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One-pot preparation of boronic acid and PEG bi-functionalized silica particles for separation and purification of catecholamine from rat serum

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A facile one-pot preparation of aminophenyboronic acid (APBA) and poly(ethyleneglycol) (PEG) bi-functionalized silica particles named SiO_2@APBA@PEG by the aid of epoxy silanes was described in this work. The as-prepared particles could not only selectively extract the catecholamine through boronate binding but also effectively resist protein adsorption with PEG brushes, FT-IR spectra and contact angle measurements proved that APBA and PEG groups were successfully introduced to the surface of silica. The maximum adsorption capacities towards dopamine (DA) and levodopa (L-DOPA) were found to be 254.45 and 104.71 μmol/g, respectively. The as-prepared material was used as a solid phase extraction (SPE) sorbent for extraction of catecholamine in rat serum. The recoveries of DA and L-DOPA were 95 ± 3.21% and 93 ± 3.57%, in the meanwhile, proteins were eliminated. The obtained results indicated the SiO_2@APBA@PEG particles were qualified for efficient purification of catecholamine from complex biological samples.

Introduction

Catecholamines, such as dopamine (DA), levodopa (L-DOPA), epinephrine (E) play a major role in the central nervous system. They have been used as specific markers for some diseases in clinical diagnostics. So it is of great significance to determine catecholamine in biological fluids (serum, plasma or urine).[1] In the literatures, a few analytical method such as high-performance liquid chromatography (HPLC),[2, 3] fluorescence measurements[4] and electrochemical analysis[5] have been reported for the determination of catecholamine in biological fluids. In all cases, sample preparation is essential, because there are many coexisting proteins in biological fluids, which generate interferences for further analysis.[6] Generally, techniques such as solid-phase extraction (SPE),[7] liquid-liquid extraction (LLE),[8] and protein precipitation (PPT)[9] are employed in sample preparation. However, when PPT is used during sample preparation, part of the analytes co-precipitate with the protein sediment, causing the loss of the analytes.[10] In LLE procedures, large amounts of toxic solvents are needed. So, compared to the above methods. SPE is preferred. Because it is simpler, more convenient, and easier to automate.[11] In the SPE procedures, the analytes are retained on column, while interfering substances are washed away. Then the analytes are eluted for further analysis. The adsorbents routinely used in SPE usually lack specificity, such as Sephadex G10,[12] cation exchange adsorbents,[13] C18 matrix[14] and alumina.[15] Recently, boronic acid-functionalized materials as the sorbents for the extraction of catecholamine have been successfully used in some researches.[16-18] Boronic acid can form boronate ester with *cis-diol* group in alkaline conditions. This complex formation is reversible. When the medium is adjusted to an acidic pH, the complex dissociates.[19, 20] Boronate affinity materials with high affinity and selectivity toward *cis-diol*-containing compounds have gained great attention.[21, 22] However, in a practical biological sample, non-specific adsorption of contaminants such as proteins or cells on the boronate affinity materials is a serious problem, which causes poor sensitivity and selectivity of the SPE technique. Hydrophobicity and electrostatic interaction are the main causes contributed to the nonspecific adsorption of proteins.[23] Therefore, to resist or reduce protein absorption, the two factors should be taken into account. Poly (ethylene glycol) (PEG) with neutral and hydrophilic characteristics exhibits excellent biocompatibility. For this reason, PEG-based materials have been widely used in biological samples to resist protein adsorption and cell adhesion.[24-27] Several strategies have been reported to immobilize PEG onto the substance surfaces, such as self-assembly,[28] physisorption,[29] surface polymerization[30, 31] and covalent grafting.[32] Among these strategies, covalent grafting of PEG onto the activated surfaces is considered to be the most effective method to prepare stable PEG surfaces.[33] However, the PEGylated materials are only used to resist protein adsorption in these literatures and there are few reports on the coupling of PEG with other active groups on the surface of materials.

In this study, we report a facile one-pot preparation of boronic acid and PEG bi-functionalized silica particles named SiO_2@APBA@PEG by the aid of epoxy silanes. To the best of

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our knowledge, there have been no reports on synthesis of boronic acid and PEG bi-functionalized silica materials through one-pot method. The novel boronate sorbent have been successfully used in solid phase extraction for selectively isolation of DA and L-DOPA from rat serum. The obtained results indicate that SiO_2@APBA@PEG particles are promising sorbents for sample preparation.

Experimental

Materials

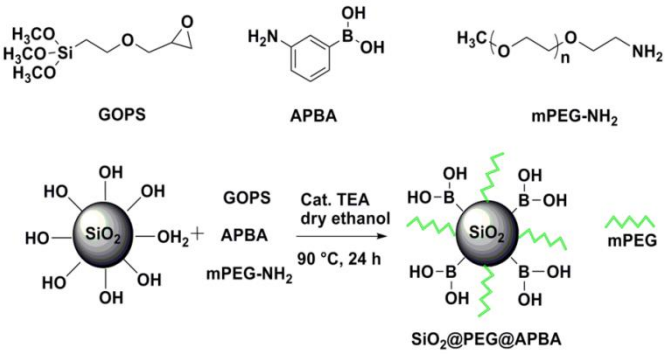
Silica purchased from Suzhou Nanomicro Technology Company (UniSil10-300, 10 μm) was activated by refluxing for 6 h in 20% HCl, and then dried at 150 ^oC under vacuum overnight. 3-aminophenylboric acid (APBA, 98%) and Sodium Pyrosulfite were purchased from Energy chemical (Shanghai, China) and used without further purification. Methoxypolyethylene glycol amine (mPEG-NH_2, MW = 2000) 3-glycidoxypropyltrimethoxysilane (GOPS), dopamine (DA) and levodopa (L-DOPA) were all purchased from Aladdin (Shanghai, China). Lysozyme (Lyz), bovine serum albumin (BSA), ovalbumin (OVA) and trypsin (Try) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were at least of analytical grade. Ethanol (99.7%, AR) was dried with magnesium turnings and iodine. Triethylamine (TEA, ≥99.0%, AR) was dried by potassium hydroxide. Deionized water was prepared with a Milli-Q water Purification system (Millipore, Milford, MA).Rat serum was obtained from SLAC laboratory Animal Co. Ltd. (Hunan, China) and stored at − 20 ^oC until use after gentle thawing. Catecholamine solution was prepared with 0.01 M phosphate buffer solution (pH = 8.5) containing 0.15 mg/mL antioxidant sodium metabisulfite to prevent the oxidation of analytes. Proteins solution were prepared with 0.01 M phosphate buffer solution (pH = 8.5).

Instrumentation and characterizations

Fourier transform infrared (FT-IR) spectra of the functionalized silicas were measured by a Nicolet-6700 FT-IR spectrometer using KBr pellets in the 4000-400 cm^{-1} region with a resolution of 4 cm^{-1}, by accumulating 32 scans. Samples (0.014±0.06 g) were intimately mixed with previously dried KBr (1.176±0.05 g) by gentle grinding so as to minimize particle break up. Static contact angle measurements were carried out on a JC2001C contact angle measurement system with DI water drops of 3 μL on the surface of the materials at room temperature. All as-prepared materials were tabletted and dried at least 36 h prior to measurements. The morphology of the pure SiO_2 and SiO_2@APBA@PEG were examined by a MIRA3 TESCAN Field-emission scanning electron microscopy (SEM) at an accelerating voltage of 20kV. UV-vis adsorption spectra were recorded on the SHIMADZU UV-2450 spectrophotometer. HPLC apparatus (Dionex Ultimate 3000, USA) and a reversed phase Water Spherisorb ODS2 (250 mm×4.6 mm, Water, Milford, MA, USA) column were employed for analysis. The chromatographic separation was carried out using a mixture of acetonitrile (A) and water containing 0.4% (v : v) (B). The gradient program was used according to the following profile: 5% A (0-20 min), 5%-20% A (20-30 min). The flow rate was 1.0 mL/min while the ambient temperature was controlled at 30 ℃. Spectra were recorded from 190 to 400 nm while the chromatogram was acquired at 280 nm.

Synthesis of SiO_2@APBA@PEG

The synthesis route of SiO_2@APBA@PEG particles is illustrated in Scheme 1. The particles were synthesized according to previous method with some modifications.[34] Typically, silica particles (0.2 g) were dispersed in 10 mL of dry ethanol in a one-neck round-bottom flask (100 mL) equipped with a condenser, followed by the addition of APBA (0.1 g), mPEG-NH_2 (0.025 g), GOPS (40 μL), TEA (20 μL). The reaction mixture was stirred for 24 hours at 90 ℃. The obtained product was separated by centrifugation, washed by ethanol and dried at 60 ℃ under vacuum for 8 h for further use. For comparison, SiO_2@APBA was also prepared under the identical conditions without the addition of mPEG-NH_2.



Scheme1. Synthetic strategy for the SiO_2@APBA@PEG particles

Binding studies

Effect of medium pH on the binding capacity was explored in the pH ranged from 7 to 9. The adsorption kinetics was investigated by changing the adsorption time from 0 to 30 min while adopting the same initial concentration of DA or L-DOPA at 0.05 mg/mL. Isothermal adsorptions were performed through changing the concentrations of DA or L-DOPA from 0.01 to 0.16 mg/mL while keeping the incubating time constant for 30 min. Specific adsorption tests were verified using a solution of quinol or phenol at a concentration of 0.05 mg/mL. Protein adsorptions were studied by using glycoprotein (OVA) and non-glycoproteins (Lyz, BSA, Try) with a known initial concentration (1 mg/ml). In all of the above experiments, the mass of SiO_2@APBA@PEG or SiO_2@APBA particles was 20 mg, the volume of the adsorption solution was 10 mL, the SiO_2@APBA@PEG or SiO_2@APBA particles were separated by centrifuging at 10000 rpm for 5 min, and the concentration of catecholamine or proteins in the supernatant was determined by UV-vis spectrometer at the wavelength of 280 nm. At least three replicates of each experiment were performed.

The adsorption capacities of the catecholamine and proteins were calculated according to the following equation (1) and (2):

$$Q = \frac{(C_0 - C_t)V \times 10^3}{WM} \qquad (1)$$

Where C_0 and C_t (mg/mL) are the initial catecholamine concentrations and the catecholamine concentrations after adsorption, V (mL) is the volume of catecholamine solution, W (g) is the weight of the as-prepared silica particles, and M is the molecular weight of catecholamine.

$$Q = \frac{(C_0 - C_t)V}{W} \qquad (2)$$

Where C_0 is the initial proteins concentration (mg/mL), C_t is the proteins concentration in the remaining supernatant, V (mL) is the volume of the initial solution, and W (g) is the weight of the SiO_2@APBA@PEG or SiO_2@APBA particles.

SPE procedure

100 mg of the SiO_2@APBA@PEG particles were packed into 1 mL SPE column. Frits were placed at the bottom and on the top of the column. 2 mL of PBS (pH = 8.5) was passed through the column in order to equilibrate it. Then 2 mL of catecholamine or protein solution was loaded into the column at a flow rate of 0.1 mL/min. After loading, column was washed with 1 mL of PBS (pH = 8.5) and a full of vacuum was applied through the column for 2 min, followed by 2 mL of 5% acetic acid elution. Finally, 20 µl of the catecholamine elute was injected into HPLC system for analysis after filtering through a 0.45 µm membrane filter. All experiments were carried out in triplicate.

Solid phase extraction of catecholamine from the rat serum

2 mL of rat serum spiked with 2 mL of DA and L-DOPA solution to provide working concentration of 0.01 mg/ml. Then the mixture was vortexed for 1 min. The SPE procedure applied to rat serum involved the same conditions mentioned above in the SPE procedure, except that the loading fraction, washing fraction and eluting fraction were measured by biuret reaction to guarantee the complete washing of the nonspecific adsorption of protein. For this, 1 mL sample solution was added into 4 mL biuret reagent ($CuSO_4$ $5H_2O$ 1.5 g/L, $NaKC_4H_4O_6$ $4H_2O$ 6g/L, NaOH 30 g/L). The mixture was shaken for 15 min, and kept to room temperature for 30 min. The absorbance was recorded at 540 nm.

Results and discussion

Characterizations of the materials

The as-prepared materials were characterized by FT-IR and static water angle experiments. Fig. 1 showed the FT-IR spectra of APBA (a), SiO_2@APBA@PEG (b), pure silica (c). In the FT-IR spectrum of SiO_2@APBA@PEG (Fig. 1b), the peaks at 1572, 1492, 1450 and 714 cm^{-1} contributed to benzene ring stretching vibrations and *m*-benzene ring distorting vibrations, and the band of B-O adsorption at 1360 cm^{-1} was observed, corresponding to the FT-IR spectrum of APBA (Fig. 1a), which demonstrated the successful functionlization of APBA onto the surface of silica. The typical bands of PEG were the stretching vibrations of C-H (2800-3000 cm^{-1}) and C-O-C (1100 cm^{-1}), which were easily influenced by other factors. So there was not sufficient evidence to indicate the PEGlation onto the surface of silica.

Compared with the static water contact angle of 52 ° for SiO_2@APBA, the angle fell down to 41 ° for the as-prepared SiO_2@APBA@PEG, which verified the successful grafting of PEG on the surface of the materials.

SEM images of pure SiO_2 and SiO_2@APBA@PEG were shown in Fig. 2. From the SEM images, the pure SiO_2 and the as-prepared particles had uniform, well-dispersed and spherical morphologies with the average diameters of approximately 10µm. There was no distinct change in shape and size after modification, which suggested that there was a very thin molecular layer coating on the silica particles.

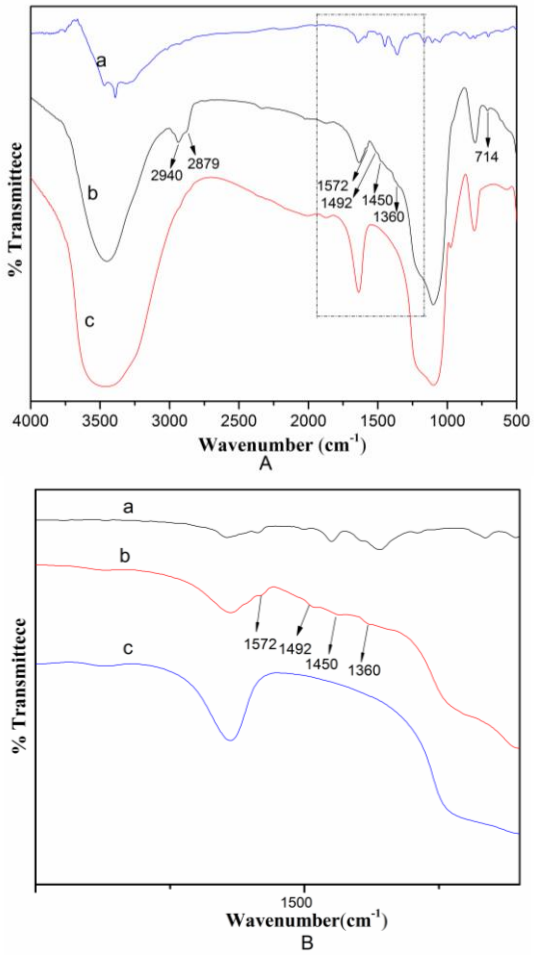


Fig. 1 (A) FT-IR spectra of APBA (a), SiO_2@APBA@PEG (b), pure silica (c). (B) Enlarged image of the marked region in (A).

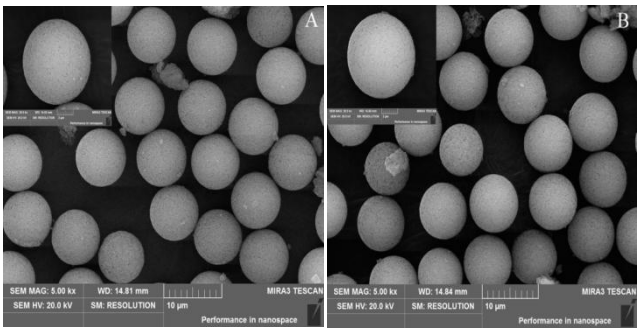


Fig. 2. SEM images of the pure silica (A), SiO2@APBA@PEG (B).

The selection of the mass ratio of APBA to mPEG-NH_2

The mass ratio of APBA to mPEG-NH_2 was a critical factor in the synthesis of SiO_2@APBA@PEG particles. The influence of different mass ratios of APBA to mPEG-NH_2 on the synthesis of SiO_2@APBA@PEG particles was investigated. The mass ratios of APBA to mPEG-NH_2 were ranged from 1:1 to 32:1. The results showed that the adsorption amounts of catecholamine had a fast increase, while the values for protein maintained constant with increasing the mass ratio of APBA to mPEG-NH_2 from 1:1 to 4:1. When the mass ratio reached more than 4:1, the adsorption amounts of catecholamine kept unchanged, while the resistance to protein was gradually weakened, this phenomenon might be explained that APBA and mPEG-NH_2 were competitively reacted with the epoxy groups on the surface of SiO_2. So the mass ratio of 4:1 was selected for the synthesis of SiO_2@APBA@PEG particles.

Effect of pH on the binding capacity

Effect of medium pH on the binding capacity was explored in the pH ranged from 7 to 9. As shown in Fig. 3, the binding amounts of DA and L-DOPA increased when increasing pH. In the pH range of 8.5-9, the binding capacity reached Maximum, which was related to the pK_a of the APBA (pK_a 8.7). However, the catecholamine was easily oxidized in the alkaline medium, so 8.5 was selected as the optimum pH.

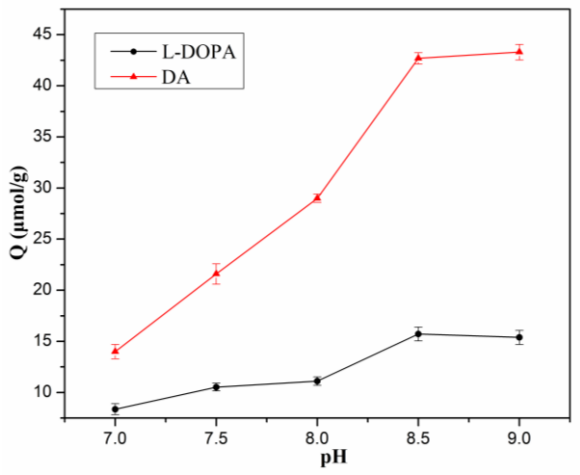


Fig. 3 Effect of pH on the binding capacity of DA and L-DOPA

Adsorption kinetics and isotherms

The adsorption kinetics of 0.05 mg/mL DA and L-DOPA solution onto SiO_2@APBA@PEG particles were introduced in Fig. 4 A. The binding amounts of DA and L-DOPA had a fast increase within 10 min, and then slowed down as the time increased. After 20 min, the adsorption almost reached equilibrium. The adsorption rate of catecholamine on the as-prepared materials was faster than that on other boronic acid-functionalized adsorbent.[16]

Isothermal adsorptions were carried out at different initial concentrations of DA and L-DOPA from 0.01 to 0.16 mg/mL. As can be seen from Fig. 4 B, the adsorption amount of DA and L-DOPA increased with the increase of the initial concentrations. The maximum equilibrium binding capacities were found as 112 μmol/g and 38 μmol/g for DA and D-DOPA respectively. The as-prepared SiO_2@APBA@PEG particles had much higher adsorption than other boronic acid-functionalized adsorbents reported in literature.[18] We can learned from the adsorption kinetics and isotherms results that the PEGylation on the surface of boronic acid-functionalized materials did not had adverse effects on the adsorption rate and capacity of the materials, while the biocompatibility of materials was improved at the same time.

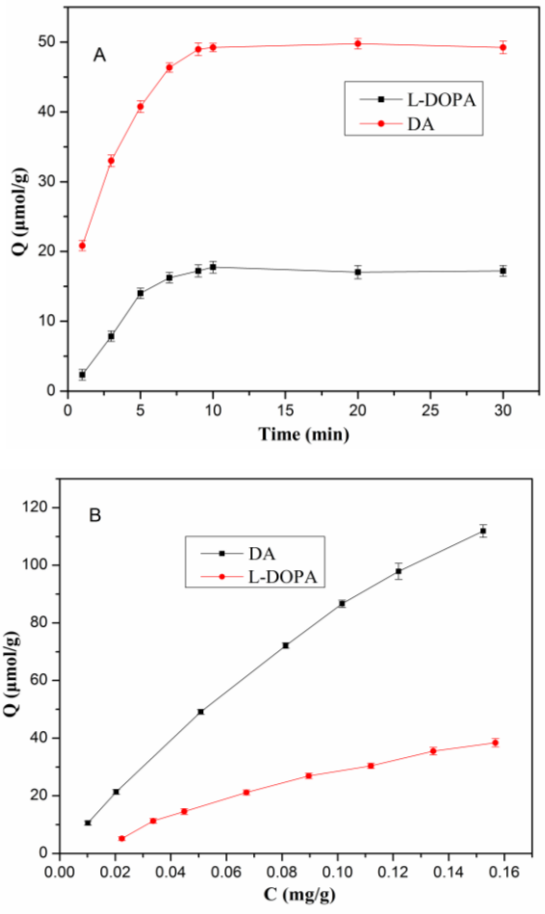


Fig. 4 Adsorption kinetics (A) and adsorption isotherms (B) of DA and L-DOPA on the SiO_2@APBA@PEG particles.

Langmuir adsorption equation was used to estimate the experimental data of adsorption isotherms. The equation is expressed as below:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{KQ_m} \qquad (3)$$

Where C_e (mg/mL) is the equilibrium concentration of DA or L-DOPA , Q_e (μmol/g) is the adsorption amount at equilibrium, Q_m (μmol/g) is the theoretical maximum adsorption capacity and K (g/mg) is the Langmuir constant. The Q_m and K can be calculated from the slope and intercept of the linear plot C_e/Q_e versus C_e. The results were given in Table. 1. The results in the table indicated that the Langmuir equation was well fitted for catecholamine adsorption within the concentration range

studied. It might be concluded that the adsorption of catecholamine on the as-prepared materials may be all monolayer adsorption, indicating catecholamine was adsorpted by covalent binding.

Table.1 Equations and parameters of Langmuir model

Analyte	Equation	K (g/mg)	Q_m(μmol/g)	R^2
DA	$C_e/Q_e = 0.00393C_e + 0.541$	7.27×10-3	254.45	0.996
L-DOPA	$C_e/Q_e = 0.00955C_e + 2.27$	4.21×10-3	104.71	0.971

Extraction specificity of SiO_2@APBA@PEG

0.05 mg/ml of DA , L-DOPA, quinol or phenol solution was used to text the specificity of the SiO_2@APBA@PEG (Fig. 4), It was seen from Fig. 5 the adsorption amounts of DA and L-DOPA were 49.3 and 15.2 μmol/g, respectively, much higher than quinol and phenol. Therefore, the as-prepared material exhibited well specificity to the catecholamine.

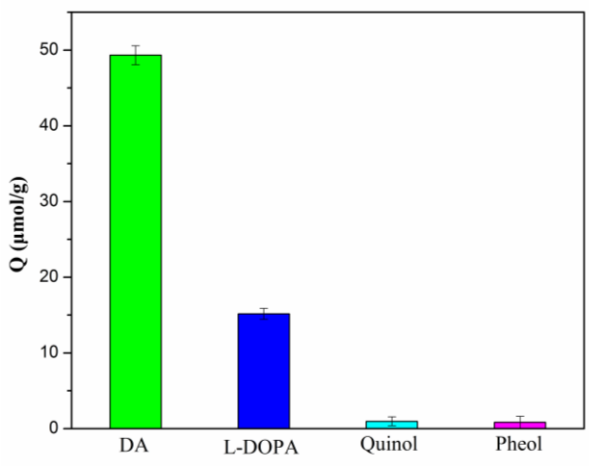


Fig. 5 Adorption of DA, L-DOPA, Quinol, Phenol on SiO_2@APBA@PEG particles.

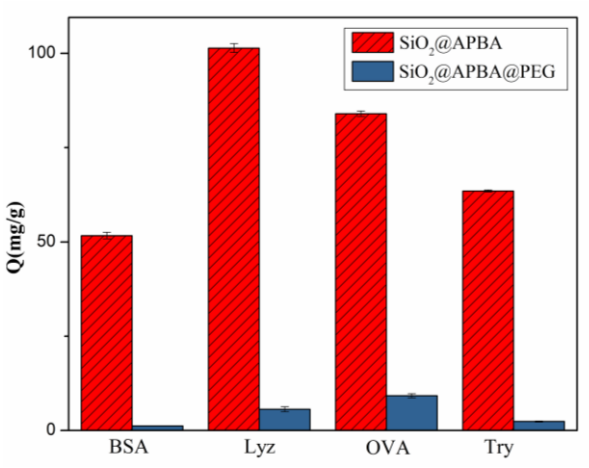


Fig. 6 Adsorption of four proteins (BSA, Lyz, OVA, Try) with concentrations of 1 mg/mL on SiO_2@APBA and SiO_2@APBA@PEG particles.

Effect of PEGylation to resist nonspecific adsorption of proteins

BSA, Lyz, OVA and Try were employed as model proteins for nonspecific adsorption, the concentration of proteins in the study was 1 mg/ml. The adsorption amounts of the BSA, Lyz, OVA and Try onto the SiO_2@APBA and SiO_2@APBA@PEG were shown in Fig. 6. It was clearly seen that the adsorption amounts of BSA, Lyz, OVA and Try onto the SiO_2@APBA were 51.7, 101.4, 83.9 and 63.5 mg/g, respectively. After PEGylation, they dropped to 1.2, 5.6, 9.2 and 2.4 mg/g, with the reduction of 97.7, 94.5, 89.0 and 96.3%. This observation demonstrated that PEGylation could dramatically reduce non-specific adsorption of proteins as well as the previous report.[35]

Performance of SiO2@APBA@PEG as SPE adsorbents

The efficacy of SiO_2@APBA@PEG as a selective sorbent in SPE of catecholamine was firstly explored by standard catecholamine solutions of 0.01 mg/ml. The percent recoveries of DA and L-DOPA were found to be 98% (RSD = 2.61%) and 99% (RSD = 3.02%).

Then BSA was employed as a model protein to evaluate the ability to resist protein adsorption of the SPE column. 2 mL of BSA solution (1 mg/mL) was loaded into the SPE column, and the UV-vis spectra of each fraction were shown in Fig. 7. It was very clear that most of the protein was flowed out in the loading procedure, after washing and eluting procedure, almost no protein was existed on the SPE column.

The reusability is one of the most important properties for the as-prepared adsorbents in practical applications. To evaluate the reusability of the as-prepared adsorbents, the adsorption-desorption cycles of catecholamine and protein on the same SiO_2@APBA@PEG particles were performed five times. The results showed that the recoveries for DA and L-DOPA decreased only about 5% and 3% at the end of the five reuses, while the ability to resist protein adsorption remained almost unchanged. The results indicated the as-prepared adsorbents had good reusability.

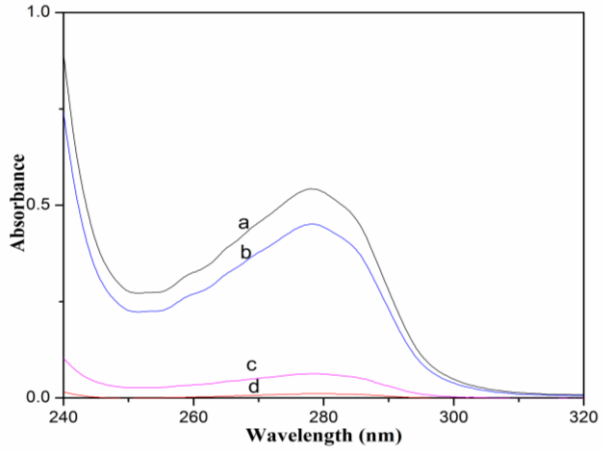


Fig. 7 UV-vis spectra of BSA in the (a) initial solution, (b) loading fraction , (c) washing fraction, (d) eluting fraction

Finally, the SiO_2@APBA@PEG sorbents were applied to extraction of catecholamine from rat serum. The catecholamine solutions spiked in rat serum were loaded into the SPE column without any pretreatment. 1mL of PBS (pH = 8.5) was followed to wash the nonspecific adsorption of protein. The absorbances

at 540 nm of the loading fraction, washing fraction and eluting fraction after biuret reaction were shown in the Fig. 8. As we can seen, there were hardly any proteins retaining onto the SPE column after SPE procedure. Fig. 9 showed the chromatograms of catecholamine in spiked rat serum after SPE protocol (A) and the standard catecholamine solution with the same concentration (0.01 mg/mL) (B), the recoveries of DA and L-DOPA in the serum sample were 95 ± 3.21% and 93 ± 3.57%. The results indicated that the novel SiO_2@APBA@PEG sorbents could successfully eliminate the contaminants in the rat serum, which avoided the fouling of the SPE column and HPLC column.

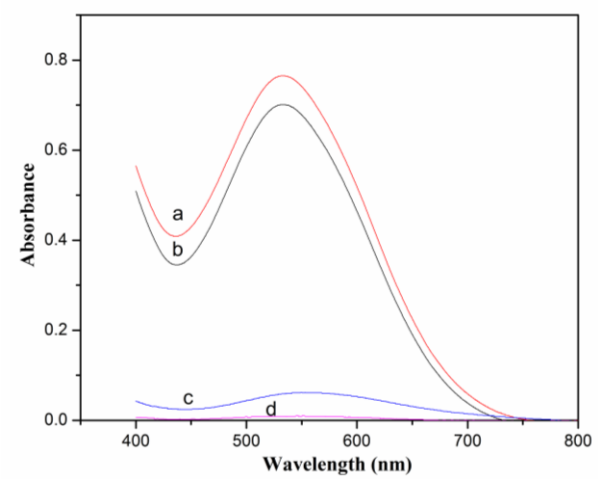


Fig. 8 UV-vis spectra of rat serum after biuret reaction (a) initial rat serum, (b) loading fraction (c) washing fraction, (d) eluting fraction

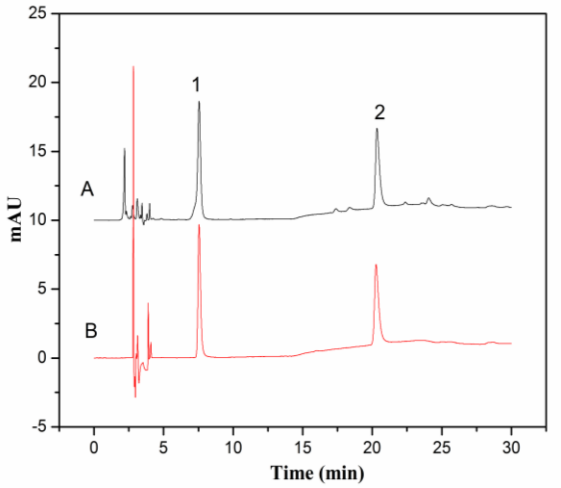


Fig. 9 HPLC chromatograms of catecholamines in rat serum after SPE procedures (A) in standard solution (B), Peak designation: 1, L-DOPA; 2, DA

Conclusions

A novel PEGylated boronate sorbent was prepared in one-pot method in this paper for the first time. The as-prepared material had the high affinity towards catecholamine and low nonspecific adsorption of protein. Based on the material, we developed a rapid, simple, selective and reproducible preparation method for extraction of catecholamine in rat serum, with the recoveries of DA and L-DOPA were 95 ± 3.21% and 93 ± 3.57%. The results showed that the as-prepared material was promising to be applied for extraction of catecholamine in complex biological samples.

Acknowledgement

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Notes and references

1 J. Bicker, A. Fortuna, G. Alves and A. Falcao, *Analytica chimica acta*, 2013, **768**, 12-34.

2 C. Muzzi, E. Bertocci, L. Terzuoli, B. Porcelli, I. Ciar, R. Pagani and R. Guerranti, *Biomedicine & Pharmacotherapy*, 2008, **62**, 253-258.

3 F. Bugamelli, C. Marcheselli, E. Barba and M. A. Raggi, *Journal of pharmaceutical and biomedical analysis*, 2011, **54**, 562-567.

4 L. Silva, K. Duarte, A. C. Freitas, T. S. L. Panteleitchouk, T. A. P. Rocha-Santos, M. E. Pereira and A. C. Duarte, *Analytical Methods*, 2012, **4**, 2300.

5 C. L. Weaver, H. Li, X. Luo and X. T. Cui*, *J. Mater. Chem. B*, 2014, **2**, 5209-5219.

6 X.-S. Li, G.-T. Zhu, Y.-B. Luo, B.-F. Yuan and Y.-Q. Feng, *TrAC Trends in Analytical Chemistry*, 2013, **45**, 233-247.

7 H. He, E. Carballo-Jane, X. Tong and L. H. Cohen, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 2015, **997**, 154-161.

8 S. Pedersen-Bjergaard and K. E. Rasmussen, *TrAC Trends in Analytical Chemistry*, 2008, **27**, 934-941.

9 C. Ji, J. Walton, Y. Su and M. Tella, *Analytica chimica acta*, 2010, **670**, 84-91.

10 A. L. Dawidowicz, E. Fornal and A. Fijalkowska, *Chromatographia* 1998, **47**, 524-528.

11 C. W. Huck and G. K. Bonn, *Journal of Chromatography A*, 2000, **885**, 51-72.

12 B. H. C. Westerlnk, F. J. Bosker and J. F. O'Hanlon, *CLIN. CHEM.*, 1982, **28**, 1945-1948.

13 K. N. Frayn and P. F. Maycock, *CLIN. CHEM.* , 1983, **28**, 1426-1428.

14 A. M. Hansen, J. Kristiansen, J. L. Nielsen, K. Byrialsen and J. M. Christensen, *Talanta*, 1999, **50**.

15 P. Maycock and K. N. Frayn, *CLIN. CHEM.* , 1987, **33**, 286-287.

16 N. Özdemir, A. Çakır and B. Somtürk, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2014, **445**, 40-47.

17 C. Cakal, J. P. Ferrance, J. P. Landers and P. Caglar, *Analytica chimica acta*, 2011, **690**, 94-100.

18 J. Du, M. He, X. Wang, H. Fan and Y. Wei, *Biomedical chromatography : BMC*, 2015, **29**, 312-320.

19 S. Zhang, X. He, L. Chen and Y. Zhang, *New J. Chem*, 2014, **38**, 4212-4218.

20 F. Yang, Z. Lin, X. He, L. Chen and Y. Zhang, *Journal of chromatography. A*, 2011, **1218**, 9194-9201.

21 X. Zhang, X. He, L. Chen and Y. Zhang, *Journal of Materials Chemistry*, 2012, **22**, 16520.

22 X. Zhang, X. He, L. Chen and Y. Zhang, *Journal of Materials Chemistry B*, 2014, **2**, 3254.

23 X.-F. Xiao, X.-Q. Jiang and L.-J. Zhou, *Chinese Journal of Analytical Chemistry*, 2013, **41**, 445-453.

24 J.-C. Yang, C. Zhao, I. F. Hsieh, S. Subramanian, L. Liu, G. Cheng, L. Li, S. Z. D. Cheng and J. Zheng, *Polymer International*, 2012, **61**, 616-621.

25 G. Zhou, C. Ma and G. Zhang, *Polymer Chemistry*, 2011, **2**, 1409.

26 T. Riedel, Z. Riedelova-Reicheltova, P. Majek, C. Rodriguez-Emmenegger, M. Houska, J. E. Dyr and E. Brynda, *Langmuir : the ACS journal of surfaces and colloids*, 2013, **29**, 3388-3397.

27 H. Hlidkova, D. Horak, V. Proks, Z. Kucerova, M. Pekarek and J. Kucka, *Macromolecular bioscience*, 2013, **13**, 503-511.

28 L. D. Unsworth, H. Sheardown and J. L. Brash, *Langmuir : the ACS journal of surfaces and colloids*, 2005, **21**, 1036-1041.

29 H. Bi, S. Meng, Y. Li, K. Guo, Y. Chen, J. Kong, P. Yang, W. Zhong and B. Liu, *Lab on a chip*, 2006, **6**, 769-775.

30 M. Zhao, X. Chen, H. Zhang, H. Yan and H. Zhang, *Biomacromolecules*, 2014, **15**, 1663-1675.

31 H. Ma, D. Li and X. Sheng, *Langmuir : the ACS journal of surfaces and colloids*, 2006, **22**, 3751-3756.

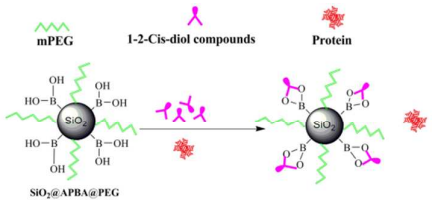
32 S. J. Sofia, V. Premnath and E. W. Merrill, *Macromolecules* 1998, **31**, 5059-5070.

33 R. Murthy, C. E. Shell and M. A. Grunlan, *Biomaterials*, 2009, **30**, 2433-2439.

34 Y. Wang, C. Zhou, L. Sun, B. Yu, M. Cao and S. Zhong, *Applied Surface Science*, 2015, **351**, 353-357.

35 S. Qin, L. Wang, X. Zhang and G. Su, *Applied Surface Science*, 2010, **257**, 731-735.

Graphical Abstract



A novel boronate adsorbent with the ability to resist protein adsorption was synthesized by one step method