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Hydrolytically stable amino-silica glass coating material for manipulation of the electroosmotic flow in capillary electrophoresis

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Abstract

A hydrolytically stable amino-silica glass coating material was fabricated inside fused-silica capillaries, using sol–gel technology. Aminopropyltriethoxysilane was used as the precursor in the glass formation process. The net charge at the surface of the coating material depends on the degree of protonation of the amino groups and the degree of ionization of the silanol groups, thus enabling manipulation of the magnitude and direction of the electroosmotic flow (EOF). At low pH (<6.0), the coating bears a net positive charge, which results in an electroosmotic flow from the cathode toward the anode and minimizes the wall-solute interactions of basic species. At high pH (>6.5), the coating surface bears a net negative charge and the coated capillary behaves like an uncoated one, having an EOF in the cathodic direction. The amino-silica glass coating has also been shown to be extremely stable under both acidic and basic conditions. The reproducibility of the electroosmotic mobility of five different capillaries was found to be 7% R.S.D. The utility of the material is shown with the separation of basic proteins, peptides and basic compounds.

Keywords: Electroosmotic flow; Amino-silica glass coating; Coating; Capillary columns; Peptides; Proteins; Amino acids; Epinephrine; Norepinephrine

1. Introduction

Capillary electrophoresis (CE) has been proven to be a powerful separation technique with unique advantages over other separation techniques (e.g., high separation efficiency and short analysis time) [1,2]. One area of considerable interest is the analysis of basic compounds by CE, especially basic peptides and proteins [3,4]. It has been realized that the degradation of the separation performance is largely due to adverse interaction between positively charged analytes (e.g., basic peptides and proteins) and the negatively charged inner walls of the fused-silica capillaries. The wall adsorption can lead to zone broadening, peak tailing and poor reproducibility of the separation. To diminish the interaction between basic analytes and the silica surface, three main approaches have been taken: (1) suppression of the wall interaction by using running electrolytes at extreme pH values [5,6] or with very high ionic strengths [7,8]; (2) shielding the negative charges on the inner walls of the capillary by adding nonionic surfactants to the running electrolyte [9], or by coating the wall with a polymeric layer, such as polyacrylamide [10,11], polyethylene glycol [12], hydroxylated polyether [13], polysiloxane [14], or crosslinked polymers [15,16]; (3) imparting positive charges to the inner walls of the capillary by using cationic electrolyte additives such as alkylamines [17], or tetraazao- macrocycles [18], or by coating the inner walls either dynamically or permanently with cationic polymers, such as chitosan [19], poly-

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arginine [20], polybrene [21], or polyethyleneimine [22,23].

The use of the first approach is limited by the fact that high ionic strengths can generate excessive Joule heating in the capillary, which can degrade the separation efficiency; and extreme pH values can denature proteins of biological interest. The second approach is very effective in blocking the adsorption sites on the inner walls of the capillary, thus minimizing the wall adsorption. In the capillary coated with hydrophilic polymers, the negative charges on the silica surface are covered by neutral polymer layers, thereby suppressing the electroosmotic flow (EOF). The migration and separation of positively charged species are completely based on their electrophoretic mobilities. In the third approach, the silica surface bears net positive charges, which repel positively charged analytes from the surface. Because of the positively charged surface, the EOF is reversed from the cathodic to the anodic direction. This approach is useful in the analysis of some difficult protein samples; however, electrolyte additives can bring interferences to some separations, and the stability of the cationic polymer coating needs to be further investigated.

Fused-silica capillaries with cationic or neutral polymer coatings have the EOF in only one direction or nearly suppressed. This limits the possibility of using a single capillary for the separation of a wide range of species. Recently, Smith and El Rassi [24] described a surface modified capillary with switchable EOF. The inner walls of the capillary were coated with a composite material of quaternary ammonium and polyether layers. The direction of the EOF could be switched depending upon the pH range of the separation electrolyte. However, the coating procedure involved several steps of chemical reactions; moreover, the hydrolytic stability of the coating material was not reported. In another procedure, Thorsteinsdóttir et al. have demonstrated similar alternation of the EOF by amino-silylating the inner walls of fused-silica capillaries [25]. Due to the unstable siloxane bonds, the modified silica surface lacked hydrolytic stability at high or low pH values.

In this report we describe the preparation and performance of an amino-silica glass material to coat fused-silica capillaries, which can provide the switchable characteristics mentioned above [24]. This coating, however, is extremely stable at low and high pH values, and its fabrication is relatively simple. The fabrication procedure is based on the recently reported sol–gel procedure to prepare stationary phases for open tubular liquid chromatography (OT-LC) and electrochromatography [26,27]. Since both amino and silanol groups are present on the surface of the amino-silica glass coating, the magnitude and direction of the EOF are dependent on the net charge of the surface at a given pH value; hence, manipulation of the EOF direction can be achieved. However, the unique sol–gel preparation procedure permits the fabrication of a hydrolytically stable material, thus stabilizing the reversed EOF at low pH values, compared with the amino-silylated capillaries previously reported. The one-step coating procedure also reduces capillary preparation time. The utility of the amino-silica glass coating is demonstrated by the separation of small basic molecules (i.e., epinephrine and norepinephrine), basic peptides and proteins with the reversed EOF at low pH values. In addition, five dansylated amino acids are also separated with the normal EOF at high pH values.

2. Experimental

2.1. Chemicals and materials

Fused-silica capillaries (50 μm I.D.×365 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). 3-Aminopropyltriethoxysilane (APTEOS) was supplied by United Chemical Technologies (Bristol, PA, USA) and used without further purification. Ribonuclease A (from bovine pancreas, p*I* 9.3), trypsinogen (from bovine pancreas, p*I* 8.7), lysozyme (from turkey egg white, p*I* 11.0), and dansylated amino acids were purchased from Sigma (St. Louis, MO, USA) and used as received. Protein and amino acid stock solutions were prepared in water purified with an Ultra-Pure water system (Millipore, Bedford, MA, USA) and were diluted to the desired concentration with running electrolytes prior to sample injection. Basic peptides were generously provided by Mr. Xiongwei Yan and Jason Wood from Professor Lawrence's group, Department

of Chemistry, State University of New York at Buffalo, and supplied as individual aqueous solutions. The solutions were stored in a freezer at −20°C and thawed before use. Heptakis (2,6-di-O-methyl)-β-cyclodextrin, norepinephrine and (±)-epinephrine were obtained from Aldrich (Milwaukee, WI, USA). Sodium dihydrogenphosphate, sodium hydrogenphosphate, sodium phosphate, phosphoric acid (HPLC grade), and tris(hydroxymethyl)-aminomethane (Tris) (Fisher Scientific, Pittsburgh, PA, USA) were used to prepare the running electrolyte solutions. All the solutions were filtered through a 0.45 μm syringe filter before use.

2.2. Instrumentation

All the EOF measurements and several CE separations were performed in a P/ACE Model 2200 CE unit (Beckman, Fullerton, CA, USA). The unit allowed the capillary to be thermostatted at 22°C. UV detection was carried out at 214 nm for mesityl oxide and epinephrine. Sample injection was made by applying 0.5 p.s.i. for 2 s (1 p.s.i.=6894.76 Pa). Data acquisition and analysis were facilitated using the Beckman Gold software (version 712).

Other CE experiments were performed in a homemade CE system, using either a positive or a negative high voltage power supply (Glassman High Voltage, Whitehouse Station, NJ, USA) capable of delivering 0–30 kV. All high voltage components were housed in a Plexiglass box fitted with a safety interlock. This system did not provide temperature control of the capillary. On-column UV detection was performed with a variable-wavelength UV absorbance detector (Model CV^4, ISCO, Lincoln, NE, USA). Proteins and peptides were monitored at 210 nm and dansylated amino acids at 214 nm. The signal from the UV detector was fed into an A/D converter board (DT2804, Data Translation, Marlboro, MA, USA) for data acquisition, and was controlled by means of GRAM 386 for Chromatography software (Galactic Industries, Salem, NH, USA).

2.3. Coating procedure

The capillary coating procedure was adapted from our previous work [26]. To expose the maximum number of surface silanol groups on the inner walls of the fused-silica capillary, the capillary was first flushed with 1 *M* NaOH for 1 h and then rinsed with water for another hour. The capillary was then dried at 180°C in an oven under nitrogen flow overnight. The sol–gel solution was prepared by mixing 0.5 ml APTEOS, 0.375 ml ethanol, 0.054 ml water and 0.1 ml 1.2 *M* HCl. HCl was added as catalyst and ethanol as solvent. The sol solution was stirred at room temperature for 10 h; it then was introduced into the pretreated capillary by a syringe. The sol–gel solution was allowed to stay inside the capillary for about 10 min before it was forced out by pressure, leaving a thin coating on the inner walls of the capillary. The coated capillary was dried overnight at 90°C in the oven under nitrogen flow. The capillary coated with the amino-silica glass material was first washed with acetone and then with methanol for 30 min each, then rinsed with water for 20 min.

The amino-silylated fused-silica capillary was prepared using conventional silane chemistry [25]. First, the capillary was pretreated as described above. The capillary was then filled with APTEOS solution (20%, v/v, in dry toluene). The capillary was kept at 90°C in the oven for 10 h with both capillary ends capped. The excessive reagent was forced out of the capillary, and the capillary was rinsed with acetone, methanol, and water as described above.

2.4. Electrophoretic conditions

The capillaries coated with the amino-silica glass material were equilibrated with the running electrolytes for about 1 h prior to use. If the pH of the running buffer was changed, the capillaries were flushed with the new running buffer for at least 1 h before starting new runs. The capillaries were rinsed for 2 min between runs with the running electrolyte, and were stored in water when not in use. Before use, uncoated capillaries were treated with 1.0 *M* sodium hydroxide for 30 min and flushed with water for 20 min, followed by equilibration with the running electrolyte for 30 min.

The pH of the 40 m*M* Tris running electrolyte was adjusted to 3.9 using 1.2 *M* HCl and was used for the separation of proteins and peptides. 10 m*M* Heptakis(2,6-di-O-methyl)-β-cyclodextrin was

added to 30 m*M* phosphate buffer (pH 2.5) to effect chiral selection of epinephrine enantiomers. These separations were performed using the negative power supply. A mixture of epinephrine and norepinephrine was separated in both amino-silica glass coated capillaries and uncoated capillaries using 10 m*M* phosphate solution (pH 3.4). The separation of a mixture of dansylated amino acids was also performed in both coated and uncoated capillaries using a positive power supply in 10 m*M* phosphate electrolyte (pH 9.0).

The electroosmotic mobility was determined by measuring the migration time of mesityl oxide in the amino-silica glass coated capillaries using a negative power supply at pH<6.3 and a positive power supply at pH>6.3. The running electrolytes were 20 m*M* phosphate solutions of different pH values, adjusted to a constant ionic strength. All the measurements of electroosmotic mobility were made in triplicate.

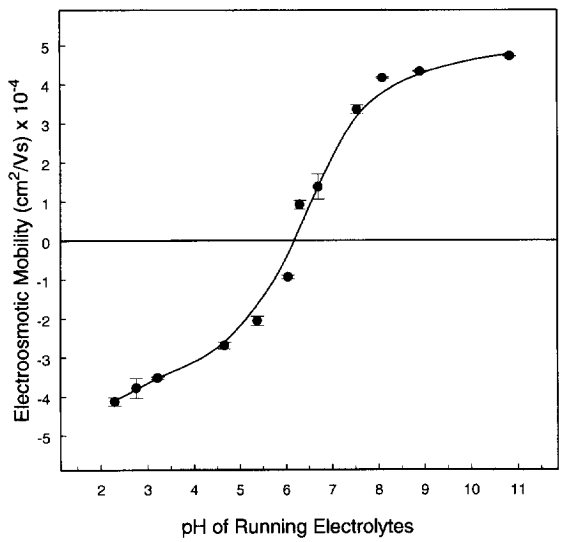

Fig. 1. Plot of electroosmotic mobility vs. the pH of the running electrolyte (20 m*M* phosphate, constant ionic strength). Amino-silica glass coated capillary: 47 cm (40 cm effective length)×50 μm I.D. Upper panel EOF towards the cathode, lower panel EOF towards the anode.

3. Results and discussion

We fabricated the amino-silica glass coating material using sol–gel technology, a low-temperature glass fabrication method [28]. Typically, this method uses tetramethoxysilane and tetraethoxysilane as precursor materials to fabricate silica glasses. The glass material is produced through hydrolysis and condensation of the precursor, aging, and drying steps, resulting in a three dimensional network material with high surface area. In our procedure, we substituted the commonly used precursor (i.e., tetraethoxysilane) with the silane reagent, APTEOS, which contains three ethoxy groups that undergo hydrolysis. By subsequent polycondensation reactions, an amino-silica glass material is obtained having a transparent glass appearance. Because aminopropyl groups are not involved in the hydrolysis and condensation reactions, a great number of them is indeed present at the surface of the coating material, along with silanol groups.

3.1. Electroosmotic flow

The effect of having both amino and silanol groups on the coating surface can be illustrated by measuring the EOF in the capillary coated with the amino-silica glass material. Fig. 1 depicts the electroosmotic mobility obtained in 20 m*M* phosphate solutions (constant ionic strength) in the pH range of 2–11. It is apparent that both magnitude and direction of the EOF change with the pH of the running electrolyte. At pH above 6.3, the direction of the EOF is from anode to cathode, as is the case in uncoated capillaries, and the electroosmotic mobility increases as the pH increases. However, the direction of the EOF is reversed (from cathode to anode) at pH below 6.3, and the electroosmotic mobility increases as the pH decreases. Furthermore, the EOF is suppressed at about a pH of 6.3. The pH value for zero EOF (pH~6.3) was estimated by curve-fitting the electroosmotic mobility data at different pH values. The estimated pH value for the zero EOF was then checked by monitoring the EOF of the coated capillary using a phosphate buffer (pH~6.3). The level of running electrolytes and the ends of the capillary were carefully adjusted to prevent any height differential that might produce siphoning. The EOF was monitored for about 2 h using both positive and negative polarities of the power supply. A signal corresponding to the neutral marker was not ob-

served during the time monitored. The pattern of the EOF change with the pH of the running electrolyte is similar to those of amino-silylated fused-silica capillaries [25] and the surface modified capillaries previously reported by Smith and El Rassi [24].

The magnitude and direction of the EOF are dependent on the number and sign of net charges on the inner surface of the capillary [1]. The pK_a of propylamino groups is 10.7 [29]; hence the amino groups become protonated at pH<10.7. The silanol groups start ionizing at pH 3.0 and become fully deprotonated at pH>8.0. The magnitude and direction of the EOF are determined by the relative concentrations of the positive amino and negative silanol groups at the surface, which in turn depend on the degree of protonation and ionization of the amino and silanol groups respectively at a particular pH. At pH values above 6.3, the number of negative silanol groups is higher than that of the protonated amino groups; therefore, the coating surface bears a net negative charge and the EOF is from anode to cathode. At pH values below 6.3, there are more positive amino groups than ionized silanols, due to the protonation of the amino groups concurrent with a decrease in the degree of ionization of silanol groups at low pH. This results in a net positive charge on the coating surface, leading to the reversal of the EOF. At approximately pH 6.3, the coated capillary exhibits no electroosmotic flow, indicating a net zero charge on the coating surface. These results indicate that a desirable EOF direction (towards the cathode or anode) can easily be achieved by adjusting the pH of the running electrolyte.

3.2. Stability and reproducibility of electroosmotic flow

The capillary coated with the amino-silica glass material exhibits a strong EOF (from cathode to anode) in the low pH range (pH<4.0), which increases as the pH of the running electrolyte is decreased. To utilize the positively charged surface and the reversed EOF for the separation of basic analytes, the hydrolytic stability of the material is extremely important to yield a reliable EOF, particularly at low pH values. Capillary columns coated with the amino-silica material were washed with 1% trifluoroacetic acid (pH~0.8), exposing the amino-silica coating to harsh conditions. The EOF was monitored after exposing the capillary to 1% trifluoroacetic acid for a certain period of time. Then, the capillary was rinsed with water and 20 m*M* phosphate solution (pH 3.4), which was the running electrolyte used to monitor the EOF. Once the EOF was recorded (at least in triplicate), the capillary was again exposed to 1% trifluoroacetic acid. The procedure was repeated until the capillary was exposed to the acidic conditions for a total period of 45 h. An amino-silylated capillary column was also subjected to the same test; however, this capillary was exposed to the acidic conditions for only 18 h. The results are shown in Fig. 2. The electroosmotic mobility of the capillary coated with the sol–gel material remained unchanged in the 45 h of the test period, whereas the electroosmotic mobility of the amino-silylated capillary decreased by almost 80% of its original value in 18 h. This indicates that most amino groups chemically bonded through the silylation procedure were removed from the silica surface. On the other hand, the sol–gel derived amino-silica glass exhibited excellent stability. The good hydrolytic stability of this material can be attributed to the fact that the aminopropyl groups are introduced by bulk modi-

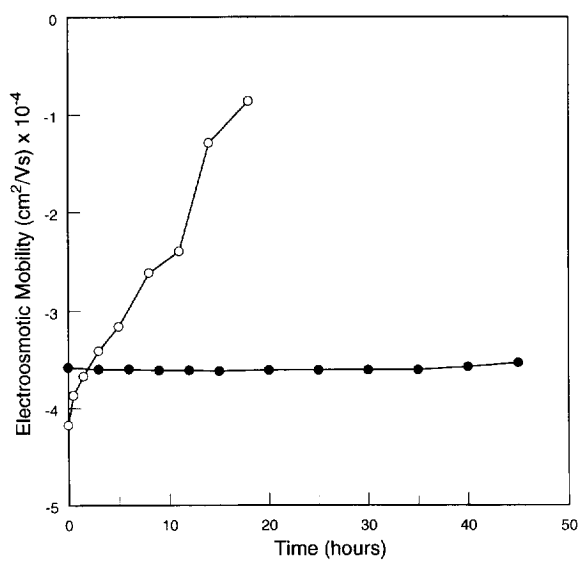

Fig. 2. Electroosmotic mobilities measured in 20 m*M* phosphate (pH 3.4) as a function of exposure time to acidic conditions (1% trifluoroacetic acid). (●) Amino-silica glass coated capillary (50 μm I.D.), (○) amino-silylated fused silica capillary (50 μm I.D.).

fication during the glass formation process. They are not attached to the surface through hydrolytically unstable siloxane bonds, as is the case in the amino-silylated capillary. Instead, the aminopropyl groups are attached to the coating surface through Si–C bonds, which are hydrolytically stable at low pH values [11].

The EOF was also examined at a basic pH in the amino-silica coated capillary and in the amino-silylated one. After preparation, each capillary was rinsed with the running electrolyte (10 m*M* phosphate, pH=9.0) for about 4 h. Then, to mimic a capillary cleaning procedure, the capillaries were washed with 0.1 *M* NaOH for 1 min and rinsed with the running buffer for 10 min. A neutral marker was injected and the EOF (from anode to cathode) was monitored. This procedure was repeated for 25 injections in a time period of over 8 h. The electroosmotic mobility for each injection in both capillaries is shown in Fig. 3. Since the number of amino groups present at the inner surface of the coated capillary directly influences the EOF, a change in electroosmotic mobility (higher EOF under this basic conditions) would be an indication of a loss of amino groups at the surface. In our case, the coated capillaries were exposed to 0.1 *M* NaOH for 25 min throughout the test period. Furthermore, it should be pointed out that dissolution of pure silica can occur under the pH conditions of the running electrolyte (pH~9.0). However, the amino-silica glass material was stable and maintained its performance under these conditions. The electroosmotic mobility of the amino-silica coated capillary remained fairly constant over the 25 injections of the test. This is seen by the reproducibility of the EOF, which was 4.5% R.S.D., even after washing with 0.1 *M* NaOH. The electroosmotic mobility of the amino-silylated capillary, however, increased over the first nine injections and started levelling off at the tenth injection. The increase in the electroosmotic mobility of the amino-silylated capillary is attributed to an increase in the number of surface silanol groups as the aminopropyl groups are removed from the inner walls of the capillary through hydrolysis. When most of the aminopropyl groups are removed, the number of silanol groups approaches a constant value and the EOF starts levelling off. After testing the EOF under basic conditions, the capillaries were exposed to low pH (10 m*M* phosphate, pH 2.9) to monitor the reversal of the EOF. The capillary coated by the sol–gel procedure showed reversal of the EOF and was used for the pH cycling experiments (see below). The EOF in the amino-silylated capillary was not observed to be reversed. This further illustrates removal of the amino groups from the surface.

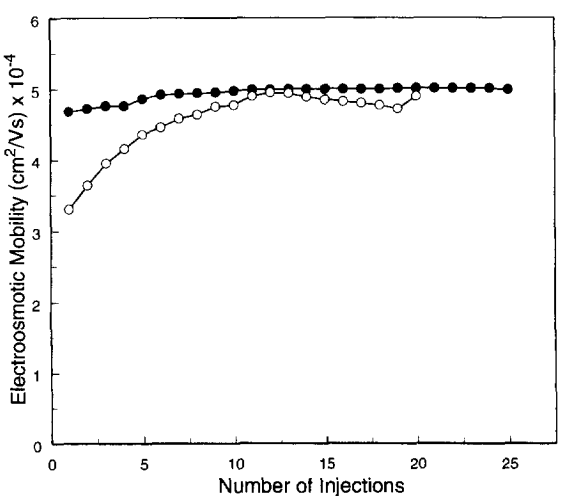

Fig. 3. Electroosmotic mobilities measured in 10 m*M* phosphate (pH 9.0) as a function of number of injections. (●) Amino-silica glass coated capillary (50 μm I.D.), (○) amino-silylated capillary (50 μm I.D.). The capillaries were rinsed with the running electrolyte for 4 h prior to injections and washed with 0.1 *M* NaOH between injections.

The amino-silica coated capillary provides a switchable EOF by adjusting the pH value of the running electrolyte. To reliably utilize this characteristic, it is very important that the EOF is not irreversibly affected when the separation is performed at different pH values, especially at high or low pH where the EOF is in the opposite direction. To investigate the effect of high and low pH of the running electrolyte on the EOF, we first measured the electroosmotic mobility of an amino-silica coated capillary (from anode to cathode) at pH 9.0 in 10 m*M* phosphate; then the capillary was rinsed continuously with 10 m*M* phosphate at pH 2.9 for 2 h, and the reversed EOF (from cathode to anode) was monitored. The capillary was rinsed with 10 m*M* phosphate at pH 9.0 for 2 h and the electroosmotic mobility was measured again. This cycling procedure was repeated four times. The electroosmotic mo-

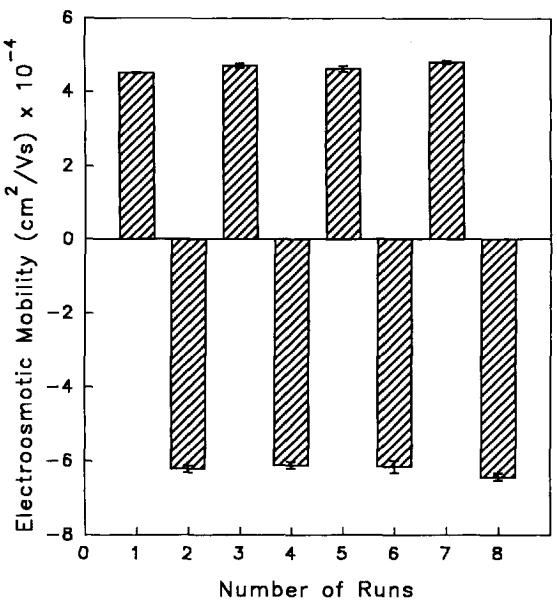

Fig. 4. Electroosmotic mobilities measured in 10 m*M* phosphate at pH 9.0 (anode to cathode) and pH 2.9 (cathode to anode) in an amino-silica glass coated capillary (50 μm I.D.).

bilities measured at pH 9.0 and 2.9 (in the opposite direction) are shown in Fig. 4. The EOF at either pH 9.0 or 2.9 does not seem to be affected by a drastic change in the pH value of the running electrolyte, indicating good stability of the EOF in both directions at different pH values.

The run-to-run reproducibility of the EOF in the capillary modified with the amino-silica coating was investigated at both low (2.9) and high pH (9.0) by measuring the migration time of the neutral marker. At a pH of 2.9 where the EOF direction was from cathode to anode, the relative standard deviation (R.S.D.) of the migration time was around 2.0% (n=40); at a pH of 9.0, where the EOF direction was from anode to cathode, the R.S.D. of the migration time was approximately 2.5% (n=35). The column-to-column EOF reproducibility was studied by determining the electroosmotic mobility of five different capillaries coated at different times using the same preparation protocol. The runs were performed using 10 m*M* phosphate buffer (pH 2.9). The reproducibility of the EOF was found to be approximately 7% R.S.D. These results demonstrate the excellent reproducibility of our process, even though the capillaries were coated manually, by means of a syringe. In addition, this performance was obtained without an accurate control of the coating time. Different coating times can provide different coating thickness. However, we do not expect that changes in the coating thickness will cause any major variation in the performance of the capillary. This is because the sol–gel procedure is a bulk modification method, the change in the coating time may affect the thickness but not the surface chemistry of the coating material. It is the chemistry at the surface of the amino-silica glass material that changes the charge character of the column inner surface due to the exposed amine groups. The stability and reproducibility of the coated capillaries show the ruggedness of the coating procedure.

3.3. Applications

The effectiveness of the positively charged amino-silica coating surface in minimizing the wall adsorption of basic species was first demonstrated in the separation of two basic drug compounds, epinephrine and norepinephrine. Fig. 5 shows electropherograms of the separation of the two compounds using an amino-silica coated column and an uncoated capillary under similar experimental conditions. The EOF in the two capillaries was in opposite directions, but the EOF velocity was adjusted to be the same. The two basic compounds eluted before the EOF in the uncoated capillary; a tailing was observed for the epinephrine peak, indicating the interaction of the basic compound with the negatively charged walls. In the amino-silica coated capillary, the two compounds eluted as sharp peaks after the EOF, indicating that solute-wall interactions were diminished effectively. The separation efficiency for the two compounds was about 110 000 theoretical plates, and the elution order of the two compounds was also reversed because of the reversed EOF in the amino-silica coated capillary. The addition of a chiral selector will facilitate separation of enantiomeric mixtures. We used the sol–gel coated capillary (EOF from cathode to anode) to separate two epinephrine enantiomers using 10 m*M* heptakis (2,6-di-O-methyl)-β-cyclodextrin in the running electrolyte. The electropherogram is shown in Fig. 6. The two enantiomers of epinephrine were base-line separated within 10 min

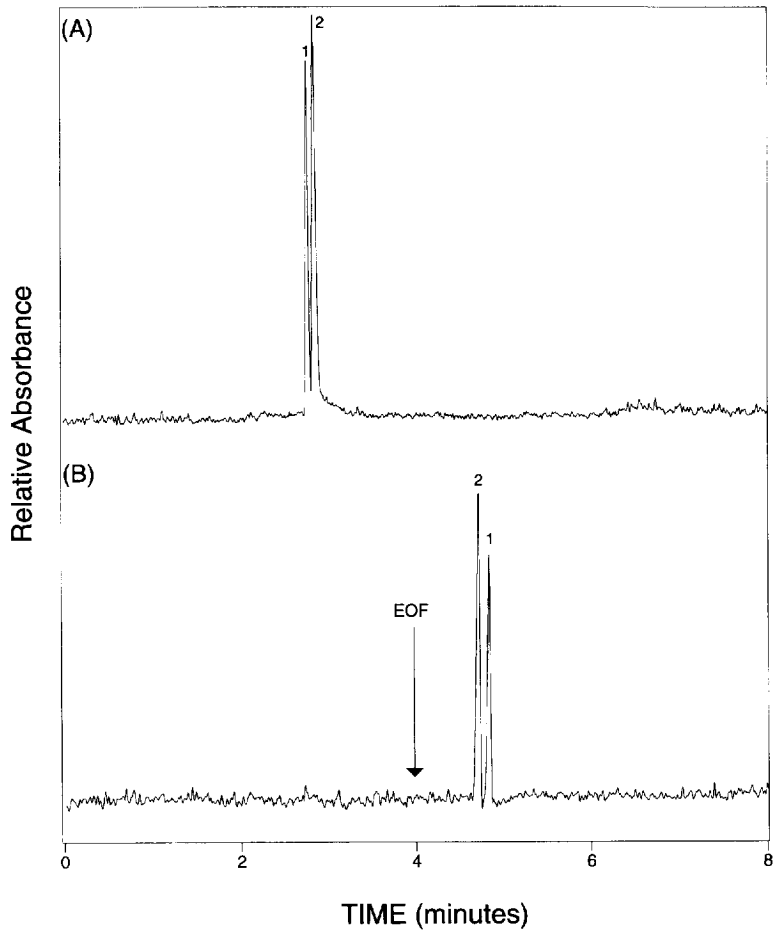

Fig. 5. Separation of two basic drug compounds, 0.2 m*M* (1) norepinephrine and (2) epinephrine. (B) Sol–gel derived amino-silica glass coated capillary: 65 cm (50 cm effective length)×50 μm I.D. (A) Fused silica capillary: 65 cm (50 cm effective length)×50 μm I.D. Running electrolyte: 10 m*M* phosphate, pH=3.4. Separation voltage: 27 kV in (A) and −24 kV in (B). Hydrodynamic injection: 5 s (10 cm height), UV detection at 214 nm.

with separation efficiencies of about 35 000 plates. The small fronting observed can be attributed to a high sample concentration relative to the concentration of the running electrolyte [30].

The positively charged surface of the amino-silica coating maintained at a pH below 6 is also useful in reducing the wall adsorption of large biomolecules, such as basic proteins and peptides. Fig. 7 illustrates a typical electropherogram of the separation of three model basic proteins obtained in a sol–gel coated capillary. The three basic proteins were separated with relatively high efficiencies; for example, 140 000 plates and 160 000 plates were obtained in the 45 cm coated capillary (injection to detection) for trypsinogen and lysozyme, respectively. The relative standard deviation (R.S.D.) of the migration time for the proteins tested, based on four consecutive injections, was ≤1%; the reproducibility for the separation efficiencies was about 4% R.S.D. The high separation efficiencies suggest that the interaction between positively charged proteins and negative surface silanol groups has been greatly reduced. This is due to the fact that the amino groups on the coating surface are protonated at pH~3.9, which promotes columbic repulsion between the positively charged amino groups and the basic proteins.

The amino-silica coated capillary is also suitable for the separation of other types of basic biomole-

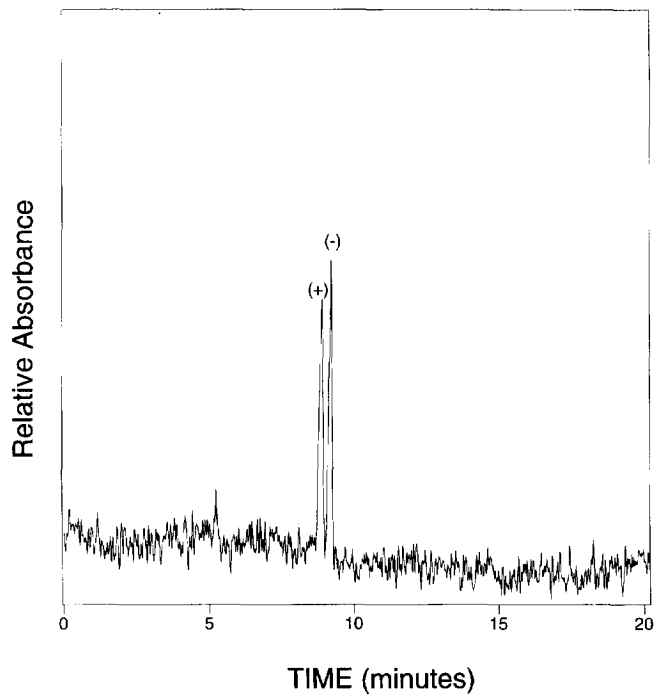

Fig. 6. Separation of epinephrine enantiomers (2 m*M*). Amino-silica glass coated capillary: 47 cm (40 cm effective length)×50 μm I.D. Running electrolyte: 30 m*M* phosphate, pH 2.5, 10 m*M* heptakis (2,6-di-O-methyl)-β-cyclodextrin. Separation voltage: −10 kV. Injection: 2 s at 0.5 p.s.i. UV detection at 214 nm.

cules. Fig. 8 shows the separation of four synthetic peptides at pH 3.9. These four peptides were designed as potential protein kinase inhibitors with the following amino acid sequences:

Pep 15: Phe–Ala–Phe–$(Arg)_3$–Phe–Ala–Phe–$(Arg)_3$–Phe–Ala–Phe
Pep 9: $(Arg)_3$–Phe–Ala–Phe–$(Arg)_3$
Pep 8: (D)–Leu–$(Arg)_4$–(D)–Phe–Ala–Phe
Pep 7: $(Arg)_4$–(D)–Phe–Ala–Phe

There are four arginine residues in two peptides (Pep 7 and Pep 8) and six in the other two peptides (Pep 9 and Pep 15). The high percentage of basic arginine residues (>40%) present in the sequences makes the peptides very basic. The four peptides were baseline separated under the experimental conditions, despite of the fact that Pep 8 and Pep 7 are only different by a leucine residue. Some impurities from the peptide sample were also observed in the separation. The elution order of the four peptides follows the increasing percentage of arginine residues in the amino acid sequence of the peptides, i.e., Pep 15 (40%), Pep 8 (50%), Pep 7 (57%), and Pep 9 (67%). Slight tailing was observed for some of the peptides; the asymmetry factors for Pep 8, Pep 7, and Pep 9 are 2.6, 3.2, and 3.0, respectively. This can be attributed to large positive charge-to-mass ratios of these peptides, which may introduce the adverse interaction between remaining silanol groups at the surface and the positively charged peptides.

In addition to the hydrolytic stability of the sol–gel derived amino-silica material, the coated capillaries offer the advantage of having a switchable EOF. This provides the capability for tuning the direction of the EOF, with respect to specific applications. This feature should enable the separation of a wider range of species with a single capillary. Fig. 9A shows the separation of a group of dansylated amino acids in a coated capillary using 10 m*M* phosphate containing 10% methanol (pH~9.0). For comparison, the separation of the same mixture in an uncoated capillary is also shown in Fig. 9B. At pH 9.0, the EOF in the coated capillary was in the same

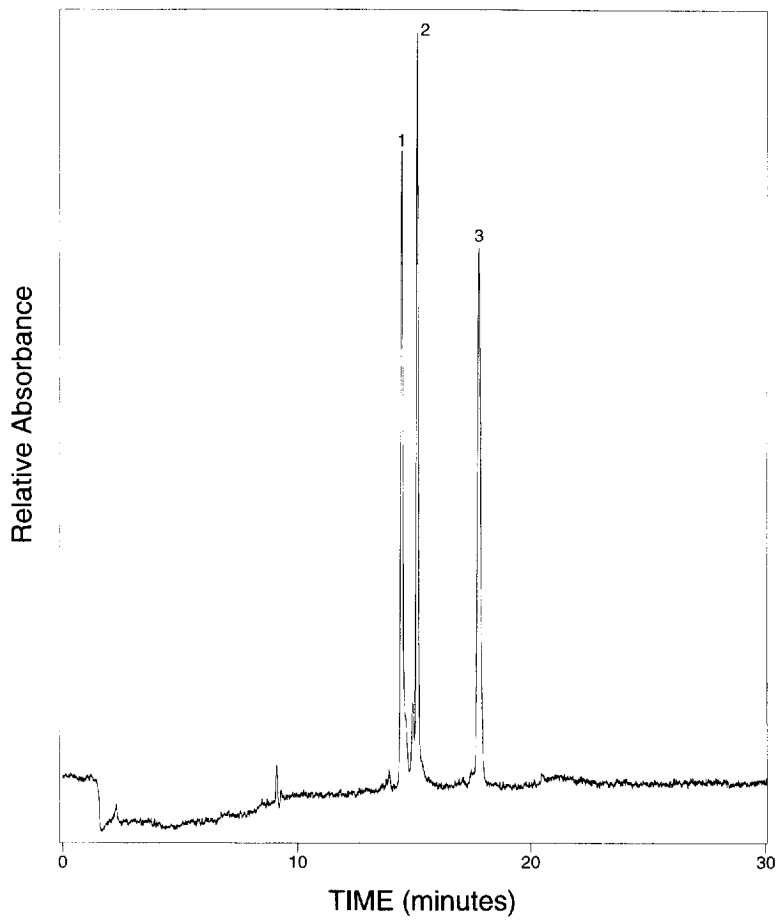

Fig. 7. Separation of three basic proteins, 0.75 mg/ml (1) trypsinogen, (2) ribonuclease A, (3) lysozyme. Amino-silica glass coated capillary: 60 cm (45 cm effective length)×50 μm I.D. Running electrolyte: 40 m*M* Tris–HCl, pH 3.9. Separation voltage: −10 kV. Hydrodynamic injection: 2 s (5 cm height). UV detection at 210 nm.

direction as in the uncoated capillary (i.e., anode to cathode); however, the electroosmotic mobility in the coated capillary was reduced by approximately 30%. Since all the dansylated amino acids were negatively charged at pH 9.0, a positive power supply was used in both separations. The EOF velocities were adjusted to be similar by increasing the applied voltage in the case of the amino-silica coated capillary. There was no indication of interactions between negatively charged analytes and the amino groups on the coating surface. The separation efficiencies in Fig. 9 range from 160 000 to 220 000 theoretical plates in the uncoated capillary, and 110 000 to 190 000 theoretical plates in the amino-silica coated capillary. In the separation with the amino-silica coated capillary, a higher voltage was applied to increase the EOF velocity to the same level as in the uncoated capillary due to the smaller electroosmotic mobility in the coated capillary. Because of the smaller electroosmotic mobility, but the same EOF velocity, the efficiencies of negative analytes are lower in the coated capillary, even at a higher applied voltage [30]. However, the efficiency can be improved and the analysis time reduced by applying a higher voltage in the amino-silica coated capillaries. Moreover, an improved resolution was ob-

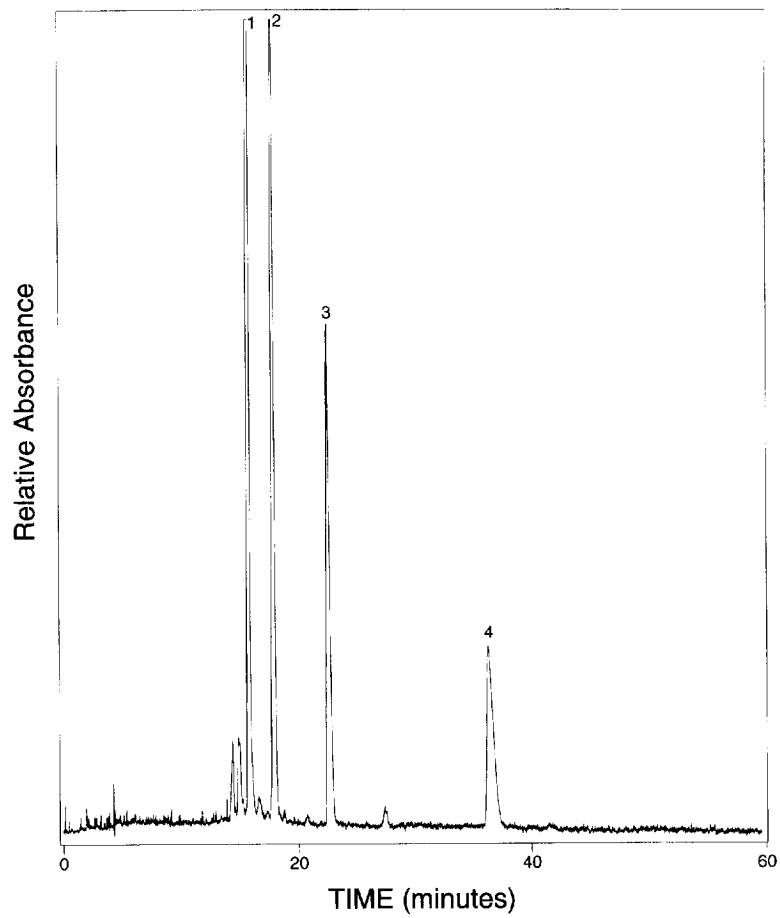

Fig. 8. Separation of four synthetic peptides. Separation conditions are the same as described in Fig. 7, except that voltage was −20 kV. Sample: (1) Pep 15; (2) Pep 8; (3) Pep 7; (4) Pep9.

tained in the coated capillary within similar analysis time, which can be attributed to the smaller electroosmotic mobility in the coated capillary [30].

4. Conclusion

The sol–gel method was used to prepare a hydrolytically stable amino-silica coating material for fused-silica capillaries. The coating procedure was simple and reproducible. The sol–gel coating material was proven to be very stable under both basic and acidic conditions. Both the magnitude and direction of the EOF in the coated capillary can be manipulated by changing the pH of the running electrolyte, due to the presence of both positively and negatively charged groups on the coating surface. This feature makes it possible to separate both positively and negatively charged species in a single capillary by changing the direction of the EOF. Two structurally close basic compounds and epinephrine enantiomers were successfully separated without adverse wall adsorption on the coated capillary. High separation efficiencies, similar to what has been reported with other systems, were achieved in the separation of basic proteins and peptides. The amino-silica coated capillary could be used in the same way as uncoated ones in separating negatively charged species at high

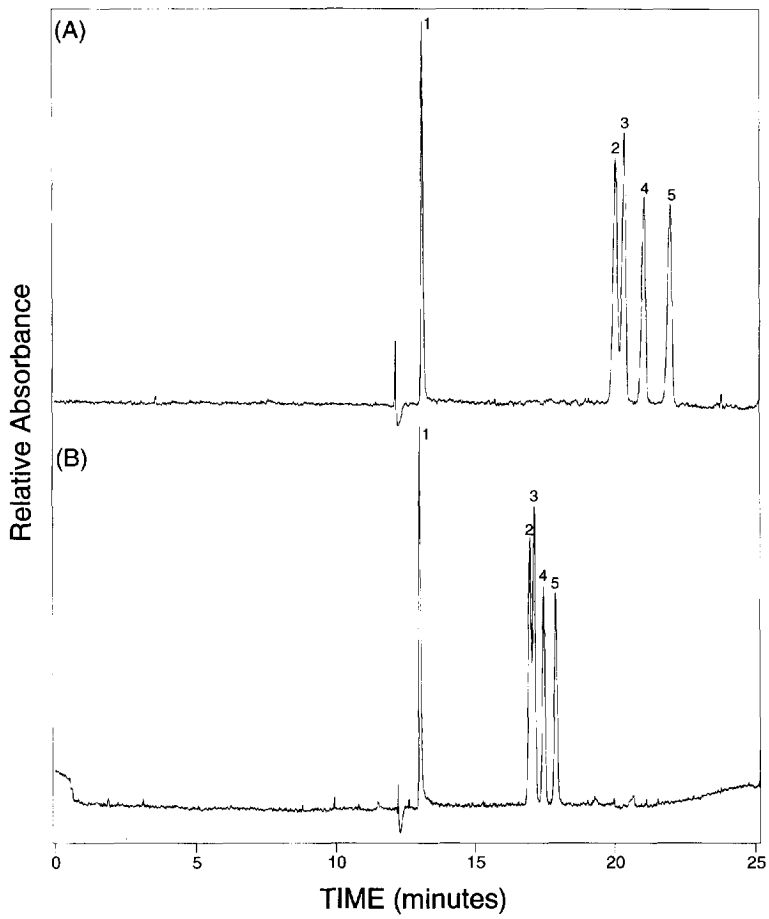

Fig. 9. Separation of dansylated amino acids in (B) amino-silica glass coated capillary and (A) uncoated capillary. Capillary: 47 cm (40 cm effective length)×50 μm I.D. Running electrolyte, 10 m*M* phosphate, pH 9.0, 10% methanol. Separation voltage: 6 kV in (A) and 8.5 kV in (B). Injection: 5 s at 0.5 p.s.i. UV detection at 214 nm. Sample: (1) lysine; (2) leucine; (3) serine; (4) methionine; (5) glycine.

pH values. The reduced electroosmotic mobility of the coated capillaries allows for an improved resolution.

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