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Silicate Polymerization for the Preparation of Bed-Retention Frits in Capillary Electrochromatography

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Bubble formation, which is associated with bed-retention frits, is a critical experimental problem in capillary electrochromatography systems. In this investigation, porous silica frits were prepared via spot-heating of a silicate solution, and the effects of several experimental parameters on their performance were studied. The optimal sodium silicate concentrations were 10.8% and 5.4% (w/v) for outlet and inlet frits, respectively. The heating times were 5−6 s for outlet frits and <1 s for inlet frits. Under optimized conditions, outlet frits were 75 μm (±12 μm) and the heat treatment did not make the capillary fragile at the frit location. Bubble formation was affected by frit length, density, and silanization of the frits with trimethylchlorosilane. Packed capillaries with optimized frits were used successfully in a commercial CE instrument over a normal working day without pressurization, at relatively high ionic strengths (10 mM), and over a wide range of acetonitrile compositions (20%−80%). Currents were also stable for ≥3 h under very high current (27 μA) conditions. As part of this study, the efficiency and reproducibility of packed capillaries were also briefly evaluated.

Since capillary electrochromatography (CEC) combines the desirable features of both capillary electrophoresis (CE) and liquid chromatography,[1,2] it has received increasing attention over the past few years.[3−5] However, its application has been restricted by difficulties associated with column preparation and instrument stability. Early publications showed that CEC had problems associated with bubble formation,[6−10] and this continues to be a serious limitation. Bubbles invariably form near bed-retention frits and are commonly thought to arise from either Joule-heating or a change in the electroosmotic flow (EOF) velocity on moving from the packed bed through the frit and into the open capillary.[6,11] Pressurization is widely used to reduce bubble formation, either by pressurizing both buffer vials[12−14] or by applying an additional pressure at the inlet side of the capillary.[15,16] However, both methods complicate the design of homemade and commercial CE instruments, and pressurized flow might lower column efficiency. Bubble formation is often suppressed by using low concentrations of electrolytes, a high proportion of organic solvent (normally about 70%−80%[3,5,17,18]), zwitterionic buffers (for example, 4-morpholineethanesulfonic acid (MES) and tris(hydroxymethyl)-methylamine (TRIS)),[7,19] or sodium dodecyl sulfate (SDS).[20] Also, experimental conditions are generally chosen to reduce Joule-heating, with typical currents being ≤10 μA. Unfortunately, all of these conditions limit the range of application of CEC.

The performance of bed-retention frits in CEC is likely the most important experimental parameter influencing bubble formation. Consequently, the method used for the preparation of frits is crucial,[21] and Rebscher et al.[10] have described three methods for frit preparation. The most common approach is to fabricate the frits by sintering the packing material as described by Smith et al.[22] and Boughtflower et al.[7] However, this approach has several disadvantages: the high fusion temperature and long heating time make the capillary fragile at the frit position, bonded stationary phase is removed from the silica surface of the frit, frit properties

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are difficult to reproduce, and frits made by this method are relatively long (~500−1000 μm).[23−25]

Despite the improvements reported in frit fabrication, the research reported in the literature avoids bubble formation via the use of restrictive experimental conditions in terms of pressure applied,[26,27] voltage, current, ionic strength, and solvent composition. In this paper, a new approach for making frits and preparing packed capillaries is described. The frits were made by heating sodium silicate solutions to form porous sodium polysilicate, which was later silanized with trimethylchlorosilane. Although a silicate-based frit has been used in a pressurized flow system,[16] this approach has not been investigated for CEC. In this paper, this approach is optimized, and the effects of the characteristics of the frit on bubble formation are studied in an effort to produce long-term stability for a wide range of CEC operational conditions. The reproducibility of packed CEC capillaries, in terms of current, retention time, and efficiency, produced under these optimized conditions, is also briefly examined.

EXPERIMENTAL SECTION

Instrumentation and Apparatus. All the experiments in this report were performed on a 4000 CE instrument with a UV detector (214 nm) (Waters, Milford, MA). A Waters model 510 HPLC pump was used to pack and flush capillaries. The capillaries (375 μm o.d., 75 μm i.d.) were obtained from Polymicro Technologies (Phoenix, AZ). They were normally cut to 35−45 cm. A bypass capillary (45 cm, 375 o.d., 50 μm i.d.) was connected to a T-fitting at the outlet of the HPLC pump to permit the use of the pump for packing and flushing CEC columns without modification of the pump for the low volumetric flows required for CEC columns. A stereomicroscope (SMZ 30-150 x, Nikon, Mississauga, ON, Canada) was used to visually inspect the CEC capillary and the frits. The ultrasonic bath (50 HT) was purchased from the VWR Scientific Co. (Buffalo, NY). Temperature was not controlled in any of the experimental procedures.

Reagents and Chemicals. All chemicals were of analytical grade and were used without further purification or drying. All solutions were prepared from distilled, deionized water. Acetonitrile and methanol were obtained from the EM Science Co. (Gibbstown, NJ). Cyclohexane, acetone, toluene, benzene, thiourea, benzyl alcohol, and *N*,*N*-dimethylformamide (DMF) were from BDH Inc. (Toronto, Canada). Thiourea (5.57 mM), benzyl alcohol (3.86 mM), and benzene (0.0449 M) were dissolved in mobile phases. Sodium silicate solution (~14% (w/v) NaOH, ~27% (w/v) SiO_2) and imidazole were from Aldrich (Milwaukee, MI). Trimethylchlorosilane (99.9%) was obtained from Huls Petrach Systems (Bristol, PA). A stock potassium dihydrogen phosphate buffer in water (50 mM, adjusted to pH 7.0 with ammonia) was mixed with acetonitrile and water to obtain the desired compositions of mobile phases and a constant ionic strength (5 or 10 mM). In this paper, the percentages of acetonitrile and phosphate buffer refer to v/v units and the concentration of SiO_2 (%) refers to w/v units. The mobile phases were filtered through a membrane filter (Varian Canada Inc., Mississauga, ON, Canada; nylon-6,6 filters, pore size 0.2 μm). Every day, before use, the mobile phases were filtered and degassed for 4 min under vacuum. The packing material ODS2 (5 μm) was obtained from Rainin Instrumental Co. Inc. (Woburn, MA).

Preparation of Outlet Frits. A sodium silicate solution (approximately 5.6% (w/v) NaOH and 10.8% (w/v) SiO_2) was injected into an ~35−45 cm capillary with a syringe (1 mL) connected to the capillary with polyethylene tubing (0.38 mm i.d.). When a drop of sodium silicate solution came out of the capillary, the syringe was removed, and the capillary was heated 10 cm from the outlet of the capillary by touching it onto a Nichrome heater wire (0.33 mm and approximately 27 cm in length). The temperature was controlled by the voltage adjustment across the wire (about 3.5 V). When the color of the segment of the capillary touching the wire became white (after 5−6 s), the capillary was removed from the wire and allowed to cool. A frit of about 75 ± 12 μm was obtained. Longer frits were made by heating for longer times. Water was then pumped through the capillary at 1−2 mL/min to wash away sodium silicate.

Silanization of Outlet Frits. Empty capillaries with outlet frits were flushed with methanol for 0.5 h and then dried in an oven at 40 °C for 10 h. The silanization procedure was a slight modification of one reported previously.[28] Solutions of 0.02 mol/L trimethylchlorosiliane in DMF were prepared in 1.5 mL polypropylene centrifuge tubes, and imidazole was added as an acid acceptor (0.04 mol/L). These solutions were filtered through a 0.2 μm nylon-66 membrane syringe filter into another centrifuge tube, and a 100 μL Eppendorf pipet tip was inserted into the cap of the tube. No attempts were made to dry the solvents. A capillary was inserted through the pipet tip into the solution, and the solution was covered with Parafilm. The inlet of the capillary that was inserted into the silane solution was held to a height of about 20 cm above the outlet end of the capillary, and for frits prepared by the recommended procedure (50−100 μm in length), the silane solution flowed easily through the capillary. For longer frits, the silane solution was sucked through the dried capillary with a syringe connected to the outlet with a piece of polyethylene tubing. After silanization overnight, the capillary was rinsed with pure DMF followed by methanol.

Preparation of Packed Capillaries. A schematic of the packing equipment, which consisted of an HPLC pump, an ultrasonic bath, a slurry reservoir, and a capillary, is shown in Figure 1. The stationary phase (5 μm ODS2) was slurried in cyclohexane/toluene (1:1, v/v) at a concentration of 0.2 g/mL, and the slurry was placed in the ultrasonic bath for 5 min. Then 0.05 mL of the slurry was placed in a 1 mL syringe, and the syringe was attached via a union to the stainless steel reservoir (1.6 mm o.d. tubing, 9 cm in length, 0.38 mm i.d.). The slurry was then injected into the reservoir, the inlet of reservoir was connected to the pump, and the outlet was connected to the inlet of a capillary containing an outlet frit. The inlet of the capillary was pushed into the connecting union until it almost touched the outlet end of the reservoir. Then both the reservoir and the capillary were placed in an ultrasonic bath and the pump pressure was adjusted to ~5000 psi. Even though the capillary was packed to its full length almost immediately, the pressure and ultrasound were maintained

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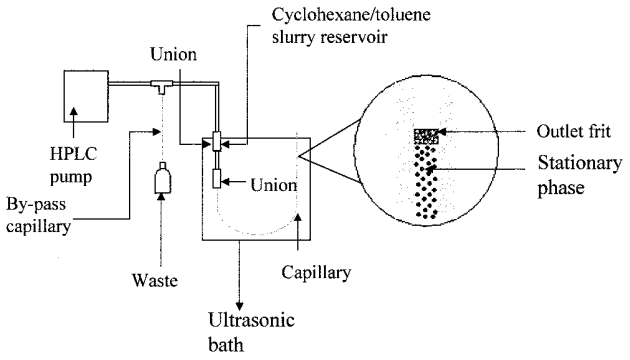

Figure 1. Packing setup. Experimental conditions: capillary, 35−45 cm in length, 75 μm i.d., 375 μm o.d.; pressure, ~5000 psi; flow rate, ~0.6−0.9 mL/min; bypass capillary, 45 cm in length, 375 μm o.d., 50 μm i.d.

for ~30 min to ensure maximum packing density in the column. Then the flow rate of the driving solvent (methanol) was switched to zero, the pressure was allowed to drop to zero, and water was flushed through the capillary for 2 h prior to making an inlet frit (see below).

Before the first separation, the capillary was rinsed with the degassed mobile phase for at least 2 h by pressurizing (~3000 psi) the capillary inlet. This procedure conditioned the stationary phase and removed any bubbles. Finally, a detection window was made by burning off the protective layer about 1 cm after the outlet frit. It was found that acetonitrile slowly attacked the polymide coating at the end of the capillary, and thus for storage, both ends were immersed in water instead of electrolyte. The efficiencies of packed capillaries were calculated from $N = 16(t_R/t_w)^2$, where N is the theoretical plate number, t_R is the retention time, and t_w is the peak width. All samples were injected into the capillaries electrokinetically (5 kV, 2 s).

Preparation of Inlet Frits. After the capillary was packed, an inlet frit was made by dipping the inlet end of the capillary into a dilute sodium silicate solution (~5.4% SiO_2) for <1 s. The end of the capillary was then immediately touched for <1 s to a preheated Nichrome wire. The capillary was then immediately flushed in the reverse direction to remove extra sodium silicate from the end of the capillary at a flow rate of 0.5 mL/min for ~10 min.

RESULTS AND DISCUSSION

Outlet Frit Preparation and Structural Properties. Outlet frits were made by touching the capillary at one spot on a preheated Nichrome wire. Compared to heating with a wire wrapped around the capillary, this method was experimentally simpler, and we could control heating time more accurately. The criteria we established for the initial stage of frit preparation were that the frits should be short, porous, and rigid. Carley et al.[24] studied frits from 1000 to 6000 μm and indicated that the length of the frit can influence bubble formation. Also, low porosity could affect local heating due to high resistance and create pressure differences at the frit if a packed column could create a sufficiently large EOF; both of these effects might enhance bubble formation. Since CEC capillaries are packed and/or conditioned by high pressure, the frit must be rigid. The parameters examined to optimize frit properties were heating time, silicate concentration, and heating temperature.

The length, density, and shape of the outlet frits were influenced by the concentration of the silicate solution. With high concentrations of sodium silicate solutions (SiO_2 > 10.8%), the frits were longer than 300 μm and the color observed under a microscope was gray to black; darker frits were found to always exhibit poor permeability. It was found that for a frit to exhibit good permeability, it had to be nearly transparent. With low concentrations of sodium silicate solutions (SiO_2 < 10.8%), frits were irregular in shape and the capillary had to be heated for a relatively long time (>10 s) to form a frit; this long heating also destroyed the polymeric coating of the capillary. The heating temperature (voltage applied across the heating wire) affected the quality of the frits in a similar manner. Low temperatures resulted in irregular frits, while with high temperatures, the frits were not very permeable. The optimal temperature was obtained when the voltage across the Nichrome wire was 3.5 V, which produced an almost invisible dull-red glow. This relatively low temperature caused minimal damage to the protective layer on the capillary, and thus the capillaries were not fragile at this position. Generally the heating time was selected at 5−6 s to improve reproducibility and minimize heat damage. Change in the heating time produced similar effects as change in silicate concentration or change in temperature.

To maximize permeability, frits should be as short as possible but they should also be able to withstand high pressure. Permeability and pressure stability were evaluated by connecting empty capillaries with frits (~40 cm capillary; frits located 10 cm from outlet) directly to an HPLC pump, and water was flushed through these capillaries at ~2.8 mL/min. Capillaries with short frits (75−200 μm) had the same back-pressures (~4300 psi) as an open capillary. However, longer frits (700 μm) had larger back-pressures (4500−4600 psi). All of the frits withstood these high pressures and high flows, and thus it can be concluded that these frits should have both the permeability and rigidity required for CEC columns. The reproducibility of making frits under the optimum conditions (as per the experimental procedure) was examined by preparing 20 frits, and the average length of the frits was 75 ± 12 μm (1σ).

Inlet Frit Preparation. In most CEC systems, inlet frits are necessary. This is because the stationary phase carries a negative charge and thus can move out of the capillary. A previous study[10] suggested that using silicate to bind packing material was not suitable for preparing frits. However, since our study with outlet frits showed that optimization of the experimental conditions was important, a brief study was carried out to see if this approach could also be used for inlet frit preparation. Satisfactory results were obtained when we dipped the inlet end of a capillary into a dilute sodium silicate solution (5.4% SiO_2) and touched the end of the capillary to the preheated Nichrome wire (heating voltage 3.5 V) for <1 s. These inlet frits were about 75 μm in length, and the appearance was similar to the outlet frit. To minimize void formation during preparation of the inlet frits, an aqueous mobile phase should be present in the packed capillary. The mechanical strength of inlet frits was tested under pressures of 4000−5000 psi in a reversed-flow direction. The total flow rate through the packed capillary was about 0.7−0.8 mL/min, and all inlet frits were stable under these conditions. Since the frits are small and located at the end of the capillaries, caution should be taken to avoid dislodging the inlet frits when capillaries

Table 1. Reproducibility of Packed Capillaries[a]

packed length (cm)	n[b]	H (μm)	δ_H (%)	k'	$\delta_{k'}$ (%)	$\delta_{k'}$ (%) column to column	current (μA)
Silanized Frits, 70% Acetonitrile (5 mM Phosphate)							
32	12	6.98	5.6	0.266	0.7	6.2	3.9
28	11	6.99	8.8	0.255	0.7		4.4
33	12	7.69	10.2	0.288	1.8		4.0
Silanized Frits, 40% Acetonitrile (10 mM Phosphate)							
23	5	10.88	4.6	0.914	3.3	10.6	12.2
32.5	3	12.96	4.4	0.980	1.8		9.3
33	3	12.86	3.1	0.793	1.5		9.5
Unsilanized Frits, 80% Acetonitrile (5 mM Phosphate)							
30	3	8.39	5.1	0.162	0.8	1.9	3.2
30	3	7.34	3.6	0.160	0.8		3.2
30	3	8.94	4.1	0.164	0.9		3.2

[a] Experimental conditions: capillaries ~10 cm longer than the packed bed length indicated in the table; 15 kV; pH 7.0; sample injection 2 s at 5 kV. [b] Number of data points.

are connected to and disconnected from fittings and instrumentation.

Preparation of Packed Capillaries. Initially capillaries were packed without immersing the capillary and reservoir in the ultrasonic bath. However, under these conditions the capillaries could not always be packed to the required length. We also found that the slurry reservoir and connecting union needed to be cleaned carefully after every use to avoid the formation of stationary phase blockage in the reservoir tube, which would cause incomplete packing of the capillary. Acetone and methanol have been the most commonly used slurry solvents.[17,19,27] In our experiments, methanol, acetone, and cyclohexane/toluene (1:1, v/v) were tested, with methanol as the driving solvent. Cyclohexane/toluene (1:1, v/v) was finally selected, since it created more stable suspensions of the packing material, required <5 min for packing, and gave a success rate of ~100%.

The concentration of packing material used in the slurry was tested from 0.1 to 0.5 g/mL. It was found that if the concentration was less than 0.2 g/mL, the packing material did not always fill the capillary. To minimize packing consumption and to prevent blockage, slurry concentrations were kept at 0.2 g/mL. Later in this investigation, it was discovered that more efficient transfer of the packing material into the capillary could be obtained when the inlet of the capillary was placed very close to the outlet of the tubing used as the slurry reservoir. Consequently, with this configuration it may be possible to use slurry concentrations of <0.2 g/mL, but this was not investigated. The packing reproducibility was evaluated for three sets of three capillaries prepared on different days. The results of this evaluation are summarized in Table 1. The k' values for benzyl alcohol were measured using thiourea as the unretained marker. The results in Table 1 show that good column-to-column reproducibility was observed. Slightly poorer reproducibilities were obtained with 40% acetonitrile, and this may be related to heating effects; note the appreciable current (12 μA) for the 23 cm capillary (Table 1). Overall, these efficiencies and reproducibilities are comparable to values reported by other workers.[6,17,21] Almost all data in the literature on k' reproducibility refer to low percentages of water, and more studies are needed over a wide range of electrolyte compositions to evaluate reproducibility. While long-term stability was not specifically evaluated, some columns were used periodically over a 4 month period (storage in water and operation for ~30 h) with no noticeable change in performance or loss of frits. Often the most important factor in determining the long-term stability of CEC columns is the breakage at the frit, and this has never happened with the frit systems described in this paper.

Current Stability in Unpacked Capillaries. To initially examine the relationship between frit preparation and bubble formation, outlet frits with lengths from 100 to 1000 μm were tested in unpacked capillaries by monitoring the current as a function of time at 20 kV in an 80% acetonitrile electrolyte (5 mM phosphate, pH 7.0). The initial current, 9 μA, was found to be the same for all the frits. Stable currents were observed for frits ≤300 μm, but for frits >300 μm, the current decayed to a very small value (<0.1 μA) in 2−40 min with the rate of current decay directly proportional to the frit length.

One of the explanations proposed for the formation of bubbles is that there is a difference in the EOF between the packed bed and the frit due to destruction of the reversed phase during the frit preparation.[24] If this were a contributing factor to bubble formation, then resilanization of the packing in the frit zone should reduce this effect. One reported study evaluated this briefly, and it was shown that, over the short time periods examined (40 min), there was noticeable improvement for long (6000 μm) frits.[24] Although these frits are up to 100 times as long as the ones we used, we also examined the effect of silanization. For this study, the electrolyte was 100% aqueous and had a relatively high ionic strength (25 mM phosphate buffer); these conditions were used to increase the probability of bubble formation. The results obtained for frits in open capillaries showed there was a significant reduction in bubble formation. Even for 800 μm frits, stable high current levels (50 μA) were obtained.

Current Stability in Packed Capillaries. Since CEC performance over a wide range of experimental conditions was of interest, several CEC capillaries with unsilanized 75 μm frits were tested with different acetonitrile compositions, voltages, and ionic strengths. Representative results are shown in Figure 2, which gives plots of the current observed as the acetonitrile concentration was lowered from 80% to 20% at a phosphate buffer concentration of 5 mM (curves a−d). These curves show good stability until the acetonitrile content was lowered to 20% (curve d), at which point the current dropped quickly due to bubble formation.

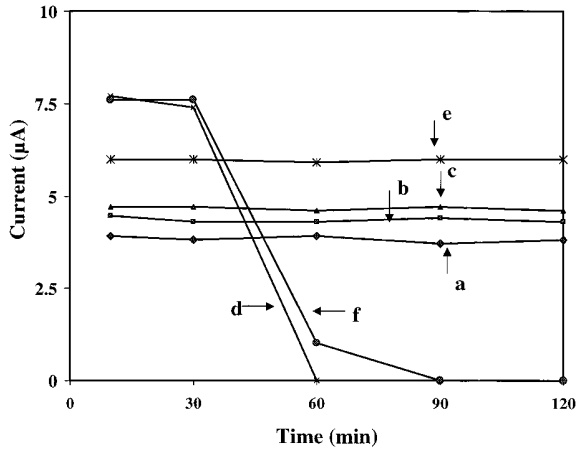

Figure 2. Current as a function of time for packed capillaries with different compositions of mobile phases (pH 7.0). Experimental conditions: capillary with unsilanized frit, 38 cm total length, 28.1 cm packed length, 75 μm frit length; curve a, 80% acetonitrile (5 mM phosphate), 15 kV; curve b, 70% acetonitrile (5 mM phosphate), 15 kV; curve c, 50% aetonitrile (5 mM phosphate), 15 kV; curve d, 20% acetonitrile (5 mM phosphate), 15 kV; curve e, 50% acetonitrile (10 mM phosphate), 15 kV; curve f, 50% acetonitrile (10 mM phosphate), 20 kV.

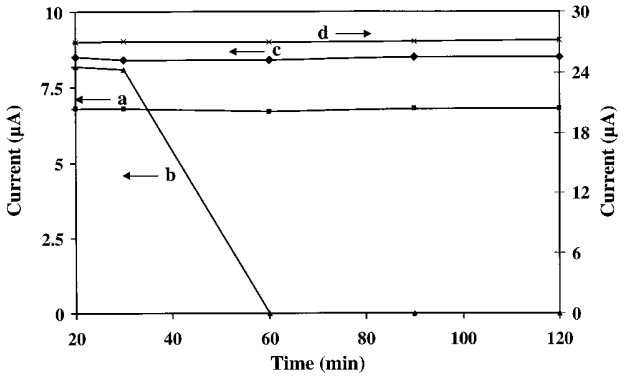

Figure 3. Current as a function of time for packed capillaries with different lengths of silanized outlet frits. Experimental conditions: capillary with 800 μm outlet frit (curves a and b), 40.3 cm total length, 29.6 cm packed length; capillary with 75 μm outlet frit (curves c and d), 34.5 cm total length, 24.5 cm packed length; mobile phases, 50% acetonitrile (10 mM phosphate) for curves b and c, 80% acetonitrile (5 mM phosphate) for curve a, 20% acetonitrile (10 mM phosphate) for curve d; working voltage, 20 kV for curves b and c, 30 kV for curves a and d.

Curve e shows that minor increases in current (from increased buffer concentration) could be tolerated at 50% acetonitrile, but if current was further increased by raising the voltage (curve f), then bubble formation was observed. These results show that while unsilanized frits offer an improved range of operating conditions without the use of external pressure, stable currents can only be obtained when the current is $\leq$7 μA and when solvent composition is $\geq$50% acetonitrile.

To evaluate the effect of silanization, packed capillaries with 75 and 800 μm silanized frits were tested. Curve a in Figure 3 shows that an 800 μm silanized frit (30 kV) gave a stable current during a 2 h test (80% acetonitrile, 5 mM phosphate). However, when the proportion of organic solvent was decreased to 50%, the current decreased quickly (curve b, Figure 3) because of bubble formation. However, current was stable with 75 μm frits under

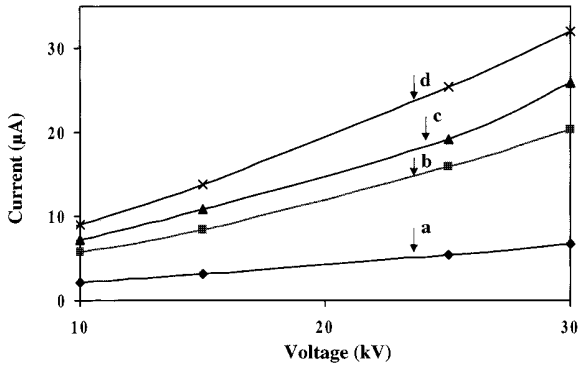

Figure 4. Current as a function of voltage for different solvent compositions. Experimental conditions: capillary (silanized 75 μm outlet frit) as in Figure 3; mobile phases, 80% acetonitrile (5 mM phosphate) for curve a, 50% acetonitrile (10 mM phosphate) for curve b, 20% acetonitrile (10 mM phosphate) for curve c, 10% acetonitrile (10 mM phosphate) for curve d.

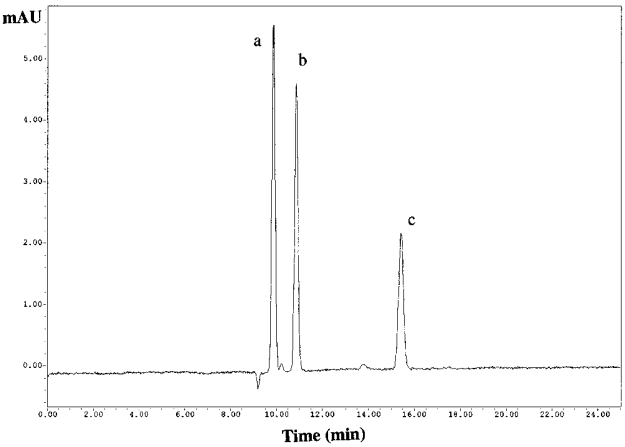

Figure 5. Typical electrochromatogram on a packed capillary with a silanized frit. Experimental conditions: total capillary length, 40 cm; packed length, 30 cm; injection, 5 kV, 3 s; separation voltage, 15 kV; mobile phase, 80% acetonitrile (5 mM phsphate, pH 7.0). Solutes: a, thiourea; b, benzyl alcohol; c, benzene.

the same conditions (curve c, Figure 3). To more rigorously evaluate the performance of silanized 75 μm frits, mobile phase compositions below 50% were tested at 30 kV. The currents were stable even when the percentage of acetonitrile was 20% (10 mM phosphate buffer) and the value of the current was ~27 μA (curve d, Figure 3); this test was done at 30 kV for 3 h. The silanized 75 μm frit also gave stable operation in 10% acetonitrile as long as the current was $\leq$20 μA. These stable currents are the highest reported for a CEC system thus far, particularly for high aqueous content. Other reports[6,10] have suggested that the nonwetting properties of reversed phases make it impossible to obtain stable currents for high water content unless the mobile phase is pressurized, but pressurization is not required for the frit systems developed here.

The performance of the silanized 75 μm frit was further examined via plots of current vs voltage for various proportions of acetonitrile. The phosphate buffer concentration was kept the same (10 mM) in all mobile phases except 80% acetonitrile, where solubility problems restricted the concentration to 5 mM. The results in Figure 4 show that only when the acetonitrile content is high, is the relationship of current versus applied voltage

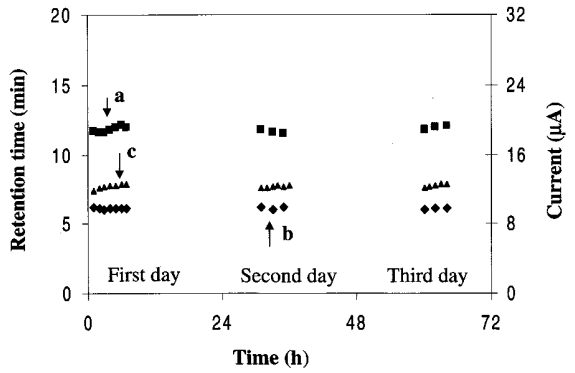

Figure 6. Reproducibility of a CEC packed capillary over three working days. Experimental conditions: total capillary length, 33 cm; packed length, 23 cm; injection, 5 kV, 3 s; separation voltage, 15 kV; mobile phase, 40% acetonitrile (10 mM phosphate). Key: a, benzyl alcohol retention time; b, thiourea retention time; c, current.

reasonably linear; in this instance, the current was below 10 μA. As the proportion of organic solvent decreased, current, and thus Joule-heating, increased and the plots became more nonlinear. However, despite this nonlinearity due to increased Joule-heating, stable CEC operation was obtained.

To further investigate the current stability of CEC capillaries with 75 μm silanized frits, three capillaries were tested over three working days. Figure 5 shows a typical separation obtained with one of the capillaries. The results of one time study are shown in Figure 6. In this test the mobile phase (40% acetonitrile, 10 mM phosphate) was degassed at the beginning of each day. The RSD values for thiourea and benzyl alcohol retention times and for current were 0.79%, 0.66%, and 1.1% respectively. Two other capillaries (~30 cm packed length) were tested once an hour over 6 h, and the reproducibilities obtained were the same (within experimental error) as those obtained in Figure 6. One of these columns was also used over a period of 3 days with only one degassing of electrolyte at the start of the 3-day test, and stable currents were observed.

CONCLUSION

Short, porous, and mechanically strong frits can be made by thermal polymerization of silicate solution. In addition to a number of minor advantages (less fragile capillaries, simplified CEC column preparation, shorter frits, no destruction of reversed phase), the major advantage is stable operation (current) over a normal working day for a wide variety of compositions with no application of pressure for bubble suppression. Any remaining limitations for these CEC columns appear to be related to high currents (perhaps primarily Joule-heating effects), and silanization of inlet frits may also improve performance marginally. While these results suggest that both Joule-heating and mismatched EOF contribute to bubble formation, nucleation of bubbles is a complex process. Bubble formation may also arise from other phenomena, such as high localized currents, and from the presence of impurity particles that are conductors or semiconductors. For a 20 cm column operated at 30 kV, a 20 μm impurity particle that is a semiconductor or a conductor would have a potential field of 3 V across it, and this is sufficient to cause electrolysis of water. We have observed black impurity particles in one column that exhibited anomalously high sensitivity to bubble formation.

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