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A novel amphipathic block copolymer coating forming micelle-like aggregates for separation of steroids in open tubular capillary electrochromatography

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ABSTRACT

A new amphipathic block copolymer, poly(tert-butyl acrylate)$_{127}$-block-poly(glycidyl methacrylate)$_{86}$, was developed for the coating in open tubular capillary electrochromatography. The self-assembly characters of the coating, which could form micelle-like aggregates under proper conditions, were observed by atomic force microscopy. Compared with bare capillary, this coating could act as surfactant and lead to improve the separation of steroids. In addition, the influence of pH, buffer concentration and organic solvents on the separation was investigated. The best separation of the three model steroid analytes could be achieved using 20.0 mM borate buffer at pH 10.5. For covalent bonding, the coating showed good repeatability and stability with RSD of u_{EOF} less than 3.3%. Then, this proposed method was well validated with good linearity ($\geq$0.999), recovery (91.0–94.0%) and repeatability, and was successfully used for separation of steroids in spiked serum samples, which indicated that this new OT-CEC method could provide a potential tool to determine steroids in real biological system without interference.

1. Introduction

As one kind of non-glyceride lipids, steroids are essential to living system and have important biological activity. According to their function, steroids can be generally divided into corticosteroids, sex steroids, cholesterol, bile acids, vitamin D, phytosteroids and others [1]. It has been reported that steroids can be widely employed in disease therapy. For example, hydrocortisone and prednisone are both glucocorticoids that can be used in treatment for pediatric renal-transplant [2], and medroxyprogesterone acetate can be employed to treat endometriosis [3]. Therefore, the determination of steroids individual or simultaneous is indispensable for disease treatment. So far, a numerous methods [1,4–6], such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrohporesis (CE), have been used to analyze steroid samples. Owing to the high separation efficiency, fast analysis and small sample consumption, CE is gaining more attention [7]. Either micellar electrokinetic chromatography (MEKC) or capillary electrochromatography (CEC) is considered as the preferable method for separation of steroids because most of them are neutral [4–6]. However, only few papers about CEC method for the analysis of pharmaceuticals in body fluids have been reported [8].

It is well-known that CEC has good selectivity and favors separation of neutral substances. Thus, CEC is a promising technique used to analyze many compounds, such as proteins [9–11], peptides [12–14], nucleic acids [15], and pharmaceuticals [16,17]. Generally, CEC possesses three modes: packed CEC (p-CEC), mono-

Abbreviations: GMA, glycidyl methacrylate; t-BA, butyl acrylate; MAN, maleic anhydride; St, styrene; EBIB, ethyl-2-bromoisobutyrate; APTES, 3-aminopropyltriethoxysilane; SDS, sodium dodecyl sulfate; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; HPCE, high performance capillary electrophoresis; ^{1}H NMR, ^{1}H nuclear magnetic resonance; PDI, polydispersity index; GPC, gel permeation chromatography; SEM, scanning electron microscope; AFM, atomic force microscopy; ATRP, atom transfer radical polymerization; GC, gas chromatography; HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; CEC, capillary electrochromatography; OT-CEC, open tubular capillary electrochromatography; p-CEC, packed capillary electrochromatography; MEKC, micellar electrokinetic chromatography; EOF, electroosmotic flow; RSD, relative standard deviation.

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lithic CEC and open tubular CEC (OT-CEC). Although p-CEC and monolithic CEC have high phase ratio and excellent separation power, frit fabrication and bubble formation are still the challenging problems in the operation. As a result, OT-CEC is an essential separation mode based on its faster and simpler preparation procedures. To fully gain insight into its performance, the comprehension and exploration of the surface chemistry in capillary are significant.

Recently, it has been found that the copolymer modified capillary could act as one kind of OT-CEC which can improve on separation. For example, Wang and colleagues have applied poly(ethylene oxide)-block-poly(4-vinylpyridine) and hydroxyethylcellulose-graft-poly(4-vinylpyridine) as the physical coatings. Then they used the coatings in CEC to successfully separate basic protein samples [18,19]. Zheng and co-workers have proposed the use of hydroxyethylcellulose-graft-poly(N,N-dimethylacrylamide) as a novel dynamic coating for well separation of basic proteins [20]. However, most of these coatings were hydrophilic copolymers used for reduction in electrostatic absorption of proteins, which had limited their application in separation of various substances, especially uncharged hydrophobic analytes. Furthermore, these coatings were mostly fixed by physical absorption that would result in their irrepeatability.

Although we have exploited a new copolymer coating P(MAn-alt-St)$_{127}$-b-PSt$_{592}$ anchored onto the capillary wall which acted as the surfactants in organic solvents [21,22] for the OT-CEC separation of the aromic amines, few studies about the amphipathic block copolymers used as coatings have been reported in spite of some on glass and Si wafers [23]. Thus, exploring and investigating the property of the new covalently bound amphipathic block copolymer coatings in OT-CEC mode are interesting and meaningful.

In this work, we explored a novel amphipathic block copolymer, poly(tert-butyl acrylate)$_{127}$-block-poly(glycidyl methacrylate)$_{86}$ (PtBA$_{127}$-b-PGMA$_{86}$), as the coating for OT-CEC. Atomic force microscopy (AFM) was used to study the structural transformation of the amphipathic block copolymer under different separation conditions. Meanwhile, the OT-CEC separation mechanism with the copolymer coating was discussed. Furthermore, the application of the new block copolymer coating for separation of steroid samples in OT-CEC and its potential usage in biological analysis have been studied.

2. Materials and methods

2.1. Materials

Cuprous bromide and cuprous chloride (CuBr and CuCl, Beijing Chemical Plant, Beijing, China) were firstly washed by acetic acid and subsequently washed by methanol before use. 1,1,4,7,7-Pentamethyldiethylene-triamine (PMDETA) was obtained from JK Chemical Ltd. (Tokyo, Japan). Ethyl-2-bromoisobutyrate (98%, EBIB) (98%) was gotten from Shanghai Crystal Pure Reagent Co. Ltd. (Shanghai, China). tert-Butyl acrylate (t-BA), glycidyl methacrylate (GMA), sodium dodecyl sulfate (SDS) and 3-aminopropyltriethoxysilane (APTES) were purchased from Acros Company (New Jersey, USA). Dimethyl sulfoxide (DMSO) was analytical grade and obtained from Beijing Xinjing Chemical Plant (Beijing, China). Sodium tetraborate pentahydrate was analytical grade and was obtained from Tianjin Guangfu Science & Technology Development Co. Ltd. (Tianjin, China). Sodium hydroxide, hydrochloric acid, anhydrous ethanol, anhydrous tetrahydrofuran (THF), acetone, 1,4-dioxane and acetonitrile were analytical grade and produced by Beijing Chemical Factory (Beijing, China). Medroxyprogesterone acetate and prednisone acetate were obtained from Zhejiang Xianju Pharmaceutical Ltd. (Zhejiang, China). Hydrocortisone was obtained from Tianjing Jinyao Amino Acid Ltd. (Tianjing, China).

2.2. Instrumentation

The CE experiment was carried out with a 1229 high performance capillary electrophoresis (HPCE) analyzer (a commercial equipment from Beijing Institute of New Technology and Application, Beijing, China) equipped with a UV-detector (detection at 254 nm). Unless otherwise mentioned, separations were performed at room temperature in the block copolymer coated capillary of 75 μm I.D. × 70 cm (56 cm effective). Bare fused-silica capillaries (75 μm I.D. × 360 μm O.D.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

^{1}H nuclear magnetic resonance (^{1}H NMR) observation of the block copolymers was performed on Bruker Avance 400 spectrometer (Bruker biospin, Switzerland) (400 MHz).

Scanning electron microscope (SEM, Hitachi, Japan) was used to characterize the morphology of the block copolymer coating.

Atomic force microscopy (AFM; Nanoscope III a SPM controller, Veeco Metrology, USA) studies were performed using tapping mode at room temperature. Samples for AFM measurement were coated on silica wafers (UV grade, polished, Sinoma Advanced Jiangsu Silica Materials Co. Ltd., Jiangsu, China) by the same treating process as the capillary. Before measurement, the samples were dipped in corresponding solutions for 30 min and then air dried at room temperature.

2.3. Synthesis of the copolymer

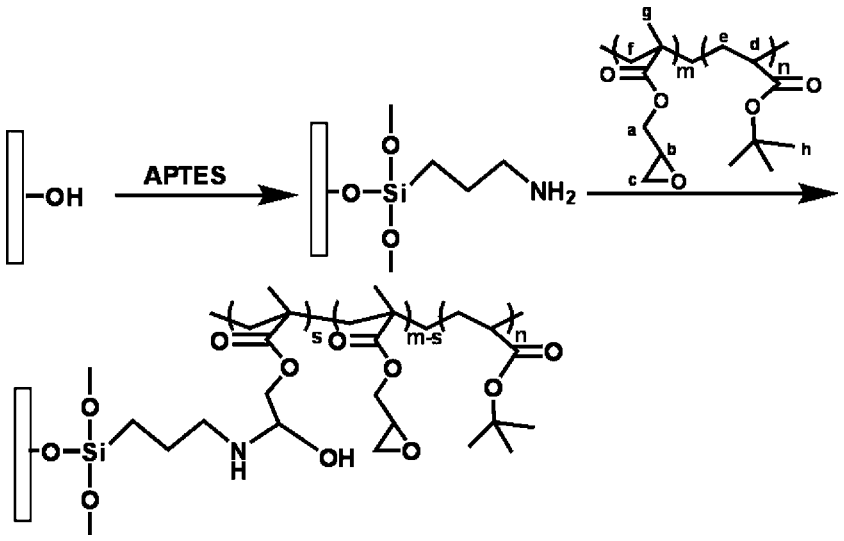
PtBA$_{127}$-b-PGMA$_{86}$ was synthesized by atom transfer radical polymerization (ATRP) in our Lab as follows: EBIB (0.2 mmol, 39.0 mg), PMDETA (0.2 mmol, 42 μL), tBA (40 mmol, 5.12 g) and 1.5 mL acetone were added into a flask. The mixture was degassed by three freeze–evacuate–thaw cycles and then CuBr (0.2 mmol, 28.8 mg) was added into the flask. Subsequently, the flask was sealed tightly with rubber plugs and filled with N_2 after evacuation. The solution was allowed to 60 °C in an oil bath and reacted for 11 h under the protection of N_2. The crude product was passed through a column filled with neutral alumina and isolated by precipitating into large amount of methanol and water mixture (1:1 volume ratio) three times. The yield of the product was 63.5% and polydispersity index (PDI) was 1.10 with Mn = 1.62 × 10^4.

Then PtBA–Br (0.03 mmol, 0.486 g), PMDETA (0.03 mmol, 6.3 μL), GMA (6 mmol, 0.853 g) and 1.5 mL acetone were added into another flask. CuCl (0.03 mmol, 3.0 mg) was added after three freeze–evacuate–thaw cycles for degassing. Then, the flask was disposed as above and the mixed solution was reacted at 50 °C in oil bath for 2 h in the atmosphere of N_2. The resulted product was isolated by precipitating into large amount of methanol and water mixture (2:1 volume ratio) three times.

To confirm the composition of the block copolymer, nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) were performed. Signal assignment δ (ppm) of the ^{1}H NMR spectroscopy is displayed in Fig. S-1: 4.33, 3.86 (CH_2, a in Scheme 1), 3.24 (CH, b in Scheme 1), 2.86, 2.65 (CH_2, c in Scheme 1), 2.24 (CH, d in Scheme 1), 2.10–0.844 (CH, e–h in Scheme 1). The GPC result of the block copolymer (Fig. S-2) shows that Mn was 2.84 × 10^4 and the polydispersity index (PDI) was 1.15.

2.4. Coating preparation

A bare fused-silica capillary was first rinsed with 1 M NaOH for 3 h, and next flushed with water for 30 min, and then washed with 1 M HCl for 30 min, water for 1 h, and acetone for 30 min. After blown dry with nitrogen, the capillary was flushed with 200 μL



Scheme 1. The coating process of the block copolymer.

silanization solution comprising THF and APTES (50/50, v/v) for yielding a thin layer. Then the capillary was kept at room temperature (20 °C) for 20 h and flushed later by THF for 1 h. Similarly, in order to produce a thin layer, the capillary was subsequently flowed with 50 μL coating solution (30 mg $PtBA_{127}$-b-$PGMA_{86}$ in 1 mL THF), and then kept for 20 h at room temperature. Finally, the capillary was continuously washed with THF for 1 h to remove the residual copolymer solution. The specific coating process is displayed in Scheme 1. After the capillary was coated, the detection window (about 2 mm) was prepared by burning off the block copolymer coating inside and the polyimide coating outside. For storage, the prepared capillary was filled with water and both ends were sealed with rubbers.

2.5. Solution preparation

Stock solutions of the steroids were prepared in anhydrous ethanol with concentrations ranging from 2.8 to 5.2 mM. Then the standard solutions of hydrocortisone and prednisone acetate were diluted with 0.9% saline in the defined concentration range. Notably, the mixed solution consisted of medroxyprogesterone acetate, hydrocortisone and prednisone acetate with the concentrations of 1.7 mM, 0.5 mM and 2.5 mM respectively. All these solutions were stored at 4 °C in refrigerator.

Serum samples were obtained from healthy volunteers and were prepared by three steps as follows: (1) 100 μL of the stock solutions of two steroids was added into 400 μL serum and mixed by vortex for 3 min to get the serum sample, in which the final concentration of hydrocortisone was 27.6 μM and prednisone acetate was 50.0 μM; (2) proteins in the serum sample were denatured by adding 2 mL of acetonitrile to 500 μL of the serum sample. After well mixed, the mixture was centrifuged at 7043 × *g* for 10 min to remove denatured proteins; (3) 1 mL of the supernatant in the centrifuged serum sample was taken out and blown dry with nitrogen. Then, the dried sample was redissolved with mixed solvent consisting of 45 μL ethanol and 5 μL buffer, and ready for injection. Additionally, the blank serum sample was prepared by the same method as before, except addition of steroids.

2.6. EOF measurement and CE separations

DMSO was used as the neutral marker in the determination of electroosmotic flow (EOF). The EOF measurement and CE separation were achieved under conditions as follows: voltage +20 kV, sample injection by siphoning 8 s at 25 cm height. Before each run, the coated capillary was rinsed with water followed by buffer solution for 2 min. The EOF mobility was calculated using the equation as following [24]:

$$\mu_{EOF} = \frac{L_d L_t}{t_m V} \quad (1)$$

where L_d is the effective length of the capillary and L_t is the total length of the capillary, t_m is the migration time of the EOF marker, V is the applied separation voltage. The effective mobility in CEC was defined as [25]:

$$\mu_{eff} = \mu_{obs} - \mu_{EOF} \quad (2)$$

where μ_{obs} is the observed mobility of an analyte in CEC experiment.

3. Results and discussion

3.1. Characterization of the block polymer coating

The well-defined block copolymer $PtBA_{127}$-b-$PGMA_{86}$ has been synthesized by atom transfer radical polymerization (ATRP) method in our Lab. Then we prepared the coated capillary by the method described in Section 2.4. It is well-known that the morphology of the copolymer coatings on the capillary surface is an important parameter to directly evaluate the existence of the coatings [26,27]. Thus SEM has been utilized to observe the surface morphologies of the coated capillary (Fig. 1a) and the bare capillary (Fig. 1b). Obviously, compared to the bare one, the coated capillary demonstrates a thin layer closely adhered to the inner wall with a wrinkly surface. Additionally, the produced epoxy group in the $PtBA_{127}$-b-$PGMA_{86}$ modified capillary inclined to hydrolyze under basic conditions by ring-opening reaction [28], which could generate EOF in alkaline environment. Our investigation displayed that the EOF mobility in the uncoated capillary slightly increased along with the raise of pH (8.0–11.0). Meanwhile, the coated capillary suppressed EOF effectively compared to the uncoated one, which also could indirectly prove the successful coating process.

3.2. Column stability and repeatability

Stability is an important parameter to evaluate the lifetime of coatings in capillary. High stability could make coatings used for a long time. As a key indicator of column stability, EOF

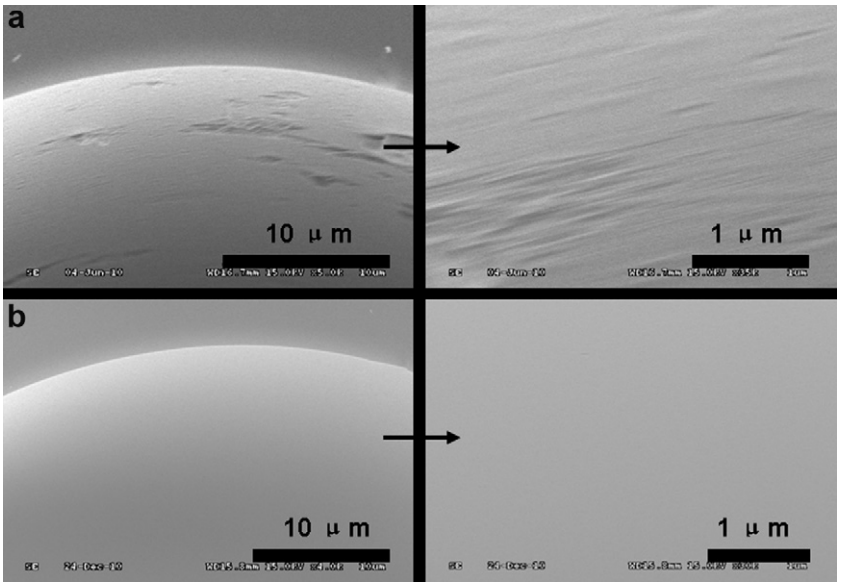


Fig. 1. SEM images of $PtBA_{127}$-b-$PGMA_{86}$ coated capillary (a) and bare capillary (b).

was investigated by 30 runs in three days at room temperature (20 °C) with 20 mM sodium tetraborate buffer solution at pH 10.5, which was adjusted by 1.0 M sodium hydroxide, and DMSO was used as the EOF marker. The result demonstrated that the block copolymer coating had a good endurance with $u_{EOF} = 3.2 \times 10^{-8}\ m^2\ V^{-1}\ s^{-1} \pm 3.3\%$ ($n = 30$). Furthermore, the coating stability was also evaluated based on degradation ratio, which was expressed as a percentage of the EOF difference before and after solvent flowing over the coated capillary [29]. After continuously flushed with pH = 2.0, pH = 7.0 or pH = 12.0 buffer solution for 15 min, the degradation ratio of the coated capillary was 0.24%, 0.53%, 0.15% respectively. This result indicated that the copolymer coating had good stability under acid, neutral and alkaline solutions.

Repeatability is another significant factor in evaluation of column performance and can be estimated by the relative standard deviation (RSD) of u_{EOF}. The result testified that the block copolymer coated capillary showed good run to run repeatability with RSD of 3.0% ($n = 30$) and excellent column to column repeatability with RSD of 3.0% (6 successive runs in each of three capillaries).

3.3. Optimization of the separation conditions

3.3.1. Effect of buffer pH

Buffer pH is a vital factor in affecting the dissociation of substances including the coating and the analytes, which is important in OT-CEC separation. As a result, in consideration of the best performance of the copolymer coating on separation, it was desirable to study the effect of buffer pH. Fig. 2a demonstrates that the effective mobility of the test steroid samples decreased with the increase of pH at the range from 8.0 to 11.0 except medroxyprogesterone acetate. Meanwhile, Fig. 2b shows the increasing trend for the number of plates with the increased pH. For getting the better separation efficiency in short time and for avoiding the higher current produced in the OT-CEC process, finally, the optimal pH at 10.5 was chosen for further separation in this work.

Furthermore, the migration order of the three steroid samples (medroxyprogesterone acetate, hydrocortisone, prednisone acetate) is worth mentioning. According to the literatures, relative molecular mass, charge and hydrophobic property are the important factors influencing the migration order of neutral compounds in CE and CEC [5,30]. Also, it has been reported [5] that the steroids containing diols were inclined to form anionic chelate compounds in the basic borate buffer solution. In consequence, the uncharged medroxyprogesterone acetate ($\log P = 4.11$, $\log P$ represents the hydrophobicity of compounds with the logarithm of n-octanol/water partition coefficient) moved fastest with the EOF which is the main driving force in the OT-CEC separation of the neutral compounds (Fig. 2a). Simultaneously, negatively charged hydrocortisone ($\log P = 1.61$) migrated faster than negatively charged prednisone acetate ($\log P = 3.30$) because the former has lower hydrophobic property than the later. The results revealed

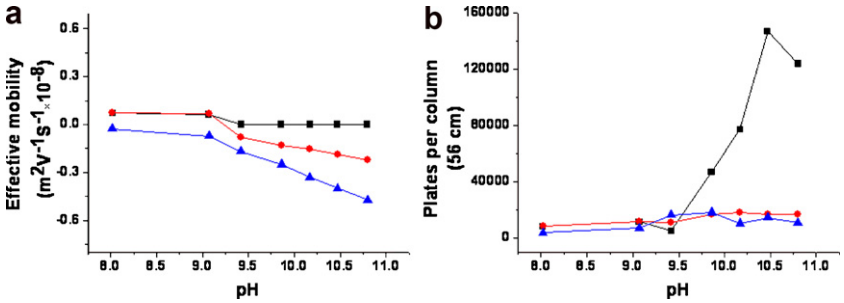


Fig. 2. Impact of pH on the effective mobility (a) and the efficiency (b). Experimental conditions: 20.0 mM borate; capillary: 75 μm I.D. × 70 cm (56 cm effective); injection was siphoned for 8 s in 25.0 cm height; voltage: +20 kV; UV detection: 254 nm. Symbols for the different analytes: square for medroxyprogesterone acetate; circle for hydrocortisone; triangle for prednisone acetate.

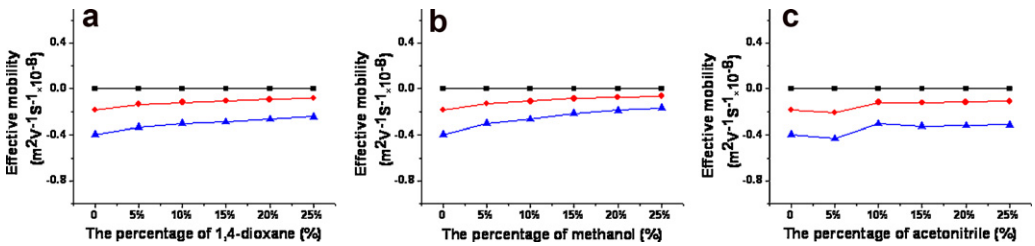


Fig. 3. Impact of 1,4-dioxane (a), methanol (b) and acetonitrile (c) content on the effective mobility. Experimental conditions: 20.0 mM borate at pH 10.5; other conditions are the same as in Fig. 2.

Table 1
The influence of buffer concentration on the separation.[a]

Buffer concentration (mM)	10.0	15.0	20.0	25.0	30.0
R_{s1}	0.0	1.6	3.2	3.5	3.6
R_{s2}	1.0	1.5	2.7	2.7	3.1

R_{s1}, resolution between medroxyprogesterone acetate and hydrocortisone; R_{s2}, resolution between hydrocortisone and prednisone acetate.

[a] Experimental conditions: pH 10.5; other conditions are the same as in Fig. 2.

that in the OT-CEC process, the hydrophobic interaction between the test steroid samples and the PtBA segment which could act as a hydrophobic part of the copolymer (Scheme 1) might play an important role in separation.

3.3.2. Effect of buffer concentration

For optimizing separation, the buffer concentration from 10.0 mM to 30.0 mM was studied. With the increase of buffer concentration, the intensified ion strength would lead to the decrease in thickness of the electrical double layer. Then the reduction of electrokinetic potential could lessen EOF [31]. The reduced EOF usually can improve the separation of the neutral and negative charged compounds. Table 1 indicates that the resolutions of the test steroid samples were improved obviously as the buffer concentration increased from 10.0 mM to 20.0 mM. However, the resolution did not change so much when the buffer concentration increased from 20.0 mM to 30.0 mM. Meanwhile, the raised buffer concentration could lead to the elevation of current which was not helpful for getting the good reproducible results in OT-CEC. Thus, 20.0 mM borate buffer was finally selected for the separation in this work.

3.3.3. Effect of organic solvents

In order to understand the effect of organic modifiers on separation of the test steroids, organic solvents with varied polarities, including 1,4-dioxane (P = 1.5), methanol (P = 5.7) and acetonitrile (P = 11.8) [32], were investigated. As shown in Fig. 3a, the effective mobility of the test steroid samples increased with the elevated content of 1,4-dioxane except medroxyprogesterone acetate which was neutral and co-migrated with the EOF. The same phenomenon was also observed in the investigation of methanol and acetonitrile (Fig. 3b and c), which may be contributed to the lessened EOF with the increased organic solvents [33]. However, adding organic solvents into the buffer solution did not improve the separation efficiency obviously. In addition, to consider it should be friendly to the environment, the buffer solution without organic solvents was selected as the optimal condition for further separation.

3.4. The separation of steroids by using the coated and the bare capillary

It is possible that the copolymer coating plays the role as surfactant in CE due to its amphipathic structure. In order to confirm this speculation, the common surfactant SDS was investigated on separation of the steroids by using a bare capillary. As displayed in Fig. 4b, the three test steroid samples could not be baseline separated by CZE method using a bare capillary. However, when 5.0 mM SDS was added into the buffer solution (the critical micelle concentration of SDS was 3.0 mM in borate buffer [34]), the separation efficiency of the test steroids obviously improved by MEKC

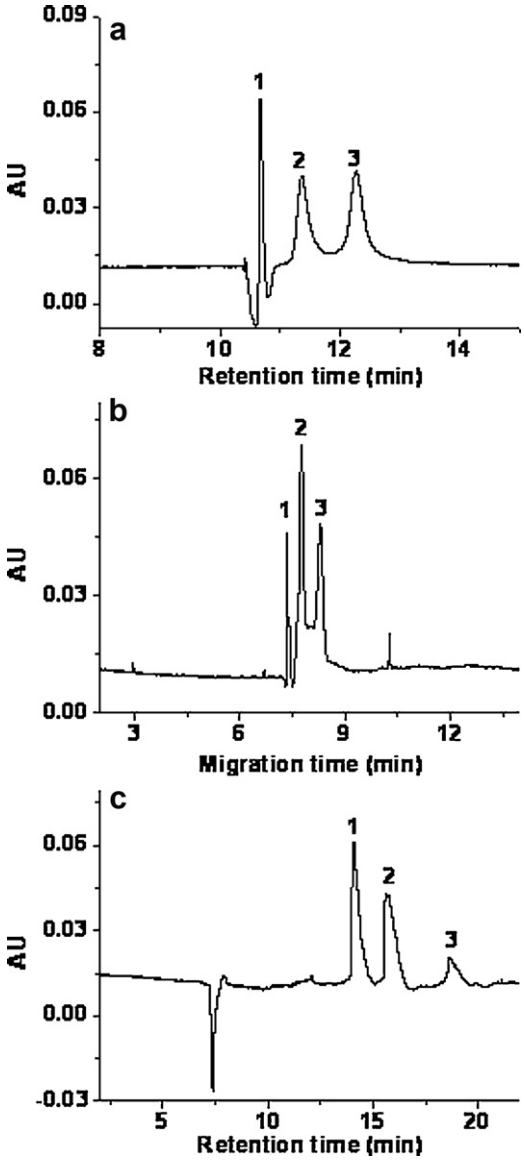


Fig. 4. Separation of steroid samples (a) in coated capillary; (b) in bare capillary; (c) in bare capillary with 5.0 mM SDS. Experimental conditions: 20.0 mM borate at pH 10.5, other conditions are the same as in Fig. 2. Peak identity: 1. medroxyprogesterone acetate, 2. hydrocortisone, 3. prednisone acetate.

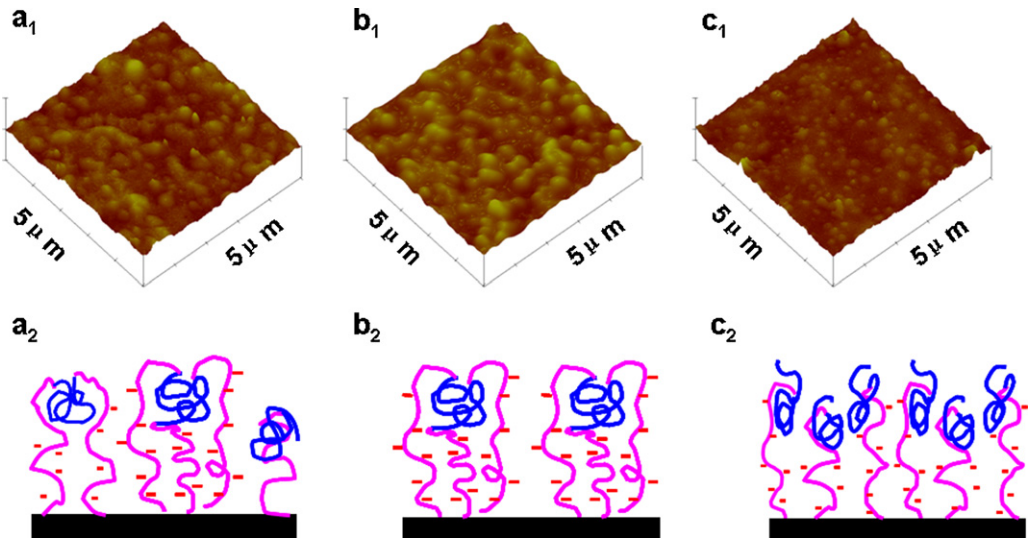


Fig. 5. AFM images of the block copolymer coating after dipped in different solutions (a_1–c_1) and schematic mechanism of block copolymer morphologies in different CE procedures (a_2–c_2). (a_1) Borate buffer (pH 8.0); (b_1) borate buffer (pH 10.5); (c_1) borate buffer with 50% methanol (pH 10.5) for 30 min.

method (Fig. 4c). Interestingly, the baseline separation of the three test steroid samples could be obtained when the block copolymer coating capillary was used (Fig. 4a). Meanwhile, Fig. 4a also shows that medroxyprogesterone acetate, which was uncharged, migrated together with the EOF.

The results indicated that this amphipathic block copolymer could exhibit surfactant behavior and play the same role as SDS.

3.5. The mechanism of separation

As we all know, the block copolymer coating possessing amphipathic structure could direct the self-assembly behavior [35]. The PtBA segment of the copolymer could act as a hydrophobic part. Meanwhile, the PGMA part of the copolymer could hydrolyze under alkaline conditions and contribute to a hydrophilic tail. Thus, at different pH, the hydrolysis degree of epoxy groups in hydrophilic segment is distinctive, which may influence the structure of the coating.

The experimental results obtained in Section 3.4 indicated that the block copolymer coating could act as surfactant. For exploring the separation mechanism, AFM was used for investigating the morphology of this coating under different conditions (Fig. 5).

At pH 8.0, medroxyprogesterone acetate and hydrocortisone could not be separated (Fig. 2, Fig. S-3). Meanwhile, the resolution between hydrocortisone and prednisone acetate was worse with the decrease of pH value (Fig. S-3). These results could be ascribed to the fact that the epoxy groups only partially hydrolyze (Fig. 5a_2) at alkalescent condition and the amount of the micelle-like aggregates was relatively less and the size of the aggregates was smaller as performed in Fig. 5a_1.

However, when the pH was increased to 10.5, the resolutions of the test steroids were higher than 2.0 (Fig. S-3). In this condition, the epoxy groups might hydrolyze mostly and the hydrophilic chain could extend straight in order to farthest decrease the repelling force. Meanwhile, the hydrophobic chain outside would curl for avoiding undesired interactions between water molecules and the hydrophobic region (Fig. 5b_2), which resulted in the formation of more micelle-like aggregates as shown in Fig. 5b_1. This result further confirmed that the coating could form micelle-like aggregates and play the same role as surfactant in the steroids separation (Fig. 4a).

It is well known that the conformation of amphipathic block copolymer is prone to be impacted by solvents. Additionally, methanol is a selective solvent for PtBA and PGMA, and can influence the morphology of block copolymer [36,37]. When 50% methanol (v/v) was added into the buffer solution, the size of the formed micelle-like aggregates obviously decreased (Fig. 5c_1). We also found that the thickness of the coating as shown in Fig. 5c_1 was about 10 nm higher than that shown in Fig. 5b_1. The phenomenon can be explained by the fact that the hydrophobic segment might straighten slightly when methanol was added (Fig. 5c_2). Meanwhile, because the structure of the block copolymer was disrupted by organic solvent, the separation efficiency decreased.

3.6. Analytical performance

To further reveal the application of this new method in actual system, the quantificational validation of hydrocortisone and prednisone acetate was performed. A standard calibration curve for peak area versus concentration (μM) reveals the linearity of the analyte and typical regression equations were as follows: $y = 1857x + 2.0 \times 10^4$ ($r^2 = 0.999$) for hydrocortisone, $y = 951.3x + 1.6 \times 10^4$ ($r^2 = 0.999$) for prednisone acetate. The limit of detection (LOD) and the limit of quantification (LOQ) were 3.8 μM and 11.4 μM for hydrocortisone, 5.5 μM and 16.6 μM for prednisone acetate respectively.

Then, the recovery of this proposed method assessed through adding the standard analytes into the serum samples was 91.9% ± 2.6% for hydrocortisone and 93.1% ± 1.7% for prednisone acetate over five injections. The repeatability of the method was determined by repeating five measurements of mixed standard solution containing 68.5 μM hydrocortisone and 125.0 μM prednisone acetate. The run to run RSD of retention time was less than 0.5% and that of peak area was less than 1.0%.

Method validation indicated that this developed method was desirable for quantificational analysis. To investigate its potential of practical applications, the proposed OT-CEC method was used to analyze the blank serum sample and the serum sample spiked with hydrocortisone (27.6 μM) and prednisone acetate (50.0 μM). Fig. 6b reveals that no peak had been found in the electrochromatogram of the blank serum sample. Meanwhile, the steroids could be separated and detected in the spiked serum sample with-

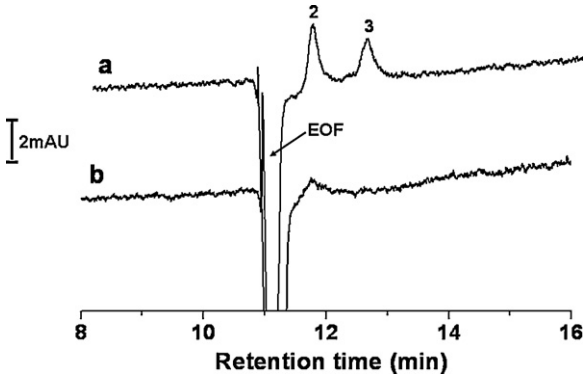


Fig. 6. Electrochromatograms of the serum samples in coated capillary. (a) Serum spiked with 27.6 μM hydrocortisone and 50.0 μM prednisone acetate; (b) blank serum. Experimental conditions are the same as in Fig. 4 except sample injection for 5 s.

out interference (Fig. 6a). Consequently, the OT-CEC method by using the well-defined copolymer coated capillary could provide a new way for direct and easy determination of steroids in biological samples.

4. Conclusion

A new amphipathic block copolymer was proposed and successfully modified in capillary. The copolymer coating could form micelle-like aggregates under proper conditions and act as surfactant, which could improve on the separation of steroids. It had been found that pH had vital influence on the separation efficiency. Moreover, method validation showed good levels of performance in terms of linearity, recovery and repeatability. Meanwhile, the coating could be effectively used to detect the steroids without interference in mimic blood samples, which provided a possible method for body fluid analysis. Additionally, the proposed chemical bonding method for coating was preferable for good stability and repeatability. The application of separation science for biological samples analysis also could be extended by using this kind of amphipathic block copolymer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.01.039.

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