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Electroosmotic flow controllable coating on a capillary surface by a sol–gel process for capillary electrophoresis

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Abstract

A simple coating procedure employing a sol–gel process to modify the inner surface of a bare fused-silica capillary with a positively charged quaternary ammonium group is established. Scanning electron microscopic studies reveal that a smooth coating with 1~2 μm thickness can be obtained at optimized coating conditions. With 40 m*M* citrate as a running electrolyte, the plot of electroosmotic flow (EOF) versus pH shows a unique three-stage EOF pattern from negative to zero and then to positive over a pH range of 2.5 to 7.0. At pH above 5.5, the direction of the EOF is from the anode to the cathode, as is the case in a bare fused-silica capillary, and the electroosmotic mobility increases as the pH increases. However, the direction of the EOF is reversed at pH below 4.0. Over the pH range of 4.0 to 5.5, zero electroosmotic mobility is obtained. Such a three-stage EOF pattern has been used to separate six aromatic acids under suppressed EOF and to separate nitrate and nitrite with the anions migrating in the same direction as the EOF. The positively charged quaternary ammonium group on the coating was also utilized to minimize the adsorption problem during the separation of five basic drugs under suppressed EOF and during the separation of four basic proteins with the cations migrate in the opposite direction as the EOF. Also, the stability and reproducibility of this column are good.

Keywords: Electroosmotic flow; Coated capillaries; Sol–gel; Basic drugs; Proteins; Inorganic anions; Organic acids

1. Introduction

Capillary electrophoresis (CE) has recently emerged as one of the most powerful separation techniques [1–3]. However, the irreproducibility of migration behavior due to surface adsorption and the lack of control of electroosmotic flow (EOF) limit its use in real sample analysis. In order to improve the separation performance or expand the types of application in CE, a great number of studies have focused on the modification of capillary surface for better controlling EOF and/or reducing surface adsorption. The approaches to the modification of capillary surface include: (1) controlling the ionic strength or pH of the electrolyte [4–7]; (2) modifying the capillary surface by dynamic coating with ionic or nonionic surfactants [8–12]; (3) modifying the capillary surface permanently by physical sorption [13–17]; and (4) modifying the capillary surface permanently by covalent bonding [18–36]. The last approach normally provides the coated columns with higher efficiency, better lifetime and fewer side effects in separation. By this approach, a stable

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surface with a neutral, positive, or negative charge is normally created, hence EOF is only in one direction or almost suppressed. This kind of surfaces limits possibility of employing a single capillary for the separation of a wide range of species.

There are very few reports mentioned the procedure to prepare a capillary column with switchable EOF, i.e., the direction of the EOF can be manipulated by controlling the experimental parameters [18–23,33]. Pesek and co-workers used etched, chemically modified capillaries to reverse the EOF at low pH [18,19]. Bruin et al. coupled an aminopropyl group with maltose to prepare a carbohydrate-modified silica capillary [20]. At low pH, the aminopropyl group protonates and leads to a positively charged surface and hence a reversal of the EOF. Maa et al. employed an amphoteric compound, α-lactalbumin, to deactivate the capillary surface so that both the direction and magnitude of the EOF can be controlled by adjusting the pH of the running electrolyte [21]. Huang et al. immobilized a cryptand-containing polysiloxane onto the capillary surface to create a positively charged surface [22]. Due to the formation of a highly selective and stable complex between cryptand and alkali metal ions, the positively charged polysiloxane coating provides a switchable EOF from low to high pH. Smith and El Rassi reported a procedure to prepare a capillary column with a switchable EOF by covalently bonding a quaternary ammonium functionalized layer and a hydroxylated polyether layer in sequence [23]. As such, the coating consists of unreacted silanol groups and the positively charged quaternary ammonium group. Thus, they were able to manipulate the magnitude and direction of the EOF by changing the pH of the running electrolyte. However, such surface modification methods normally involve several steps of surface reaction. They are time consuming and may even lead to significant column-to-column variation because of the tedious procedures. Sol–gel processes have been proposed as an alternative approach for modifying the capillary surface with a wide variety of functional groups due to the simplicity in the coating process and diversity in the types of materials available [31–36]. By the sol–gel approach, a capillary column with a switchable EOF was also obtained by modifying the capillary surface with an amino group via a one-step coating procedure [33].

In this report, a sol–gel coating process was used to modify the capillary surface with a positively charged quaternary ammonium group. The resulting quaternary ammonium group and the uncapped silanol groups can provide the switchable EOF mentioned above. A unique three-stage EOF pattern versus the pH of the running electrolyte has been observed with this coating [37]. To demonstrate the applications of such a three-stage EOF pattern exhibited by this column, the uses of this column for the separation of six aromatic acids, nitrate and nitrite, five basic drugs, and four basic proteins are presented.

2. Experimental

2.1. Chemicals and materials

Bare fused-silica capillaries (75 μm I.D.×365 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). *N*-Trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMSPTMA) was obtained from Gelest (Tullytown, PA, USA). Tetramethyl orthosilicate (TMOS), pyrometallitic acid, trimetallitic acid, terephthalic acid, isophthalic acid, and phthalic acid were obtained from Tokyo Chemical Industry (Tokyo, Japan). Benzoic acid was obtained from Riedel-de Haën (Seelze, Germany). Amphetamine, methylamphetamine, morphine, cocaine, and heroin were purchased from Radian (Austin, TX, USA). Cytochrome *c* was obtained from Merck (Darmstadt, Germany). Lysozyme, myoglobin, and α-chymotrysinogen A were purchased from Sigma (St. Louis, MO, USA). Sodium dihydrogen phosphate, acetic acid, sodium acetate, citric acid, disodium citrate, and hydrochloric acid (Merck) were employed to prepare the running electrolytes. All running electrolytes were filtrated through a 0.45 μm syringe filter (Alltech, Deerfield, IL, USA) before use. All chemicals were used as purchased without further purification.

2.2. Instrumentation

All CE separations were performed using a Waters Quanta 4000 capillary electrophoresis system (Milford, MA, USA) equipped with a built-in 0–30 kV

positive or negative power supply and a circulating fan for temperature control. A Zn lamp with 214 nm band pass filter was employed for UV detection. Hydrodynamic injections of 10 s were employed throughout. Data acquisition was achieved with an EZChrom Chromatography data system (Scientific Software, San Ramon, CA, USA). The pH values of the running electrolytes were measured with an Orion Model 420A pH meter (Boston, MA, USA). All CE experiments were performed at an ambient temperature of about 26 °C. The micrographs of the coated capillaries were taken with a Hitachi S2400 scanning electron microscopy (SEM) system (Tokyo, Japan).

2.3. Preparation of a coated capillary column by the sol–gel process

The use of TMSPTMA to prepare propyl-*N,N,N*-trimethylammonium functionalized silica (PTMAFS) has been described elsewhere [37]. In this report, the method to coat the inner capillary surface by PTMAFS is described. Pre-hydrolysis of TMOS was performed by adding 180 μl deionized water and 15 μl 0.1 *M* catalytic amount of hydrochloric acid to 500 μl TMOS. The mixture was sonicated in an ice bath for approximately 30 min and then stored in the ice bath for an additional 6 h. Subsequently, 340 μl TMSPTMA was added to the mixture. The mixture was then placed in a laboratory-made coating device immediately. One end of a bare fused-silica capillary was allowed to dip into the sol–gel mixture solution and the other end was connected to an aspirator vacuum system. Immediately after the capillary was filled with the sol–gel solution, the capillary was removed from the sol–gel solution and water was flushed through the capillary. This procedure was employed to avoid cracks during aging [38] and to give a more reproducible column. However, uneven coating resulted if the flushing time was longer than necessary. Since the gelation time for this sol–gel solution is about 8 min, the capillary would be clogged if the sol–gel solution were left in the column for a time longer than necessary. The best coating was obtained by forcing the sol–gel solution through the capillary for 10 s and then flushing with water for 30 s. Subsequently, the capillary was filled with deionized water, removed from the coating device, sealed at both ends, and annealed at 50 °C for 24 h.

Prior to use, each new PTMAFS-coated capillary went through a treatment sequence. The coated capillary was flushed with 1.0 *M* HCl for about 30 min to hydrolyze the remaining methoxy groups, rinsed with water for 3 min to allow further condensation of the newly formed surface silanol groups, flushed with 1.0 *M* HCl for 5 min again, and then purged with a running electrolyte for at least 30 min. Randomly spiked signals were observed during the treatment, probably because the treatment causes some PTMAFS debris to detach from the coating. This sequence was repeated if necessary until a smooth baseline was obtained. Between each run, the coated capillary was pretreated by purging with 0.1 *M* NaOH solution for about 10 s to clean the inner coating, rinsing with the deionized water for 5 min, purging with 1.0 *M* HCl for 3 min so that the PTMAFS layer was ion exchanged to a chloride form [37], and then purging with a running electrolyte for about 15 s (here we refer it as pretreatment sequence I). Alternatively, the last two steps were replaced by purging only with a running electrolyte for 10 min (here we refer it as pretreatment sequence II). At the end of each day, the coated capillary was purged with 0.1 *M* NaOH for about 10 s, rinsed with deionized water for 5 min, filled with deionized water, sealed at both ends, and then stored under room conditions.

3. Results and discussion

3.1. Characterization by SEM

The advantages of the sol–gel process for capillary coating are the simplicity and reproducibility of the process. Also, sol–gel coatings in general are thicker than that by other coating processes so that it offers a higher sample loading capability [31]. In order to obtain a coated capillary reproducibly by the sol–gel process at optimized conditions, SEM was employed to examine the coating at various coating conditions. As shown in Fig. 1a, a coating with 1~2 μm thickness of PTMAFS on the inner capillary surface is normally obtained at optimized coating conditions: coating the column with sol–gel solution

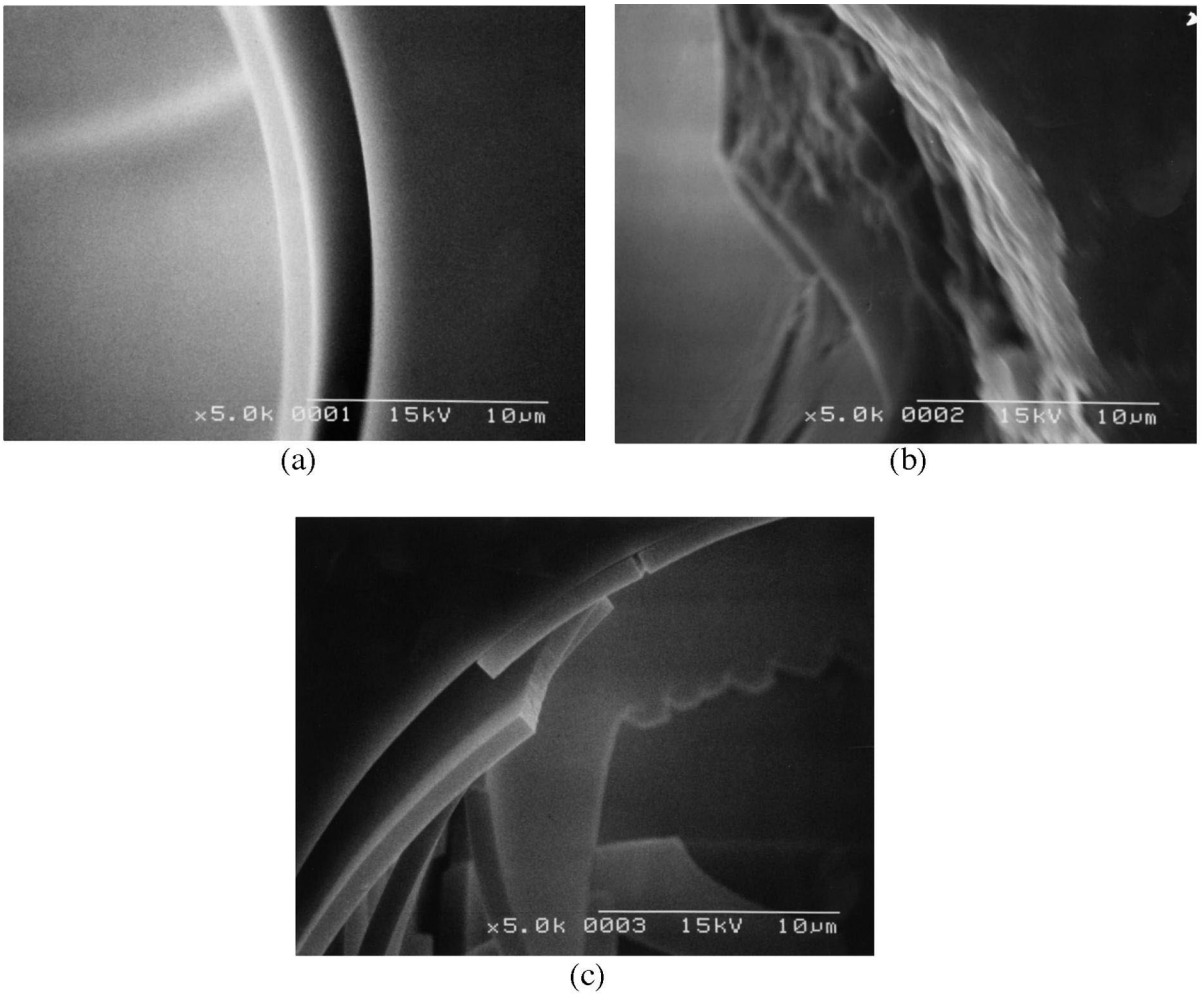


Fig. 1. Electron micrographs of a PTMAFS coating inside a fused-silica capillary. Coating conditions: (a) sol–gel coating time: 10 s, water flushing time: 30 s, and aging in water; (b) sol–gel coating time: 10 s, water flushing time: 4 min, and aging in water; (c) sol–gel coating time: 10 s, water flushing time: 30 s, and aging in air.

for 10 s and flushing with water for 30 s afterward. Prolong flushing process with water immediately after the coating process reduces the thickness of the coating and leads to a rough surface, as shown in Fig. 1b. Rough surface (not shown here) is also obtained for flushing the column with air only after the sol–gel coating. The aging process also affects the morphology of the coating. Fig. 1c reveals that cracking occurs when the coating has been subjected to dry aging, as contrast to the smooth coating after wet aging (Fig. 1a). Such SEM micrographs indicate that a smooth and thick PTMAFS film on the capillary wall can be obtained by the relatively simple sol–gel procedure. Homogeneous coated surface has been shown to be important to reduce the adsorption of basic proteins on capillary wall [30].

3.2. Characterization of surface charge by electroosmotic flow

In order to understand the surface charge of the coated capillary and the chemistry of the coated material, the measurement of the direction and magnitude of the EOF was performed. Capillary

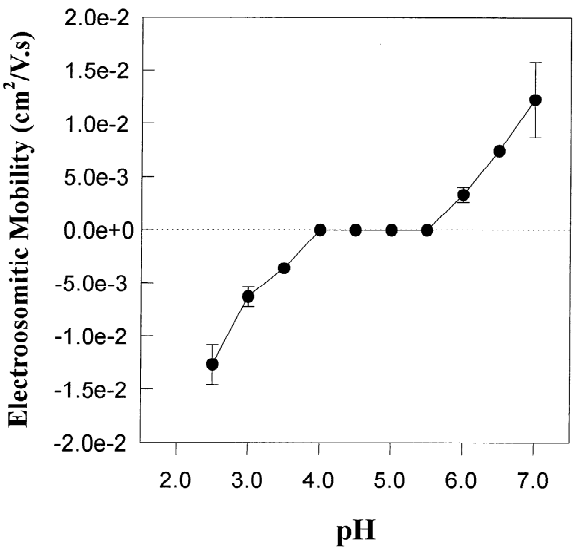


Fig. 2. Plot of electroosmotic mobility versus pH of the running electrolyte at a PTMAFS-coated capillary. Conditions: mobile phase, 40 m*M* citrate; capillary, 30 cm×75 μm I.D.; applied voltage, −5 kV, +5 kV; detection wavelength: 214 nm; EOF marker: DMSO.

columns with a smooth coating as shown in Fig. 1a were employed to examine the effect of solution pH on the EOF. Fig. 2 depicts the electroosmotic mobility obtained with 40 m*M* citrate at different pH under constant ionic strength. Both direction and magnitude of the EOF change with the pH of the running electrolyte. At pH above 5.5, the direction of the EOF is from anode to cathode, as is the case in bare fused-silica capillaries, and the electroosmotic mobility increases as the pH increases. However, the direction of the EOF is reversed at pH below 4.0, and the electroosmotic mobility increases as the pH decreases. Interestingly, over the pH range of 4.0 to 5.5, the electroosmotic mobility is suppressed ($<4.0\cdot 10^{-5}$ $cm^2/V\,s$). A signal corresponding to the neutral marker of dimethyl sulfoxide (DMSO) cannot be detected in 2 h with both positive and negative polarities of the power supply. So, electroosmotic mobilities at this pH range are assumed to be zero.

Based on the direction of the EOF, we can assume that the net surface charge of the coating is about zero for pH 4.0–5.5, negative at pH above 5.5 and positive at pH below 4.0. As proposed previously, the net surface charge is determined by the relative concentration of SiO^- and $-N(CH_3)_3^+$ at the surface [37]. Since the degree of ionization of surface silanol groups depends on pH while the quaternary ammonium group has a positive charge over the whole pH range in this study, one will expect that the EOF switches at a single pH value as previously reported by other groups [18–23,33]. Yet, we observed no EOF over a wide pH range of 4.0–5.5 for the PTMAFS-coated capillary with 40 m*M* citrate as the running electrolyte. To the best of our knowledge, no CE columns were reported that show zero EOF over a wide pH range. The possible reason is that the pK_2 of citric acid is about 4.8, which is close to the isoelectric point of the PTMAFS surface [37]. With the increase in solution pH around 4.8, the thickness of the electrical double layer decreases due to the increased number of higher charged citrate molecules in the diffuse layer. On the other hand, the number of surface $Si–O^-$ also increases in the same pH range. Consequently, the two effects cancel each other at pH around 4.8 and results in zero EOF over the pH range of 4.0–5.5. Thus, the PTMAFS-coated capillary with citrate as the running electrolyte provides the feasibility of controlling EOF from negative to zero and then to positive by simply adjusting the pH of the running electrolyte.

3.3. Applications

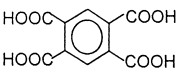
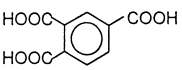
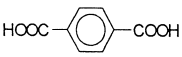
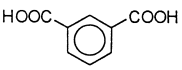
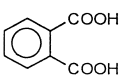
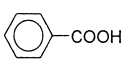
PTMAFS-coated capillary offers the advantage of manipulating the magnitude and direction of the EOF. Thus, potential applications of employing the PTMAFS-coated capillary in CE for both negatively and positively charged species are explored. By capillary electrophoresis, negatively charged species can be separated under either counter-EOF or co-EOF conditions. That is, an anion migrates to the detector, respectively in the opposite or in the same direction as the EOF. For bare fused-silica capillaries, the capillary wall exhibits a negative charge at solution pH above ~2. Hence, anions migrate to the detector under counter-EOF conditions at solution pH above ~2. As a result, the separation of anions with a bare fused-silica capillary generally requires a relatively long separation time.

To establish co-EOF conditions for anions in bare fused-silica capillaries, dynamic coatings with positively charged EOF modifiers (e.g., quaternary am-

monium ions) [39] or permanent coatings with positively charged functional groups [18–23,33] have been used to reverse the direction of the EOF. However, the former method has a severe problem when CE is coupled with mass spectrometry [40]. Separation of anions under co-EOF conditions leads to short separation time but the time window available for separation is limited by the average electroosmotic velocity of the system [41]. It has been shown that a large component of EOF in the same direction as the electrophoretic migration will decrease the resolution of two zones [1]. For this reason the PTMAFS-coated capillary could be beneficial to separation of anions through manipulation of the magnitude and direction of the EOF to improve the efficiency or to enhance the resolution. In particular, separation of anions in the PTMAFS-coated capillary with citrate as the running electrolyte provides a wide pH range with zero EOF so that anions can be separated simply by differences in their electrophoretic mobilities, and hence results in a compromise between separation time and available time window. Below we shall use two examples, separation of aromatic acids under zero EOF and separation of nitrate and nitrite with the EOF moves from cathode to anode, to demonstrate the applications of the PTMAFS-coated capillary in separation of anions using such a three-stage EOF pattern.

Aromatic acids are used as preservatives in food industry. These aromatic acids have a wide range of acid dissociation constants, as shown in Table 1. The migration time of each aromatic acid in CE depends on the degree of dissociation (charge) and the size of each anion. To manipulate the separation selectivity of organic acids, variation of the solution pH is the most common approach [42]. The electropherograms of six aromatic acids at different solution pH values are shown in Fig. 3. Excellent separations are obtained at pH below 4.5. However, a longer migration time is required for all the anionic species to migrate through the column at pH lower than 4.5. The optimum separation pH of the six aromatic acids is about 4.5, at which the EOF of the system is suppressed. Thus, the anions are separated simply by differences in their electrophoretic mobilities or by a combination of ion-exchange interaction with the stationary phase and their differences in apparent electrophoretic mobilities. Our previous work [37] shows that PTMAFS is an anion-exchange material. Considering the mobilities of an anion in a bare

Table 1
Acid dissociation constants of six aromatic acids

Compound	Structure	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
Pyromellitic acid		1.92	2.87	4.49	5.63
Trimellitic acid		2.52	3.84	5.20	
Terephthalic acid		3.54	4.46		
Isophthalic acid		3.62	4.60		
Phthalic acid		2.95	5.41		
Benzoic acid		4.21			

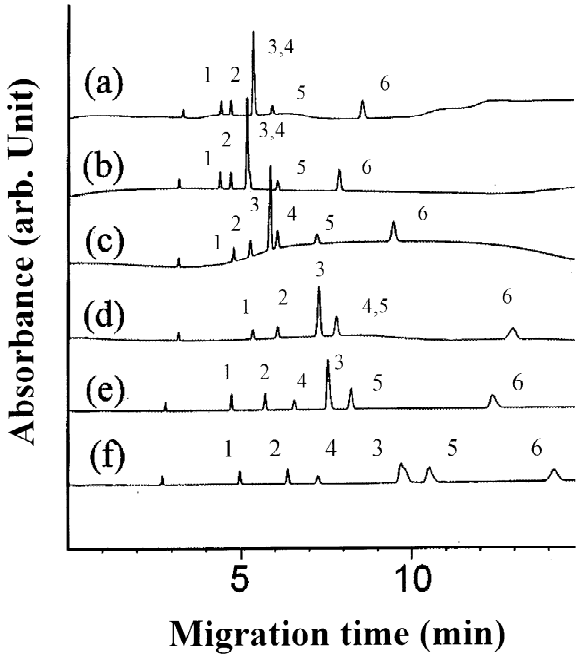


Fig. 3. The pH effect of the running electrolyte (40 m*M* citrate) on the separation of six aromatic acids: (a) pH 5.5, (b) pH 5.0, (c) pH 4.5, (d) pH 4.0, (e) pH 3.5, and (f) pH 3.0. Conditions: capillary, 30 cm×75 μm I.D.; separation voltage, −5 kV; detection wavelength: 214 nm. Peaks: 1=pyromellitic acid, 2=trimellitic acid, 3=terephthalic acid, 4=isophthalic acid, 5=phthalic acid, 6=benzoic acid.

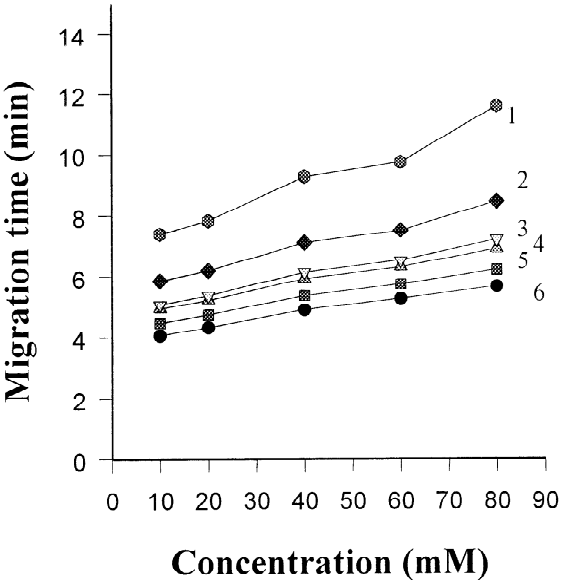


Fig. 4. Concentration effect of citrate buffer (pH 4.5) on the migration time of six aromatic acids: (a) 80 m*M*, (b) 60 m*M*, (c) 40 m*M*, (d) 20 m*M*, and (e) 10 m*M*. Conditions: capillary, 30 cm×75 μm I.D.; separation voltage, −5 kV; detection wavelength: 214 nm. Analytes: 1=pyromellitic acid, 2=trimellitic acid, 3=terephthalic acid, 4=isophthalic acid, 5=phthalic acid, 6=benzoic acid.

fused-silica capillary (μ_{CE}) and in the PTMAFS-coated capillary (μ_{CEC}), then the difference between these values ($\Delta\mu$) describes the degree to which ion-exchange effects influence the migration of the anion [43]. Since mobility is inversely proportional to migration time and retention factor, k', is defined as the ratio of the time spent by an anion in the stationary phase to the time it spends in the mobile phase, k' of an anion in a capillary electrochromatographic system is equal to $\Delta\mu/\mu_{CEC}$. By this approach, k' of terephthalic acid, isophthalic acid, and phthalic acid are, respectively, 0.68, 0.71, and 0.77 in the PTMAFS-coated capillary with pH 4.5 citrate (40 m*M*) as the running electrolyte. Hence, even in an open-tubular format with 75 μm I.D., the PTMAFS-coating still provides some contribution of ion-exchange to the observed migration time when citrate is used as the running electrolyte.

The effect of buffer concentration on the separation of the aromatic acids at pH 4.5 was also examined. As shown in Fig. 4, the migration times of the aromatic acids decrease with the decrease of the buffer concentration. Since there is no EOF at pH 4.5 for the PTMAFS-coated capillary with citrate as the running electrolyte, the effect of buffer concentration on the migration time of the aromatic acids is mainly due to the ionic strength effect on the migration time of the analyte. This is supported by the linear relationship between electrophoretic mobility of pyrometallitic acid and the reciprocal square root of ionic strength (data not shown).

Nitrate and nitrite are widespread contaminants in foods, drinking water, and the environment. Abnormally high levels in the body have been linked to diabetes and a number of nervous system disorders. Due to the very similar electrophoretic mobilities of nitrate and nitrite, analysis times in CE are relatively long. Since nitrite has a pK_a of 3.15, the use of a low pH buffer can selectively reduce the mobility of nitrite. As shown in Fig. 5a, baseline separation of nitrate and nitrite can be achieved in about 3.5 min at

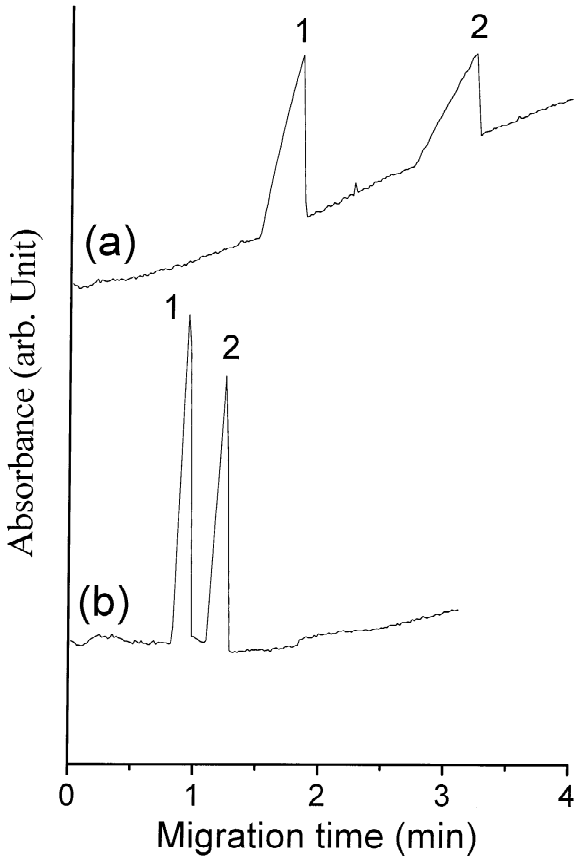


Fig. 5. Electropherograms of nitrate and nitrite with (a) a bare fused-silica capillary and (b) the PTMAFS-coated capillary. Conditions: mobile phase, 10 m*M* citrate, pH 3.5; capillary, 35 cm×75 μm I.D.; separation voltage, −25 kV; detection wavelength, 214 nm. Peaks: 1=nitrate, 2=nitrite.

pH 3.5. However, this separation is performed under counter-EOF conditions. Greater analysis speed would be achieved if the separation were performed under co-EOF conditions. For the PTMAFS-coated capillary at pH 3.5, nitrate and nitrite are expected to migrate in opposite direction as the EOF. As shown in Fig. 5b, baseline separation of nitrate and nitrite can be achieved in about 1.5 min. Since PTMAFS is an anion-exchange material, nitrate and nitrite may have ion-exchange interactions with the coating. However, k' of nitrate is only about 0.1 in the PTMAFS-coated capillary with pH 5.0 citrate (40 m*M*) as the running electrolyte, suggesting that the anions are separated mainly by differences in their apparent electrophoretic mobilities when citrate is used as the running electrolyte.

Conventionally, bare fused-silica capillaries are often inadequate for the separation of positively charged species, especially proteins, since co-EOF conditions lead to limited separation time windows. Moreover, the interaction between the negatively charged walls and the positively charged analytes sometimes results in band broadening. This decrease in separation efficiency provides a challenge for the separation of positively charged species in CE. The use of the positively charged PTMAFS-coated capillary allows separation of positively charged species under suppressed EOF or counter-EOF conditions and may also minimize the adsorption of the positively charged analytes to the wall. To overcome the adsorption problem, dynamic coatings [9,44] with EOF modifiers have often been used to modify the capillary wall to minimize adsorption of positively charged species to the capillary wall. However, the method has a severe problem when CE is coupled with mass spectrometry [40]. Hence, a permanent coating is often preferable. For the PTMAFS-coated capillary at pH below about 6, the positively charged surface is expected to minimize adsorption of positively charged species. Below we shall use two examples, separation of basic drugs under suppressed EOF and separation of basic proteins with the EOF moves from cathode to anode, to demonstrate the applications of the PTMAFS-coated capillary to minimize the adsorption problem.

Drug abuse is a serious and growing problem. Hence, simultaneous screening of drugs in body fluids is of considerable importance for the investigation of intoxications, in the identification of drug users and in the control of drug addicts enrolled in withdrawal therapy. Capillary electrophoresis is a powerful technique for the simultaneous screening of drugs [45]. However, adsorption on capillary wall for bare fused-silica capillaries can be significant for some basic drugs. The PTMAFS coating has a positively charged quaternary ammonium group. As such, basic drugs will be repelled from the surface. Fig. 6b shows an electropherogram of a mixture of five basic drugs in 100 m*M* acetate at pH 6.0. For the PTMAFS-coated capillary with pH 6.0 acetate as the running electrolyte, the EOF was also found to be zero (data not shown). Thus, the five basic drugs are

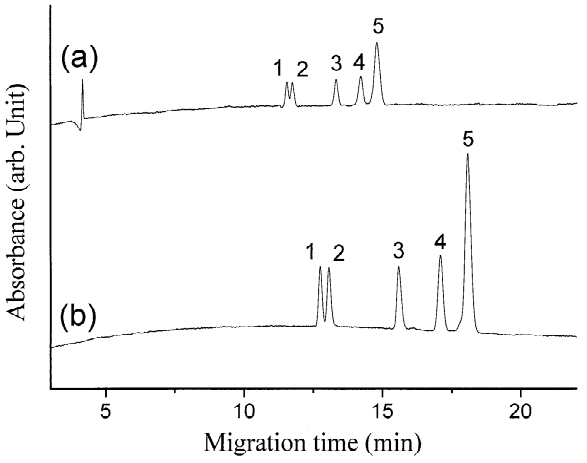


Fig. 6. Electropherograms of five basic drugs with (a) a bare fused-silica capillary and (b) the PTMAFS-coated capillary. Conditions: mobile phase, 100 m*M* sodium acetate, pH 6.0; capillary, 35 cm×75 μm I.D.; separation voltage, −10 kV; detection wavelength, 214 nm. Peaks: 1=amphetamine, 2=methylamphetamine, 3=cocaine, 4=morphine, 5=heroin.

separated simply by differences in their apparent electrophoretic mobilities. For a bare fused-silica capillary under a similar separation condition, as shown in Fig. 6a, complete separation of the five drugs is not achieved. Moreover, peak areas in Fig. 6b are about three times larger than that in Fig. 6a, suggesting that the use of the PTMAFS-coated capillary has minimized adsorption of the basic drugs.

In CE separation of proteins, interaction of the biopolymers with the capillary wall often results in loss of separation efficiency and poor reproducibility of migration time. The adsorption is believed to be due to the electrostatic interactions between positively charged residues of the proteins and the negatively charged silanol groups that are intrinsic to the fused-silica surface. The PTMAFS coating is particularly suited for the separation of basic proteins since at acidic pH the surface and proteins have the same positive charge. Under these conditions, basic proteins are repelled from the surface. Four basic proteins, myoglobin, α-chymotrysinogen A, cytochrome *c*, and lysozyme were used as the test samples. The isoelectric points (p*I*) of myoglobin, α-chymotrysinogen A, cytochrome *c*, and lysozyme are 7.3, 9.2, 10.2, and 11.0, respectively [9]. With a bare fused-silica capillary, the electropherograms were not reproducible and generally associated with poorly resolved peaks, low recovery, and broad tailing peaks. The low recovery and tailing peaks imply that positively charged proteins interact strongly with the negatively charged capillary wall. When the same separation was performed in the PTMAFS-coated capillary at pH 3.0, the resultant repulsive force between the basic proteins and the wall leads to baseline resolution and high recovery of the proteins, as shown in Fig. 7.

3.4. Column performance

Sol–gel coating for CE allows a porous and thick layer to be formed on the inner capillary wall. This type of coating process has the advantage of relative stable EOF and migration time even under a harsh condition [33]. In order to evaluate the stability of the PTMAFS-coated capillary, EOF reproducibility of the column under harsh conditions was examined. Previous report suggests that sol–gel silica tends to dissolve at pH above 8.5 [46]. Hence, exposure of the PTMAFS-coated capillary with 0.1 *M* NaOH, 1.0 *M* HCl, and a pH 9 buffer in sequence may partially dissolve the PTMAFS coating and affect the EOF. As shown in Fig. 8, however, our results show that

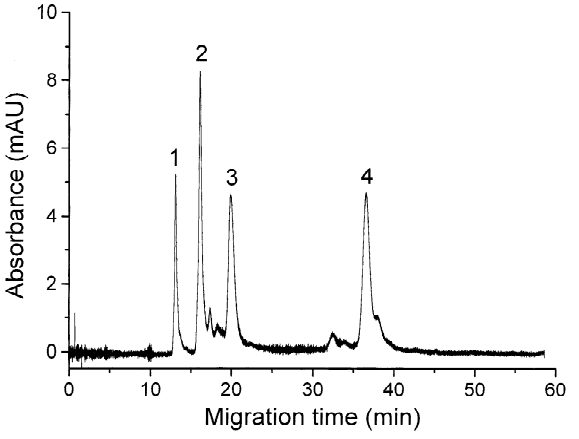


Fig. 7. Electropherogram of four basic proteins with the PTMAFS-coated capillary. Conditions: mobile phase, 30 m*M* phosphate, pH 3.0; capillary, 35 cm×75 μm I.D.; separation voltage, 10 kV; detection wavelength, 214 nm. Peaks: 1=cytochrome *c*, 2=lysozyme, 3=myoglobin, 4=α-chymotrysinogen A.

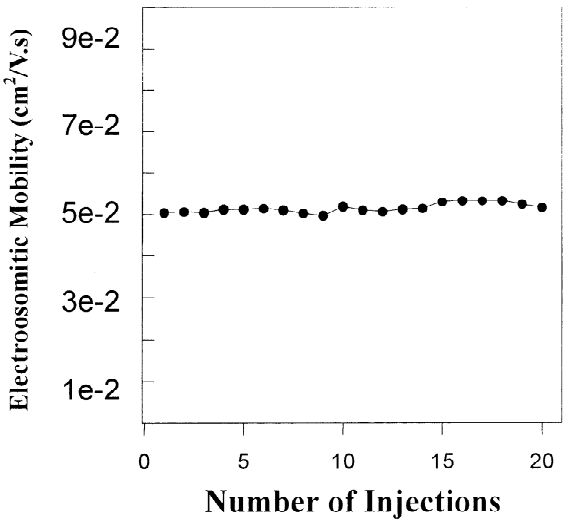


Fig. 8. Retentive characteristics of DMSO in a PTMAFS-coated capillary after repetitive injections. Each injection follows the under sequence: rinsed with 0.1 *M* NaOH for 10 s, 1.0 *M* HCl for 3 min, water for 1 min, and then pH 9.0 phosphate buffer (20 m*M*) for 3 min.

the EOF remains unchanged (less than 2.0%) from repeated exposure of the coated capillary to 0.1 *M* NaOH for 10 s, 1.0 *M* HCl for 3 min, water for 1 min, and then 20 m*M* phosphate at pH 9 for 3 min, indicating that the number of $-N(CH_3)_3^+$ groups relative to the number of $Si-O^-$ groups remains unchanged throughout the test period. This is attributed to the relatively thick PTMAFS coating from the sol–gel process as compared to that from chemical modification of the inner capillary wall through silylation. The exposure of the column to base solutions during purging and rinsing may dissolve PTMAFS to a certain extent. Nevertheless, it is reasonable to assume that the dissolution of the PTMAFS coating between each EOF measurement may act like refreshing the PTMAFS surface. As long as the bulk PTMAFS coating is homogeneous, the surface chemistry of a thick coating should be the same before and after treatment with base solutions. Consequently, reproducible EOF should be observed even though the coated material gradually dissolves when it is exposed to base solutions. Stable EOF shown in Fig. 8 implies that the bulk composition of the PTMAFS coating is homogeneous. The lifetime of the PTMAFS-coated capillary is at least 200 runs (the maximum number of runs we tested).

Table 2
Reproducibility of migration times with the same column in the same day

Sample	Migration time (min)	RSD (%)
Pyromellitic acid	4.06±0.07	1.83
Trimellitic acid	4.74±0.09	1.95
Phthalic acid	5.30±0.11	2.06
Isophthalic acid	5.84±0.10	1.67
Terephthalic acid	6.29±0.13	2.02
Benzoic acid	8.19±0.18	2.23
DMSO	11.7±0.30	2.53

Conditions: capillary, 30 cm×75 μm I.D.; mobile phase, 40 m*M* citrate, pH 3.5; separation voltage, −5 kV; detection wavelength, 214 nm; pretreatment sequence I; $n=8$.

The reproducibility of the PTMAFS-coated capillary was evaluated by measuring the relative standard deviations (RSDs) of the migration times of the six aromatic acids. Eight replicates of migration time measurement for the six aromatic acids at the same day were used to examine the short-term stability of the coated capillary. As shown in Table 2, the migration time data indicate that the short-term stability of the coated capillary is reasonably good (with RSDs in the range of 1.7–2.5%). Similarly, eight replicates of migration time measurement for the six aromatic acids on different days in 1 week were also used to examine the long-term stability of the coated capillary. As shown in Table 3, the migration time data indicate that the long-term stability of the coated capillary is also reasonably good (with RSDs in the range of 2.1–3.5%). In

Table 3
Reproducibility of migration times with the same column on different days

Sample	Migration time (min)	RSD (%)
Pyromellitic acid	5.31±0.11	2.07
Trimellitic acid	5.96±0.13	2.13
Terephthalic acid	6.70±0.16	2.34
Isophthalic acid	7.05±0.17	2.48
Phthalic acid	8.63±0.22	2.58
Benzoic acid	12.31±0.43	3.46

Conditions: capillary, 30 cm×75 μm I.D.; mobile phase, 40 m*M* citrate, pH 4.5; separation voltage, −5 kV; detection wavelength, 214 nm; pretreatment sequence I; $n=8$.

Table 4
Reproducibility of migration times with different columns in the same day

Sample	Migration time (min)	RSD (%)
Terephthalic acid	9.03±0.24	2.71
Isophthalic acid	9.37±0.26	2.77
Phthalic acid	10.66±0.32	3.01

Conditions: capillary, 35 cm×75 μm I.D.; mobile phase, 40 m*M* citrate, pH 4.5; separation voltage, −5 kV; detection wavelength, 214 nm; pretreatment sequence II; $n=4$.

practice, batch-to-batch reproducibility is a key issue. Hence, the reproducibility of four different columns was examined. The relative standard deviations of the migration time of three aromatic acids in different PTMAFS-coated capillaries, as shown in Table 4, are in the range of 2.7–3.0%, suggesting that the sol–gel process gives a good batch-to-batch reproducibility.

The method detection limit (MDL) of the PTMAFS-coated capillary was also evaluated. By the *t* distribution, $\mathrm{MDL}=t_{(n-1,\ 1-\alpha=0.95)}s$, where the *t* value can be obtained from the *t* distribution table and *s* is the standard deviation of seven replicates. The MDL for the six aromatic acids (concentration of each is 6.25 ppm) are in the range of 0.2 to 0.6 ppm.

4. Conclusions

The use of sol–gel process for capillary coating provides a relative simple way for the modification of capillary surface. The thickness of the coated-layer is about 1~2 μm and the surface is rather smooth as indicated by SEM. Also, relatively stable migration behaviors can be obtained even under harsh experimental conditions. From the plot of EOF versus pH, a three-stage EOF pattern is discovered. This property provides the benefit to manipulate the direction and magnitude of EOF with a single column. By controlling the direction of the EOF from cathode to anode, this column has been applied to the separation of nitrate and nitrite; while under a suppressed EOF mode, this column has been applied to the separation of six aromatic acids. Furthermore, with a positively charged quaternary ammonium group on the coating, this column minimizes adsorption of positively charged species and has been applied to the separation of basic drugs and basic proteins.

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References

[1] J.W. Jorgenson, K.D. Lukcas, Anal. Chem. 53 (1981) 1298.
[2] J.W. Jorgenson, K.D. Lukcas, J. Chromatogr. 218 (1981) 209.
[3] J.W. Jorgenson, K.D. Lukcas, J. High Resolut. Chromatogr. Chromatogr. Commun. 4 (1981) 230.
[4] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[5] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166.
[6] J.S. Green, J.W. Jorgenson, J. Chromatogr. 478 (1989) 63.
[7] Y. Walbroehl, J.W. Jorgenson, J. Microcol. Sep. 1 (1989) 41.
[8] X. Huang, J.A. Luckey, M.J. Gordon, R.N. Zare, Anal. Chem. 61 (1989) 766.
[9] J.K. Towns, F.E. Regnier, Anal. Chem. 63 (1991) 1126.
[10] C.A. Lucy, R.S. Underhill, Anal. Chem. 68 (1996) 300.
[11] E.L. Hult, A. Emmer, J. Roeraade, J. Chromatogr. A 757 (1997) 255.
[12] J.K. Towns, F.E. Regnier, J. Chromatogr. 516 (1990) 69.
[13] F.B. Erim, A. Cifuentes, H. Poppe, J.C. Kraak, J. Chromatogr. A 708 (1995) 356.
[14] J. Preisler, E.S. Yeung, Anal. Chem. 68 (1996) 2885.
[15] Q. Liu, F. Liu, R.A. Hartwick, J. Chromatogr. Sci. 36 (1997) 126.
[16] M. Morand, D. Blass, E. Kenndler, J. Chromatogr. B 691 (1997) 192.
[17] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[18] J.J. Pesek, M.T. Matyska, S. Cho, J. Chromatogr. A 845 (1999) 27.
[19] M.T. Matyska, J.J. Pesek, A. Katrehar, Anal. Chem. 71 (1999) 5508.
[20] G.J.M. Bruin, R. Huisden, J.C. Kraak, H. Poppe, J. Chromatogr. 480 (1989) 339.

[21] Y. Maa, K.J. Hyver, S.A. Swedberg, J. High Resolut. Chromatogr. 14 (1991) 65.
[22] M. Huang, G. Yi, J.S. Bradshaw, M.L. Lee, J. Microcol. Sep. 5 (1993) 199.
[23] J.T. Smith, Z. El-Rassi, J. High Resolut. Chromatogr. 15 (1992) 573.
[24] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[25] K.A. Cobb, V. Dolnik, M. Novotny, Anal. Chem. 62 (1990) 2478.
[26] M. Gilges, H. Husmann, M. Kleemiβ, S.R. Motsch, G. Schomburg, J. High Resolut. Chromatogr. 15 (1992) 452.
[27] H.N. Clos, H. Engelhardt, J. Chromatogr. A 802 (1998) 149.
[28] M.H. Hong, J. Sudor, M. Stefansson, M.V. Novotny, Anal. Chem. 70 (1998) 568.
[29] A. Cifuentes, J.C. Diez-Masa, J. Fritz, D. Anselmetti, A.E. Bruno, Anal. Chem. 70 (1998) 3458.
[30] X. Huang, L.J. Doneski, M.J. Wirth, Anal. Chem. 70 (1998) 4023.
[31] L.A. Colon, Y. Guo, Anal. Chem. 67 (1995) 2551.
[32] H. Engelhardt, M.A. Cunat-Walter, J. Chromatogr. A 716 (1995) 27.
[33] Y. Guo, G.A. Imahori, L.A. Colon, J. Chromatogr. A 744 (1996) 17.
[34] J.D. Hayes, A. Malik, J. Chromatogr. B 695 (1997) 3.
[35] J.-T. Wu, P. Huang, M.X. Li, M.G. Qian, D.M. Lubman, Anal. Chem. 69 (1997) 320.
[36] J.D. Hayes, A. Malik, Anal. Chem. 73 (2001) 987.
[37] P. Tien, L. Chau, Y. Shieh, W. Lin, G. Wei, Chem. Mater. 13 (2001) 1124.
[38] T. Mizuno, H. Ngata, S. Manabe, J. Non-Cryst. Solids 100 (1988) 236.
[39] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[40] W.M.A. Niessen, U.R. Tjaden, J. van der Greef, J. Chromatogr. 636 (1993) 3.
[41] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A.
[42] C.W. Klampfl, W. Buchberger, Trends Anal. Chem. 16 (1997) 221.
[43] E.F. Hilder, M. Macka, P.R. Haddad, Anal. Commun. 36 (1999) 299.
[44] D. Corradini, G. Cannarsa, E. Fabbri, C. Corradini, J. Chromatogr. A 709 (1995) 127.
[45] G.N.W. Leung, H.P.O. Tang, T.S.C. Tso, T.S.M. Wan, J. Chromatogr. A 709 (1995) 127.
[46] J. Friche, E. Emmerling, in: R. Reisfeld, C.K. Jorgensen (Eds.), Chemistry, Spectroscopy and Applications of Sol–Gel Glasses, Springer, Berlin, 1992, p. 37.