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A Sol–Gel-Modified Poly(methyl methacrylate) Electrophoresis Microchip with a Hydrophilic Channel Wall

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Abstract: A sol–gel method was employed to fabricate a poly(methyl methacrylate) (PMMA) electrophoresis microchip that contains a hydrophilic channel wall. To fabricate such a device, tetraethoxysilane (TEOS) was injected into the PMMA channel and was allowed to diffuse into the surface layer for 24 h. After removing the excess TEOS, the channel was filled with an acidic solution for 3 h. Subsequently, the channel was flushed with water and was pretreated in an oven to obtain a sol–gel-modified PMMA microchip. The water contact angle for the sol–gel-modified PMMA was $\approx 27.4°$ compared with $\approx 66.3°$ for the pure PMMA. In addition, the electroosmotic flow increased from $2.13 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ for the native-PMMA channel to $4.86 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ for the modified one. The analytical performance of the sol–gel-modified PMMA microchip was demonstrated for the electrophoretic separation of several purines, coupled with amperometric detection. The separation efficiency of uric acid increased to $74\,882.3\,m^{-1}$ compared with $14\,730.5\,m^{-1}$ for native-PMMA microchips. The result of this simple modification is a significant improvement in the performance of PMMA for microchip electrophoresis and microfluidic applications.

Keywords: electrophoresis · microchips · polymers · purines · sol–gel processes

Introduction

Recently, microfluidic analytical systems have undergone explosive growth.[1] Microfluidic devices have the potential to fundamentally change the way in which chemical analysis is conducted. More and more attention has been paid to capillary electrophoresis (CE) microchips owing to their high degree of integration, portability, minimal solvent/reagent consumption, high performance, and speed.[2–4] CE microchips are often fabricated from a range of glass substrates, from cheap soda-lime glass to high quality quartz.[5] However, their application has been limited because of high costs, harmful and complicated fabrication procedures, and limitations on the geometric modification of the chip channel.[6] Therefore, polymers have been used extensively in place of glass or silicon in microchip devices, as they offer attractive mechanical and chemical properties, low cost, simple fabrication, biocompatibility, and higher flexibility.[6,7] Polymers are becoming the most promising materials for microfluidic devices because they can be mass-produced by using technologies such as injection molding and hot embossing.[6,7] A wide variety of polymeric materials have been evaluated for fabricating microchips instead of glass. These materials include poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), polycarbonate, polyester, polystyrene, poly(ethylene terephthalate glycol), and polyolefins.[6,7] Among them, PDMS and PMMA are the two most commonly used polymers. As a rigid polymer, PMMA is inexpensive and has good mechanical properties that are suitable for many applications such as the fabrication of analytical instrumentation. PMMA has been particularly useful for the fabrication of electrophoresis chips with low price, excellent optical transparency, and excellent insulation (electri-

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. It contains a figure illustrating the photographs of a drop of water on the surfaces of the sol–gel modified and the pristine PMMA plates, the fabrication process of the detection electrode, a 3-D adjustable device for microchip CE-AD, and a SEM of the cross section of a channel in a sol–gel-modified microchip.

cal) and mechanical properties.[8,9] PMMA can directly generate a stable electro-osmotic flow (EOF) in the microchannels if an electrical field is applied.[10,11] Existing procedures for fabricating PMMA chips include laser ablation,[10] imprinting,[12–14] and injection molding.[15] However, PMMA suffers from some significant limitations. For example, the EOF of native PMMA is less than one half of that of glass owing to the negligible presence of ionizable functional groups.[8] PMMA is also very hydrophobic, so the channels in a PMMA device are difficult to wet. More importantly, hydrophobic analytes will actually adsorb onto its surface or absorb into the bulk PMMA. This significantly limits the analytes that can be separated on devices made from PMMA and generally results in separation efficiencies that are significantly lower than those reported for glass devices. Despite these limitations, PMMA remains an attractive material for microchip CE and microfluidics in general because of its ease of fabrication and low cost. A number of groups have sought to overcome the problems associated with PMMA through careful control of the surface and bulk chemistry. Johnson et al. exposed PMMA microchannels to ultraviolet (UV) light to increase the surface charge, hydrophilicty, and related electro-osmotic mobilities (EOMs).[16] The UV treatment of PMMA microchannels led to an average 4% increase in the EOM relative to the untreated surfaces. A covalent modification of channels was introduced by Soper et al. to impart amine or octadecyl groups to the surface of PMMA microchannels by way of aminolysis and reactions with *n*-octadecylisocyanate, to control the EOF and the hydrophilicity.[8,17,18] A rather different approach to the modification of PMMA surfaces was taken by Lee et al.[19] Poly(ethylene glycol) (PEG) was grafted to the surface of PMMA substrates by using atom-transfer radical polymerization, which substantially reduced the EOF and the nonspecific adsorption of proteins onto the PMMA surface. As a result, fast, highly efficient, and reproducible electrophoretic separations of proteins and peptides were achieved by using a PEG-grafted PMMA microchip. Wang et al. developed a method based on the in situ polymerization of monomers for the bulk modification of PMMA microchips.[20] This new protocol is based on the modification of the bulk microchip material by tailored copolymerization of monomers during atmospheric-pressure molding. The judicious addition of a modifier to the primary monomer solution imparts attractive properties to the plastic microchip substrate, such as high stability, a strong pH sensitivity, and significant enhancement and modulation of the EOF (to achieve flow velocities comparable to those of glass). In addition to these permanent modifications of the PMMA surfaces, dynamic coatings have also been evaluated by dissolving surfactants or hydrophilic neutral polymers in the running buffers to enhance the separation efficiency.[21,22]

Herein, the development of an alternative method for the modification of the surface of PMMA microchannels through introduction of a sol–gel layer is described. To prepare the sol–gel layer, the channels were filled with tetraethyl orthosilicate (TEOS) that diffused into and adsorbed onto the surface layer of the PMMA channel. These TEOS-treated channels were then hydrolyzed by using a dilute HCl solution and were subsequently cured in an oven. The hydrophilic sol–gel layer formed on the surface of the PMMA channels through the condensation of the hydrolyzed silanes. The fabrication details and the characterization of the sol–gel-modified PMMA microchip are reported in the following sections. Additionally, the remarkable performance of the new microchip, coupled with end-column amperometric detection, is demonstrated by its ability to separate and detect three purines.

Results and Discussion

A sol–gel modification layer was directly synthesized on the surface of PMMA to generate a hydrophilic microchip channel wall that has a high EOF. The scanning electron microscopy (SEM) images of the surface of pure and sol–gel-coated PMMA plates are shown in Figure 1A and B, respectively. It can be seen clearly in Figure 1B that a layer of rough sol–gel coating has formed and is continuously distributed on the surface. The diameter of the pores is in the 10–20 nm range. The modified surface of PMMA is wettable toward TEOS because they have similar polarities. Immersion of the modified PMMA in TEOS leads to adsorption of TEOS onto the surface; prolonged immersion results in the diffusion of TEOS into the surface layer of PMMA. The TEOS-pretreated PMMA was washed with a 0.1 M HCl aqueous solution to hydrolyze the TEOS to form the sol–gel on the channel surface. To evaluate the surface roughness of pristine PMMA and the sol–gel-modified PMMA, images of the surface were taken by using atomic force microscopy (AFM) (Figure 2). The AFM images of both samples are in agreement with the corresponding SEM images. The surface

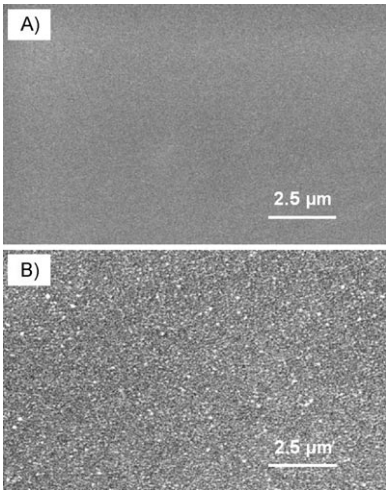

Figure 1. SEM images of A) the pure and B) the sol–gel-modified PMMA surfaces. Conditions: accelerating voltage, 20 kV; magnification, ×5000.

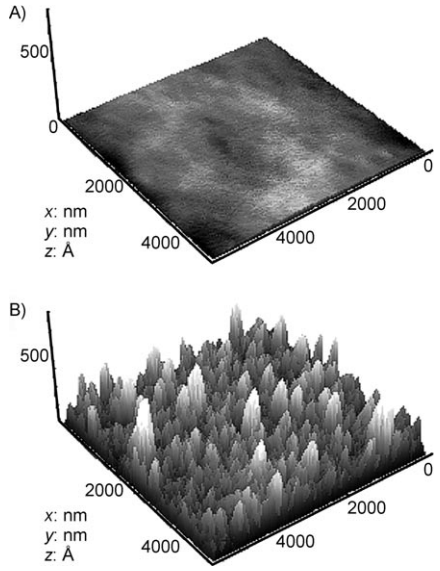

Figure 2. AFM images of A) the pristine and B) the sol–gel-modified PMMA surfaces.

of pristine PMMA is flat and smooth with a root-mean-square (RMS) roughness of 2.44 nm (Figure 2A). In comparison, the morphological features of the sol–gel-modified PMMA surface are very different. Protrusive structures with a RMS roughness of 13.9 nm can be observed in the AFM image (Figure 2B). The higher water wettability of the sol–gel-modified surface can be attributed to not only its chemical properties, but also its greater roughness.

Poly(dimethylsiloxane) (PDMS) is another polymer commonly used for the fabrication of microfluidic chips. Roman et al. developed a sol–gel method to fabricate hydrophilic channel walls in PDMS microchips by bulk modification.[23] The pristine PDMS microchip was soaked in TEOS for 30 min to allow the TEOS to reach the surface of the channels in the chip. The PDMS absorbed the TEOS that caused the chip to swell. Subsequently, the chips were placed in an aqueous solution containing 2.8% ethylamine, and after heat had been applied, nanometer-sized SiO_2 particles formed in the PDMS polymer matrix. Herein we describe a new approach to fabricate hydrophilic PMMA channel walls based on a sol–gel process using TEOS. PMMA is a rigid polymer and will not swell if immersed in TEOS. This characteristic causes a sol–gel to form on the surface rather than in the whole PMMA matrix. This layer is suitable for an electrophoresis microchip because the properties of the channel wall play a key role in the separation. This sol–gel-derived method can take advantage of the rich and diverse chemistry of organically modified sols. This opens the door for a wide range of interactions between sols and solutes and hence flexible manipulations can be achieved in electrophoresis microchips.[24,25]

X-ray photoelectron spectrometry (XPS) was employed to measure the elemental maps on the surfaces of the pristine and the sol–gel-modified PMMA. The XPS spectra of both surfaces is shown in Figure 3. As expected, the peaks of silicon (Si_{2s} and Si_{2p^3}) appeared after PMMA was modified by using silica sol–gel (Figure 3B). Decreased carbon (C_{1s}) and enhanced oxygen (O_{1s} and O_{KLL}) peaks were observed in the XPS spectrum of the modified PMMA surface, indicating that a nanoscale sol–gel layer had formed on PMMA.

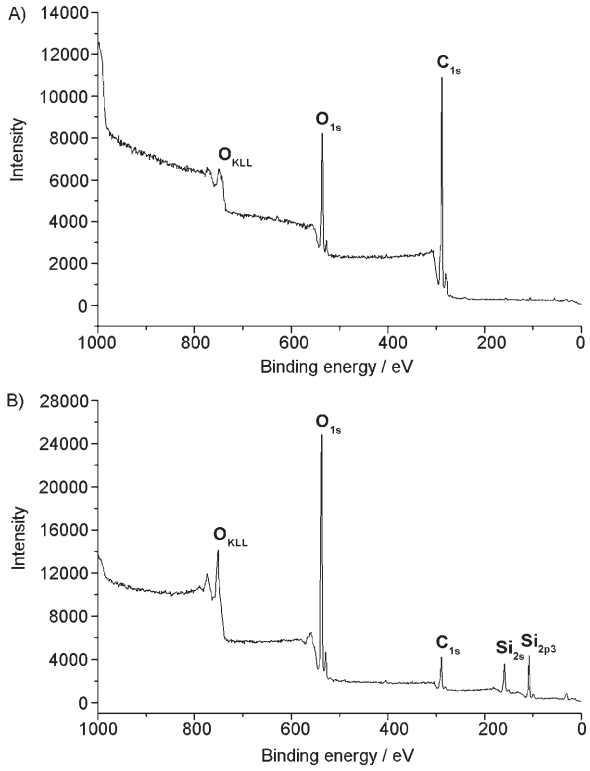

Figure 3. XPS spectra of A) the pristine and B) the sol–gel-modified PMMA surfaces.

In an effort to further characterize the surface of the PMMA, the hydrophilicity of the walls was assessed by using water contact-angle measurements. Contact angles for the pure and the sol–gel-modified PMMA were measured to be $66.3° \pm 1.7°$ ($n = 5$) and $27.4° \pm 0.6°$ ($n = 5$), respectively, in which n is the number of times that the contact angles were recorded approximately 24 hours after modification. Water contact-angle measurements revealed a significant difference (38.9°) between the native and the sol–gel-modified PMMA. Our value for the pure PMMA was close to that measured by Henry and co-workers ($\approx 66°$).[18] The contact-angles values provide preliminary proof that the hydrophilicity of the PMMA surface has been improved after the sol–gel modification. The contact-angle value recorded after 30 days, for the sol–gel-modified PMMA surface, was found to be $28.1 \pm 1.2°$ ($n = 5$), similar to that of the original measurement ($27.4° \pm 0.6°$ ($n = 5$)). A 5 μL drop of water on the surface of the pure and the sol–gel-modified PMMA plates is shown in Figure S1 in the Supporting Information. It is clearly apparent that the surface of the sol–gel-modified

PMMA is much more water-wettable than that of pure PMMA.

The direction and magnitude of the EOF within the microchannels is primarily determined by the property and density of surface charges on the channel walls. The EOFs in the microchannels were measured by using the well-established baseline monitoring technique of Huang et al.[26] At pH 8.2, the EOF mobility of a pure PMMA channel was increased from 2.13×10^{-4} (RSD = 2.9%, $n=5$) to $4.86\times10^{-4}\,cm^2V^{-1}s^{-1}$ (RSD = 2.3%, $n=5$) after sol–gel modification. The increased EOF can be attributed to the presence of the glasslike sol–gel surface and the resultant increase in surface charge. The measured EOF mobility of pure PMMA compares favorably to literature values (1.2×10^{-4}–$2.6\times10^{-4}\,cm^2V^{-1}s^{-1}$).[8,17,18]

Seen in Figure 4 are the FTIR spectra of the pure and the sol–gel modified PMMA films. Absorption bands of PMMA

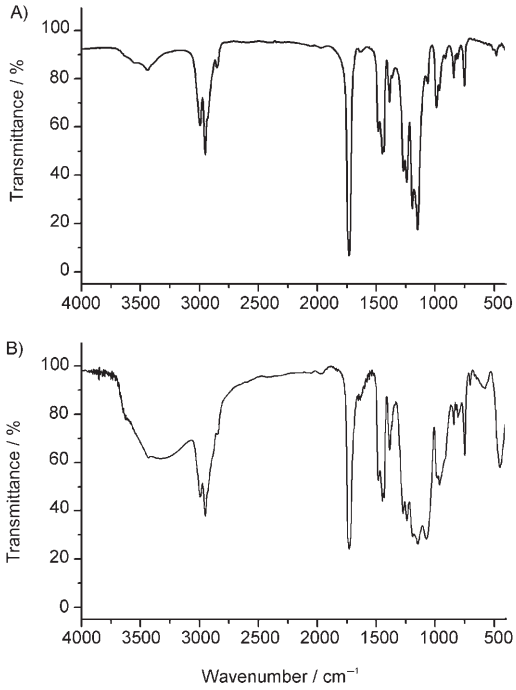

Figure 4. FTIR spectra of A) the pristine and B) the sol–gel-modified PMMA films.

before and after the sol–gel modification are observed at $\tilde{\nu}=2994$, 2951, 1732 cm^{-1}, and in the ranges of 1365–1484 cm^{-1} and 1149–1273 cm^{-1}, which are assigned to CH_3, CH_2, C=O, C–H, and C-O-C, respectively.[27] In the FTIR spectrum of the sol–gel-modified PMMA (Figure 4B), the absorption peak of the Si-O-Si asymmetric stretching found at 1075 cm^{-1} was attributed to the formation of silica structures through the sol–gel process of TEOS.[28] The presence of residual silanols in the silica networks on the surface of PMMA, which are capable of hydrogen-bond formation, is evidenced by the appearance of the hydroxyl peaks in the 3100–3600 cm^{-1} region.[29]

The analytical performance of the sol–gel-modified PMMA microchip for the electrophoretic separation of several purines was coupled with amperometric detection. Illustrated in Figure 5 are the representative electropherograms

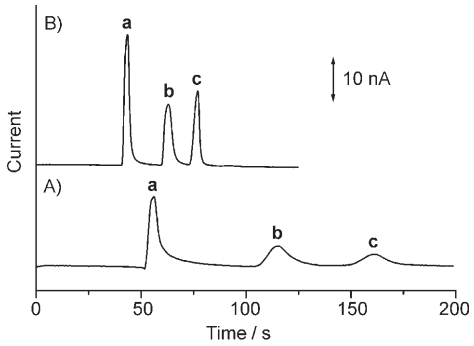

Figure 5. Electropherograms for a mixture containing a) guanine, b) xanthine, and c) uric acid (250 μM of each) using A) the pure and B) the sol–gel-modified PMMA electrophoresis microchips. Conditions: separation voltage, +2000 V; injection voltage, +1500 V; injection time, 3 s; running buffer, borate (20 mM)/phosphate buffer (10 mM, pH 8.2); detection electrode, 320 μm-diameter carbon electrode; detection potential, +0.9 V (vs. Ag/AgCl wire).

for a mixture containing guanine, xanthine, and uric acid (250 μM for each) for the pure and the sol–gel-modified PMMA electrophoresis microchips. The three purines can be separated in the sol–gel-modified PMMA microchips within 80 s and result in well-defined and resolved peaks. In comparison, broader peaks along with a longer separation time (180 s) were observed when the pure PMMA microchip was employed. The sharp and well-resolved responses result in smaller values of the half-peak widths for guanine, xanthine, and uric acid in the modified microchip relative to the pure one (2.7 vs. 4.9, 4.0 vs. 11.5, and 2.8 vs. 13.2 s, respectively). By using the modified microchip the plate number of uric acid has effectively been increased from 14730.5 to 74882.3 m^{-1}, significantly enhancing the separation efficiency. The shorter migration time and the sharper peaks can be attributed to an increase in EOF and to the lower adsorption of the analytes on the sol–gel-modified channel wall in the PMMA microchip.

Figure 6 shows the electropherograms for a mixture containing guanine, xanthine, and uric acid (250 μM for each) by using the sol–gel-modified microchip at the separation voltages of +500, +1000, +1500, +2000, and +2500 V. As expected, increasing the separation voltage from +500 to +2500 V dramatically decreases the migration time, from 177.8 to 34.2, from 258.2 to 49.9, and from 322.0 to 61.3 s for guanine, xanthine, and uric acid, respectively. Also shown (in the inset of Figure 6) is the effect of the separation voltage upon the peak current and the half-peak width ($W_{1/2}$) of guanine. Upon raising the separation voltage from +500 to +2500 V, the peak current of guanine increases to the maximum values of 28.71 nA (at +2000 V) and decreases to 24.49 nA (at +2500 V), while the half-peak width ($W_{1/2}$) of guanine significantly decreases from 11.0 to 2.2 s. Notice

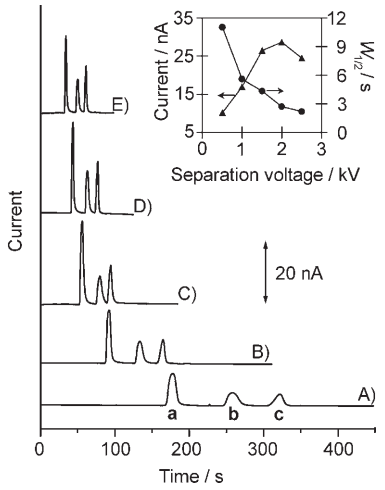

Figure 6. Electropherograms for a mixture containing a) guanine, b) xanthine, and c) uric acid (250 μM of each) using the sol–gel-modified microchip at the separation voltages of A) +500, B) +1000, C) +1500, D) +2000, and D) +2500 V. Also shown (in the inset) are the resulting plots of the dependence of the peak current and half-peak width ($W_{1/2}$) of guanine upon the separation voltage. For further conditions see Figure 5.

also the flat baseline current even at high separation voltages. Such a behavior indicates the effective isolation of the detection electrode from the high separation voltage. Moreover, higher separation voltages may result in higher Joule heat that directly affects the separation efficiency of this method. However, separation voltages that are too low will increase the analysis time considerably, which in turn causes peak broadening. Based on these experiments, +1500 and +2000 V were chosen as the optimum voltages in this work to accomplish a good compromise of resolution, sensitivity, and analysis time.

The suitability of the sol–gel-modified PMMA microchip for measuring uric acid in a biological sample is demonstrated in Figure 7, in which the electropherogram of a 1:100 diluted urine sample in the running buffer is displayed. The concentration of uric acid in the urine sample was determined to be 2.98 mM (RSD = 3.7 %, $n = 5$), which is similar

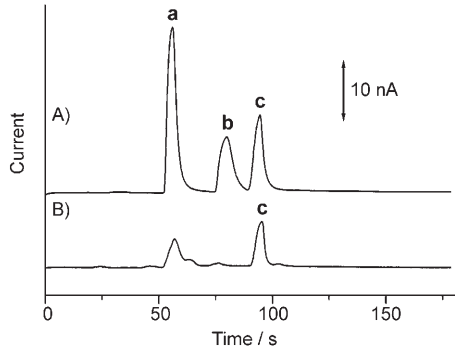

Figure 7. Electropherograms for A) a mixture containing a) guanine, b) xanthine, and c) uric acid (250 μM of each) and B) a 1:100 diluted urine sample in the running buffer. Separation voltage, +1500 V; other conditions as in Figure 5.

to the data in a previous report was obtained by using conventional CE with amperomeric detection (2.05 and 1.98 mM).[30] The results demonstrate that this method has both high accuracy and good precision for the analytes tested in the real sample.

Conclusion

In summary, we have developed a simple approach for the fabrication of silica sol–gel-modified PMMA electrophoresis microchips with highly hydrophilic channel walls. Analysis of SEM, AFM, XPS, and FTIR spectra indicated that the sol–gel had been loaded on the surface of PMMA channels by our simple sol–gel modification approach. The performance, utility, and advantages of the new microchip have been successfully demonstrated in combination with the separation and detection of purines. The hydrophilicity and the EOF of the PMMA channel have been significantly enhanced by the modification. The ease, simplicity, versatility, and low cost of the modification route hold significant promise for the generation of hydrophilic surfaces on the channel walls for use in high-performance microchip electrophoresis. This new sol–gel process may also be useful for the modification of microchips made from other polymers.

Experimental Section

Reagent and solutions: Methyl methacrylate (MMA), benzoin ethyl ether, 2,2′-azobis(isobutyronitrile), tetraethoxysilane (TEOS), guanine, xanthine, and uric acid were all purchased from SinoPharm (Shanghai, China). Graphite powder was supplied by Aldrich (Wilwaukee, WI, USA). Other chemicals were all analytical grade.

Stock solutions of guanine, xanthine, and uric acid (10 mM) were all prepared in a NaOH (50 mM) aqueous solution and then diluted to the desired concentration with the running buffer just prior to use. The running buffer for the capillary electrophoresis (CE) separation of purines was a borate (20 mM)/phosphate buffer (10 mM, pH 8.2). Sample solutions were prepared by diluting the stock solutions in the running buffer. The urine sample collected from a healthy male volunteer was diluted with the running buffer (urine/buffer, 1:100) prior to injection for analysis. The sample solutions obtained were directly injected for microchip CE analyses.

Electrode fabrication: The fabrication process for the detection electrodes is illustrated in the Supporting Information, Figure S2). A piece of copper wire (10 cm long, 150 μm diameter) was inserted into a 3.0 cm-long fused-silica capillary (320 μm (i.d.) × 450 μm (o.d.), Hebei Yongnian Ruipu Chromatogram Equipment Co., Hebei, China) and a 2 mm opening was left in the capillary for the subsequent filling of the graphite–epoxy composite. The other end of the capillary was sealed together with copper wire by thermal adhesive. Epoxy resin and hardener (Zhejian Cixi Tiandong Adhesive Co., Ningbo, China) was mixed thoroughly at a weight ratio of 2:1. The graphite powder and epoxy resin/hardener were hand-mixed in a ratio of 1:1 (w/w). The graphite–epoxy composite was then packed into the capillary by pressing the opening end of the capillary (to a depth of ≈3 mm) into a sample of the composite. The graphite–epoxy composite should touch the end of the copper wire inside the capillary tightly for electrical contact. The composite was then allowed to cure at room temperature for at least 3 h.

Apparatus: Details of the integrated microchip capillary-electrophoresis amperometric-detection (CE-AD) system have been described previous-

ly.[4,31] Briefly, a laboratory-made high-voltage dc power supply (+4000 V) provided a voltage for the electrophoretic separation and the electrokinetic sample introduction. The PMMA chips (15×75×1.5 mm) had simple cross layouts, with a four-way injection cross-connected to the three reservoirs and the separation channel. Before use, the original detection cell of the chip was cut off leaving the channel outlet at the end of the chip, thereby facilitating the end-column AD. The PMMA chip shown in the Supporting Information (Figure S3) consisted of a 56 mm-long separation channel (between the injection cross and the detection reservoir) and a 5 mm-long injection channel. The two channels crossed each other halfway between the sample and the unused reservoirs, at 5 mm from the running buffer reservoir. The channels had a trapezoidal cross section with a top width of ≈100 μm, bottom width of ≈40 μm, and depth of ≈37 μm. A SEM image of the cross section of a sol–gel-modified microchannel in the PMMA microchip is illustrated in the Supporting Information (Figure S4). The thickness of the cover sheet for the microchip was approximately 150 μm. The channel plates of the CE microchips were fabricated by the UV-initiated in situ polymerization of a MMA prepolymer molding solution between a silicon template and a commercially available PMMA plate as described in a previous report.[31] The silicon template was fabricated by standard photolithography and wet chemical etching. Short pipette tips were inserted into the holes of the various reservoirs. A three-dimensionally adjustable Plexiglass device for microchip CE-AD[32] was fabricated for housing the separation chip and the detector, allowing their convenient replacement, and facilitating the precise alignment between the outlet of the separation channel and the capillary-based microdisc electrode for end-column AD. Platinum wires, inserted into the individual reservoirs on the holder, served as contacts to the high-voltage power supply.

An alternate constant-voltage power supply (YS73-4 A-3 KVA, Shanghai Keyi Instrumental Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. To improve the repeatability of the peak current and migration time, the whole CE system was assembled in a room that was air-conditioned at 25 °C to reduce the temperature fluctuation. The surface morphologies of the pure and the sol–gel-modified PMMA surfaces were observed by using a scanning electron microscope (PHILIPS XL 30, Eindhoven, The Netherlands) and a Pico-SPM atomic force microscope (Molecular Imaging, Tempe, AZ, USA) in tapping mode. The XPS spectra were recorded by using a Perkin–Elmer PHI 5000C ESCA system with $Mg_{K\alpha}$ radiation ($h\nu$ = 1253.6 eV). In general, the X-ray anode was run at 250 W and the high voltage was kept at 14.0 kV with a detection angle at 54°. The pass energy was fixed at 93.9 eV to ensure sufficient resolution and sensitivity. The base pressure of the analyzer chamber was about 5×10^{-8} Pa.

Chip modification: Prior to modification, the channel in the PMMA microchip was flushed with isopropanol and dried in an oven at 60 °C for 1 h. Subsequently, TEOS was injected into the channel and was allowed to diffuse into the surface layer for 24 h. After the extra TEOS in the channel was removed under a stream of nitrogen, the channel was flushed with water for 2 min and was filled with 0.1 M HCl aqueous solution by using a syringe. After 3 h, the channel was flushed with water and was kept in an oven at 60 °C for 6 h to obtain the sol–gel modification layer on the PMMA channel wall.

Characterization measurements

Contact-angle measurements: The static-state water contact angles were measured on the pure and the sol–gel-modified PMMA plates. Two small PMMA plates were flushed with isopropanol and dried in the air. One plate was immersed in TEOS for 24 h. Then the extra TEOS on the plate was flushed away with pressed nitrogen and water successively, followed by emersion in HCl (0.1 M) aqueous solution for 3 h. Subsequently, the plate was flushed with water and was kept in an oven at 60 °C for 6 h to form the sol–gel modification layer on the PMMA surface. Another PMMA plate was only dipped in 0.1 M HCl aqueous solution for 3 h for comparison. The material upon which the contact-angle measurements were to be made was placed on a platform. A 5 μL drop of water was then dropped on the material with a syringe and allowed to rest on the surface at room temperature (≈25 °C). After 30 s, the contact angle was determined by using an OCA15 optical contact angle meter (Data Physics Company, Germany). Each value reported here was the average of five measurements.

EOF measurements: The EOF mobility (μ_{eo}) of the microchips was measured by using a modified current monitoring technique.[29] For this purpose, the baseline current of the amperometric detector was monitored while switching between a borate (15 mM)/phosphate buffer (7.5 mM, pH 8.2) and a borate (20 mM)/phosphate buffer (10 mM, pH 8.2). A separation voltage of +1500 V (corresponding to a field strength of approximately 267.8 V cm^{-1}) was used to minimize Joule heating in the channel and the increased dispersion of the peaks by thermal diffusion. The EOF velocity was determined according to Equation (1):

$$v_{EOF} = L/t \quad (1)$$

In which L is the channel length and t is the time required for the lower-concentration buffer to fill the microchannel. The EOM is given by the ratio of v_{EOF} to the applied electric field intensity (E) of 267.8 V cm^{-1}.

FTIR spectra measurements: The FTIR spectra of pure and sol–gel-modified PMMA films were measured by using a FTIR spectrometer (NEXUS470, NICOLET). To prepare PMMA films, PMMA (0.1 g) was dissolved in chloroform (10 mL) and sealed in a bottle overnight to obtain a clear solution. The solution was poured on a 10×10 cm glass plate and was allowed to dry in air and in vacuum successively to form a film. This plate was then cut into 2×2 cm pieces to perform the contact angle measurements mentioned above. The film on the plate was very thin so the effect of the sol–gel modification layer on the IR spectrum of the PMMA film became more pronounced.

Electrochemical measurements: The microdisc detection electrode was prepared by polishing it with emery paper and alumina powder, sonication in doubly distilled water, and finally the surface of the detection electrode was positioned carefully opposite the channel outlet of the separation channel through the guiding metal tube. The gap distance between the disc electrode and the channel outlet was adjusted approximately to 50 μm by comparison with the top width (100 μm) of the channel while being viewed under a microscope. Amperometric detection for microchip CE was performed with a CHI 830B electrochemical analyzer (Shanghai Chen-Hua Instruments Co., Shanghai, China) in combination with a three-electrode electrochemical cell consisting of a laboratory-made disc detection electrode, an auxiliary electrode, and an Ag/AgCl wire reference electrode. The electropherograms were recorded with a time resolution of 0.1 s (without any software filtration) using the "amperometric *i–t* curve" mode while applying the detection potential. Sample injections were performed after stabilization of the baseline. All experiments were performed at room temperature.

Procedures: The channels of the microchip were treated before use by rinsing then with doubly distilled water and the running buffer for 10 min each. The buffer reservoir and unused reservoir were filled with the running buffer solution, while the sample reservoir was filled with a sample solution. The detection cell was filled with the running buffer solution. A high voltage was applied for 20 s to the sample reservoir to facilitate the filling of the injection channel, while the detection cell was grounded and all the other reservoirs were floating. The sample solution was loaded into the separation channel by applying an injection voltage to the sample reservoir for 3 s, whereas the detection cell was grounded and the other reservoirs were floating. The separation was performed by applying a separation voltage to the running buffer reservoir with the detection cell grounded and the other reservoirs floating.

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