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ORIGINAL PAPER

Boronic acid-modified polyhedral oligomeric silsesquioxanes on polydopamine-coated magnetized graphene oxide for selective and high-capacity extraction of the catecholamines epinephrine, dopamine and isoprenaline

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Abstract

Amino-functionalized polyhedral oligomeric silsesquioxanes (POSS-$8NH_2$) were covalently bound to the surface of polydopamine-coated magnetized graphene oxide. It was then reacted with 4-formylphenylboronic acid to prepare a "cubic boronic acid"-bonded magnetic graphene oxide adsorbent. The new adsorbent exhibits better selectivity and much higher adsorption capacity for ortho-phenols over adsorbents where small boronic ligands are directly bound to the surface of the material. It is shown to enable selective and faster enrichment of the catecholamines epinephrine (EP), dopamine (DA) and isoprenaline (IP) with high selectivity over many potential interferents that can occur in urine. The analytes were then quantified by HPLC with fluorometric detection. Under optimal conditions, response is linear ($R^2 \geq 0.9907$), limits of detection are low (0.54–2.3 ng·mL^{-1}), and reproducibility is acceptable (inter- and intra-day assay RSDs of≤10.9%). The method was successfully applied to the determination of endogenous EP and DA and exogenous IP in urine samples.

Keywords Boronate affinity adsorbent · Magnetic assisted miniaturized dispersive solid-phase extraction · Ortho-phenols · Receptor agonists · Neurotransmitters · Adenosine · Urine sample · HPLC with fluorometric detection

Introduction

A large number of *cis*-diol-containing compounds (*cis*-diols) and ortho-phenols play significant physiological functions and pharmacological characteristics in the body, such as glycoproteins, carbohydrates, catecholamines and nucleosides. As such, the separation and analysis of *cis*-diols and ortho-phenols are of great significance [1]. At present, boronate affinity chromatography is viewed as one of the effective technologies to separate and enrich *cis*-diols and ortho-phenols. In this technology, boronic acids on the surface of adsorbent can form five- or six-member cyclic esters with *cis*-diol groups in alkaline solution and these cyclic esters dissociate at acidic pH [1]. Based on this principle, boronate affinity materials (BAMs) can specifically adsorb *cis*-diols and ortho-phenols from the complex biological samples. Besides, boronate affinity technique is easy-to-manipulate capture/release, low cost and good compatibility with mass spectrometry. For these reasons, the boronate affinity technique has drawn increasing attention in the separation and enrichment of *cis*-diols and ortho-phenols.

As a key in boronate affinity technology, BAMs should possess the two features of good adsorption selectivity and high adsorption capacity. Adsorption selectivity mainly comes from the structure of boronate, whereas adsorption capacity depends on the binding amount of boronate [2]. So far, the

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prevailing method for preparation of BAMs is to activate the matrix with silane coupling agents (act as spacers) and then to immobilize various boronic ligands on various matrix [3]. By this method, the BAMs usually have low adsorption capacity. To increase the binding amount of boronate, the new matrix with high specific surface area tends to be used, such as porous organic and inorganic materials [2], nanoparticles [4], magnetized graphene [5] and metal-organic frameworks [6]. Besides the high specific surface area, magnetized graphene can endow the BAMs with the advantage of magnetic separation. Anyway, the adsorption capacities of this type of BAMs are usually in the range from 0.46 to 97 $\mu mol \cdot g^{-1}$ towards adenosine [7–11] and remain to be improved.

Catecholamines epinephrine (EP), dopamine (DA) and isoprenaline (IP) are three receptor agonists acting on adrenergic receptors. The former two belong to endogenous neurotransmitters released from neurons, and their levels are related with human health, and thus can serve as biomarkers for the diagnosis, therapy and prognosis of several neurological and cardiovascular disorders [12]. Whereas IP is often employed to treat bronchial asthma, cardiac arrest and atrioventricular block [13]. For these reasons, the measurement of EP, DA and IP in biological samples is of great importance. However, they are usually present in very low abundance in biological systems and co-exist with high-abundance interferents, so the effective sample pretreatment procedure is necessary before the instrument analysis. As one of the most popular sample preparation methods, solid-phase extraction has been widely used. Alumina [14], cation exchanger [15], BAMs [16], Cu(II)-immobilized [17] and reversed-phase [18] adsorbent are the present types of solid-phase extraction materials. Among various adsorbents, BAMs exhibit special selectivity towards ortho-phenols IP, EP and DA. However, the reported BAMs had either lower adsorption capacity [7–11], or showed slow mass transfer for the analytes (30~90 min) [16, 19], limiting the extraction performance.

This work aims to use amino-functionalized polyhedral oligomeric silsesquioxanes (POSS-8NH_2) as a spacer to prepare a selective and high-capacity boronate affinity magnetized graphene oxide (magGO@POSS-BA). POSS is a family of inorganic-organic hybrid molecules which consist of a cage-like inorganic core and eight vertex groups. Each POSS molecular can provide eight reaction sites, and thus POSS reagents are ideal building blocks for functional composites. So far, various POSS reagents are usually employed as the cross-linker/monomers to prepare varieties of porous inorganic-organic hybrid polymers [16, 20, 21]. In contrary, POSS-8NH_2 was employed as an amplifier. Finally, the magGO@POSS-BA was employed to extract IP, EP and DA from human urine to show the extraction efficiency of the high-capacity adsorbent.

Experimental

Materials and chemicals

Amino-functionalized polyhedral oligomeric silsesquioxanes (POSS-8NH_2; 96%) was obtained from American Hybrid Plastics Ltd., USA (http://www.hybridplastics.com). Dopamine (DA, 98%), 4-formylphenylboronic acid (FPBA, 97%), catechol(99.0%), 5-hydroxytryptamine and adenosine (99%) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China, http://www.aladdin-e.com). Quinol (99.0%), protocatechualdehyde (98%) and p-hydroxybenzaldehyde (98%) were from Sinopharm Chemical Regent Co. Ltd. (Shanghai, China, http://www.sinopharmholding.com). Ethyl theophylline and dihydroxypropyltheophylline (98%) were from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, https://www.cnbg.com.cn). Epinephrine hydrochloride (EP, 98%) and isoprenaline hydrochloride (IP, 98%) were purchased from Xiya Reagent Ltd. (Linyi, China, http://www.xiyashiji.com). Acetic acid (HAc, 99.0%), $NaBH_3CN$ (98%) and HPLC grade methanol were supplied by Yongda Chemical Reagent Co. Ltd. (Tianjin, China, http://www.tjydhxsj.com). All other reagents were of analytical grade.

Instrumentation

The morphology and size of materials were observed by transmission electron microscopy (TEM, H-600, Hitachi, Japan, http://www.hitachi.com) and scanning electron microscope (SEM, SV8010, Hitachi, Japan, http://www.hitachi.com). The chemical composition was analyzed by Fourier-transform infrared spectrometer (FT-IR, TENSOR27, Bruker, Germany, https://www.labx.com) and X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Fisher Scientific, USA, https://www.thermofisher.com). The magnetic property was determined by a vibrating sample magnetometer (VSM, MPMS-XL-7, Quantum Design, USA, https://www.qdusa.com).

The chromatographic separation was performed on a Shimadzu HPLC system consisting of two LC-20AT pumps, a SPD-20A UV-Vis and a RF-10A fluorescence detector (Kyoto, Japan, https://www.shimadzu.com). The column (250 mm × 4.6 mm I.D., TSK-GEL ODS-100 V, 5 μm) was obtained from Tosoh Co. (Kyoto, Japan, https://www.tosoh.com). The column was maintained at 25 °C and the flow-rate of mobile phase was 1.0 $mL \cdot min^{-1}$. The injection volume was 20 μL.

Preparation of boronic acid-modified polyhedral oligomeric silsesquioxanes on polydopamine-coated magnetized graphene oxide (magGO@POSS-BA)

The preparation procedure of magGO@POSS-BA is shown in Fig. 1. Of the four steps, graphene oxide (GO), magnetized GO (magGO) and polydopamine-

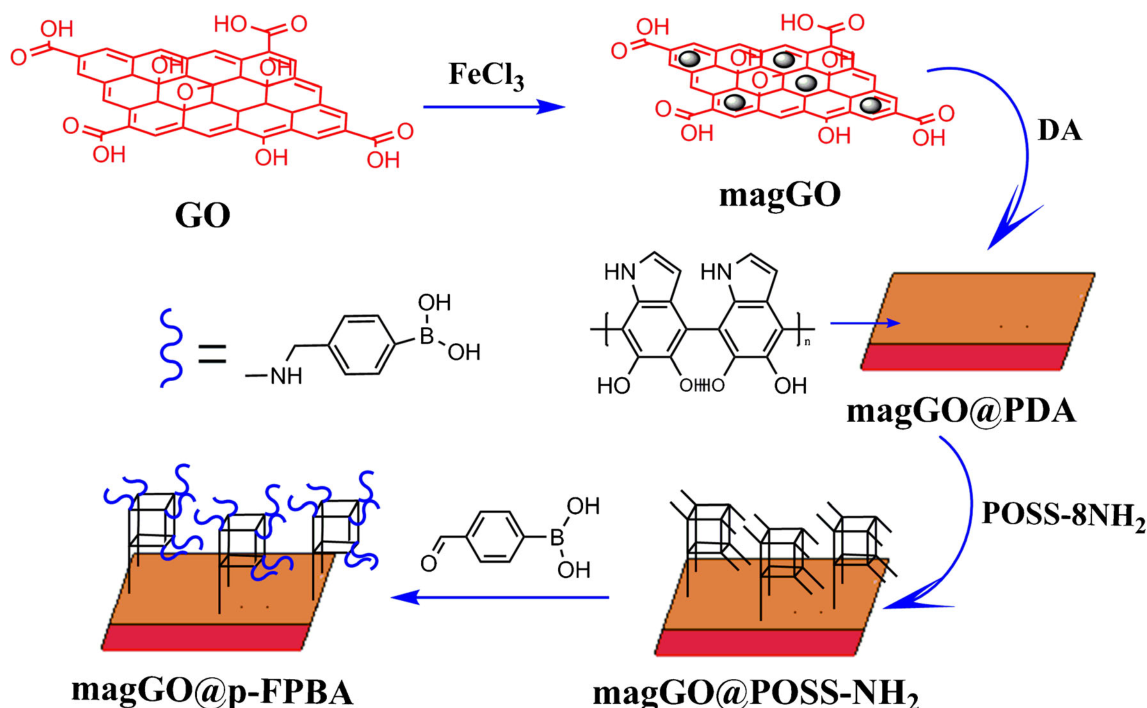

Fig. 1 Schematic for the preparation of the magGO@POSS-BA

coated magGO (magGO@PDA) were prepared according to the reference [22].

0.2 g magGO@PDA and 0.4 g POSS-8NH$_2$ were dispersed in 30.0 mL distilled water and the mixture was adjusted to pH 8.5 with 1.0 mol·L^{-1} NaOH aqueous solution. Then, the mixture was mechanically stirred for 24 h at room temperature. The magGO@POSS-NH$_2$ was separated, washed with distilled water and ethanol under the aid of a magnet and finally dried in a vacuum.

0.2 g magGO@POSS-NH$_2$, 0.4 g FPBA and 0.5 g NaBH$_3$CN were dispersed in 30 mL of methanol under ultrasonication. After the mixture was stirred at room temperature for 24 h, the magGO@POSS-BA was separated via magnetic separation operation. It was washed with methanol, 5% NaHCO$_3$ and distilled water, respectively, and finally was dried in a vacuum. The magGO@POSS-BA was prepared three times in parallel to investigate the batch-reproducibility.

Preparation of the control magGO@BA

The adsorbent without amino-functionalized polyhedral oligomeric silsesquioxane as a spacer, namely magGO@BA, was prepared as a control. Briefly, 0.2 g magGO@PDA, 0.4 g FPBA and 0.5 g NaBH$_3$CN were dispersed in 30 mL of methanol under ultrasonication. After the mixture was stirred at room temperature for 24 h, the magGO@BA was separated and washed with methanol, 5% NaHCO$_3$ and distilled water, respectively, and finally was dried in a vacuum.

Adsorption selectivity of the adsorbent

Three pairs and two groups of compounds were chosen to assess the selectivity of the adsorbent. Three pairs were quinol and catechol; DA and 5-hydroxytryptamine; protocatechualdehyde and p-hydroxybenzaldehyde, in which two compounds had similar molecular structure. The first group consisted of quinol, resorcinol, catechol and phenol, and the second was of DA, IP, 5-hydroxytryptamine, ethyl theophylline and dihydroxypropyltheophylline. Each pair and group of compounds were dissolved into 20 mM NH$_3$-NH$_4$Cl buffer (pH 8.5) to make 10.0 μg·mL^{-1} of each compound. Five solutions were subjected to magnet-assisted miniaturized dispersive solid-phase extraction (M-d-μSPE) procedure. Briefly, 10.0 mg of the adsorbent was dispersed in 3.0 mL of each solution. After the solution was shaken at 25 °C for 1.0 min, the adsorbent was separated and washed twice with 1 mL of NH$_3$-NH$_4$Cl buffer under the aid of a magnet. Next, the adsorbent was immediately immersed in 1.0 mL of 5% HAc to release the analytes. Then, the supernatant was collected under a magnetic field, and filtered with 0.45 μm membrane before HPLC analysis.

For enrichment factors (EFs) of compounds, 10.0 mg of the adsorbent and 5 mL of 0.3 μmol·mL^{-1} catechol, IP and adenosine were respectively subjected to the same M-d-μSPEM procedure as the above. EFs are calculated according to the following equation:

$$EF = \frac{C_i}{C_0} \quad (1)$$

Where C_0 and C_i are the concentration of the analyte in the initial solution and final supernatant, respectively.

Adsorption capacity of the adsorbent

The adsorption capacity of the adsorbent was tested by catechol, IP and adenosine. Briefly, 10.0 mg of the adsorbent was dispersed in a series of 5.0 mL standard solutions of each analyte at different concentrations in 20 mM NH_3-NH_4Cl buffer. After the adsorption equilibrium was reached, the supernatant was collected under a magnetic field. The supernatant was analyzed by HPLC-UV, and the adsorption capacity (Q_e, μmol·g^{-1}) is calculated according to the following equation:

$$Q_e = \frac{(C_0 - C_e) \times V}{M \times m} \times 1000 \qquad (2)$$

Where C_0 (μg·mL^{-1}) and C_e (μg·mL^{-1}) are the concentrations of analyte in the initial and supernatant, respectively. V (mL) is the volume of the initial solution, m (mg) is the amount of the adsorbent, and M (g·mol^{-1}) is the molecular weight of the analyte. Adsorption isotherms were obtained by plotting of Q_e against C_e.

Extraction of catecholamines from urine

The human urine samples from healthy volunteers in our group and patients from Shaanxi University of Chinese Medicine (Xi'an, China) were collected under the guidelines of the Ethics Committee of the Institute. The urine samples were stored at −20 °C before further treatment. To obtain the blank urine, the urine from a healthy volunteer was kept at 37°Cfor 48 h to degrade the endogenous catecholamines oxidatively below detectable levels. 4.9 mL of blank urine was spiked with 0.1 mL of a series of standard solutions of DA, IP and EP at different concentrations in 20 mM NH_3-NH_4Cl buffer to make the concentration range from 10 to 500 ng·mL^{-1}. 0.1 mL of acetonitrile was added to the spiked solution; the solution was shaken vigorously on a rotary mixer and centrifugated at 12,863 g for 10 min to complete the deproteinization process. The supernatant was separated and adjusted to pH 8.5 with ammonium hydroxide. Then, 10.0 mg of magGO@POSS-BA was dispersed in the solution. The mixture was shaken for 1 min; the adsorbent was separated and washed twice with 1 mL of NH_3-NH_4Cl buffer (pH 8.5) with the aid of a magnet. Next, the adsorbent was immediately immersed in 1.0 mL of 5% HAc for 1 min. The supernatant was collected under a magnetic field and was dried under a nitrogen stream. The residue was redissolved in 0.2 mL of mobile phase and filtered with 0.45 μm membrane before HPLC with a fluorescence detector (FLD). The FLD excitation and emission wavelength were set at 280 nm and 330 nm, respectively. The calibration plots were plotted by the peak areas (y) versus the concentrations of analytes (x).

The limits of detection (LODs) and limits of quantification (LOQs) of the method are calculated using Eqs. 3 and 4, respectively:

$$\mathrm{LOD} = 3\sigma/\mathrm{S} \qquad (3)$$

$$\mathrm{LOQ} = 10\sigma/\mathrm{S} \qquad (4)$$

Where σ is the standard deviation of the blank and S is the slope of calibration plot.

The extractions of three spiked urine samples with different concentration levels (20, 100 and 500 ng·mL^{-1}) were performed under optimal conditions. The recoveries of the analytes are calculated according to the following equation:

$$\mathrm{Recovery} = \frac{C_i \times V_i}{C_0 \times C_0} \times 100\% \qquad (5)$$

where C_i (ng·mL^{-1}) and V_i (mL) are the concentration and volume of the treated solution, respectively; C_0 (ng·mL^{-1}) is the spiked concentration, and V_0 (mL) is the added volume.

Results and discussion

Preparation and characterization of the adsorbent

Figure 1 shows the preparation steps and the structure of magGO@POSS-BA. GO, magGO and magGO@PDA can be easily prepared according to the reference [22]. Because the coated poly(dopamine) can easily react with amines [23], POSS-8NH_2 is bonded onto the magGO@PDA to produce magGO@POSS-NH_2. There are two kinds of -NH_2, one coming from POSS-8NH_2 and the other from poly(dopamine). The -NH_2 groups from the octahedral POSS-8NH_2 can react with FPBA to form a "cubic boronic acid" around POSS core. Therefore, POSS amplifies boronic acids on the surface of the adsorbent. Since POSS-8NH_2 is of nanoparticles, the introduction of POSS also roughens the surface of the adsorbent, alleviating the steric hindrance as the target approaches the boronate sites. As for the -NH_2 groups from poly(dopamine), they can also react with FPBA. To distinguish the contribution from the two kinds of -NH_2, a control magGO@BA was prepared by reacting magGO@PDA with FPBA.

In the Electronic Supplementary Material (ESM), Figs. S1 and S2 show the SEM and TEM of the adsorbents, respectively. It can be seen that GO has a layered structure with wrinkles and folds (Fig. S1a and S2a), and Fe_3O_4 nanoparticles with average diameters of approximately 250 nm are well dispersed on a GO sheet (Fig. S1b and S2b). After being successively modified with PDA, POSS-8NH_2 and FPBA, Fe_3O_4 nanoparticles on the magGO@POSS-BA show a distinct core-shell structure with a shell thickness of about 20 ± 3 nm (Fig. S2c).

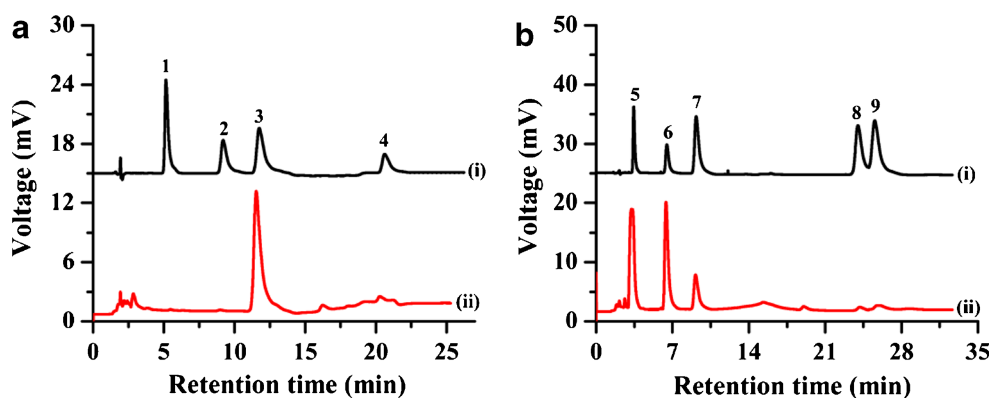

Fig. 2 Chromatograms of the untreated solution (i) and the eluate treated with magGO@POSS-BA (ii). Condition: a, 50 mM HAc-methanol (75:25); b, Mobile phase is 10% methanol+90% 10 mM KH_2PO_4 (pH 3.0) at the first 10 min, and then was changed to 25% methanol in 5 min. UV wavelength: 280 nm. Peaks: 1, quinol; 2, resorcinol; 3, catechol; 4, phenol; 5, DA; 6, IP; 7, 5-hydroxytryptamine; 8, ethyl theophylline; 9, dihydroxypropyltheophylline

The magnetic hysteresis loops of materials indicate that the saturation magnetization values of magGO and magGO@POSS-BA are 32.7 emu·g^{-1} and 26.3 emu·g^{-1}, respectively (Fig. S3). The saturation magnetization of magGO@POSS-BA is reduced to 6.4 emu·g^{-1}. However, the nanocomposites can still be separated from the mixture within 1 min with the aid of a magnet.

The chemical composition of materials was characterized by FT-IR and XPS (Fig. S4), and the results are discussed in ESM. From the B content of materials measured by XPS, the binding content of boronate on magGO@POSS-BA is calculated to be 5.6 mmol·g^{-1}, being 2.5 times as much as the control magGO@BA (Table S1). All results confirm the successful preparation of magGO@POSS-BA.

Selective adsorption of ortho-phenols under a competitive environment

The adsorption selectivity of magGO@POSS-BA and magGO@BA are first evaluated with three pairs of compounds. The ortho-phenols are catechol, DA and protocatechualdehyde, and their corresponding analogs are quinol, 5-hydroxytryptamine and p-hydroxybenzaldehyde, respectively. Fig. S5 shows both adsorbents can extract the ortho-phenols efficiently while minimizing the adsorption of their analogs. Considering that the ortho-phenols often coexist with many interferents in real samples, magGO@POSS-BA was tried to capture ortho-phenols from a multicomponent mixed solution to evaluate the selectivity of the adsorbent under competitive adsorption environment. Figure 2 indicates that magGO@POSS-BA can also enrich the ortho-phenols while removing other phenols almost completely, showing a good selectivity towards ortho-phenols in a complex solution.

Effect of polyhedral oligomeric silsesquioxanes (POSS) on enhancing the adsorption capacity

The adsorption capacity is another crucial factor for evaluating an adsorbent. The adsorption capacities of BAMs are often determined via the adsorption isotherm method. In the structure of magGO@POSS-BA, POSS acts as the core of a "cubic boronic acid". To highlight the effect of POSS on enhancing the adsorption capacity of the adsorbent, magGO@BA is employed as a control. Regarding that the adsorption capacities of BAMs are often characterized by catechol and adenosine, the two compounds as well as IP were selected as model analytes. From the adsorption isotherms (Fig. S6), the saturation adsorption capacities of magGO@POSS-BA are 197.3 μmol·g^{-1} for catechol, 137.0 μmol·g^{-1} for adenosine and 212.5 μmol·g^{-1} for IP, respectively, which are remarkably higher than those of magGO@BA (70.9 μmol·g^{-1} for catechol; 48.6 μmol·g^{-1} for adenosine; 74.7 μmol·g^{-1} for IP). Such high capacities are contributed to

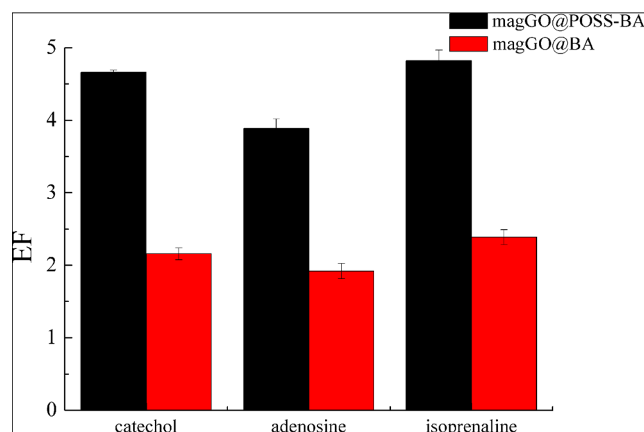

Fig. 3 EF of catechol, adenosine and isoprenaline on magGO@POSS-BA and magGO@BA

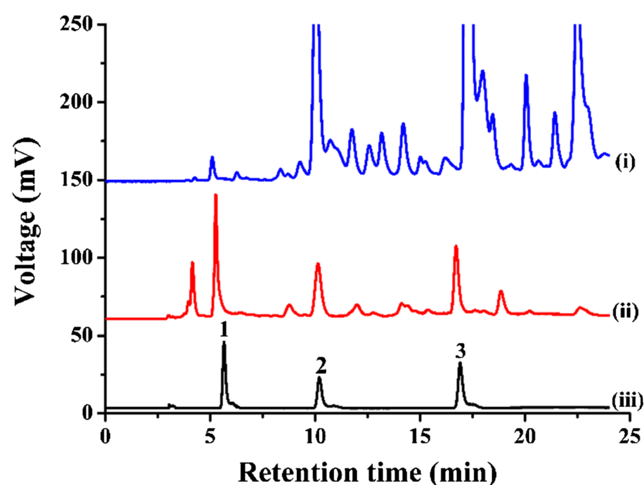

Fig. 4 Chromatograms of (i) urine sample spiked with 100 ng·mL^{-1}, (ii) eluate of the spiked urine sample after extraction by magGO@POSS-BA and (iii) standard solution of EP, DA and IP. Mobile phase: methanol (solvent A) and 10 mM NaH_2PO_4 (solvent B), gradient: 2% A in 0–5 min, then 2% A-15% A within 10 min. The FLD excitation and emission wavelength were set at 280 nm and 330 nm, respectively. Peaks: 1. EP; 2. DA; 3. IP

the grafting of POSS-8NH_2, which greatly amplifies boronic acids on the surface of magGO@POSS-BA. As a result, the enrichment ability towards the analytes is improved. Figure 3 shows that magGO@POSS-BA can give much higher enrichment factors (EFs) towards catechol, adenosine and IP than the control magGO@BA. In Fig. 3, the used amounts of both adsorbents are the same, whereas the amounts of analytes in solution slightly exceed the amounts that magGO@POSS-BA can adsorb according to its maximum adsorption capacity. In this case, high-capacity magGO@POSS-BA can adsorb much more targets than low-capacity magGO@BA, achieving higher EF. The result suggests that it had better use the high-capacity magGO@POSS-BA in real sample because it can extract the targets more completely than the same amount of low-capacity adsorbent.

Comparison of adsorption capacity with other adsorbents

To date, there are many BAMs for the enrichment of *cis*-diols and ortho-phenols. According to the surface structure, the preparation methods of BAMs are generally categorized into two categories. The first kind is to bind small boronic ligands directly on various matrix, i.e. Fe_3O_4@SiO_2, magGO, attapulgite, titania [7–11]. This method is popularly used, and this type of BAMs generally give the adsorption capacity in the range from 0.46 to 97.2 μmol·g^{-1} towards adenosine [7–11] and from 50 to 96 μmol·g^{-1} towards catechol [24, 25], respectively. In comparison with the type of BAMs, magGO@POSS-BA shows the highest adsorption capacity. The second kind is to modify boronic-functionalized polymer on the surface of the matrix. The second type of BAMs usually contain more binding sites due to the use of polymer [2, 5, 26–29], and thus some of them show higher adsorption capacities than magGO@POSS-BA [2, 5, 26]. For instance, polymer brush-modified silica [2] and magGO [5] via ATRP had adsorption capacities of 513.6 and 1111 μmol·g^{-1} to catechol, respectively; polymer-modified monolith via two-step ATRP had adsorption capacity up to 303 μmol·g^{-1} towards catechol [26]. However, the three polymer-modified BAMs were rather difficult to make as ATRP reaction needs an anaerobic operation in the preparation procedure, and polymer-modified BAMs usually provide low mass-transfer [2, 16]. For polymer brush-modified silica, it took 60 min to reach the adsorption equilibrium [2]. Feng' group also prepared a BAM via co-polymerization of octavinyl POSS and 3-acrylamidophenylboronic acid on the surface of Fe_3O_4 nanoparticles [16]. In their work, octavinyl POSS acted as the cross-linker, and the enrichment of analytes needed extraction time of 60 min and desorption time of 30 min. However, our adsorbent with POSS-8NH_2 as amplification scaffold is structurally of the first type and has fast mass-transfer, so it can finish the extraction process within 1.0 min, being more faster over the polymer-modified adsorbents. Overall, magGO@POSS-BA shows lower adsorption capacity than some of the second type of BAMs, but it is easily made and can extract the analytes rather fast. Based on the boronic affinity principle, BAMs can specifically adsorb ortho-phenols and *cis*-diols, i.e., catecholamines and *c*is-diol-containing biomolecules, glycoproteins, saccharides and nucleosides [1]. Predictably, magGO@POSS-BA can also extract these substances quickly. In the following, magGO@POSS-BA is employed to extract catecholamines from the urine samples to show its potential in the pretreatment of complex biological sample.

Table 1 Urinary excretion of analytes from volunteers

*Sample No.	Age	Sex	EP (ng·mL^{-1})	DA (ng·mL^{-1})	IP (ng·mL^{-1})
1	24	male	5.1 ± 0.4	32.2 ± 2.2	ND
2	23	female	6.4 ± 0.6	24.7 ± 2.5	ND
3	22	female	6.0 ± 0.3	23.8 ± 1.8	ND
4	51	male	5.5 ± 0.4	50.3 ± 4.2	4.3 ± 0.3

*1–3, urine samples from healthy volunteers; 4, urine sample of a bronchial asthma patient at about 2 h after 10 mg IP was taken via sublingual administration. ND, not detected

Table 2 Comparison of the method with other methods in references

Detection system	Matrix	Extraction (min)	Linear range ($ng \cdot mL^{-1}$)	LOD ($ng \cdot mL^{-1}$)	Recovery (%)	Reproducibility (RSD, %)	Ref.
HPLC/FLD	Urine	2	10–500	EP: 0.54 DA: 2.28 IP: 0.73	88.7–101.7 81.3–94.9 87.1–97.7	7.4–9.7 6.9–9.7 7.3–9.6	This work
HPLC/ECD	Urine	12	10–2000	EP: 2.0 DA: 7.9	94–108 98–104	3.6–6.8 3.2–5.3	[4]
HPLC/FLD	Urine	6	3–100	EP: 2.15 DA: 4.5 IP: 0.32	103.6–109.4 94.3–107.3 86.2–104.4	4.6–8.6 4.4–9.5 3.8–10.0	[5]
LC–MS/MS	Plasma	–	0.02–2	DA: 0.02	99.1–108.2	2.0–3.8	[14]
HPLC/UV	Urine	90	10–500	EP: 0.89	89.8–101.7	3.8–9.2	[16]
HPLC/FLD	Plasma	30	0.7–50	EP: 0.33 DA: 0.2 IP: 0.31	102.5–109.4 94.3–107.3 86.2–104.4	4.6–8.6 4.4–9.5 3.8–10.0	[17]
HPLC/UV	Serum	31	5–100	EP: - DA: -	95.7–107 87–112	8.2–9.3 7.8–10.2	[19]
HPLC/UV	Plasma	–	0.8–2000	DA: 0.1	92.6–95.7	–	[33]
CE/UV	Urine	–	50–300	DA: 4.8	92–103	–	[34]
HPLC/FLD	Urine	–	2.0–50	EP: 0.5 DA: 0.45	91.7–92.2 81–104.2	4.0–10.5 2.5–4.3	[35]

Reusability and inter-batch reproducibility of boronic acid-modified polyhedral oligomeric silsesquioxanes on polydopamine-coated magnetized graphene oxide (magGO@POSS-BA)

It was reported that POSS-8NH_2 is unstable in water due to the hydrolysis but the rate is rather slow. Adding longer aliphatic chains in POSS-8NH_2 can protect the siloxane cages and can better prevent the degradation of POSS cage [30]. Consequently, the stability of magGO@POSS-BA in water is improved significantly due to the introduction of phenylboronic in POSS-8NH_2. To prove the stability and assess the recycle of magGO@POSS-BA, it was subjected to magnet-assisted miniaturized dispersive solid-phase extraction (M-d-μSPE) for eight cycles. The recoveries of EP are shown in Fig. S7. The recovery of EP only drops by 9.1% after eight cycles of M-d-μSPE. To evaluate the batch-reproducibility, magGO@POSS-BA was prepared three times in parallel, and the adsorption capacities of three batches of adsorbents were determined (Table S2). The RSDs for the adsorption capacities are 8.8% for catechol, 4.8% for IP and 11.8% for adenosine, respectively. Since there are four steps in the preparation of adsorbents, such RSDs are acceptable. These results indicate the adsorbent is reproducible and recyclable for use as an eligible adsorbent.

Extraction conditions of catecholamines

Since EP, DA and IP are ortho-phenols, they can be enriched by magGO@POSS-BA. Firstly, the following parameters affecting enrichment performance were optimized: (a) pH value of the sample solution; (b) type of eluent; (c) adsorption time; (d) desorption time; (e) adsorbent dosage. Respective data and Figures are given in Fig. S8, and the detail discussion is in ESM. Finally, the following experimental conditions are found to give best results: pH of sample solution is 8.5; 5% HAc aqueous solution is selected as eluent; adsorption and desorption time are 1 min, and the adsorbent dosage is 10 mg for 3.0 mL of the sample solution, respectively.

Validation of magnet-assisted miniaturized dispersive solid-phase extraction (M-d-μSPE) coupled with HPLC with fluorometric detection

To achieve reliable quantification, it is desired to establish calibration plots by using a matched matrix that spiked with standard analytes. According to the reported literatures [31], there is a normal range for endogenous DA and EP in the human urine. Therefore, endogenous DA and EP must be removed in the matched matrix, otherwise their presence can result in a false positive value. Zhang et al. reported that the blank plasma was prepared by degrading oxidatively the endogenous catecholamines in plasma [14]. This method is feasible because endogenous DA and EP are of ortho-phenols and can be easily oxidized in the solution. Similarly, this method was employed to prepare the blank urine samples to well-match the real urine matrix. Then, the calibration plots were established using the spiked samples for quantification.

Analytical performance characteristics of the method, including linear range, LODs, LOQs, recovery and precision,

were performed to validate the analytical method (Table S3 - S5). The linear range is between 10 and 500 ng·mL^{-1} for three targets with a correlation coefficient greater than 0.9907. LODs are calculated to be 0.54 ng·mL^{-1} for EP, 2.28 ng·mL^{-1} for DA and 0.73 ng·mL^{-1} for IP, respectively. The recoveries of the method were measured by analyzing the spiked human urine at three different concentrations (20, 100 and 500 ng·mL^{-1}). Table S4 and S5 show that the intra- and inter-day assay recoveries are in the range of 85.7–101.7% with the RSDs less than 10.9%. The values of precision and accuracy meet the requirement that RSDs should not exceed 15% for bioanalytical test methods according to the international recommendation [32].

To evaluate the application of the M-d-μSPE-HPLC system in the analysis of biological samples, the urine samples from three healthy volunteers and one patient were treated by the M-d-μSPE and analyzed by HPLC-FLD. Figure 4 is the typical chromatograms of the untreated urine, extracted urine and standard solution. For the untreated urine, very sensitive FLD was employed, but there are still so many interfere peaks that the quantitative determinations of EP, DA and IP are disturbed. After extraction with magGO@POSS-BA, the peaks of the targets are strengthened and most interfering peaks are eliminated, indicating the contribution of enrichment protocol in the removal of interferents from the complicated biological samples. Table 1 is the concentrations of three analytes in the urine of the volunteers. It was reported that DA is usually from ten to one hundred ng·mL^{-1}, and EP is from a few to dozens ng·mL^{-1} in the urine of health people [14, 31]. The mean values of EP and DA for three healthy volunteers are in good agreement with the normal levels, and are also similar to those obtained previously by other researchers [14, 16]. For healthy people, there is no IP in urine, whereas IP in the urine of the patient is found to be 4.3 ng·mL^{-1} at about 2 h after 10 mg of IP was taken via sublingual administration. These results indicate our method is potential in the analysis of endogenous EP and DA and exogenous IP in urine.

Table 2 lists a comparison between the method and other methods in the literature. In comparison, the practicability and superiority of the method are considered. The precision and accuracy of the method are comparable to most references. The linear range of the method is also wider and covers the normal range, especially the abnormal higher concentration range. When it comes to LODs, the proposed method is comparable or superior to most of the methods except that using more sensitive mass spectrometer detector [14]. Such a low LOD completely meets the analysis of EP, DA and IP in urine. In particular, the costed time (only 2 min) of the method is greatly shorter than the previously reported methods, favoring the rapid analysis of sample in clinical diagnosis. Therefore, our method has potential in the analysis of complicated biological samples.

Conclusions

A high-capacity boronate affinity adsorbent was constructed by taking advantage of GO and the POSS-8NH_2. The special structure of POSS-8NH_2 plays an important role in the improvement of adsorption capacity as well as the mass transfer of analytes. This method using POSS as an amplifier can be extended to the preparation of other affinity and ion-exchange adsorbents since these two types of adsorbents have special requirements for high capacity. As an application of the new adsorbent, the magnet-assisted miniaturized dispersive solid-phase extraction (M-d-μSPE-HPLC) is a fast, selective and sensitive method in the determination of catecholamines in human urine. Because of the same boronic affinity principle, magGO@POSS-BA has also potential in the extraction of glycoproteins, saccharides, and nucleosides from the real biological sample.

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