Sol–gel-derived fluorinated stationary phase for open tubular electrochromatography

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Abstract

We report on the preparation of a sol–gel-derived fluorinated stationary phase using a 13 μm I.D. fused-silica capillary for open tubular electrochromatography (OTEC). The sol–gel-derived fluorinated column is prepared by hydrolyzing a mixture of tetraethoxysilane (TEOS) and tridecafluoro-1,1,2,2-tetrahydrooctyl-1-triethoxysilane (F_{13}-TEOS) followed by coating a thin film onto the inner walls of a fused-silica capillary. The retention characteristics of the sol–gel-derived fluorinated column are compared with a sol–gel-derived octylhydrocarbonaceous (C_8) column. Using the sol–gel-derived fluorinated column, we report on the successful separation of six model fluorinated organic compounds which cannot be separated using the C_8 column prepared either via sol–gel method or by conventional methods. We achieved high efficiencies of 100 000–300 000 plates/m for the various model fluorinated compounds using the sol–gel-derived fluorinated column. We have also investigated the separation performance of the sol–gel-derived fluorinated column using different TEOS:F_{13}-TEOS ratios and the optimum reaction time before column coating. Optimum selectivity was obtained with a TEOS:F_{13}-TEOS molar ratio of 1:2 reacted for 24 h prior to column coating.

Keywords: Electrochromatography; Stationary phases, LC; Fluorinated stationary phases; Fluorinated compounds

1. Introduction

Reversed-phase high-performance liquid chromatography (HPLC) is generally practiced using straight chain hydrocarbonaceous bonded phases (e.g., C_{18} and C_8) because of their versatility and selectivity in separating a wide variety of organic compounds [1–6]. Recently, there has been great interest in using fluorinated hydrocarbonaceous stationary phases (more commonly known as fluorinated stationary phases) which offer certain advantages over the conventional hydrocarbonaceous

stationary phases [6–12]. Fluorinated stationary phases have been demonstrated to possess high selectivity and specificity for fluorinated compounds [13]. They have also been used for separation of esters and ketones [6]. Perfluorocarbon bonded phases have been used to separate substances of high chemical reactivity such as metal fluorides, halogens, interhalogen compounds and halides of sulfur, hydrogen and phosphorus [13]. In general, these compounds can destroy conventional stationary phases and thus, can only be separated on chemically inert perfluorocarbon stationary phases. Another important application of fluorinated stationary phases is the separation of perfluorocarbon compounds and freons

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which are difficult to separate using conventional hydrocarbonaceous stationary phases [12]. Fluorinated stationary phases have also been used for the enhanced recovery of active proteins during their separation [8]. In their report, Xindu and Carr demonstrated that the retention of non-polar solutes on fluorocarbon bonded phases is considerably lower compared to hydrocarbonaceous stationary phases under identical mobile phase conditions [8]. Therefore, a minimum amount of organic modifier is required in the mobile phase, which leads to the reduced denaturing of the proteins and thus, a higher yield of the active protein separated.

Most of the work so far with fluorinated stationary phases has been performed using HPLC. Mao et al. have prepared a fluorocarbon-coated capillary for the separation of proteins using capillary electrophoresis [14]. They used conventional coating procedures for preparing the stationary phases. The practical constraints of regular HPLC include: (1) the inability to separate and detect low quantities of material, typically contained in low volume samples (e.g., nl); and (2) high back pressures generated in the column [15]. The smaller the particle size used for packing an HPLC column, the greater the back pressure developed. A more recent separation technique, open tubular electrochromatography (OTEC) [15–17] alleviates some of the problems encountered in HPLC. OTEC is a hybrid technique between open tubular liquid chromatography (OT-LC) [18–20] and capillary electrophoresis (CE) [21]. OTEC can be performed using capillaries of various inner diameters (5–50 μm), which enables separation of samples to nl volumes. In OTEC, the solvent is transported through the column by an electrically driven flow, known as the electroosmotic flow (EOF), instead of the hydraulic flow used in HPLC or OT-LC. The major advantages of the EOF over hydraulic flow are: (1) practically no column back pressure and (2) a reduced band broadening, due to the EOF plug-flow profile, hence high efficiency [15,22].

Recently, in our laboratory, a new coating material was developed based on the sol–gel process to prepare stationary phases for OTEC [16,17]. It was demonstrated that the sol–gel-processed stationary phases offer many advantages over conventional methods to prepare stationary phases. These include high hydrolytic stability, high mass loadability, high

surface area leading to a higher retention, high column efficiency and a relatively simple preparation procedure [16,17].

The sol–gel process has been described in detail in many recent review articles [23,24]. Briefly, the sol–gel process is a low temperature procedure to prepare glasses, and can provide materials with high optical quality. The sol–gel process consists of five major steps: hydrolysis; condensation and polycondensation; casting; aging and drying. The first step involves the hydrolysis of a metal alkoxide under acidic or basic conditions to form the corresponding metal hydroxide which further undergoes condensation and polycondensation to form "sol" particles. The "sol" particles crosslink to form a "gel". Before the formation of a "gel", one can cast the "sol" solution in the form of a thin film or mold it into any desired shape, such as a disc, slab, etc. Finally, the "gel" is kept for aging under room temperature and pressure conditions to form a glassy material. At this stage, it is termed as a "xerogel". If desired, one can dry the "xerogel" under elevated temperatures to completely remove the solvent from the pores of the "gel".

In this paper, we describe the first attempt to use a fluorinated stationary phase prepared by the sol–gel process for the separation of fluorinated organic compounds using OTEC. Two conventional methods to prepare stationary phases for OTEC are: (1) direct chemical attachment of the organically modified silane onto the inner walls of the capillary; and (2) a two step process where first an active porous silica layer is coated onto the inner walls of the capillary followed by chemical attachment of the functional groups. Both of these methods are prone to disadvantages [16], such as lower hydrolytic stability (limited pH working range), limited retentive and mass loadability characteristics, and in some cases tedious preparation protocols; these limit the use of such columns for chromatographic applications.

The sol–gel-derived fluorinated column described here was prepared by hydrolyzing a mixture of tridecafluoro - 1,1,2,2 - tetrahydrooctyl - 1 - triethoxysilane (F_{13}-TEOS) and tetraethoxysilane (TEOS) (2:1 molar ratio) followed by casting a thin film onto the inner walls of the fused-silica capillary. We report on the separation of a mixture of six model fluorinated organic compounds using this fluorinated

sol–gel-derived stationary phase. In contrast, octylhydrocarbon stationary phases prepared by either the sol–gel process or by conventional methods did not separate the model fluorinated organic compounds. We observed that the sol–gel-derived fluorinated stationary phase separated not only the fluorinated compounds but also model halogenated organic compounds. The sol–gel-derived fluorinated stationary phase is demonstrated to be very efficient as we are able to baseline separate even a mixture of *ortho-*, *meta-* and *para-*difluorobenzenes.

2. Experimental

2.1. Instrumentation

All the OTEC experiments performed in this work were performed on an in-house CE system constructed in our laboratory. The electric field was supplied by a Glassman (Whitehouse Station, NJ, USA) high voltage power supply (0–30 kV). The high voltage was housed in a plexiglass box for safety purposes. On-column detection was performed using a UV detector (Model CV^4) at 214 nm. The signal from the UV detector was fed into an A/D converter board (DT2804, Data Translation, Marlboro, MA, USA) mounted on an IBM personal computer. Data acquisition was controlled by means of GRAMS 386 for chromatography software (Galactic Industries, Salem, NH, USA). Samples were injected into the column using electrokinetic injection. To measure the electroosmotic mobility, a small amount of ethanol was added to the sample solution. Ethanol served as the unretained neutral marker as it generated a deflection in the baseline when passing by the detector.

2.2. Chemicals

Tetraethoxysilane (TEOS), *n*-octyltriethoxysilane (C_8-TEOS) and tridecafluoro-1,1,2,2-tetrahydrooctyl-1-triethoxysilane (F_{13}-TEOS) were purchased from United Chemical Technologies (Horsham, PA, USA). Water was purified using a Milli-Q UV Plus water purification system, fed from a Milli-RO 10 Plus reversed osmosis system (Millipore, Bedford, MA, USA). The mobile phase was prepared by mixing methanol and phosphate buffer (pH 7.0; 10 m*M*) in a 1:1 ratio. Fluorinated benzenes were used as test compounds and were obtained from Aldrich (Milwaukee, WI, USA). For all our work described here, the concentration of all the fluorinated compounds used was 50 m*M*. The test stock solutions were prepared in HPLC-grade methanol. Appropriate dilutions were performed prior to injection using the mobile phase as the solvent. All the solutions were filtered through 0.45 μm nylon membrane filters and degassed prior to use. The fused-silica capillaries used in the experiments (13 μm I.D.×350 μm O.D.) were obtained from Polymicro Technology (Phoenix, AZ, USA).

2.3. Column preparation

The column preparation involved the following steps [16]: (1) pretreatment of the capillary: the fused-silica capillary was flushed with sodium hydroxide (1 *M*) and then with distilled water for 1 h each. The capillary was then kept under a flow of nitrogen at 180°C to dry overnight. (2) Coating procedure: the sol–gel solution was prepared by mixing F_{13}-TEOS, TEOS, ethanol, hydrochloric acid and water. Different molar ratios of silanes were mixed (TEOS:F_{13}-TEOS; 1:1, 1:1.5, 1:2) with appropriate amounts of the catalyst and the solvent. The water to silane ratio was kept at 4:1 for all the sol–gel solutions prepared. For example, to prepare a 1:1 (TEOS:F_{13}-TEOS) sol–gel solution, the following amounts of each chemical were used: 0.5 ml TEOS, 0.846 ml F_{13}-TEOS, 0.0896 ml HCl (0.1 *M*), 0.486 ml ethanol and 0.282 ml water.

The pH of the sol–gel solution was observed to be about 5, as measured by a short-range Alkacid test paper (Fisher Scientific, USA). The sol–gel solution was stirred for 24 h at room temperature before coating the capillary. This sol–gel solution was allowed to react with the walls of the capillary for 15–20 min. The excess solution was then flushed out under nitrogen flow for a few minutes. The coated capillary column was subsequently dried overnight in an oven at 70°C under nitrogen flow. After drying, this column was washed with acetone, methanol, and distilled water. The column was equilibrated with the mobile phase for 30 min prior to use. The thickness

of a sol–gel coating material inside of a capillary is about 0.5 μm [17].

3. Results and discussion

The sol–gel process commonly involves the use of one precursor, a metal alkoxide, which is hydrolyzed with water under acidic or basic conditions [23,24]. In this work, we mixed two precursors, TEOS and F_{13}-TEOS to prepare the sol–gel matrix. TEOS was used for the formation of a silica (SiO_2) backbone and F_{13}-TEOS was used to introduce the fluorinated moiety as the functional group into the sol–gel matrix. Upon hydrolysis of the mixture of these two precursors, the resulting sol–gel solution was cast as a thin film onto the walls of the capillary to prepare the sol–gel-derived fluorinated column.

A mixture of up to six model fluorinated organic compounds were used to illustrate the selectivity of the sol–gel-derived fluorinated column and compared to a hydrocarbonaceous column. This mixture includes fluorobenzene, three isomers of difluorobenzene (*ortho*, *meta* and *para*), trifluorobenzene and tetrafluorobenzene. We investigated the separation performance for the model compounds in three different sol–gel-derived columns: (1) a fluorinated column; (2) a hydrocarbonaceous column (C_8) and (3) a thin porous silica film-base column (no functional group added). The columns were fabricated by coating 13 μm I.D. fused-silica capillaries with the appropriate sol–gel solution. The solutions were prepared by hydrolysis of a mixture of TEOS and F_{13}-TEOS in 1:1 molar ratio for the fluorinated column, a mixture of TEOS and octyltriethoxysilane (C_8-TEOS) in a 1:1 molar ratio for the C_8 column, and only TEOS for the porous silica film column. In all cases, the hydrolysis was carried out under acidic conditions with a silane-to-water molar ratio of 1:4. We injected a mixture of five model fluorinated organic compounds (fluorobenzene, *ortho*-, *meta*- and *para*-difluorobenzene and trifluorobenzene) into each of three columns. Similar experimental conditions were used for each column. Typical electrochromatograms are presented in Fig. 1.

Fig. 1a represents an electrochromatogram for the sol–gel porous silica film column. As expected in this case, we observed no separation and all five
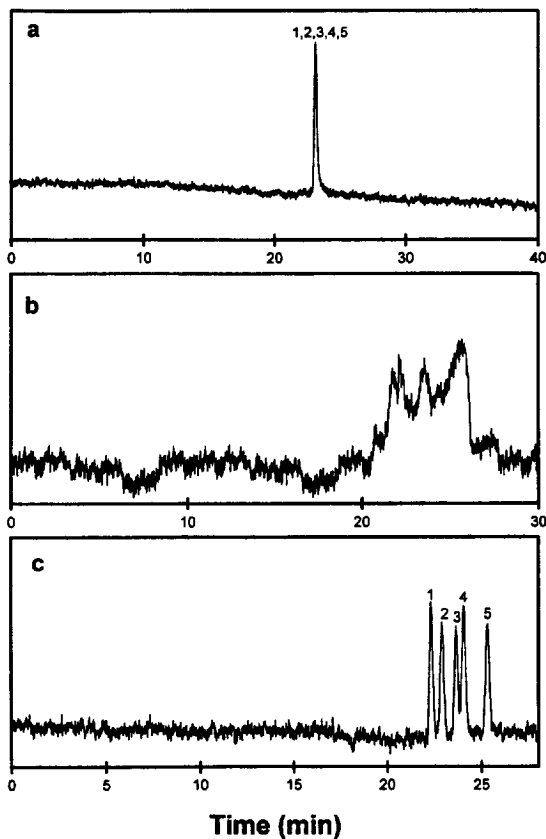

Fig. 1. Separation of a mixture containing five fluorinated organic compounds by OTEC in three different capillaries: (a) capillary coated with TEOS, (b) capillary coated with C_8-TEOS:TEOS (1:1) and (c) capillary coated with TEOS:F_{13}-TEOS (1:2), all solutions were reacted for 24 h before coating. Separation conditions: capillary column, 39 cm injection to detection; total length=60 cm×13 μm I.D.; mobile phase, methanol–10 mM phosphate buffer pH 7 (50:50); separation voltage, 12 kV; electrokinetic injection, 4 s at 6 kV; detection, UV at 214 nm. The test mixture contained: (1) fluorobenzene; (2) 1,4-difluorobenzene; (3) 1,3-difluorobenzene; (4) 1,2- difluorobenzene; (5) 1,2,4-trifluorobenzene.

fluorinated compounds co-eluted as a single peak. Fig. 1b represents an electrochromatogram for a column where C_8-TEOS was used as one of the precursors. Here, we observed an apparent interaction of the fluorinated organic compounds with the stationary phase; however, the separation efficiency is very poor, none of the compounds are baseline separated, and it is difficult to identify the peaks. It should be noted that the type of sol–gel-derived C_8-column used here was similar to the one used to

separate polycyclic aromatic hydrocarbons (PAHs) [16,17]; we were also able to separate the same PAHs using our C_8-column (see below). In contrast to the sol–gel C_8-column, the sol–gel-derived fluorinated column shows baseline separation for all the five model compounds (Fig. 1c). In the electrochromatogram shown in Fig. 1c, the order of elution for the compounds is as follows: fluorobenzene; 1,4-difluorobenzene; 1,3-difluorobenzene; 1,2-difluorobenzene; 1,2,4- trifluorobenzene. The elution order of the peaks was determined by performing injections of each individual compound. From the elution order of the compounds, we can infer that the higher the degree of fluorination on the compound, the longer it is retained in the column.

Using the sol–gel-derived C_8-column, we separated a mixture of PAHs containing naphthalene, fluorene and dimethylnaphthalene; these compounds were used as models to test sol–gel derived columns [16,17]. We also used this sample mixture to test the sol–gel-derived fluorinated column; all three sample components were separated as shown in Fig. 2. In order to investigate the selectivity of the sol–gel-derived fluorinated column toward halogenated compounds, we injected a mixture of fluorobenzene bromobenzene, dichlorobenzene, diflurobenzene and tetrafluorobenzene into the sol–gel-derived fluori-
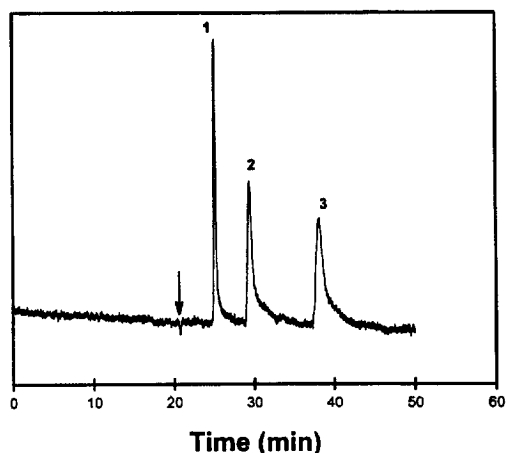
nated column. The resulting electrochromatogram is shown in Fig. 3. All model compounds are separated, demonstrating that the fluorinated phase is capable of separating the different halogenated species. This suggests that there are sufficient fluorinated moieties available on the sol–gel layer to undergo strong fluorine–halogen interactions. The separations shown in Figs. 2 and 3 show that a fluorinated sol–gel-derived column can effectively separate fluorinated and non-fluorinated organic compounds. The separation of the fluorinated compounds achieved in the fluorinated column but not in the C_8 one (see Fig. 1), is indicative that retention is primarily due to fluorine–fluorine interactions in the fluorinated column.

For optimization of a chromatographic separation, the ratio of the time spent by the solute in the stationary phase to the time it spends in the mobile phase is fundamentally more important; this is the solute retention factor (k). In order to illustrate the effect of reaction time of the silane precursors on the
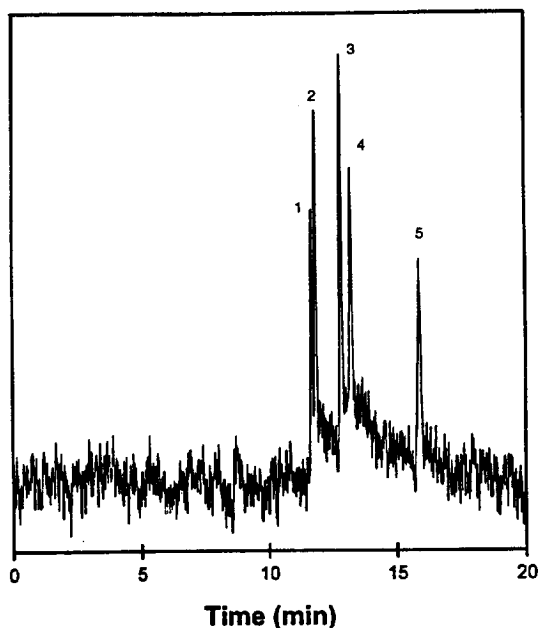
Fig. 2. Separation of a mixture containing three polycyclic aromatic hydrocarbons by OTEC. Capillary coated with a TEOS:F_{13}-TEOS (1:2) sol–gel solution, reacted for 24 h. The test mixture contained: (1) naphthalene, (2) fluorene and (3) dimethylnaphthalene. The arrow indicates the EOF marker. Separation conditions as in Fig. 1 but separation voltage of 20 kV.

Fig. 3. Separation of a mixture containing halogenated organic compounds by OTEC. Capillary coated with TEOS:F_{13}-TEOS in the ratio of 1:2. The test mixture contained: (1) fluorobenzene; (2) bromobenzene; (3) 1,2-dichlorobenzene; (4) 1,2-difluorobenzene and (5) 1,2,4-trifluorobenzene. Separation voltage was 20 kV. Other separation conditions as in Fig. 1.

retention characteristics of the column, we prepared sol–gel-derived fluorinated columns by reacting a mixture of TEOS and F_{13}-TEOS (molar ratio 1:2) and coating the columns at different times. We then compared the retention factors of three model fluorinated compounds (1,2,4-trifluorobenzene, 1,2,3,5-tetrafluorobenzene and para-difluorobenzene) on these sol–gel-derived fluorinated columns. Fig. 4 represents the effect of reaction time on the retention factors of the three fluorinated organic compounds. In the first 12 h of reaction, there is minimal change in the retention factor for all three cases. But after 12 h, we observe an increase in the retention factor that seems to remain relatively constant after 24 h of reaction. Therefore, in all subsequent studies, at least 24 h of reaction time were used before coating capillaries with the fluorinated sol–gel solution.

The retention characteristics of the solutes can be changed by varying the molar ratio of the precursors used in the sol–gel material [16,17]. We investigated the retention characteristics of columns prepared with 1:1, 1:1.5 and 1:2 mixtures of the two silanes, TEOS and F_{13}-TEOS. A mixture of the six model fluorinated organic compounds was used to investigate these columns. Fig. 5 represents the electrochromatograms for two columns, one prepared by

Fig. 5. Separation of a mixture containing six model fluorinated organic compounds by OTEC in two different capillaries coated with sol–gel solutions containing (A) 1:1 and (B) 1:2 TEOS:F_{13}-TEOS ratios. Separation voltage was 20 kV. Other separation conditions as in Fig. 1. The mixture contained: (1) fluorobenzene; (2) 1,4-difluorobenzene; (3) 1,3-difluorobenzene; (4) 1,2-difluorobenzene; (5) 1,2,4-trifluorobenzene and (6) 1,2,3,5-tetrafluorobenzene.
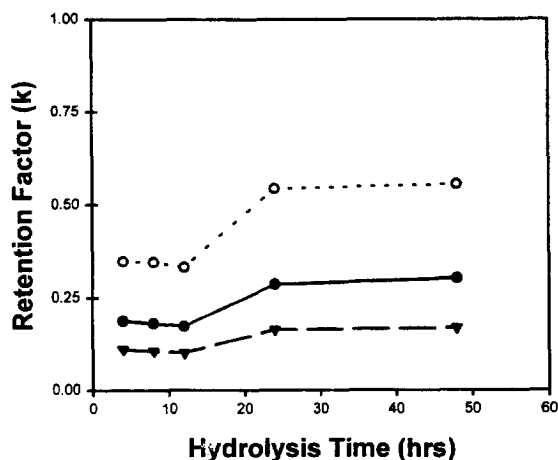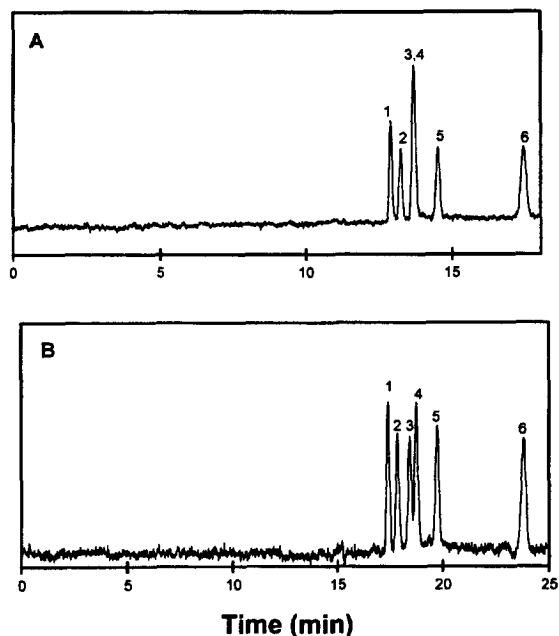

Fig. 4. Effect of reaction time of the precursors on the retention factor for three model compounds separated by OTEC. The capillary column was coated with a solution containing a TEOS:F_{13}-TEOS ratio of 1:2. Separation voltage was 20 kV. Other separation conditions as in Fig. 1. Test compounds were: 1,2,3,5-tetrafluorobenzene (O); 1,2,4-trifluorobenzene (●); para-difluorobenzene (▼).

mixing the silanes in a 1:1 molar ratio (Fig. 5A), and the second, prepared by mixing TEOS and F_{13}-TEOS in the molar ratio of 1:2 (Fig. 5B). The electrochromatogram in Fig. 5A clearly shows that the column coated with a 1:1 silane ratio cannot separate ortho- and meta-difluorobenzene from the given mixture of six compounds. For the column coated with a 1:2 silane ratio (Fig. 5B), a higher degree of separation is achieved for the six fluorinated compounds, thus, showing a higher selectivity than the column coated with a 1:1 silane ratio. The separation of the ortho- and meta-difluorobenzenes was not achieved with columns prepared with neither 1:1 nor 1:1.5 ratios of TEOS:F_{13}-TEOS.

The higher the amount of fluorinated silane used, the more effective the separation. This result suggests that upon addition of higher amounts of fluorinated silanes to the precursor mixture, the resulting sol–gel matrix will have a higher number

of fluorinated moieties readily available for chromatographic interactions. These fluorinated moieties are accessible to the solute molecules, which results in an improved separation selectivity for the fluorinated compounds in the mixture. We attempted to prepare sol–gel solutions with a $TEOS:F_{13}$-TEOS ratio higher than 1:2 (such as 1:3), but the resulting sol–gel solution turned milky-white after 24 h of mixing; eventually, a precipitate was observed. Presumably, the excess of the precursor F_{13}-TEOS does not allow formation of a strong silica backbone because of the reduced quantities of TEOS. This leads to a polymeric material with somewhat different characteristics; we did not attempt to fabricate columns with such a material.

The column efficiency in electrochromatography is also dependent on the voltage drop across the capillary column. To investigate the optimum voltage for obtaining the best efficiency with the 1:2 $(TEOS:F_{13}$-TEOS) column, we injected the mixture of the six model compounds at different voltages (varying from 5 kV to 30 kV). Fig. 6 represents the electrochromatograms obtained for the mixture of the six model fluorinated compounds at the different voltages (Fig. 6a–d). The electrochromatograms clearly show sharper peaks (indicating an increase in the separation efficiency) as the voltage is raised. The analysis time is also reduced considerably. The electrochromatograms for the separation of the six fluorinated organic compounds show good resolution for all six compounds. The best column efficiencies were obtained above 20 kV (200 000 to 300 000 plates/m).
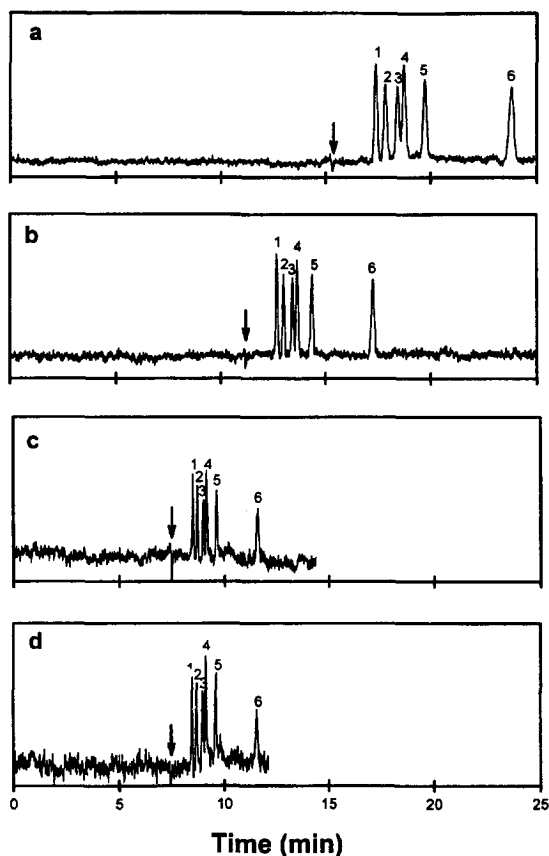
Acknowledgments

Fig. 6. Electrochromatograms for a test mixture containing six fluorinated compounds by OTEC in a 1:2 $(TEOS:F_{13}$-TEOS) column at different separation voltages: (a) 15 kV; (b) 20 kV; (c) 25 kV; (d) 30 kV. Other separation conditions as in Fig. 1. The test mixture contained: (1) fluorobenzene; (2) 1,4-difluorobenzene; (3) 1,3-difluorobenzene; (4) 1,2-difluorobenzene; (5) 1,2,4-trifluorobenzene; (6) 1,2,3,5-tetrafluorobenzene. The arrows indicate the EOF marker.

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