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Polyethyleneimine-grafted boronate affinity materials for selective enrichment of *cis*-diol-containing compounds

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ABSTRACT

Polyethyleneimine (PEI)-grafted and 3-acrylamidophenylboronic acid (AAPBA)-functionalized SiO_2 boronate affinity materials were synthesized for the selective enrichment of *cis*-diol-containing compounds. Characterization results of scanning electron microscopy, Fourier transform infrared spectroscopy, elemental analysis, zeta potential, and X-ray photoelectron spectroscopy indicated the successful fabrication of SiO_2@PEI–AAPBA materials. Chromatographic separation of test mixtures reveals that SiO_2@PEI–AAPBA has high selective enrichment ability for *cis*-diol-containing compounds. The binding pH between SiO_2@PEI–AAPBA and catechol was found to be as low as pH 4.5, while that between SiO_2@PEI–AAPBA and adenosine was only ~7.5. This difference might be attributed to the strong electrostatic repulsion between the solid phase and analytes at a low pH. Furthermore, a diphasic separation column was fabricated based on boronate affinity chromatography, C18-reversed-phase chromatography and applied in pressurized capillary electrochromatography (pCEC). Results showed that four polar nucleosides could be well captured by the boronate affinity chromatography (BAC) section and separated by reversed phase pCEC. Finally, SiO_2@PEI_{600}–AAPBA-based solid-phase extraction technology was applied to the purification of ribonucleosides in real urine samples, and results of UHPLC–MS/MS revealed that the intensities of the extracted ions (a neutral mass loss of *m*/*z* 132.04 Da) of the ribonucleosides were significantly enhanced after the enrichment.

1. Introduction

cis-Diol-containing biomolecules such as glycoproteins, glycolipids, nucleosides, carbohydrates, and catechols play a crucial role in many fields of biotechnology and medicine. For example, glycosylation is one of the most common post-translational modifications, and glycoproteins play fundamental roles in many biological processes [1]. Numerous studies have shown that many potential biomarkers and clinical therapeutic targets are *cis*-diol-containing biomolecules [2–7]. Therefore, their selective detection in biological samples will facilitate the discovery of potential biomarkers. Unfortunately, most *cis*-diol-containing biomolecules in real samples are of low abundance and difficult to analyze quantitatively because of the matrix effect in mass spectrometric detection [8]. Therefore, effective enrichment and purification of *cis*-diol-containing biomolecules are essential.

Boronate affinity chromatography (BAC) is a powerful tool for selective separation and enrichment of *cis*-diol-containing compounds. The fundamental principle of BAC is based on the reversible covalent complexation between boronic acids and *cis*-diols in an alkaline aqueous solution (pH ≥ 8.5) to afford the corresponding cyclic boronate esters that dissociate in acidic environment. However, conventional boronic acids, taking phenylboronic acid for example, cannot strongly bind to *cis*-diol-containing compounds in near-neutral or weakly acidic solutions because of the requirement of an alkaline pH for significant binding; furthermore, conventional boronic acids exhibit limited binding affinity for trace amounts of compounds. In general, the properties of BAC are determined by both the boronic acid ligands and support materials [9], that is to say, materials with strong boronate affinity binding ability at lower pH condition and high-density boronate affinity groups are needed. Therefore, based on these requirements, several strategies have been developed for synthesizing appropriate support materials and aromatic boronic acids or their derivatives with a lower pKa value [10–15].

Polyethyleneimine (PEI) polymers have been widely used as coating materials for inorganic nanoparticles (NPs) and polymeric carriers for gene delivery because of their strong electrostatic affinity for polynucleic acids [16–18]. Due to a high density of

Abbreviations: PEI, polyethyleneimine; AAPBA, 3-acrylamidophenylboronic acid; BAC, boronate affinity chromatography; pCEC, pressurized capillary electrochromatography; SPE, solid-phase extraction

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amino groups, PEI polymers can supply a large number of active binding sites for grafting, which can greatly increase the amount of functional ligands in the chemical modification of a polymer surface [19], known as brushes or tentacles. Moreover, PEI significantly improved the hydrophilicity of bonded materials facilitating applications [20] in the separation and detection of biological samples, particularly reducing nonspecific adsorption significantly. Thus, these unique features of PEI make it an ideal support material for improving the binding density of interesting functional monomers.

Based on the excellent features of both PEI and boronic acids, Peng et al. [21] reported a phenylboronic acid-modified PEI, which significantly improved gene transfection efficiency compared to unmodified PEI. The efficiency of PEI was attributed to a high charge density at physiological media and a high buffer capacity at weakly acidic media. Li et al. [22] reported a new type of magnetic NPs, namely, Fe_3O_4@SiO_2@PEI–FPBA (FPAB=4-formylphenylboronic acid), which was found to show a much higher binding capacity for *cis*-diol-containing compounds.

The objective of this study is to combine the features of PEI and BAC to synthesize a novel boronate affinity material with strong binding capacity for isolating and purifying *cis*-diol-containing biomolecules, particularly those at a low concentration. Thus, a novel SiO_2@PEI–AAPBA affinity material was prepared, and 3-acrylamidophenylboronic acid (AAPBA) was selected as the boronate affinity ligand to grow on PEI, which was covalently immobilized on a SiO_2 material, by the Michael addition reaction between the amino groups of PEI and the α,β-unsaturated carbonyl groups of AAPBA. SiO_2@PEI–AAPBA was characterized and applied for chromatographic separation to confirm its utility and specificity. Further, the separation mechanism of this material was investigated, and the effect of the molecular weight of PEI on the binding ability of the material was elucidated for the first time. Moreover, a single column packed serially by boronate affinity materials and C18-reversed-phase was utilized in pressurized capillary electrochromatography (pCEC) for the separation of complex mixtures containing *cis*-diols. Finally, a solid-phase extraction–high-performance liquid chromatography–mass spectrometry (SPE–HPLC–MS) platform based on SiO_2@PEI_{600}–AAPBA was applied to the enrichment and separation of urinary ribonucleosides in real urine samples.

2. Materials and methods

2.1. Materials

3-Aminophenylboronic acid (APBA) monohydrate was purchased from Beijing Zhongsheng Huateng Technology Co., Ltd. Acryloyl chloride, stabilized with 400 ppm phenothiazine, as well as the polyethyleneimine molecules with four different molecular weights (PEI, MW~600 Da, ~1800 Da, 10,000 Da, 99%, and ~70,000 Da, 50%, marked as PEI_{600}, PEI_{1800}, $PEI_{10,000}$, and $PEI_{70,000}$, respectively) was bought from J&K Chemical Ltd. and used directly without further purification. Tetraethylorthosilicate (TEOS), γ-(2,3-epoxypropoxy) propytrimethoxysilane (KH-560), methyl alcohol, ethyl alcohol, formic acid, ammonium formate, anhydrous ether, and toluene were purchased from the Sinopharm Chemical Reagent Co., Ltd. The toluene must be used after the dehydration treatment. Adenosine, deoxyadenosine, catechol, resorcinol, hydroquinol, uridine, guanosine, and uracil were purchased from the Aladdin Industrial Inc. Ultrapure water was purified using a Molelement water purification system (Molecular, Chongqing, China). Other reagents utilized were of analytical grade or better. Silica spheres (particle size ~3 μm and ~20 μm, pore diameter ~12 nm) and C18–SiO_2 spheres (particle size ~3 μm, pore diameter ~12 nm) were achieved from the Global Chromatography (Suzhou, China). Fused-silica capillary tubes (100 μm ID) were obtained from the Yongnian Fiber Plant (Hebei, China).

2.2. Instruments

Scanning electron microscopy (SEM) of the morphology for sub-2 μm SiO_2 was carried out on a Hitachi S-4800 SEM instrument. The samples were prepared by dispensing drops of aqueous suspension onto aluminum foil fragments which were adhered onto the objective table by the conductive tapes. A Nano ZS Zetasizer (Mlavern Instruments Ltd.) was used to determine the zeta potential of the materials using the aqueous suspension at pH=7. Fourier-transform infrared spectrometric (FT-IR) spectra were recorded on a Nicolet 6700 (Thermo Fisher, USA) FT-IR spectrometer using KBr pellet, which were used to further prove the coating of the functional groups. X-ray photoelectron spectroscope (XPS, AXIS UltraDLD, Kratos, Japan) with Al Kα X-ray (energy=1486.3 eV) as excitation source was used to probe the surface element. The elemental analysis was performed with a Vario EL Cube (Elementar, Germany) type apparatus. The capillary chromatographic operations were completed on a Trisep-2100 pCEC (Unimicro Technologies, USA) instrument with a UV absorbance detector. The SPE part was performed on a CNW 12 Position Vacuum Manifold Set. And the UHPLC–MS/MS system consisted of an ACQUITY ultra performance liquid chromatographic system (Waters, Milford, MA, USA) coupled with an API 5500 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a Turbo Ion Source (ESI) operating with positive mode was used.

2.3. Synthesis of 3-acrylaminophenylboronic acid

The functional ligand AAPBA was synthesized by reacting APBA with acryloyl chloride in an aqueous solution containing sodium hydroxide according to the method previously reported [23]. First, APBA (1.14 g) was dissolved in aqueous sodium hydroxide (1.2 M, 25 mL), and the resulting solution was cooled in the ice-bath. Then, acryloyl chloride (1.2 mL) was added dropwise over a period of ~2 min under vigorous stirring. After 1 h, the mixture was warmed to room temperature, and the pH of the mixture was adjusted to ~1 by a hydrochloric acid solution (1 M). The resulting beige precipitates were filtered and washed several times with cold water (~5 mL). Then the precipitates were dissolved in ultrapure water (20 mL) at 85 °C, and the impurities were filtered off. The filtrate was left to stand overnight at room temperature, and the resulting light needle-like crystals of the product were filtered and dried in a desiccator. ^{1}H NMR spectrum of the product was consistent with previously published data. ^{1}H NMR (400 Hz): δ ppm 9.21 (s, 1H, NH), 8.01 (s, 2H, B–OH), 7.90 (d, 1H, Ar–H), 7.57 (d, 1H,Ar–H), 7.29 (t, 1H, Ar–H), 7.13 (s, 1H, Ar–H), 6.46 (dd, 1H, CH), 6.33 (dd, 1H, C=CH_2), 5.68 (dd, 1H, C=CH_2).

2.4. Preparation of SiO_2@PEI–AAPBA microspheres

2.4.1. Procedure for preparing sub-2 μm SiO_2@PEI–AAPBA microspheres with four different molecular weights

Procedure for preparing sub-2 μm SiO_2@PEI–AAPBA microspheres with four different molecular weights: (i) nonporous sub-2 μm silica (SiO_2) microspheres were synthesized by the modified two-phase sol–gel method [24]. The obtained SiO_2 microspheres were activated in a hydrochloric acid solution (12 wt%) for 24 h, washed thoroughly with ultrapure water until neutral pH, and dried under vacuum at 45 °C after filtration. (ii) To modify the SiO_2 surface with epoxy groups, the activated SiO_2 microspheres (1 g) were refluxed with KH-560 (4 mL) in anhydrous toluene (60 mL) for 24 h under nitrogen. The resulting epoxy-modified SiO_2 microspheres (denoted as SiO_2–epoxy) were isolated and washed in sequence with toluene, acetone, and

anhydrous ether and then dried at room temperature. (iii) After the dissolution of PEI (1.0 g) with four different molecular weights (PEI_{600}, PEI_{1800}, $PEI_{10,000}$, and $PEI_{70,000}$) in ultrapure water/anhydrous ethanol (80 mL, 1:1, v/v) by stirring and ultrasonic treatment, the SiO_2–epoxy microspheres were added and dispersed by ultrasonic treatment. Then, the mixture was magnetically stirred for 12 h at 65 °C under nitrogen. The resulting PEI–decorated SiO_2–epoxy microspheres (denoted as SiO_2@PEI) were isolated and washed in sequence with ultrapure water, anhydrous ethanol, and anhydrous ether to remove the excess PEI and then dried at room temperature. (iv) AAPBA (0.25 g) was dissolved in ultrapure water/anhydrous methanol (80 mL, 1:1, v/v) by ultrasonic treatment, and then the SiO_2@PEI particles were added under ultrasonic treatment. Next, AAPBA–grafted SiO_2@PEI (denoted as SiO_2@PEI_{600}–AAPBA, SiO_2@PEI_{1800}–AAPBA, SiO_2@$PEI_{10,000}$–AAPBA, and SiO_2@$PEI_{70,000}$–AAPBA) microspheres were obtained by reacting at 65 °C in an oil bath under magnetic stirring and nitrogen. Finally, the resulting materials were isolated and washed in sequence with ultrapure water, anhydrous methanol, and anhydrous ether to remove excess AAPBA and then dried at room temperature.

2.4.2. *Procedure for preparing 3 μm SiO_2@PEI_{1800}–AAPBA microspheres and 20 μm SiO_2@PEI_{600}–AAPBA microspheres*

Procedure for preparing 3 μm SiO_2@PEI_{1800}–AAPBA microspheres and 20 μm SiO_2@PEI_{600}–AAPBA microspheres: Same as that in Section 2.4.1, except that silica particles with different particle sizes and PEI molecules with different molecular weights were used.

2.5. *Boronate affinity capacity measurements*

All the chromatographic experiments for the boronate affinity capacity measurements were conducted using fused-SiO_2 capillary (100 μm ID, total length 26 cm, and effective length 11 cm) packed with sub-2 μm SiO_2@PEI_{600}–AAPBA, SiO_2@PEI_{1800}–AAPBA, SiO_2@$PEI_{10,000}$–AAPBA, and SiO_2@$PEI_{70,000}$–AAPBA materials, respectively, by traditional slurry packing procedure, under high pressure (45 MPa) supplied by a Butech high pressure pump. The UV detection wavelength was set at 254 nm, and the sample injection volume was 1 μL. 100 mM ammonium acetate (NH_4OAc) buffer with different pH values and 200 mM formic acid were used as the mobile phases during the gradient elution. A pump flow rate of 0.1 mL/min was used throughout the experiments unless otherwise specified. A four-port splitter was used between the injection valve and the monolithic capillary column to split the flow into a desirable and stable flow rate. Adenosine and deoxyadenosine were dissolved and diluted in an ammonia solution (25 mM) with a concentration of 0.25 mg/mL. Catechol, resorcinol, and hydroquinone were dissolved and diluted in methanol with a concentration of 0.1 mg/mL. All the solutions were stored at 4 °C prior to use.

2.6. *BAC–C18 RPC–pCEC separation system*

To develop an effective method for the separation of complex mixtures containing *cis*-diol compounds, a biphasic BAC–C18 RPC column was prepared in a capillary packed serially with 3 μm SiO_2@PEI_{1800}–AAPBA and C18–SiO_2 by the traditional slurry packing procedure. The 3 μm mesoporous SiO_2@PEI_{1800}–AAPBA materials were first packed into the capillary to a length of 5 cm, and then the following 15 cm was packed by 3 μm mesoporous C18–SiO_2 particles. Finally, the

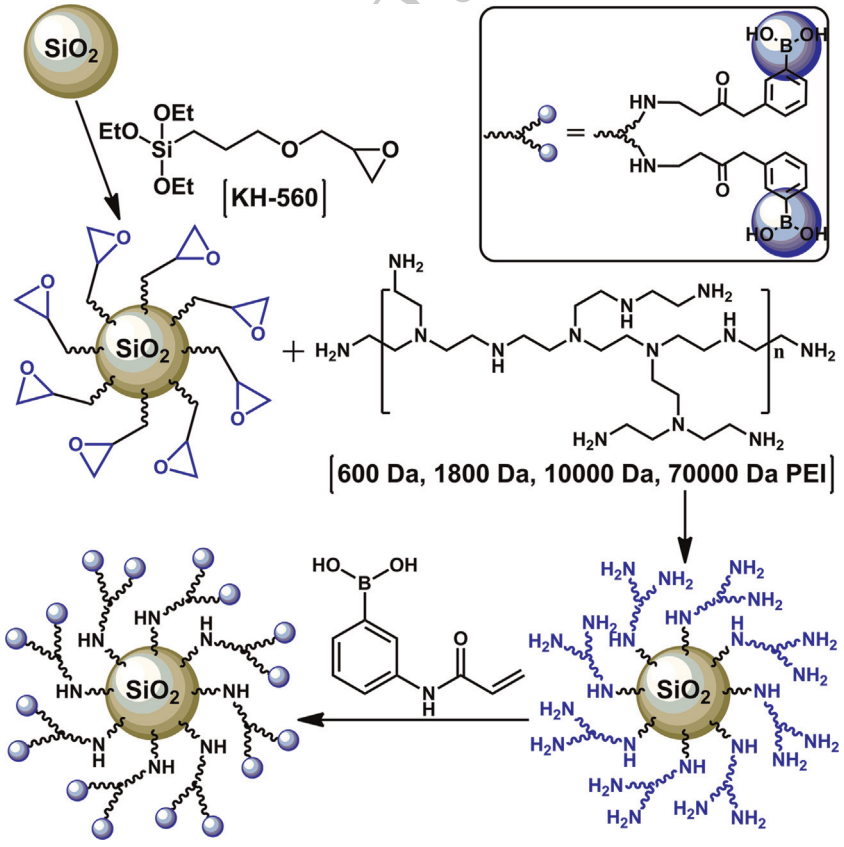

Fig. 1. Schematic of preparation of SiO_2@PEI–AAPBA boronate affinity materials.

biphasic BAC–C18 RP capillary column was obtained with a 40 cm total length (20 cm effective length) and used in the pCEC system. This BAC–C18 RPC–pCEC system was investigated with four nucleosides, as 10 mM NH_4OAc (pH=8.95)–acetonitrile (ACN) (96:4, v/v) and 200 mM formic acid–ACN (96:4, v/v) were prepared as the mobile phase.

2.7. Biological sample analysis

To apply this material to the enrichment and analysis of *cis*-diol-containing compounds in a biological sample, urine from a healthy male was selected as the test sample. For the ease of operation, 20 μm mesoporous SiO_2 spheres were used in the preparation, affording SiO_2@PEI_{600}–AAPBA microspheres. Then, 80 mg of the prepared 20 μm mesoporous SiO_2@PEI_{600}–AAPBA microspheres were packed into a 1 mL SPE tube for the offline enrichment of the ribonucleosides in the urine sample; ribonucleosides have been used for the diagnosis of cancer [25].

The SPE process was performed as follows: (i) The SPE column was equilibrated with 0.5 mL × 3 methanol and 0.5 mL × 3 alkaline buffer ($NH_3 \cdot H_2O$ solution, pH=9). (ii) After the urine was thawed and centrifuged at 12,000 rpm for 10 min, 950 μL of the urine was alkalized to a pH of 9 and loaded onto the SPE column under a 20 kPa vacuum. (iii) 0.5 mL × 2 0.1 wt% $NH_3 \cdot H_2O$ solution was applied to wash the unretained compounds. (iv) 0.5 mL × 3 formic acid solution (200 mM) and 0.5 mL × 2 methanol were used to elute the retained analytes. The final eluates were collected and combined. After evaporating to dryness, the residue was redissolved with 50 μL dilute formic acid solution (10 mM). The final solution was centrifuged at 12,000 rpm for 10 min, and the supernatant liquid was subjected to the HPLC–MS system.

Chromatographic and mass conditions: The determination was carried out on a C18 column (5 μm, 4.6 × 250 mm^2) under a gradient elution condition (0–5 min, 2% B; 5–22 min, 2–10% B; 22–27 min, 10% B; 27–35 min, 10–100% B; 35–40 min, 100% B; 40–50 min, 2% B), where the mobile phase A was 10 mM NH_4OAc, and the mobile phase B was acetonitrile, at a flow rate of 1 mL/min. The injection volume of sample was 20 μL, and the UV detection wavelength was set as 254 nm. The mass scan type was set as Neutral Loss (NL) in the positive mode. The ion spray voltage was kept at 5500 V, and the ion source temperature was set at 550 °C. The ion source gases 1 and 2 and the curtain gas were at pressures of 60, 05, and 35 psi, respectively. The collision energy (CE) was 10 eV, and the declustering potential was maintained at 100 eV.

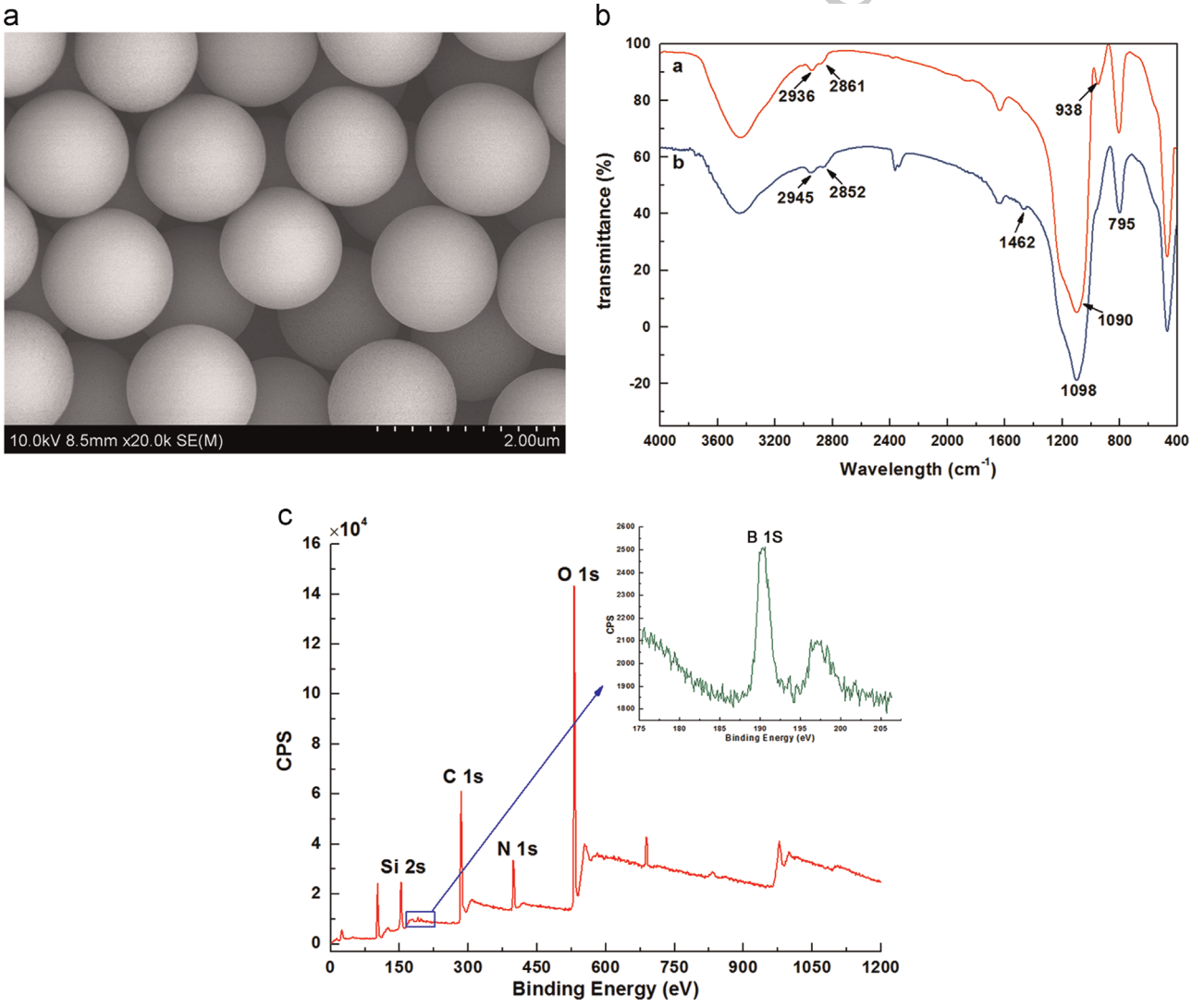

Fig. 2. (A) SEM photograph of the silica spheres, (B) FT-IR spectra of (a) KH-560 modified SiO_2 and (b) PEI_{1800} grafted SiO_2, and (C) XPS result of SiO_2@$PEI_{10,000}$–AAPBA.

3. Results and discussion

3.1. Characterization of SiO_2@PEI–AAPBA

Fig. 1 shows the schematic of the preparation of SiO_2@PEI–AAPBA. These SiO_2@PEI–AAPBA materials were fabricated mainly through a "two-step" method: modification of PEI molecules onto SiO_2 surface by the ring-opening reaction and graft of AAPBA functional groups on the chains of PEI by the Michael addition reaction. This synthetic strategy has the advantages of simple operation plus post treatment, mild reaction condition, and low consumption of reagents.

Fig. 2A shows the representative scanning electron microscopy (SEM) micrograph of the SiO_2 spheres prepared by the modified two-phase sol–gel method. The product is spherical and monodispersed, with an average size of ~1.4 μm. The FT-IR spectrum shown in Fig. 2B(a) indicates the successful modification of glycidyl groups onto the SiO_2 spheres by the hydrolysis of KH-560, as the bands at 2936 and 2862 cm^{-1} can be attributed to the characteristic stretching vibration of methylene C–H bonds. The characteristic absorption peak of the epoxy group also appears at 938 cm^{-1}. This peak disappeared after the grafting of PEI (Fig. 2B(b)), indicating the opening of epoxy rings by the amino groups of PEI on the surface of the SiO_2 matrix. The zeta potential changed from −35.7 to 53.3 mV

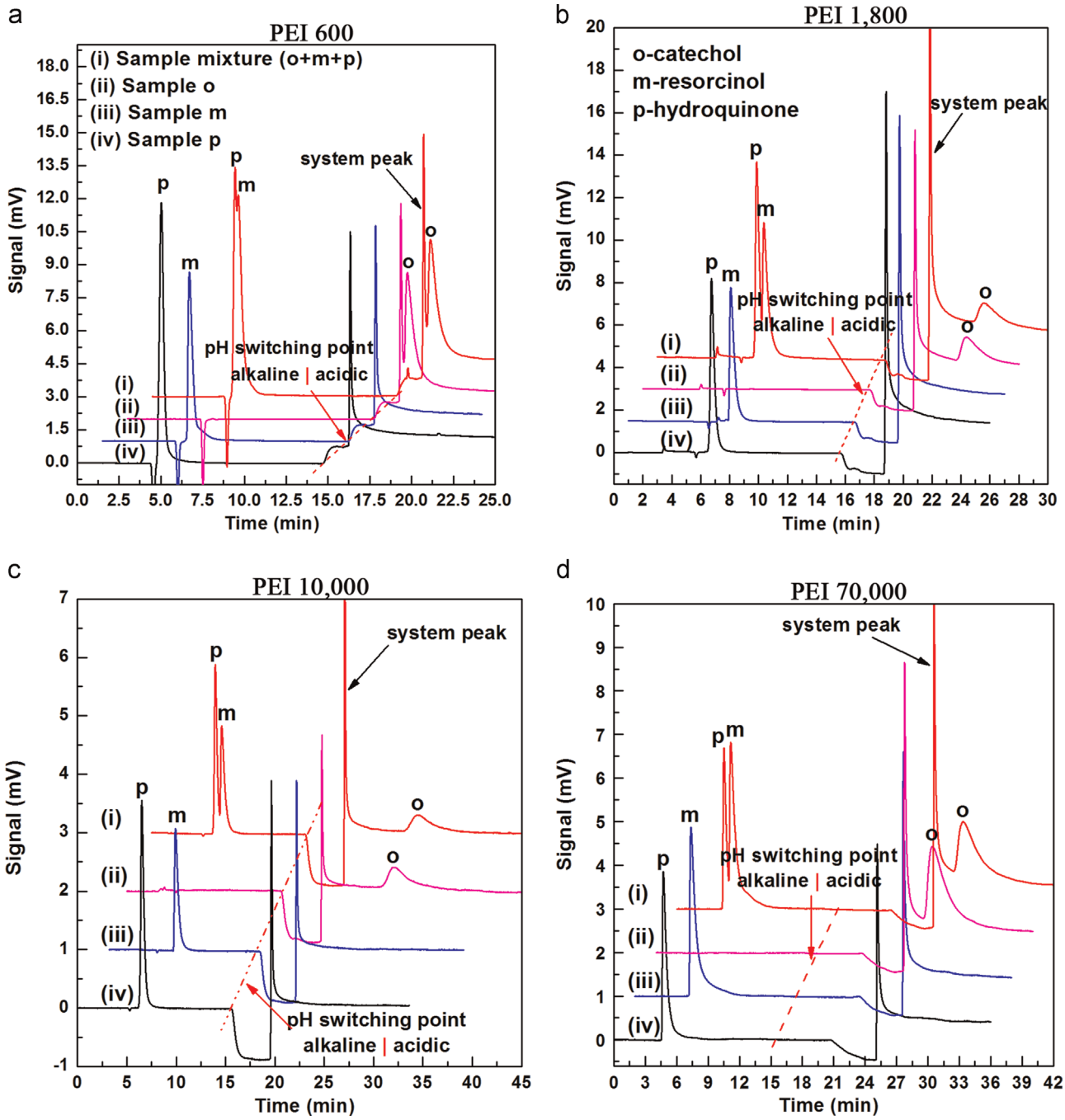

Fig. 3. Chromatograms of the separation of a mixture of hydroquinone (p), resorcinol (m), and catechol (o) on packed capillary columns of (a) SiO_2@PEI_{600}–AAPBA, (b) SiO_2@PEI_{1800}–AAPBA, (c) SiO_2@$PEI_{10,000}$–AAPBA, and (d) SiO_2@$PEI_{70,000}$–AAPBA. Mobile phase is 100 mM NH_4OAc (pH=8.95) before the pH switching point, and 200 mM formic acid solution after the pH switching point.

after the grafting of PEI, indicating that a large number of amino groups were immobilized on the surface of SiO_2 spheres. The elemental analysis results (wt%) for PEI_{1800}-grafted SiO_2 are as follows: C, 2.48; H, 1.48; and N, 0.38, confirming the immobilization of amino groups. The X-ray photoelectron spectrum (XPS) of SiO_2@$PEI_{10,000}$ @AAPBA exhibits a B 1s peak at 191.5 eV (Fig. 2C), indicating the successful attachment of boronic acid groups onto the PEI-grafted SiO_2 material. Moreover, the presence of an N 1s peak indicates that there are still a large amount of unreacted amino groups due to steric hindrance, which may affect chromatographic separation.

3.2. Boronate affinity

3.2.1. Boronate selectivity

To evaluate the covalent binding capacity of the materials with *cis*-diol-containing compounds on the packed SiO_2@PEI–AAPBA boronate affinity capillary columns, a mixture of hydroquinone, resorcinol, and catechol was selected as the analytes. Fig. 3 shows the different retention behavior of the analytes. The boronate affinity capillary columns were first equilibrated with the loading buffer (100 mM NH_4OAc, pH = 8.95). At the starting mobile phase conditions, hydroquinone and

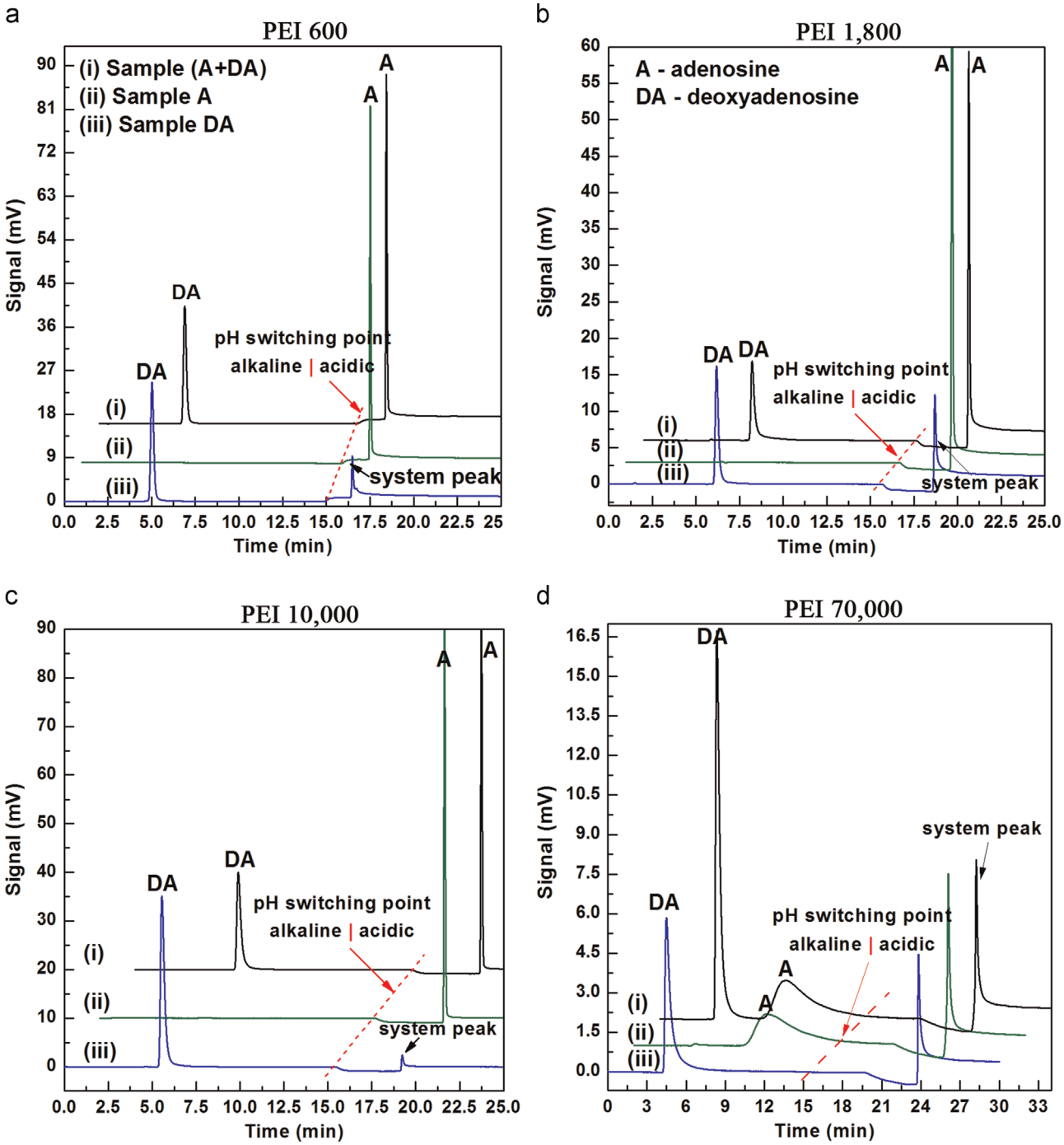

Fig. 4. Chromatograms of the separation of adenosine (A) and deoxyadenosine (DA) on packed capillary columns of (a) SiO_2@PEI_{600}–AAPBA, (b) SiO_2@PEI_{1800}–AAPBA, (c) SiO_2@$PEI_{10,000}$–AAPBA, and (d) SiO_2@$PEI_{70,000}$–AAPBA. Mobile phase is the same as in Fig. 3.

resorcinol had no retention on all the four columns and were eluted at the void time, while catechol molecules, containing the *cis*-diol groups, were completely captured by the boronate affinity solid phase. When the mobile phase was changed to the acidic mobile phase (200 mM formic acid), the captured catechol molecules were eluted. These results indicate that the prepared SiO_2@PEI–AAPBA columns exhibited a high selectivity toward *cis*-diol-containing compounds over other non-*cis*-diol-containing molecules. Moreover, from the partial separation of hydroquinone and resorcinol and the increasing duration between the mobile phase switching point and the system or catechol peak with increasing molecular weights of PEI, namely, ~1 min for PEI_{600}, ~3.2 min for PEI_{1800}, ~3.8 min for $PEI_{10,000}$, and ~7.5 min for $PEI_{70,000}$, it can be concluded that there are excess amino groups on the solid phase, facilitating the anion-exchange chromatographic interactions with the analytes and prolonging the duration of buffer when changing the mobile phase. This phenomenon may negatively affect the enrichment of *cis*-diol-containing compounds with acidic groups (e.g., acetate ion) due to the irreversible adsorption.

Similar results were obtained when using the SiO_2@PEI–AAPBA boronate affinity material packed capillary columns for the specific capture of *cis*-diol-containing biomolecules. Adenosine and deoxyadenosine were selected as the analytes. The chromatographic retention behavior of the two analytes shown in Fig. 4(a–c) is in accordance with the principle of boronate selectivity on all the boronate affinity columns. Deoxyadenosine was eluted, while adenosine was totally captured at the alkaline mobile phase of pH 8.95 because of the presence of a *cis*-diol group. Adenosine was eluted by changing to the acid mobile phase (200 mM formic acid). These results confirm the boronate affinity selectivity of the column towards compounds containing *cis*-diol groups.

The different result obtained on the SiO_2@$PEI_{70,000}$–AAPBA-packed capillary column (Fig. 4(d)), namely that adenosine was eluted before the pH switching point, can be explained by the strong electrostatic repulsion between the positively charged ammonium ions from both the solid phase and analytes, thus reducing the boronate affinity interaction. In addition, Fig. 4 shows that all the eluted adenosine peaks merged with the system peaks, clearly different from the catechol peaks shown in Fig. 3, also confirming the reduction of boronate affinity interaction. SiO_2@$PEI_{70,000}$–AAPBA solid phase should have the strongest positive-charge electrostatic repulsion with adenosine because of the largest number of amino groups; therefore, the above undesired result in Fig. 4(d) was observed. This can be solved by increasing the pH of the mobile phase to inhibit the generation of positively charged ammonium ions.

3.2.2. *Binding pH measurement*

Binding pH plays an essential role in BAC. The pKa value of boronic acids is a key parameter that affects the boronate affinity. Usually, a lower pKa value results in a stronger affinity and requires a lower environmental pH for binding [9]. The binding pH in conventional BAC columns should be larger than the pKa values of the boronic acid ligands. Besides, characteristics of the support materials also can significantly affect the binding pH. To date, multiple strategies have been proposed to reduce the binding pH, thus facilitating the applications of BAC under practical conditions, by reducing the pKa of the boronic ligand and selecting the appropriate support materials [9,11–14,22].

In this study, AAPBA was selected as the functional ligand. The pKa of AAPBA was ~8.2, and the binding pH of the monolithic column using AAPBA as the boronic acid ligand prepared by Li et al. [14] was found to be as low as 6.5. The retention of catechol on SiO_2@PEI_{1800}–AAPBA-packed capillary column at different pH values (Fig. 5(b)) reveals that catechol molecules can be captured by the boronate affinity material at a pH as low as 4.5 while eluting at pH 3.5. This result shows that the prepared SiO_2@PEI_{1800}–AAPBA boronate affinity materials possess a strong binding capacity for the *cis*-diol-containing compounds at even weak acidic conditions. However, the retention of adenosine at different pH values shows that the *cis*-diol-containing adenosine can be captured on the solid phase at pH 7.5, but cannot be retained at pH 6.5 (Fig. 5(a)). This relatively high binding pH value for adenosine can also be explained by the above mentioned electrostatic repulsion interactions.

3.3. *BAC–C18 RPC–pCEC separation system*

To overcome the difficulty in the separation of complex mixtures, pCEC was also utilized on SiO_2@PEI_{1800}–AAPBA and C18-RP column. A negative voltage was applied to the inlet of the capillary column. Four nucleosides were used as the mixture, namely, cytidine (C), uridine (U), guanosine (G), and adenosine (A). The four nucleosides were first adsorbed in the SiO_2@PEI_{1800}–AAPBA part under alkaline condition and then eluted to the C18-RP part by the acidic mobile phase. Finally, the immediately eluted nucleosides were separated by both the C18-RP materials and electric field. As highly polar compounds, nucleosides could not be

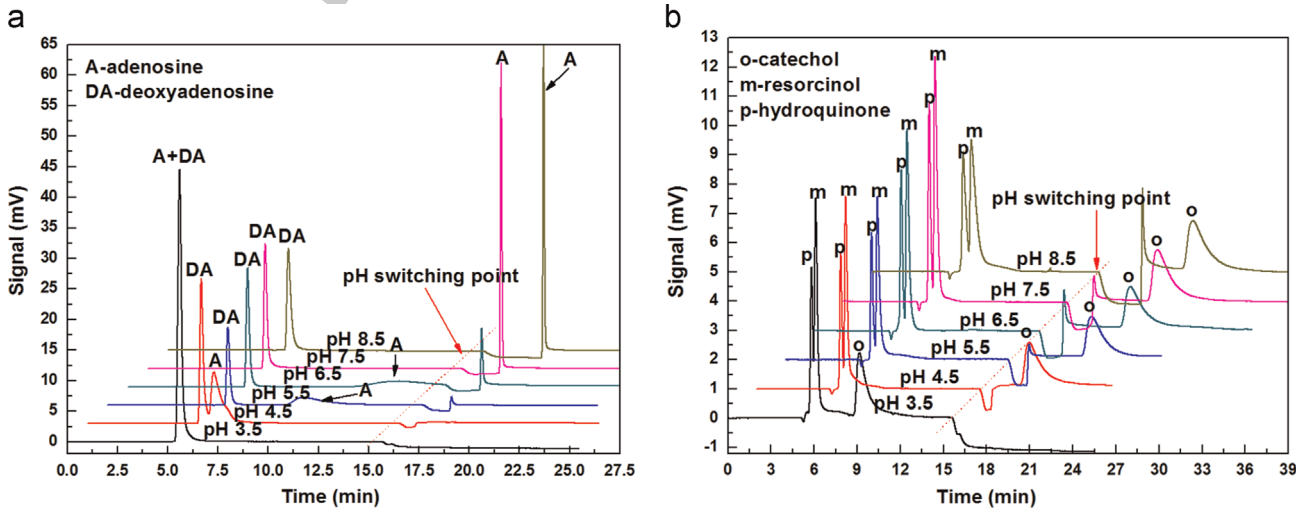

Fig. 5. Chromatograms of the separation of mixtures of (a) adenosine and deoxyadenosine, and (b) hydroquinone, resorcinol, and catechol on SiO_2@PEI_{1800}–AAPBA under different pH values. Mobile phases: 100 mM NH4OAc buffer at pH 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5, switched to 200 mM formic acid at 15 min.

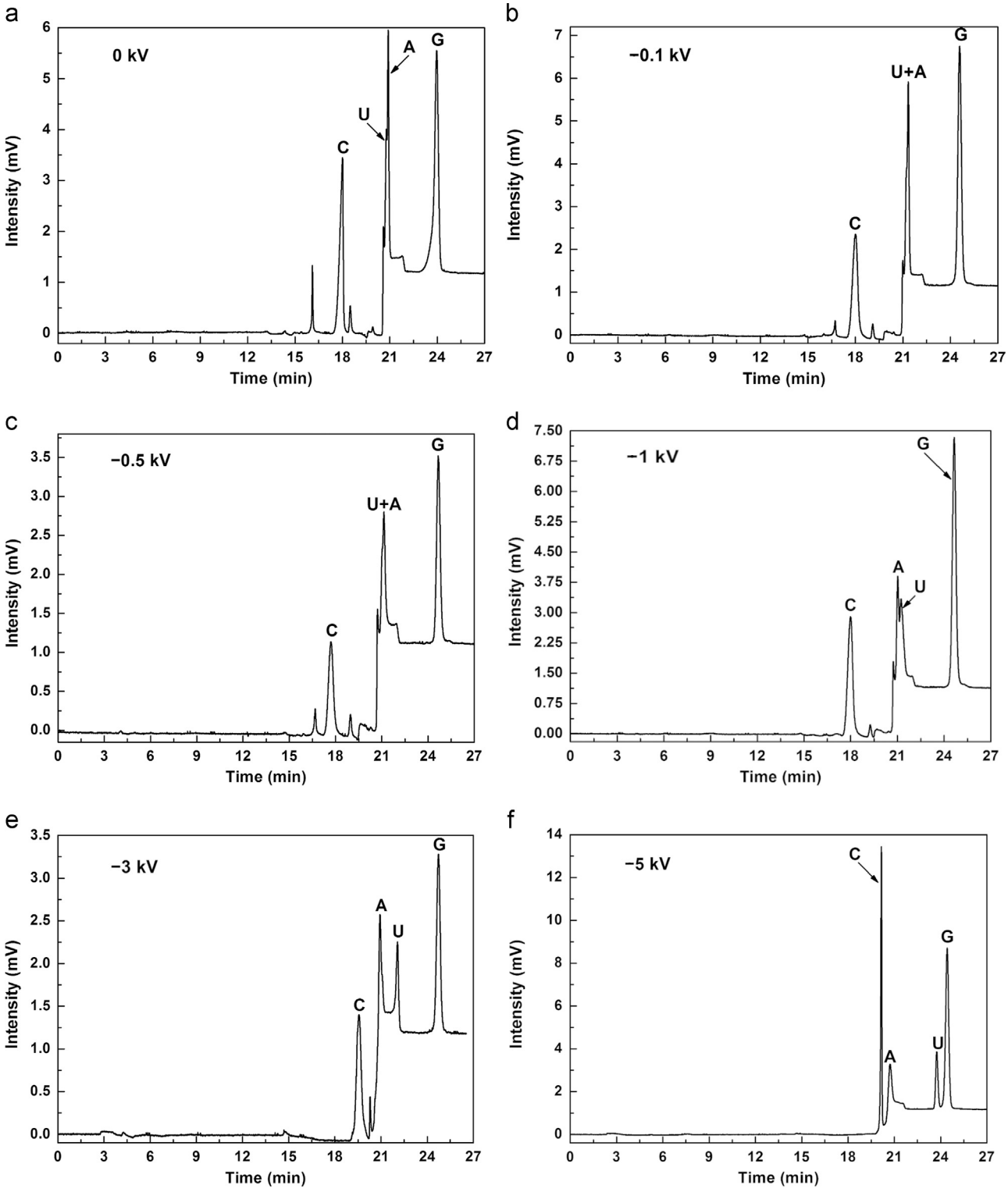

Fig. 6. Electrochromatograms of the separation of nucleosides under different voltages.

completely separated by the single reversed-phase interaction; therefore, an electric field was applied. Fig. 6 shows the electrochromatograms of the four nucleosides in the BAC–C18 RPC–pCEC system under different applied voltages. The results show that the four nucleosides can be separated by increasing the electric voltage. This indicates that the BAC–C18 RP–pCEC system is a promising tool for the separation of complex mixtures with *cis*-diol groups.

3.4. Biological sample analysis

In the analysis of biological sample, SiO_2@PEI_{600}–AAPBA material was selected rather than the other three materials for the enrichment of ribonucleosides in the urine sample to avoid the intensive electrostatic repulsion interactions between the functional materials and nucleosides of the larger molecular-weight PEI. According to the previous reports [22,26,27], urine samples contain various ribonucleosides

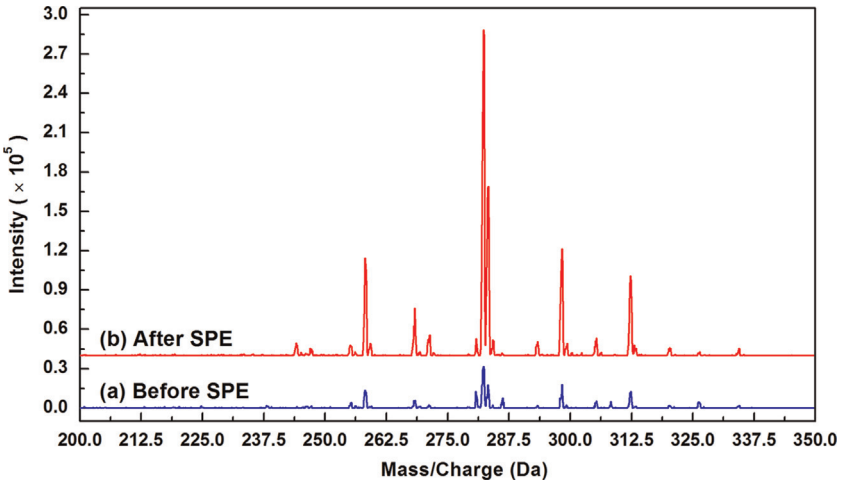

Fig. 7. Neutral mass loss (*m*/*z* 132.04 Da) extracted-ion chromatograms of the urine analyzed (a) before and (b) after the enrichment by a 20 μm mesoporous SiO_2@PEI_{600}–AAPBA boronate affinity material packed SPE column.

that act as the biomarkers of malignant diseases (e.g., cancer). Fortunately, most urinary ribonucleosides are *cis*-diol-containing biomolecules, and a neutral mass loss of the ribose moiety (132.04 Da) has been widely used for their identification. Neutral mass loss (*m*/*z* 132.04 Da) extracted-ion chromatograms of the urine analyzed before (Fig. 7(a)) and after (Fig. 7(b)) the enrichment by 20 μm mesoporous SiO_2@PEI_{600}@AAPBA boronate affinity SPE column reveal that the MS signals of these ribosylated metabolites were significantly enhanced after the enrichment. Moreover, a total of 171 ion-pairs with an intensity of >1500 were extracted by the neutral mass loss (*m*/*z* 132.04 Da) after the extraction, while only 97 ion-pairs with an intensity of >1500 were observed in the urine sample without any treatment. Therefore, the material is an attractive alternative tool for enriching and purifying the urinary ribonucleosides in urine samples.

4. Conclusions

An alternative method for the synthesis of PEI-introduced and boronic acid-functionalized SiO_2 boronate affinity materials was developed for selectively capturing *cis*-diol-containing compounds. The prepared boronate affinity materials exhibit significant binding selectivity because of the combination of BAC and PEI, which provides a large number of active binding sites and improves the hydrophilicity of the materials. Furthermore, a novel BAC–C18 RPC–pCEC separation system and an SPE–HPLC–MS technology based on these SiO_2@PEI–AAPBA materials were found to be promising in the effective separation of complex mixtures containing compounds with the *cis*-diol groups. Therefore, these systems may have promising applications in the separation and detection of biological samples containing *cis*-diol groups.

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