K. J. Reynolds, R. Alicea-Maldonado, A. M. Fermier, Electrophoresis, **18(12-13)**, 2162-2174 (1997).

4. J. H. Miyawa, M. S. Alasandro, LC-GC, **16(1)**, 36-41 (1998).

5. K. A. Kelly, M. G. Khaledi, Chem. Anal., **146**, 277-299 (1998).

6. K. D.Altria, N. W. Smith, C. H. Turnbull, Chromatographia , **46(11/12)**, 664-674 (1997).

7. M. M. Dittmann, G. P Rozing, G. Ross, T. Adam, K. K. Unger, J. Capillary Electrophor., **4(5)**, 201-212 (1997).

8. B. Behnke, J. Johansson, S. Zhang, E. Bayer, S. Nilsson, J. Chromatogr., A, **818(2)**, 257-259 (1998).

9. A. M. Fermier, L. A. Colon, J. Microcolumn Sep., **10(5)**, 439-447 (1998).

10. L. A. Frame, M. L. Robinson, W. J. Lough, J. Chromatogr., A, **798(1+2)**, 243-249 (1998).

11. K. J. Reynolds, L. A. Colon, Analyst (Cambridge, U.K.), **123(7)**, 1493-1495 (1998).

12. J. J. Pesek, M. T. Matyska, J. Capillary Electrophor., **4(5)**, 213-217 (1997).

13. J. J. Pesek, M. T. Matyska , L. Mauskar, J. Chromatogr. A, **763**, 307-314 (1997).

14. J. E. Sandoval, M. Chen, Anal. Chem., **68**, 2771-2775 (1996).

15. R. McCormic, Anal. Chem., **60**, 2322-2333 (1988).

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