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Permanent gold nanoparticle coatings on polyelectrolyte multilayer modified capillaries for open-tubular capillary electrochromatography

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ABSTRACT

This paper reports on a new strategy to coat fused silica capillaries through ionic adsorption of gold nanoparticles (AuNPs) on a polyelectrolyte multilayer (PEM) modified capillary wall. The coating was constructed in situ by alternating rinses with positively charged poly(diallydimethylammonium chloride), negatively charged poly(sodium-4-styrenesulfonate), and positively charged AuNPs. After self-assembly of n-octadecanethiol onto the surface of AuNPs, the modified capillary was investigated as a new medium for the separation of neutral analytes and proteins in open-tubular capillary electrochromatography (OT-CEC). The surface coverage of the capillary wall was increased using the high density of AuNPs which were dynamically capped with 4-dimethylaminopyridine (DMAP). The chromatographic performance of the column coated with positively charged AuNPs was remarkably improved compared with a column modified with negatively charged AuNPs. The coating was robust over more than 810 runs in this study and also showed high stability against 0.01 M NaOH, 0.01 M HCl, and electrolyte concentrations up to 70 mM. The run-to-run, day-to-day, and capillary-to-capillary reproducibilities of electroosmotic flow were satisfying with relative standard deviation values of less than 1% in all cases. The AuNP-coated PEM modified capillary column not only showed good performance for neutral analytes but also was suitable for the analysis of both basic and acidic proteins.

1. Introduction

Open-tubular capillary electrochromatography (OT-CEC) has shown a great potential to reach high efficiencies for the analysis of complex samples. Compared to traditionally packed capillary columns, OT-CEC has several advantages including the ease of preparation, the absence of bubble formation, and simple instrumental handling. However, the low-phase ratio of OT capillary columns is the primary drawback and restricts their wide application in chromatographic separations. In order to resolve this shortcoming, some new approaches including sol–gel-derived phases [1,2], etching [3,4], porous layers [5,6], and nanoparticle phases [7–13] have been developed to increase the surface area of the columns in OT-CEC separations. Among these approaches, coating of capillaries with nanoparticles provided high separation efficiencies for various analytes since nanoparticles possess the largest surface area of all above-mentioned materials. Up till now, two main methods have been employed to coat the capillary wall with nanoparticles: (1) immobilizing nanoparticles onto prederivatized fused silica capillaries through chemical bonding [8–10] and (2) deposition of a nanoparticle layer onto the capillary wall through a chemical reaction at high temperature [11–13]. Significant improvement of separation performance has been achieved using these capillary columns coated with various types of nanoparticles. However, the wide application of these methods is limited by their time-consuming procedures and tedious coating processes.

In order to simplify the preparation process, a method named dynamic or semipermanent coating based on the physical adsorption has been used to fabricate stationary phases for OT-CEC separation [14]. In both the dynamic and semipermanent cases, the stationary phases can be prepared by flushing the capillary wall with the desired coating materials. The difference between these two methods is that the adsorption of the coating material to the capillary wall is relatively weak in case of dynamic coating. Thus, the coating material must be present in the electrolyte during a separation. A semipermanent coating is formed by the strong physical adsorption of coating material to the capillary wall, thus the addi-

Abbreviations: PEMs, polyelectrolyte multilayers; OT-CEC, open-tubular capillary electrochromatography; AuNPs, gold nanoparticles; EOF, electroosmotic flow; LBL, layer-by-layer; DMAP, 4-dimethylaminopyridine; PDADMAC, poly(diallydimethylammonium chloride); PSS, poly(sodium-4-styrenesulfonate).

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tion of the coating material to the running buffer is not necessary [15]. Although physical adsorption has a simple and rapid coating procedure, the short lifetime of these coatings is a problem that cannot be overcome easily. Obviously, an ideal coating would be one that is both easy to prepare and stable.

Recently, a layer-by-layer (LBL) approach employing a polyelectrolyte to modify the inner surface of fused silica capillary was developed [16,17] and has been widely used in capillary electrophoresis (CE) to prevent the absorption of basic analytes to the capillary wall [18–28]. The polyelectrolyte multilayers (PEMs) are constructed by a sequential deposition of anionic and cationic polyelectrolyte onto negatively charged surfaces through electrostatic self-assembly. In contrast to most films prepared by the Langmuir–Blodgett (LB) method [29] or covalent attachment strategies [30], a major advantage of PEMs is their simplicity and low cost. Additionally, although adsorbed on the substrate or previous layer by noncovalent interactions, the resulting PEMs have multiple electrostatic bonds and are stable and uniform, especially when at least two layers of polyelectrolyte are used [18,19]. Because of these merits, PEMs have been gradually employed to prepare stationary phase for OT-CEC. For example, Warner et al. have successfully used a capillary coated with positively charged poly(diallydimethylammonium chloride) and negatively charged molecular micelles as stationary phase for OT-CEC [31–35,27]. Because the PEMs can offer larger surface areas and higher charge density than the inner surface of bare fused silica capillary [36], a dense and stable nanoparticle coating can possibly be formed when negatively charged nanoparticles are deposited onto a PEM surface [37]. However, to the best of our knowledge, there is no study where nanoparticles deposited onto PEMs are used as stationary phase for OT-CEC.

In all the types of nanoparticles used as coating materials, gold nanoparticles (AuNPs) have attracted most interest because of their long-term stability, high-surface area-to-volume ratio, and easy chemical modification [38]. Unlike silica nanoparticles, AuNPs are very stable under extremely basic and acidic conditions. Moreover, alkanethiols can bind to gold to form highly ordered and densely packed monolayers. AuNPs have been used to modify the surface of micron spheres [39,40] and bare fused silica capillaries [8–10,15] through a chemical bonding technique and served as good stationary phase for chromatographic separation.

Thus, it would seem that the polyelectrolyte multilayers containing AuNPs would be an ideal coating for OT-CEC separation. In this work, positively charged AuNPs are prepared and adsorbed to the capillary wall modified with two layers of polyelectrolyte. An examination of the influence of several parameters that are used to obtain more stable, dense coating and to obtain more efficient and more reproducible chromatographic separations is discussed. The AuNPs deposited on PEMs were found to be remarkably robust with a performance of more than 810 runs.

2. Experimental

2.1. Chemicals and materials

Chloroauric acid ($HAuCl_4{\cdot}3H_2O$), trisodium citrate, sodium borohydride ($NaBH_4$), 2-methylnaphthalene, toluene, fluorene, and acenaphthene were purchased from Chemical Reagent (Shanghai, China). Tetraoctylammonium bromide, 4-dimethylaminopyridine (DMAP), n-octadecanethiol, poly(diallyldimethylammonium chloride) (PDADMAC), Mw ~200,000 g/mol and poly(sodium-4-styrenesulfonate) (PSS), Mw ~70,000 g/mol, were obtained from Aldrich and used without further purification. Water used in all of the experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). All the glassware were cleaned by aqua regia and rinsed with deionized water prior to experiments.

2.2. Equipment

A Beckman MDQ P/ACE system (Beckman Fullerton, CA, USA) with an on-column UV detector was used for all experiments. The Beckman 32 Karat software Version 4.01 (1999–2000 Beckman–Coulter) allowed instrument control and data analysis. Bare fused silica capillaries (75 μm i.d., 365 μm o.d.) were obtained from Reafine Chromatographic Device Co., Ltd. (Yongnian, Hebei, China). The total length of the capillary was 60 cm (50 cm effective length). The temperature of the capillary was maintained at 25 °C. The samples were injected by pressure (0.5 psi, 1 psi = 6894.8 Pa) for 2 s. Scanning electron microscope (SEM) images were obtained on a Philips (Netherlands) XL-30 ESEM. Transmission electron microscopy (TEM) observation was conducted on a TECNAI-12 instrument (Philips, The Netherlands), operated at an accelerating voltage of 120 kV. UV/Vis adsorption spectra were measured on a TU-1800spc UV/Vis spectrophotometer (Beijing Purkinje General Instrument, China).

2.3. Synthesis of gold nanoparticles

Positively charged AuNPs (stabilized by DMAP) were synthesized according to a procedure previously reported by Gittins and Caruso and the particle concentration was ca. 1.0×10^{18} particles/L [41]. Negatively charged AuNPs (13 nm) were synthesized according to a procedure previously described by Freeman et al. and the particle concentration was estimated to be 9.0×10^{15} particles/L [42].

2.4. Column preparation

Deposition solutions contained 5% (w/v) polyelectrolyte and 0.1 M NaCl. The capillary was first conditioned by a 30-min rinse of 1.0 M NaOH, and then deionized water for 15 min. All polyelectrolyte depositions were done with 5-min rinses followed by 5-min water rinses. After the capillary was modified with a cationic layer of PDADMAC and an anionic layer of PSS, positively charged AuNPs were adsorbed onto the previously deposited negatively charged PSS film by rinsing the capillary with AuNP solution. This solution was kept in the capillary for 30 min (Type I capillary). The capillary prepared by direct adsorption of AuNPs onto the capillary wall was assigned as Type II. Type III capillary was prepared by adsorption of negatively charged AuNPs to the capillary wall modified with three layers of polyelectrolyte (PDADMAC/PSS/PDADMAC) (the negatively charged AuNPs were allowed to stay in the capillary for 12 h to ensure the complete absorption of AuNPs). The scheme of the three types of the capillaries is shown in Fig. 1. Finally, the three types of capillary columns were derivatized with n-octadecanethiol by passing an ethanol solution containing 20 mM n-octadecanethiol through the capillary, which was kept in the column for 10 min. The excess n-octadecanethiol was then removed from the capillary column by pumping with hexane and then flushing with ethanol and distilled water.

2.5. Preparation of background electrolyte and analyte solutions

Stock solutions of 1.0 mg/mL of each sample were prepared in methanol (MeOH). Standard working solution was prepared by diluting the standard stock solution with MeOH–H_2O (50/50, v/v). Prior to carrying out the electrochromatographic separation, all solutions were filtered through a 0.2 μm membrane filter (Schleicher & Schuell, Dassel, Germany). A stock background electrolyte was prepared by dissolving an exact amount of phosphate in water.

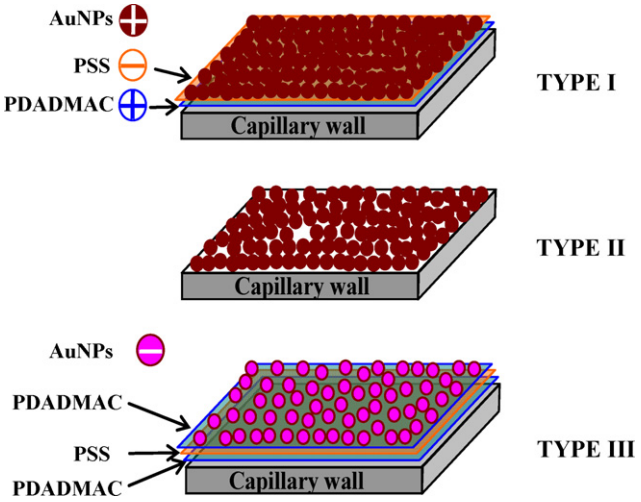

Fig. 1. Fabrication scheme of the three types capillaries.

The pH value of the phosphate solution was adjusted to the range of 2.0–12.0 by the addition of 0.1 M NaOH or 1.0 M phosphoric acid solutions. The mobile phase was obtained by mixing the phosphate solution with the appropriate amount of water and MeOH. The final mobile phase was filtered through a 0.2 μm membrane filter and degassed in an ultrasonic bath for 15 min before use. Chicken eggs were obtained from the local supermarket. The egg white and egg yolk were separated. The egg white was diluted with an 8-time volume of phosphate buffer solution (20 mM, pH 5.6, with urea in the concentration of 10 mM) and filtered through a 0.2 μm membrane filter before use.

2.6. EOF detection

The EOF (μ_{eo}) was determined from the expression $\mu_{eo} = L_d L_t / V t_0$, where L_d is the distance from injector to detector, L_t is the total capillary length, t_0 is the neutral marker (thiourea) migration velocity, and V is the applied voltage. In this work, the EOF direction is from anode to cathode since the AuNPs are uncharged after being adsorbed onto the surface of PEMs [43].

3. Results and discussion

In order to increase the phase ratio of the stationary phase used for OT-CEC, the amount of the nanoparticles adsorbed onto the capillary wall should be as much as possible. However, because of the coulombic interaction between charged nanoparticles, the surface coverage ratio is less than 0.3 when conventional assembly processes such as LB technique were applied to adsorb nanoparticles onto surfaces [44,45]. To overcome this shortcoming, in this work, DMAP capped AuNPs were synthesized and used to coat the capillary wall. The unique property of DMAP capped AuNPs is that an equilibrium exists between DMAP adsorbed onto the AuNPs surface and free DMAP in solution [43]. Hence, the coulombic interaction between charged nanoparticles prevents the formation of a dense coating. Therefore, a dense AuNP coating similarly to the one formed by repeated adsorption of nanoparticles using linking molecules can be obtained using this single nanoparticle adsorption step.

The synthesized DMAP capped AuNPs dispersed in toluene were characterized by TEM and UV/Vis spectroscopy. Fig. 2a shows the TEM micrograph of AuNPs. The particle shape of AuNPs was nearly spherical and the average diameter of them was 5.5 nm. The maximum absorption wavelength peak of DMAP capped AuNPs was 518 nm (Fig. 2b), which was in good agreement with the value

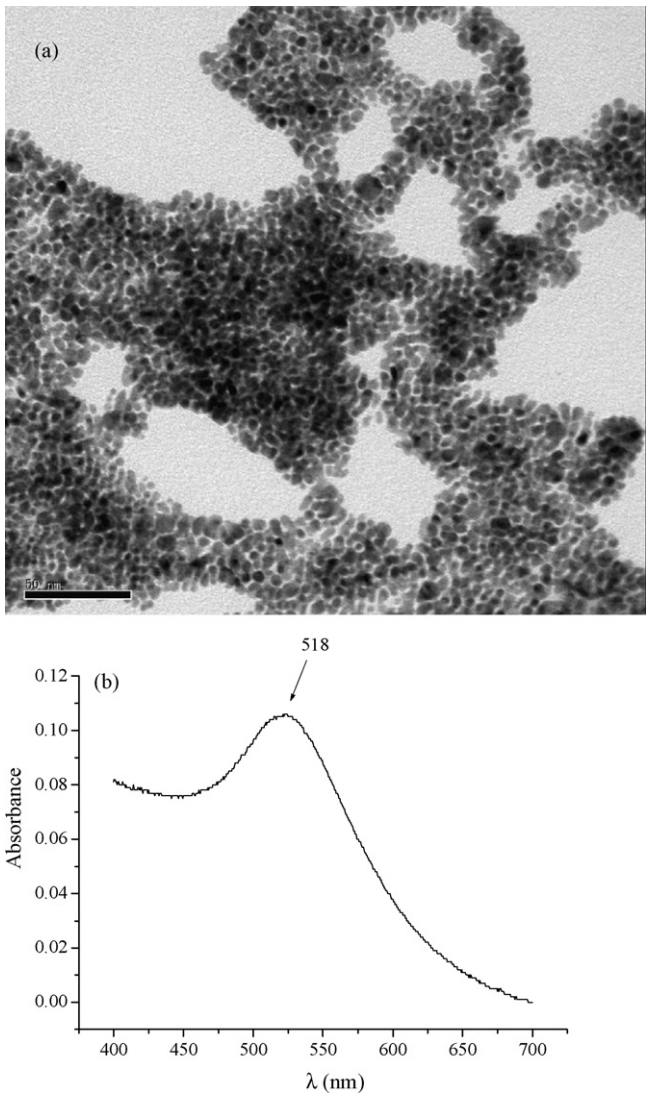

Fig. 2. Transmission electron micrographs of (a) gold nanoparticles stabilized with DMAP (b) and UV/Vis spectra of a diluted solution of these nanoparticles in toluene.

reported by Caruso et al. [41]. This result confirms that as-prepared AuNPs were stabilized by DMAP. Fig. 3a shows the AFM image of a PDADMAC/PSS–AuNPs modified quartz slide in which the modification process is the same as in the Type I column. The quartz slide is very flat with a root-mean-squared (rms) roughness of less than 0.5 nm (1 μm × 1 μm). After modification, the rms surface roughness of the film was about 5.3 nm, indicating that the aggregation of the AuNPs is very little. Besides, Fig. 3a also demonstrates that the AuNP loading of the surface is very dense. As expected, an AFM image of a quartz slide modified with negatively charged AuNPs (PDADMAC/PSS/PDADMAC–AuNPs) given in Fig. 3b shows a loose AuNPs coating on the slide.

In order to confirm the formation of a high-density layer of AuNPs in the Type I column, mixtures of thiourea, naphthalene, and biphenyl were analyzed on Type I, Type III, and a bare fused silica column. As expected, only one peak was found when bare fused silica column was used for separation. Fig. 4 shows that the mixtures were well separated on the Type I column, while serious peak overlap occurred on the Type III column. As mentioned above, we believe that a loose bonding of DMPA on the AuNP surface contributed mainly to the improved surface coverage of the Type I column, which in turn increased the resolution. It should be noted that in the column preparation process, the time for nega-

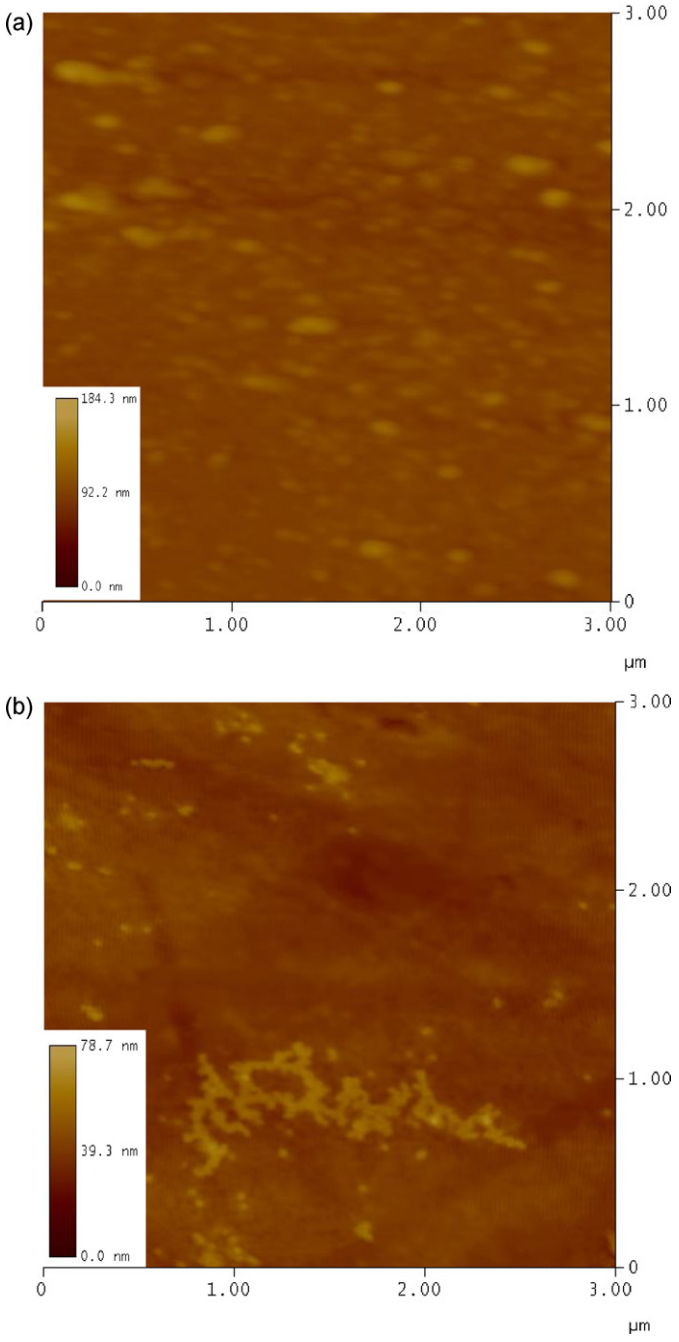

Fig. 3. AFM image of a thin film of PDADMAC/PSS–AuNPs prepared on a quartz slide. The height profile across a region of the surface is also shown.

tively charged AuNPs to stay in the capillary was 12 h, while that for positively charged AuNPs was only 30 min. Nevertheless, the surface coverage of the Type I column is still higher than that of Type III even though recent research showed that the adsorption of AuNPs onto a PEM coated capillary wall is a slow process that needs at least 1 h to reach saturation of the AuNPs [36,46]. Thus, this experimental result also indicates that the adsorption rate of DMAP capped AuNPs onto the PEM coated capillary wall is very fast. To confirm this conclusion further, an OT-CEC separation was performed using a Type I column when the residence time of the positively charged AuNPs in the capillary was changed from 30 min to 12 h. As expected, the retention time obtained in the 12 h column was nearly the same as that obtained on the 30 min column because of the unchanged surface coverage (data not shown). The increased

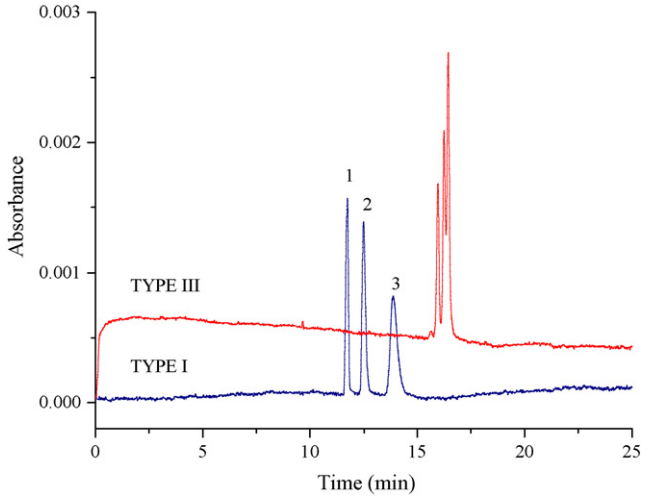

Fig. 4. OT-CEC separation of test mixtures using Type I and Type III columns. Conditions: MeOH–10 mM Na_2HPO_4 buffer (45:55), pH 7.0; separation voltage, 20 kV. Analytes peaks: 1, thiourea; 2, naphthalene; 3, biphenyl.

adsorption rate in the Type I column can be mainly attributed to the elimination of electrostatic repulsion between nanoparticles. Moreover, the high concentration of positively charged AuNPs may also contribute to the increased adsorption rate. Thus, the use of DMAP capped AuNPs as coating material can increase the surface coverage and reduce the coating time.

3.1. Stability of the coating

In OT-CEC, instability is the key factor affecting a wide application of a coating. The stability of the coating used in Type I column was examined by the use of the following procedure. First, 10 replicate separations were performed with applied voltage of 20 kV at 25 °C. The mobile phase was 10 mM phosphate buffer at pH 7.0 with 45% (v/v) MeOH. Then 390 separations were performed within 20 days. Each separation was done in a voltage range of 5–30 kV using an electrolyte concentration range of 5–70 mM, a pH range of 2.0–12.0, and a MeOH content of 35–60%. After these experiments, 10 replicate separations were performed, in which the experimental conditions were the same as in the first step. Thereafter, this column was used to perform other nearly 390 runs for PAH and protein separation. Finally, the same experiments as those performed in step 1 were repeated. Because the changing of the EOF is mainly depending on the coverage of the AuNPs on the surface of PEMs, if some AuNPs were detached from the surface of PEMs, more PEMs would be exposed and the charge density of the inner wall of the capillary would be increased which in turn increases the EOF. Therefore, the increase of the EOF can be used to indicate the detachment of the AuNPs from the surface of PEMs. Since the last layer of the polyelectrolyte coated on the column is PSS which is negatively charged, the EOF direction is from anode to cathode and this conclusion is confirmed by the experiment result. Fig. 5a shows the electrochromatograms of 1st, 400th, and 800th run and Table 1 shows the EOF, retention factor (k) and plates per column of solutes at different runs. It can be seen from Table 1 that the μ_{eo} increased from 2.12×10^{-4} cm^2 V^{-1} s^{-1} (1st run) to 2.15×10^{-4} cm^2 V^{-1} s^{-1} (800th run). However, since the density of the AuNPs on the surface of the PEMs is high, there was only a minor difference of μ_{eo} (relative error = 0.5%) between the 1st run and the 400th run ($\mu_{eo} = 2.13 \times 10^{-4}$ cm^2 V^{-1} s^{-1}) even there were some AuNPs detached from the surface of the PEMs. Considering that resolution also strongly depend on the density of the AuNPs on the surface of PEMs, resolution was used to check the stability of the AuNPs

Table 1
EOF, retention factor (k) and number of plates per column of solutes at different runs.

Number of runs	μ_{eo} ($\times 10^{-4}$ cm^2 V^{-1} s^{-1})	k		Plates per column	
		Biphenyl	Naphthalene	Biphenyl	Naphthalene
1	2.12	0.050	0.134	44,160	17,500
400	2.13	0.051	0.135	43,880	20,650
800	2.15	0.034	0.091	31,770	11,520

Conditions: same as in Fig. 4.

on the surface of PEMs. It was found that the resolution, given by $R = (t_{biphenyl} - t_{naphthalene})/(W_{naphthalene/2} + W_{biphenyl/2})$, was equal ($R = 2.68$) for these two runs. Besides, the retention factors and number of plates for biphenyl and naphthalene were nearly the same too. Considering that various pH values (including a 10-min rinse with pH 12 0.01 M NaOH and a 10-min rinse with pH 3 0.01 M HCl), electrolyte concentrations, and amounts of MeOH were used between these two runs, the minor difference of μ_{eo}, resolution, retention factors and number of plates of solutes between the 1st run and the 400th run demonstrates that the coating used for Type I column is quite stable under various conditions. Although no significant difference of the μ_{eo} between the 1st run and the 800th run was found, a decrease in resolution from 2.68 (1st run) to 1.48 (800th run) indicates the detachment of the AuNPs from the PSS film. When AuNPs were detached from the surface of PEMs the interaction between solutes and stationary phase decreased. So the retention factors and number of plates of retained samples decreased, and in turn, the resolution decreased. However, since a large amount of AuNPs were initially adsorbed onto the capillary wall, the detachment of some AuNPs from the surface of PEMs only led to the small changes of the coverage of AuNPs on the surface of PEMs. Therefore, the μ_{eo} only slightly changed and a nearly baseline separation was still achieved after using the column for at least 810 times. The great stability of this coating is possibly due to the existing of at least two interactions between AuNPs and the PEMs: electrostatic interaction and enhanced interaction of van der Waals. Therefore, the stability of AuNPs on the PEMs is greatly improved compared to the coating formed by directing adsorb AuNPs onto the surface of bare silica capillary only via electrostatic interaction. It should be noted that although μ_{eo}, resolution, retention factor, and plates per column were used to check the stability of the coating during the long time separation process, the amount of AuNPs detached from the inner surface of the capillary wall was still not quantitatively determined. Desorbing all the AuNPs from the inner surface of column by flushing aqua regia through the column and measuring the Au concentration using spectrophotometry would be a potential method to solve this problem.

To find the effect of PEMs on the formation of dense and stable coating of AuNPs, the stability of a column prepared by direct adsorption of positively charged AuNPs onto the capillary wall (Type II) was tested. Eighteen replicated separations using the same conditions as for the testing of the stability of Type I column in the first step were performed. Fig. 5b shows that the stability of this type of column decreases rapidly after only 10 runs and completely loses its separation ability after 18 runs. These results demonstrate clearly that the stability of Type I column results from the multiple attachment of the ionic polymer to the capillary wall and the AuNPs.

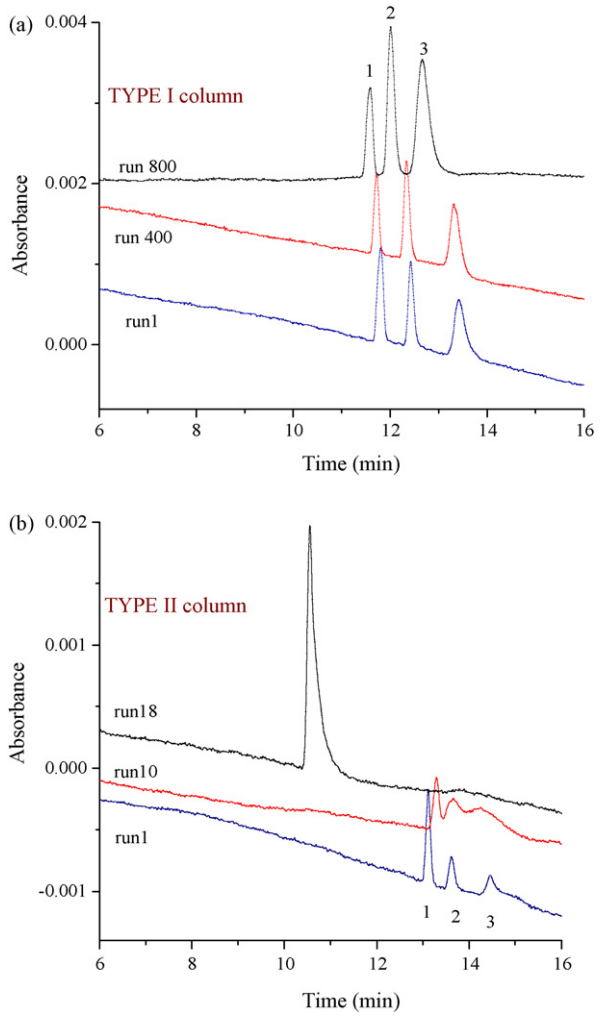

Fig. 5. Comparison of electrochromatograms obtained from Type II and Type I columns after various run times. All other conditions are the same as in Fig. 4.

3.2. Repeatability

The run-to-run, day-to-day and capillary-to-capillary repeatabilities of the Type I column were evaluated using the relative standard deviation (RSD) of EOF obtained from different times of replicated runs. The results are reported in Table 2. All RSDs of EOF were less than 0.8%. It indicates an achievement of excellent reproducibility.

Table 2
Reproducibilities of the coating for Type I column.

	EOF ($\times 10^{-4}$ cm^2 V^{-1} s^{-1})	RSD (%)
Run-to-run ($n = 10$)[a]	2.122	0.20
Day-to-day ($n = 5$)[a]	2.121	0.25
Capillary-to-capillary ($n = 25$)[b]	2.124	0.73

Conditions: same as in Fig. 4.
[a] These experiments were done in the same capillary.
[b] Five consecutive runs were performed in each capillary and the total number of runs was 25.

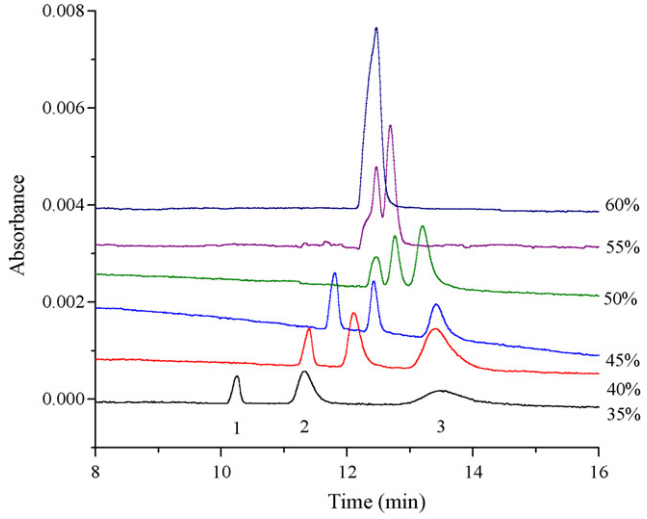

Fig. 6. Effect of MeOH percentage on the separation of test mixtures using Type I column. Conditions: MeOH–10 mM Na_2HPO_4 buffer. All other conditions are the same as in Fig. 4.

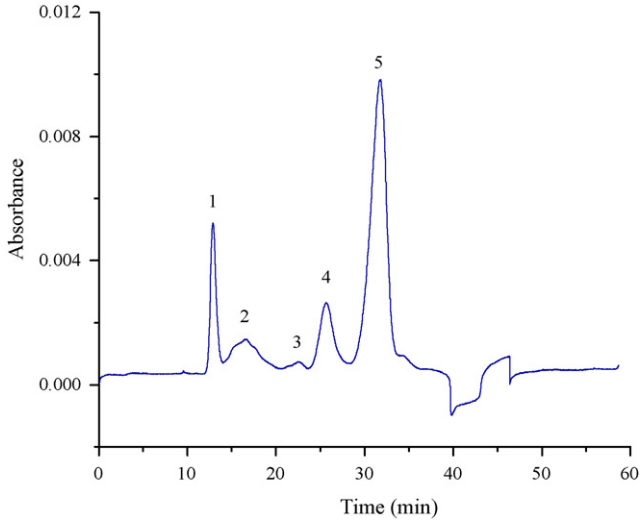

Fig. 7. Separation of egg white in a single run using Type I column. Conditions: 10 mM phosphate buffer, pH 8.6; applied voltage, 20 kV; detection 214 nm; pressure injection, 0.5 psi for 7 s; temperature, 25 °C. Analytes peaks: 1, ovomucoid; 2, lysozyme; 3, avidin; 4, ovotransferrin; 5, ovalbumin.

3.3. Retention behavior and column performance

As shown in Fig. 6, resolutions of the test compounds gradually decreased with increasing methanol concentration from 35% to 60%, while the elution order remained unchanged. This indicates that the chromatographic retention mechanism of the stationary phase is basically a reversed-phase behavior. A mobile phase with 45% (v/v) of MeOH in buffer provided the best trade-off between column performance and analysis time. The theoretical plate numbers per meter under these conditions were 46,900 for naphthalene and 18,700 for biphenyl, respectively. The separation efficiency and detection sensitivity of this new column is nearly the same as the column prepared using the traditional coating methods. However, there are two advantages of this new coating method: first, the coating process is easy although four steps were needed; second, the obtained coating is very stable since the AuNPs can be adsorbed tightly onto the inner surface of fused silica capillary column modified with polyelectrolyte via electrostatic interaction and enhanced interaction of van der Waals between AuNPs and the polyelectrolyte.

3.4. Separation of proteins

A real biological sample of egg white was selected to demonstrate the applicability of the Type I column to complex samples. The component of egg white is dominated by ovalbumin (54%, p*I* 5.16), ovotransferrin (13%, p*I* 5.65), ovomucoid (11%, p*I* 4.14), lysozyme (3.5%, p*I* 11.0), and avidin (0.05%, p*I* 10.0), which all have very different molecular weights ($(12{,}000–240)\times 10^6$ Da) [47]. After dilution and filtration, egg white was directly injected into the Type I column in phosphate buffer (10 mM, pH 8.6). As shown in Fig. 7, both basic proteins (lysozyme and avidin) and acidic proteins in egg white can be well separated in a single run. The recoveries of ovomucoid, lysozyme, avidin, ovotransferrin, and ovalbumin were 92%, 73%, 78%, 89%, and 96%, respectively. The low recoveries of lysozyme and avidin are possibly due to the electrostatic interaction between the negatively charged PSS film and the positively charged basic proteins. However, the successful elution of basic proteins indicates that this interaction is greatly suppressed by the high-surface coverage of the uncharged AuNPs on the PSS surface. Otherwise, they would be adsorbed onto the capillary wall and cannot be eluted out. These results show that AuNPs coated on PEM modified column are particularly effective in separating neutral samples and proteins.

4. Conclusions

A permanent coating was created by rinsing a capillary with PEMs and AuNPs have been developed. The prepared coating can effectively separate neutral PAHs, acidic and basic proteins in OT-CEC. The coating was stable over 810 runs. Run-to-run, day-to-day, and capillary-to-capillary repeatabilities were satisfying with RSD values below 1%. Since the AuNPs own the merit of easy chemical modification, many other species, such as biopolymers or proteins, can be bonded to the surface of AuNPs to enable, e.g., chiral separations. This new coating thus offers a promising new alternative to conventional coating techniques in OT-CEC and can be very useful in both achiral and chiral separations.

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References

[1] Y. Guo, L.A. Colon, Anal. Chem. 67 (1995) 2511.
[2] J.D. Hayes, A. Malik, Anal. Chem. 73 (2001) 987.
[3] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 255.
[4] M.T. Matyska, J.J. Pesek, A. Katrekar, Anal. Chem. 71 (1999) 5508.
[5] X. Huang, J. Zhang, C. Horváth, J. Chromatogr. A 858 (1999) 91.
[6] A.L. Crego, J. Martinez, M.L. Marina, J. Chromatogr. A 869 (2000) 329.
[7] M. Boyce, M.C. Breadmore, M. Macka, P. Doble, P.R. Haddad, Electrophoresis 21 (2000) 3073.
[8] T. O'Mahony, V.P. Owens, J.P. Murrihy, E. Guihen, J.D. Holmes, J.D. Glennon, J. Chromatogr. A 1004 (2003) 181.
[9] L. Yang, E. Guihen, J.D. Holmes, M. Loughran, G.P. O'Sullivan, J.D. Glennon, Anal. Chem. 77 (2005) 1840.
[10] F.K. Liu, Y.T. Hsu, C.H. Wu, J. Chromatogr. A 1083 (2005) 205.
[11] Y.L. Hsieh, T.H. Chen, C.P. Liu, C.Y. Liu, Electrophoresis 26 (2005) 4089.
[12] Y.L. Hsieh, T.H. Chen, C.Y. Liu, Electrophoresis 27 (2006) 4288.
[13] X.L. Dong, R.A. Wu, J. Dong, M.H. Wu, Y. Zhu, H.F. Zou, Electrophoresis 29 (2008) 3933.
[14] Z. Liu, R.A. Wu, H.F. Zou, Electrophoresis 23 (2002) 3954.

[15] Q.S. Qu, X.X. Zhang, M. Shen, Y. Liu, X.Y. Hu, G.J. Yang, C.Y. Wang, Y.K. Zhang, C. Yan, Electrophoresis 29 (2008) 901.
[16] G. Decher, J.D. Hong, J. Schmitt, Thin Solid Films 831 (1992) 210.
[17] G. Decher, Science 277 (1997) 1232.
[18] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[19] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 5272.
[20] Y. Wang, P.L. Dubin, Anal. Chem. 71 (1999) 3463.
[21] T.W. Graul, J.B. Schlenoff, Anal. Chem. 71 (1999) 4007.
[22] S.L.R. Barker, M.J. Tarlov, H. Canavan, J.J. Hickman, L.E. Locascio, Anal. Chem. 72 (2000) 4899.
[23] Y. Liu, J.C. Fanguy, J.M. Bledsoe, C.S. Henry, Anal. Chem. 72 (2000) 5939.
[24] L. Bendahl, S.H. Hansen, B. Gammelgaard, Electrophoresis 22 (2001) 2565.
[25] G. Danger, M. Ramonda, H. Cottet, Electrophoresis 28 (2007) 925.
[26] C.A. Lucy, A.M. MacDonald, M.D. Gulcev, J. Chromatogr. A 1184 (2008) 81.
[27] C.A. Luces, S.O. Fakayode, M. Lowry, I.M. Warner, Electrophoresis 29 (2008) 889.
[28] R. Nehmé, C. Perrin, H. Cottet, M.D. Blanchin, H. Fabre, Electrophoresis 30 (2009) 1888.
[29] D.R. Talham, Chem. Rev. 104 (2004) 5479.
[30] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides, Chem. Rev. 105 (2005) 1103.
[31] C.P. Kapnissi, C. Akbay, J.B. Schlenoff, I.M. Warner, Anal. Chem. 74 (2002) 2328.
[32] C.P. Kapnissi, B.C. Valle, I.M. Warner, Anal. Chem. 75 (2003) 6097.
[33] M.W. Kamande, C.P. Kapnissi, X.F. Zhu, C. Akbay, I.M. Warner, Electrophoresis 24 (2003) 945.
[34] M.W. Kamande, X.F. Zhu, C. Kapnissi-Christodoulou, I.M. Warner, Anal. Chem. 76 (2004) 6681.
[35] C.P. Kapnissi-Christodoulou, M. Lowry, R.A. Agbaria, L. Geng, I.M. Warner, Electrophoresis 26 (2005) 783.
[36] H. Kawasaki, T. Sugitani, T. Watanabe, T. Yonezawa, H. Moriwaki, R. Arakawa, Anal. Chem. 80 (2008) 7524.
[37] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293.
[38] H.Y. Koo, W.S. Choi, J.-H. Park, D.-Y. Kim, Macromol. Rapid Commun. 29 (2008) 520.
[39] K. Kobayashi, S. Kitagawa, H. Ohtani, J. Chromatogr. A 1110 (2006) 95.
[40] Q.S. Qu, S.W. Peng, D. Mangelings, X.Y. Hu, C. Yan, Electrophoresis 31 (2010) 556.
[41] D.I. Gittins, F. Caruso, Angew. Chem. Int. Ed. 40 (2001) 3001.
[42] R.G. Freeman, M.B. Hommer, K.C. Grabar, M.A. Jackson, M.J. Natan, J. Phys. Chem. 100 (1996) 718.
[43] D.I. Gittins, A.S. Susha, B. Schoeler, F. Caruso, Adv. Mater. 14 (2002) 508.
[44] R.G. Freeman, K.C. Grabar, K.J. Allison, R.M. Bright, J.A. Davis, A.P. Guthrie, M.B. Hommer, M.A. Jackson, P.C. Smith, D.G. Walter, M.J. Natan, Science 267 (1995) 1629.
[45] M.D. Musick, C.D. Keating, M.H. Keefe, M.J. Natan, Chem. Mater. 9 (1997) 1499.
[46] Q. Liu, L.H. Yao, Q.P. Shen, Z. Nie, M.L. Guo, S.Z. Yao, Chem. Eur. J. 15 (2009) 12828.
[47] E. Li-Chan, S. Nakai, Crit. Rev. Poult. Sci. 2 (1989) 21.