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Chiral separations by open tubular capillary electrokinetic chromatography

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Abstract

The inner walls of 50 μm fused-silica capillaries are etched by ammonium hydrogendifluoride and then modified by the silanization/hydrosilation method with a chiral selector. Three different types of selectors were evaluated: lactone, β-cyclodextrin and naphthylethylamine. Each of the bonded chiral stationary phases provided at least partial separation for one type of racemic solute. These results confirm that bonded organic moieties on the etched inner wall of a capillary can provided sufficient solute–bonded phase interactions to influence the retention of molecules driven through a capillary by electroosmosis or a combination of electroosmosis and electrophoretic mobility.

Keywords: Enantiomer separation; Electrochromatography; Capillary columns; Stationary phases, LC

1. Introduction

Capillary electrochromatography (CEC) is normally done in a packed column format utilizing bonded stationary phases that are similar to those used in high-performance liquid chromatography (HPLC) [1]. However, other approaches such as the formation of a monolithic structure inside the capillary [2] and deposition of a stationary phase on the inner wall through a sol-gel process [3] have proven to be viable alternatives to packed columns. Capillary electrophoresis (CE) is a method based on an open tubular approach. In CE the separation mechanism involves differences in electrophoretic migration while in CEC the mechanism is based on differences in partition coefficients (k') exclusively for neutral molecules and a combination of k' and electrophoretic mobility (μ_{ep}) differences for charged molecules. One of the main drawbacks in packed column CEC is that the stationary phases typically used are not endcapped so that strong interactions occur between the residual silanols and basic compounds. One possible solution is to use the mixed mode mechanism based on k' and μ_{ep} in the open tubular (OT) format where the number and access of solutes to silanols can be controlled better. This method, OT-CEC, when properly designed could be exploited for the solution of a variety of separation problems from small basic molecules to peptides and proteins.

One recently developed approach to OT-CEC involves etching the inner wall of fused-silica capillaries with an I.D. in the range of 20–50 μm and then subsequently chemically modifying this surface to attach the desired organic moiety [4–7]. The etching agent, ammonium hydrogendifluoride, is prepared in methanol solution which overcomes the problems associated with direct use of hydrogen fluoride (HF). The closed configuration of the capillary results in volatilization of the silica surface upon

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heating and subsequent redeposition to form a variety of morphologies depending on the time and temperature of the treatment process. The etching procedure leads to a dramatic increase in surface area as well as extensions from the inner wall of up to several microns. After chemical modification of this new surface, both of these features will facilitate solute–bonded phase interactions for solutes driven through the capillary by electroosmotic and/or electrophoretic forces.

The use of etched chemically modified capillaries has been applied to a range of small basic compounds as well as proteins and peptides [4–7]. It has already been shown that wall bonded cyclodextrins can be used for the separation of a number of chiral compounds [8–12]. Therefore, it seems reasonable to expect that the presence of chiral selectors on the etched capillary surface should be amenable to enantiomeric separations due to the favorable geometric conditions cited above. This study represents an initial investigation into the feasibility of the OT-CEC format for chiral separations based on bonding of three different types of selectors to an etched surface.

2. Experimental

2.1. Materials

The solutes D,L-doxepine, D,L-nortryptyline, D,L-clomipramine, D,L-chlorodiazepoxide, D,L-oxazepam, D,L-temazepam, D,L-diazepam, D,L-terbutaline, D,L-clenbuterol, D,L-homatropine and D,L-tropicamide were obtained from Sigma (St. Louis, MO, USA) while 3,5-dinitrobenzoyl-D,L-alanine methyl ester was prepared according to a previously described method [13]. The buffers consisted of: pH=2.14, 60 m*M* phosphate (Fisher Scientific, Pittsburgh, PA, USA) and 38 m*M* Tris (Sigma); pH=3.0, 60 m*M* citric acid (Sigma) and 50 m*M* β-alanine (Sigma); pH=3.7, 60 m*M* β-alanine and 60 m*M* lactic acid (Sigma); pH=4.41, 60 m*M* acetic acid (Aldrich, Milwaukee, WI, USA) and 60 m*M* γ-aminobutyric acid (Sigma); pH=6.0, 60 m*M* 2-(*N*-morpholino)ethanesulfonic acid (Sigma) and 45 m*M* histidine (Sigma). Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) and was filtered through a 0.20-μm nylon 66 membrane filter (Alltech, Deerfield, IL, USA). Ammonium hydrogendifluoride used to etch the capillaries was purchased from Aldrich and triethoxysilane for preparation of the hydride intermediate came from United Chemical Technologies (Bristol, PA, USA). The following compounds were used as chiral selectors: 2-hydroxyl-3-methacryloyloxypropyl-β-cyclodextrin (Wacker, Norwalk, CT, USA), (*R*)-(+)-acryloyloxy-β,β-dimethyl-γ-butyrolactone (Aldrich), and 4-allyloxybenzoyl-(*R*)-(+)-1-(α-naphthyl)ethylamine prepared as described previously [13]. Speier's catalyst, hexachloroplatinic acid (Aldrich), was used in the hydrosilation reaction. The capillary tubing used was 375 μm O.D.×50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA).

2.2. Instrumentation

OT-CEC separations and electroosmotic mobility measurements were made on an Applied Biosystems Model 270A-HT (Foster City, CA, USA) CE system. Infrared spectra were obtained on a Mattson (Madison, WI, USA) Infinity Series Fourier transform (FT) IR spectrometer equipped with a mercury cadmium telluride (MCT) detector. The spectra (1000 scans) were obtained in the diffuse reflectance mode using a Pike Technologies accessory (Madison, WI, USA) over a range of 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1}.

2.3. Capillary preparation

Procedures for preconditioning of the capillary surface with ammonia, the etching with ammonium hydrogendifluoride, the preparation of the hydride intermediate, and the hydrosilation reaction for attaching the desired organic moiety to the inner wall of the capillary are described in detail elsewhere [4]. The only change involved the preparation of the β-cyclodextrin capillary where the solvent used for hydrosilation was methanol–ethanol (75:25).

2.4. Electrochromatography

All capillaries were conditioned first by forcing at least 50 column volumes of buffer through them with

a syringe. The mobile phases were degassed by ultrasonication followed by purging with He. Injection of samples was done both hydrodynamically by vacuum and electrokinetically. Samples were detected at 254 nm and the marker (dimethylsulfoxide, DMSO) at 211 nm. Electroosmotic mobility values (an average of three measurements) were determined at constant temperature (30°C) by the two-marker injection method designed for determining low electroosmotic velocities [14]. Samples were typically prepared by dissolving 1 mg of the racemic mixture in 1 ml of deionized water and stored at 13°C. When two peaks were present for an enantiomeric compound on a chiral column, an achiral column (C_{18}) was used to verify that there was only a single peak under the same experimental conditions.

3. Results and discussion

3.1. Diffuse reflectance infrared Fourier transform (DRIFT) characterization

Both the small area and the inaccessibility of the inner bore present challenges for characterizing the internal surfaces of capillaries. For porous silica and other oxide materials, various spectroscopic methods have been developed to provide information about the surface as well as attached or adsorbed moieties [15]. However, the higher surface areas created by the etching process may allow some methods of spectroscopic analysis to be used for characterization of the organic moieties bonded to the inner wall. It has already been demonstrated that scanning electron microscopy (SEM) provides a topographical picture of this new surface [4]. For chemical information, DRIFT analysis might be possible if a sufficient amount of the inner surface is exposed and a sensitive detector like the MCT is used. Therefore, two capillaries with different chiral selectors were stripped of their polyimide coating and broken open so that the etched inner bore was exposed. These samples were further crushed and mixed with 5% spectroscopic grade KBr. The spectrum of this mixture was then taken in the DRIFT mode and referenced against a KBr blank in order to subtract out background effects.

Fig. 1a shows the partial DRIFT spectrum obtained for the cyclodextrin-modified etched capillary. It contains two essential features that have been identified on porous silica materials modified by the silanization/hydrosilation method: the Si–H stretching band at 2270 cm^{-1} and peaks in the C–H stretching region between 2800 and 3000 cm^{-1} [16]. Neither of these features are observed on an etched capillary that has not been chemically modified by the silanization/hydrosilation process. Other potential features of the cyclodextrin spectrum such as C–O, C=O and OH bands are either too weak to be observed or are masked by the residual signals from the silica matrix and/or water adsorbed on the surface. Fig. 1b shows the partial DRIFT spectrum for the lactone bonded etched capillary. Since the lactone moiety is much smaller than cyclodextrin, only a weak signal is observed in the C–H stretching region. The Si–H band is not seen in this particular sample. This result may be due to the fact that less hydride was present after the silanization process, the surface area produced in the etching process is lower on this capillary or a higher concentration of lactone than cyclodextrin per unit surface area was bonded to the inner wall of the capillary. The latter would result in fewer Si–H sites on the surface and could still produce a lower signal in the C–H region because the size of the cyclodextrin molecule is so much greater than the lactone moiety (M_r=1135 vs. 184) and contains more aliphatic carbon hydrogen groups per mole (27 vs. 6). These preliminary experiments indicate that DRIFT spectroscopy could be a useful tool for providing information about the nature of chemically modified etched capillary surfaces. Additional studies are currently underway with other bonded moieties in order to determine if better sensitivity can be achieved so that more spectral features can be observed.

3.2. Electroosmotic mobility measurements

The etching process alters not only the topography of the surface but also results in a chemical change as well [7]. The silica matrix at the etched inner wall contains some components from the ammonium hydrogendifluoride reagent. The exact nature of these species is not known at this point and is under investigation. However, the presence of species other

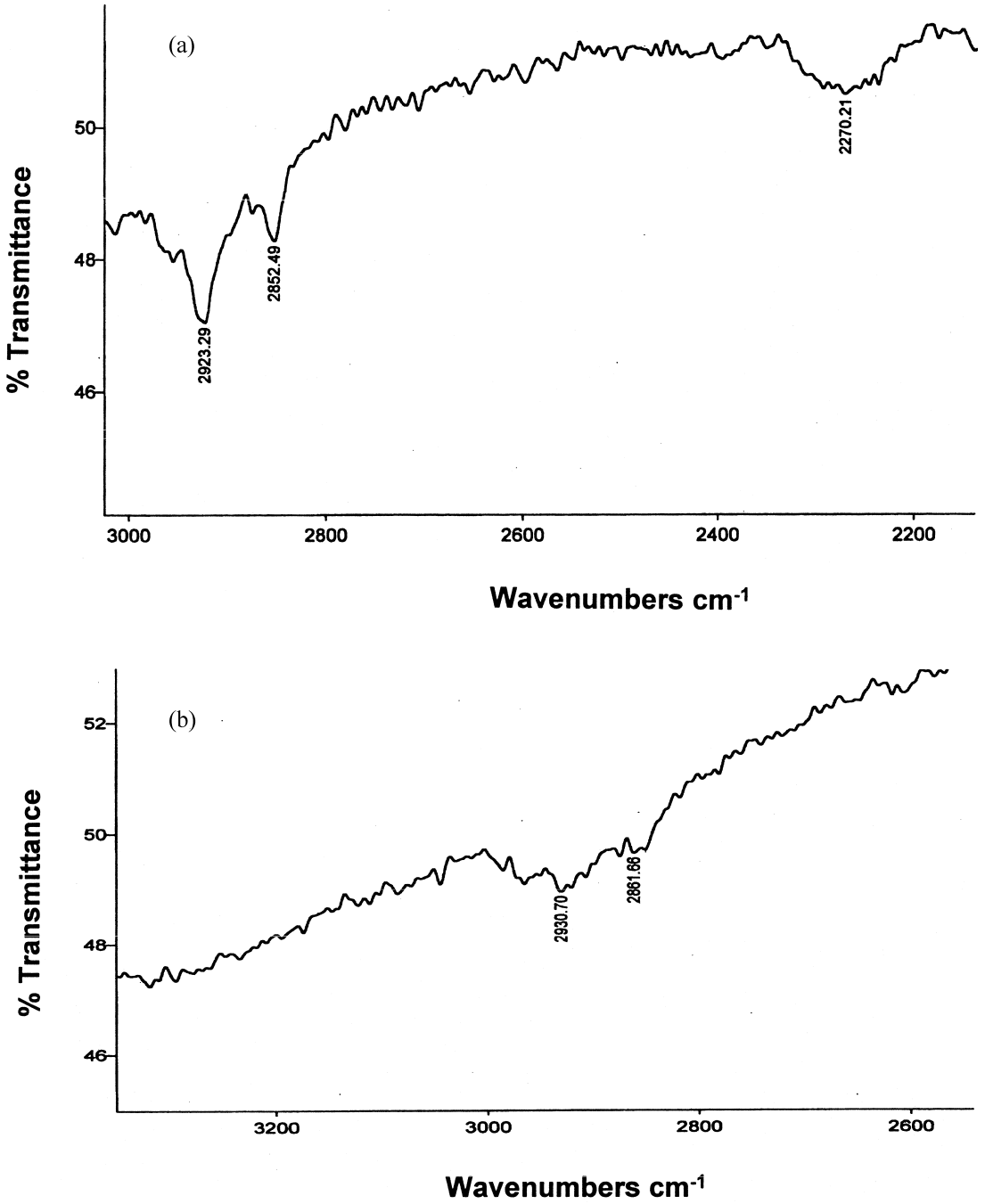

Fig. 1. Partial DRIFT spectra of the internal surface of etched 50 μm capillaries modified with (a) 2-hydroxy-3-methacryloxy-propyl-β-cyclodextrin and (b) (*R*)-(+)-α-acryloyloxy-β,β-dimethyl-γ-butyrolactone.

than siloxanes and silanols is evident from electro-osmotic mobility measurements. A typical result for etched chemically modified capillaries of μ_{eo} as a function of pH at two different electric fields is shown in Fig. 2a for the lactone column. The interesting aspect of this plot and those in a previous

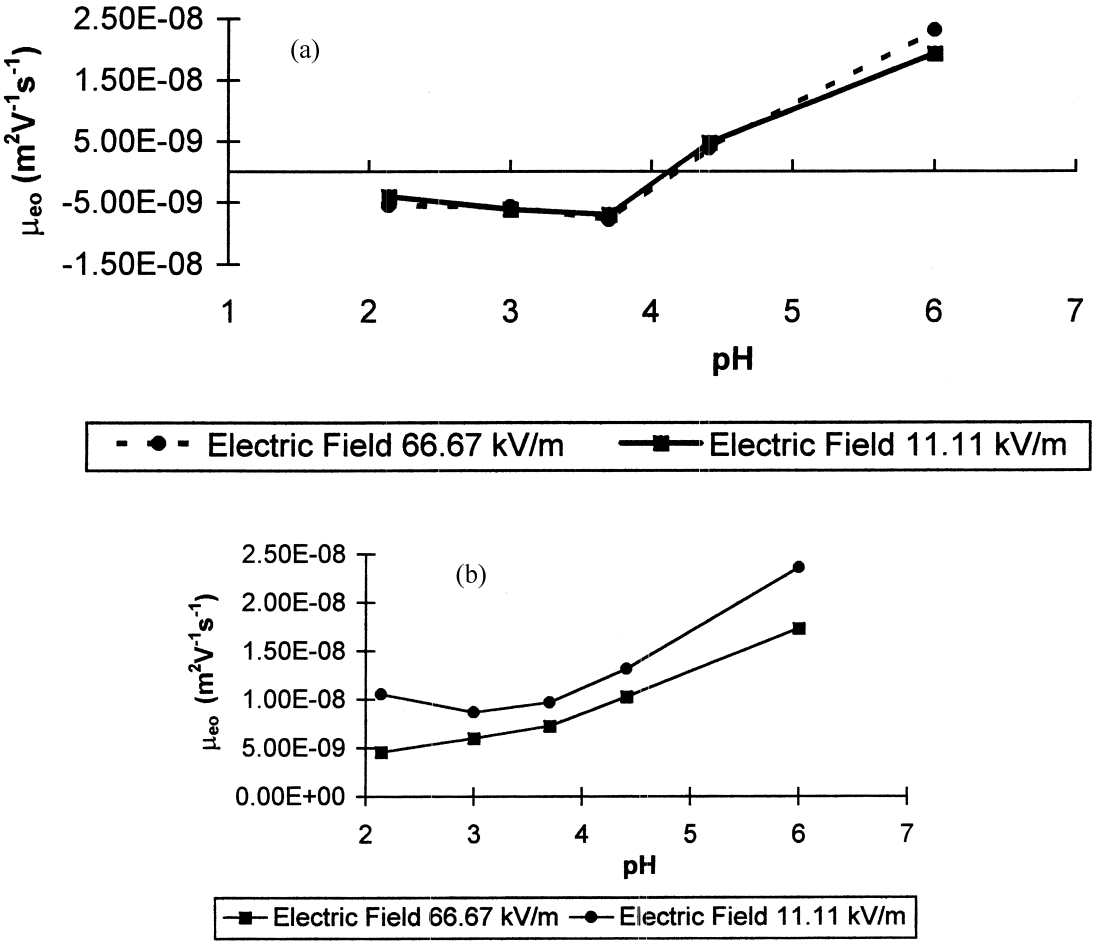

Fig. 2. Electroosmotic mobility as a function of pH at two electric field strengths for an etched 50 μm capillary modified with (a) (*R*)-(+)-α-acryloyloxy-β,β-dimethyl-γ-butyrolactone and (b) 2-hydroxy-3-methacryloxy-propyl-β-cyclodextrin. RSD=1–3%.

study [7] is the presence of an anodic electroosmotic flow (EOF) at a pH less than ≈4.5. This result suggests that a nitrogen containing species from the etching agent has become a part of the surface structure. At higher pH values the EOF switches to the cathodic direction. However, both the anodic and cathodic EOF are quite low. A somewhat different result is obtained for the etched cyclodextrin modified capillary as shown in Fig. 2b. Even though the EOF is still low, it is cathodic at all pH values over the range measured. Since the etched surface is presumably the same or similar to the lactone capillary, the reason for the change in the direction of EOF at low pH values is not known at present. More detailed studies of these surfaces by electron spectrometry for chemical analysis (ESCA) is underway to identify the exact nature of the various chemical species in the etched silica matrix.

3.3. Chiral separations

To date many chiral selectors developed for chromatographic processes successfully resolve only a limited number of racemic mixtures. Therefore, for the three phases attached to the etched capillary in this study a variety of enantiomeric compounds were used as test solutes to determine if any chiral resolution could be obtained. The selected compounds included several tricyclic antidepressants, benzodiazepines, dansyl amino acids as well as a few

miscellaneous drugs such as terbutaline, clenbuterol homatropine and tropicamide.

Each of the test solutes was injected into the lactone capillary using a variety of pH (buffer) and voltage conditions. Only some of the tricyclic antidepressants, doxepine (α=1.07), nortryptyline (α=1.02) and clomipramine (Fig. 3, α=1.06) showed any resolution of the two isomers. Injection of these three compounds on an achiral column (etched C_{18}) resulted in a single peak. While the separation is less than ideal since all are similar to the electrochromatogram in the figure, this result demonstrates that selective interaction between enantiomers and a chiral selector can be generated in the etched chemically modified capillary format. It appears that under the conditions of this experiment (pH=3), clomipramine is neutral since negative voltage is required to elute this compound from the column. As described above (Fig. 2a), the etched lactone modified capillary has an anodic EOF at this pH. Therefore, there is no contribution from electrophoretic mobility to the migration of the isomers allowing for k' interactions between the solutes and the bonded selector.

Cyclodextrins and substituted cyclodextrins in the α, β and γ forms have provided the broadest range of chiral separations in HPLC [17]. This fact and the success of some chiral separations for immobilized β-cyclodextrin on ordinary capillaries [8–12] appeared to make attachment of this moiety to the etched surface a viable option. Many of the solutes selected for testing in this study were successfully resolved by β-cyclodextrin stationary phases in HPLC [18]. For this particular etched capillary, benzodiazepines were the only class of compounds resolved among the test solutes. At least partial resolution of the two isomers was obtained for oxazepam (α=1.05), temazepam (α<1.01), chlorodiazepoxide (Fig. 4, α=1.04) and diazepam (Fig. 5, α=1.11). When these four samples were injected into the achiral column a single peak resulted in all cases except for chlorodiazepoxide where two peaks were observed because of an impurity present. The somewhat broader first peak observed in the separation of diazepam (Fig. 5) may also be due to the presence of an impurity. In contrast to the lactone capillary, the β-cyclodextrin column under the running conditions used for the separations reported

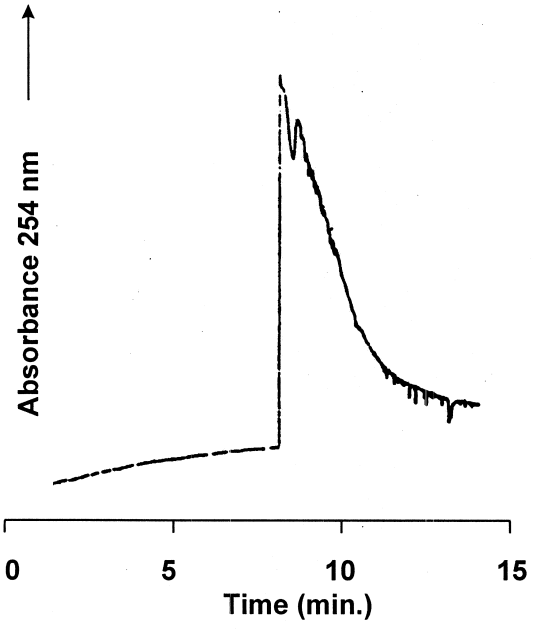

Fig. 3. Electrochromatogram for the separation of D,L-clomipramine on the etched lactone modified capillary. Capillary 32 cm (effective length 24 cm); pH=3.0; V=−10 kV.

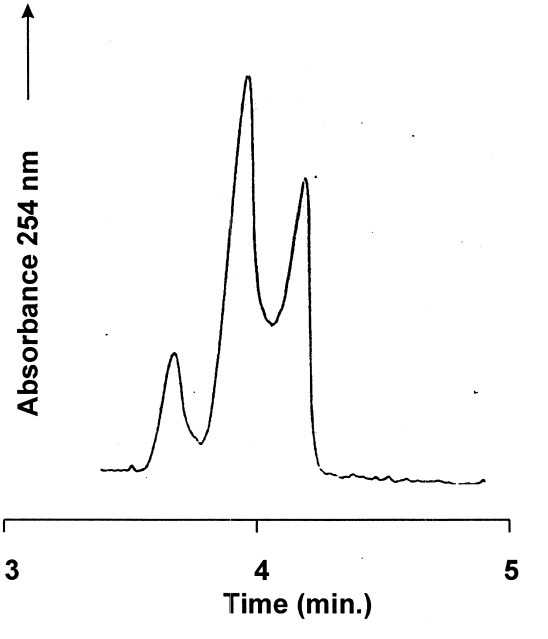

Fig. 4. Electrochromatogram for the separation of D,L-chlorodiazepoxide on the etched cyclodextrin-modified capillary. Capillary 45 cm (effective length 25 cm); pH=3.0; V=20 kV. The first peak in the electrochromatogram is an impurity.

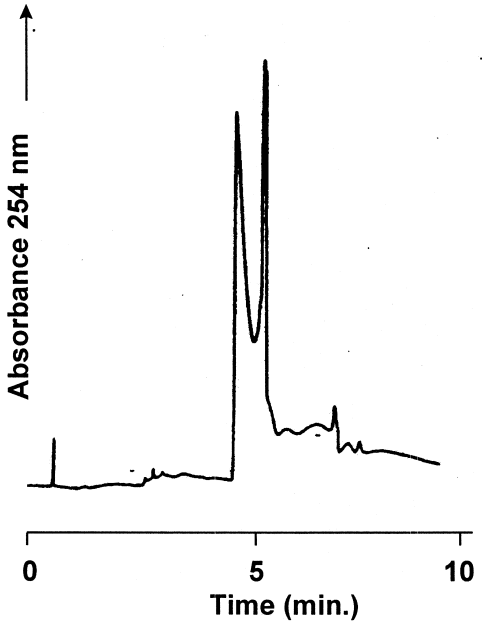

Fig. 5. Electrochromatogram for the separation of D,L-diazepam on the etched cyclodextrin-modified capillary. Capillary 45 cm (effective length 25 cm); pH=3.0; *V*=30 kV.

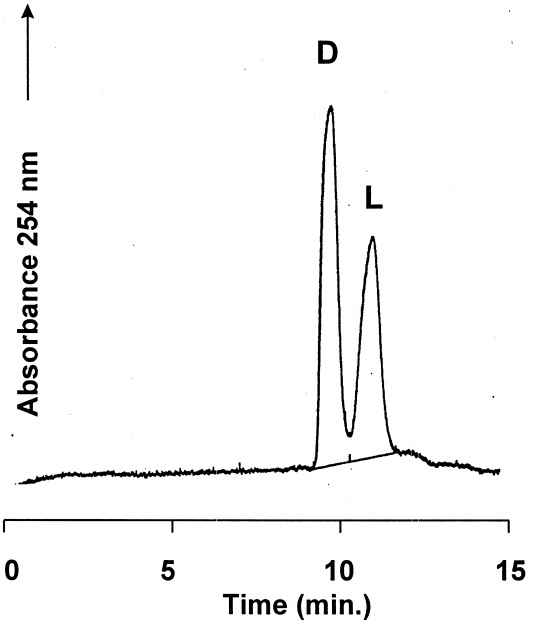

Fig. 6. Electrochromatogram for the separation of 3,5-dinitrobenzoyl-D,L-alanine methyl ester on the naphthylethylamine-modified capillary. Capillary 50 cm (effective length 37 cm); pH=2.14; *V*=30 kV.

above has a cathodic EOF as shown earlier (Fig. 2b). In comparison to the separations obtained on the lactone capillary, the two electrochromatograms shown for the β-cyclodextrin column have both better efficiency and peak symmetry.

The final etched capillary was modified with the same chiral selector reported in a previous HPLC study [13] and based on naphthylethylamine. None of the test solutes used on the other two columns were successfully resolved with this bonded phase. Finally, one of the enantiomeric compounds that was resolved by HPLC was chosen for evaluation in the etched capillary format. The electrochromatogram obtained for 3,5-dinitrobenzoyl-D,L-alanine methyl ester is shown in Fig. 6. The resolution (α=1.14), efficiency and peak symmetry is the best obtained on any of the three columns. However, as demonstrated in HPLC experiments changes in the amino acid portion of the molecule (i.e., alanine to valine) often result in a loss of chiral resolution. This is not surprising since the separation mechanism in the HPLC and electrochromatographic modes is identical.

In conclusion, this study demonstrates that etched capillaries chemically modified with a chiral selector can resolve at least some enantiomeric compounds. It appears that each selector can resolve only a limited number of optical isomers similar to many of the chiral stationary phases used in HPLC. The stability of the columns was also good since each one was used for at least 200 injections with no observable changes in the enantiomeric resolution described above. However, these are still preliminary studies and the etching conditions as well as the chiral selectors may not yet be optimized. The presence of solute–bonded phase interactions has been verified by these studies since only this mechanism can be responsible for the resolution of enantiomers. Currently other chiral selectors and various types of organic moieties are being evaluated as bonded phases for etched chemically modified capillaries in OT-CEC.

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