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ORIGINAL PAPER

Selective, fast and semi-automatic enrichment of nucleosides by using a phenylboronic acid modified hybrid material composed of graphene oxide and melamine sponge

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Abstract

A hybrid material was prepared from graphene oxide and melamine sponge, and modified with phenylboronic acid to obtain a sorbent for the enrichment of the nucleosides cytidine, uridine, inosine, guanosine and adenosine. The loading capacity typically is around 27.8 mg g^{-1} which is comparable to other sorbents, and highly selectivity for cis-diols is observed even if the concentration of potential interferents is 1500-fold higher. The sorbent was placed in an injector, and the process was operated semiautomatically by using a peristaltic pump. The sorbent is stable and can be re-used six times without decrease in efficiency. It was applied to the selective extraction of the *cis*-diols (cytidine, uridine, inosine, guanosine, adenosine) from HepG2 cells. It presents good linear between 3 to 5000 μg L^{-1} and the limits of detection (in HPLC analysis with UV detection) are 1–4 μg L^{-1}. Good recoveries of 85–101% were obtained with spiked HepG2 cells samples, with relative standard deviation of ≤9.9%.

Keywords Boronate affinity materials · Peristaltic pump · Syringe · Isotherms · Surface area · Surface morphology · HepG2 cells · Gold nanoparticles

Introduction

cis-Diols such as glycoproteins [1–4], saccharides [5, 6] and nucleosides [7–9] trigger numerous biological activities, so the quantitation of these molecules is very important. But due to the low abundance in cells and complexities of biological samples, the direct analysis of these compounds is very difficult. So different affinity absorbents were devoted to solve this problem. Among these, boronate affinity technology is a unique and promising method to analyze *cis*-diols in biological samples. The principle of this method is the reaction between boric acid and *cis*-diols compounds. In basic aqueous media, they can form five or six-membered cyclic esters. However, in acidic aqueous media, the *cis*-diols will be released into solution to realize selective enrichment and separation of *cis*-diols.

Although various and effective boronate affinity materials were studied in the analysis of *cis*-diols, searching for more absorbents with high selectivity, enrichment capacity, stability and convenient analysis process are still necessary and important. Melamine sponge is a foam-like material consisting of a formaldehyde-melamine-sodium bisulfite copolymer. It possess good ageing resistance and thermostability, and has been used as absorbent material due to its three-dimensional network structures, as well as good hydrophily, low density, fine elasticity and well capacity to reprocess. But its selectivity and enrichment capacity are not satisfied. Surface modification is an effective method including coating, grafting and growth in-situ [10–12]. Among these, grafting modification can only introduce few functional groups. Growth in-situ modification is time-consuming, so it is not suitable for large scale production. However, coating modification has advantages in large scale production. Yang and coworker [13] deposited lignins on the surface of melamine sponge with coating modification,

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then an ultra light and superhydrophobic sponge (UHS) was obtained after pyrolysis at 400 °C. This UHS sponge exhibits excellent oil/water separation performance (oil absorption capacities up to 217 times of its own weight). Liu and co-workers [14] prepared a kind of polyurethane sponge/ graphite oxide/boronic acid functionalized metal-organic framework via a three-in-one strategy, this material can selectively adsorb and effectively separate luteolin from peanut shell coarse extract.

In this work, we describe a phenylboronic acid-modified hybrid sorbent with the advantages of high specific surface area from graphene oxides and good size-memory effect from melamine sponge, respectively. For realizing convenient analysis process, the synthesized absorbent was filled in an injector. Then with the help of peristaltic pump, the whole analysis process can be operated semi-automatically. This presented absorbent exhibited good selectivity, high enrichment capacity, high stability and convenient analysis process. Based on the enrichment of the material to five *cis*-diol nucleosides (cytidine, uridine, inosine, guanosine, and adenosine), the HPLC-UV detection method was used to detect these analytes in HepG2 cells.

Experimental

Materials and reagents

Graphene oxide (GO) was purchased from Shanghai Simbatt Energy Technology Co., Ltd. (Shanghai, China, http://www.simbatt.com). Thionyl chloride, 3-aminophenylboronic acid were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China, http://macklin.company.lookchem.cn). Melamine sponges (MMS, 10 × 10 × 1 cm) was obtained from Feng Tai Nano Materials Co., Ltd. (Zhengzhou, China, http://foamtech.cn.tonbao.com). Gold nanoparticles (AuNPs, 5 nm, 277 μM) was purchased from BBI Solutions (Beijing, China, https://www.bbisolutions.com/cn). Uridine, cytidine, inosine, adenosine, and 2′-deoxyadenosine monohydrate were supplied by Aladdin Chemistry Co., Ltd. (Shanghai, China, http://www.aladdin-e.com). 2′-deoxycytidine, spongouridine, 2′-deoxyuridinewere provided by J&K Scientific (Beijing, China, http://www.jkchemical.com/index.aspx). Vidarabine was commercial available from Shanghai Future Industrial Limited By Share Ltd. (Shanghai, China, http://jianglai.company.lookchem.cn/). Cytarabine was supplied by TCI Development Co., Ltd. (Shanghai, China, http://tcisha.company.lookchem.cn/). The dichloromethane (CH_2Cl_2) and trimethylamine were analytical grade and used after evaporation to remove water. Acetonitrile (HPLC grade) was provided by Dima Technology (Richmond Hill, ON, USA, http://lasallescientific.com/dikma-technologies/). The water used in all experiments was purified with a Milli-Q Advantage A10 (Millipore, Bedford, MA, USA). Other reagents used were analytical grade.

Experimental

Preparation of MMS-GO-PBA

The commercial available Melamine sponge (MMS, 3 × 3 × 1 cm) were immersed in acetone for 6 h to remove impurities and then dried at 50 °C. After that, they were immersed in 10 mL different concentrations of GO suspension (1, 2, 4, 6, 8 g L^{-1}). They were continuous extruded the absorbed solution and then dried at 60 °C under vacuum overnight. The products were denoted as MMS-GO.

The acyl chlorination of MMS-GO (named MMS-GO-Cl) and 3-aminophenylboronic acid modified on MMS-GO-Cl (named MMS-GO-PBA) were realized according to the modified methods [15] and details were put in the Electronic Supplementary Material (ESM). The preparation and analysis process was illustrated in Fig. 1.

Extraction procedure

Before use, MMS-GO-PBA was punched into symmetrical cylindric plugs (1.0 cm in height and 1.2 cm in diameter). The sponge-based syringe was prepared by stuffing a piece of cylindric MMS-GO-PBA into the barrel of a 5 mL plastic syringe, and then sealed with a plunger. The syringe containing MMS-GO-PBA was fixed on the syringe pump. During extraction, 2.0 mL of sample solution containing 25 mM ammonium chloride-ammonia buffer (pH 10) was aspirated into the syringe by backward movement of the syringe pump plunger at a flow rate of 1.0 mL min^{-1}. Then the solution in syringe was extruded very fast by pushing syringe plunger. Elution was carried out by loading 0.6 mL of trifluoroacetic acid (TFA)/methanol (5/95, *v*/v) into the syringe at a flow rate of 0.6 mL min^{-1}. The desorption step was repeated twice. The eluates were combined into a 2.0 mL centrifuge tube and were concentrated to dryness under a nitrogen stream at 50 °C. The residue was dissolved in 100 μL of initial mobile phase and 20 μL were injected into the HPLC system for analysis.

Results and discussion

Characterization

To prove the successful synthesis of materials, the variation of elemental composition was measured by elemental analysis and ICP-OES. The results are illustrated in Table S1 (see in ESM). Compare MMS-GO with MMS-GO-Cl, the successful acylation of carboxyl acid in GO can be concluded from the decrease of H content. The achievement of modification of 3-

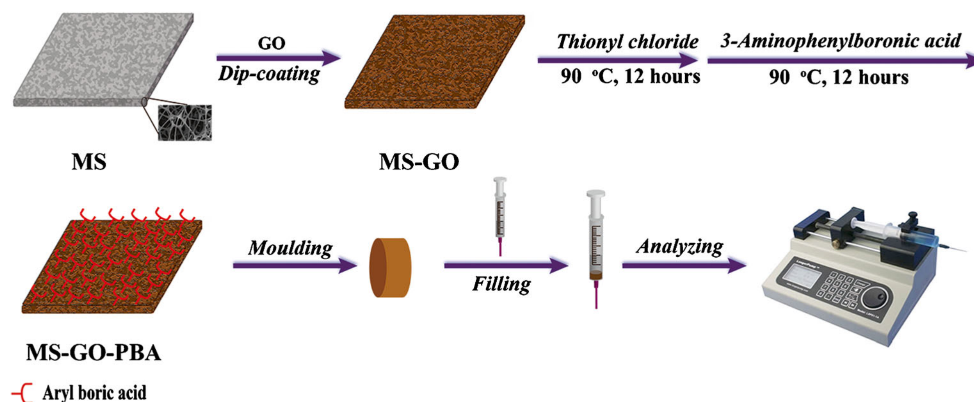

Fig. 1 Schematic for the preparation of boronic acid modified graphene oxide/ melamine sponge composite for in-syringe solid-phase extraction

aminophenylboronic acid can be demonstrated by the increase of B content.

The morphology of porous MMS and MMS-GO (4 g L^{-1}) was observed by scanning electron microscopy (see in Fig. S1 in the ESM). As can be seen from Fig. S1a, the untreated MMS has a three-dimensional honeycomb-web-like structure with pore sizes in the range of 50–100 μm. After modification with GO, the MMS maintains an intact 3D porous structure, indicating that the porous structure of MMS was not destroyed during the dip-coating process (Fig. S1b). The magnified images (Fig. S1c, d) show a wrinkled surface morphology of the GO coating on MMS. The deposition is associated with the hydrogen interaction between the NH groups in MMS and large amount of OH, COOH groups in GO, which promoted the GO coating on MMS [16].

Nitrogen sorption measurements were performed to characterize the pore parameters of the materials. (Fig. S2) Both MMS-GO and MMS-GO-PBA show type-IV isotherm. A summary of Brunauer-Emmett-Teller (BET) specific surface area and the average pore size of the materials are given in Table S2. MMS-GO has a BET surface area of 50 m^2 g^{-1} with a pore volume of 0.42 cm^3 g^{-1}, while MMS-GO-PBA has a 44 m^2 g^{-1} BET surface area with a pore volume of 0.39 cm^3 g^{-1}. MMS-GO-PBA possesses decreased surface area and pore volume comparing with MMS-GO. These results proved that PBA was successfully grafted on the acyl chlorination of MMS-GO.

Optimization of the extraction and desorption condition

In order to achieve the best extraction efficiency of the MMS-GO-PBA for selected nucleosides, the physical and chemical parameters involved during the extraction process were studied (see in Fig. S3 in the ESM). During preparation of adsorbent, different amounts of GO were deposited onto the surface of MMS, and the extraction efficiencies were investigated. As can be seen from Fig. S3a, the adsorption efficiencies increase when the concentrations of GO increased from 1 to 4 mg mL^{-1}. So, the concentration of 4 mg mL^{-1} GO was selected for adsorbent preparation. The flow rate of the extraction was studied in the range of 0.5–4 mL min^{-1}. As shown in Fig. S3b, the analyte extraction was improved by decreasing the extraction flow rate from 4 to 0.5 mL min^{-1}. Further decrease of the extraction flow rate to 1 mL min^{-1} did not led to further improvement. Consequently, the flow rate of 1 mL min^{-1} was employed for further experiment.

The influence of both desorption volume and its flow rate were investigated, to ensure effective elution of the analytes from the sorbent. Four different volumes (0.4, 0.5, 0.6 and 0.7 mL) were studied and the results are illustrated in Fig. S3c. The best extraction yield was obtained for 0.6 mL. Fig. S3d shows that the analytes responses enhanced as desorption flow rate was decreased and remained constant at flow rate of 0.6 mL min^{-1}. As shown in Fig. S3e, the desorption efficiency reaches 93% with twice elution and each time only needs 1 min. Therefore, the whole extraction and desorption procedure can be finished within 4 min.

Evaluation of adsorption isotherms

Langmuir and Freundlich isotherms were used to describe the adsorption results of adsorbents. The binding isotherms were presented in Fig. S4 (see in the ESM). The model constants and the linear regression coefficients were listed in Table S3 (see in the ESM). It showed that the adsorption of adenosine correlated better with the Langmuir model than the Freundlich model, indicating surface homogeneity of MMS-GO-PBA and monolayer adsorption. The maximum adsorption capacity calculated from Langmuir model was 27.8 mg g^{-1}. In Table 1, a comparison of enrichment capacities toward nucleosides of our work and other reported materials are listed. Although, the enrichment capacity is not the highest, the equilibrium time is shorter than those of most materials.

Table 1 Comparison of the adsorption capacities for adenosine of MMS-GO-PBA with other reported materials

Adsorbent	Capacity (mg g^{-1})	Equilibrium time (min)	Reference
boronate-affinity adsorbent prepared by SI-RAFT	26.5	<1	[7]
SiO_2@PGMA-Wulff column	21.4	–	[8]
regenerated-cellulose membrane	32.0	1	[17]
borated titania	0.2	–	[18]
boronate-affinity hybrid monolith	8.8	–	[19]
boronic-acid-functionalized magnetic attapulgite	13.8	9	[20]
PEI-amplified DFFPBA monolithic capillary	27.3	–	[21]
binary boronic acid-functionalized attapulgite	19.5	3	[9]
p(4-VPBA-co-EGDMA) coated Fe_3O_4@SiO_2	12.0	3	[22]
SCF@PEI@PBA	0.7	–	[23]
ATTA@MPS@PBA@C_{12}mimBr	30.9	5	[24]
MMS-GO-PBA	27.8	2	This work

Selectivity evaluation

Selectivity is a major parameter for absorbent materials. Accordingly, the specific selectivity of our material was evaluated by extracting cytidine, uridine and adenosine from complex samples containing some non-*cis*-diol compounds as interferences, including 2′-deoxycytidine, cytarabine, deoxyuridine, spongouridine, deoxyadenosine and vidarabine. Among them, the concentration of interferences were 1, 500, 1000 and 1500 times higher than that of analytes, respectively. As shown in Fig. 2, our material exhibited very good selectivity toward *cis*-diol compounds, even the concentration of interferences were 1500 times higher than the *cis*-diols. Therefore, material presented very satisfying specific selectivity toward *cis*-diols.

Method validation

With the optimized conditions, the calibration curves of these five nucleosides were established with their aqueous solutions, respectively. The results were summarized in Table S4 (see in the ESM). Obviously, the linear relationship between peak area (Y) and the concentration of nucleosides (X) were

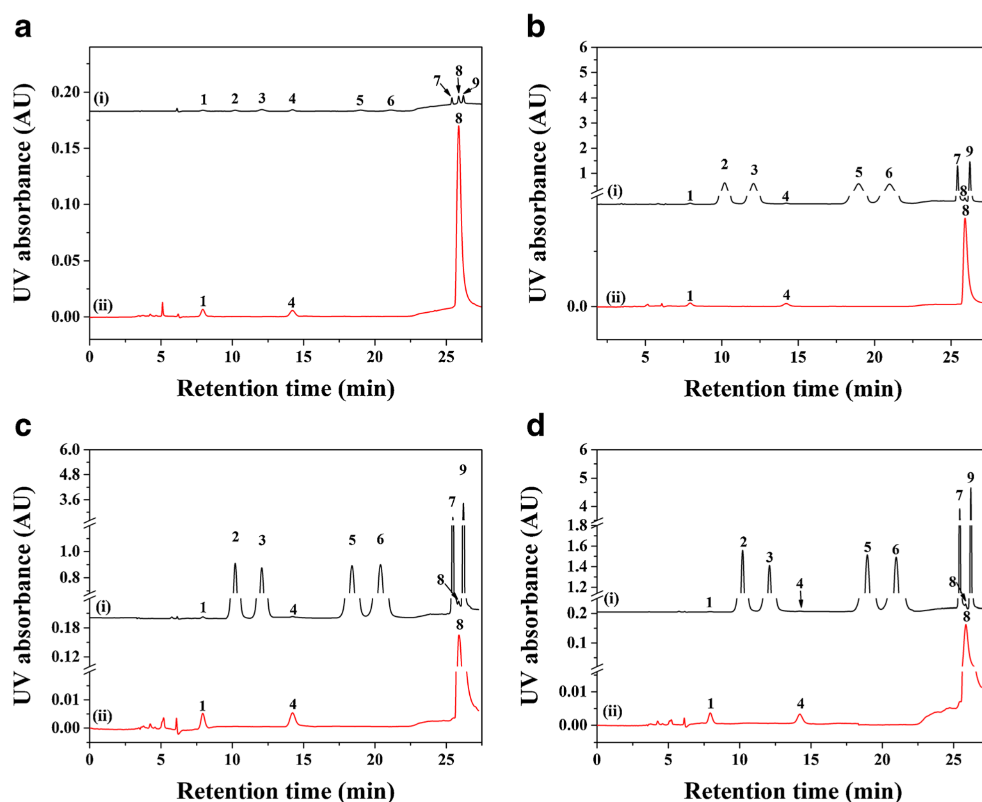

Fig. 2 Chromatograms of mixture containing non-*cis*-diols and 1,2-*cis*-diols before (i) and after (ii) enrichment by MMS-GO-PBA with molar ratios of 1:1 (**a**); 500:1 (**b**); 1000:1 (**c**); 1500:1 (**d**). Peaks: 1, cytidine; 2, cytarabine; 3, 2′-deoxycytidine; 4, uridine; 5, spongouridine; 6, 2′-deoxyuridine; 7, vidarabine; 8, adenosine; 9, 2′-deoxyadenosine

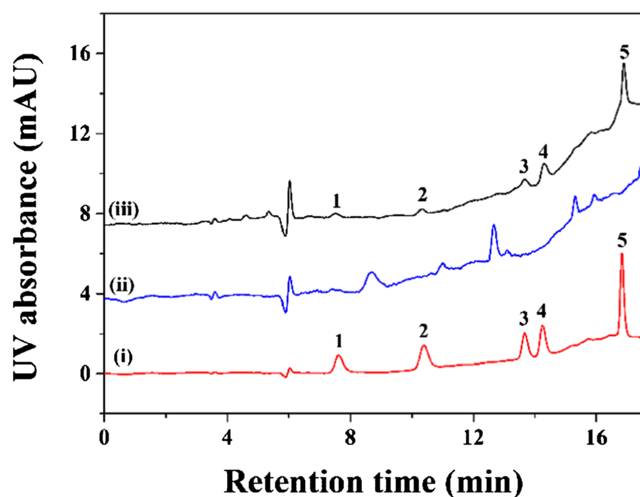

Fig. 3 Chromatograms of nucleosides in HepG2 cells. (i) Standard solution of five nucleosides (1.0 mg L^{-1}); (ii) and (iii) were blank HepG2 cells before and after enrichment with MMS-GO-PBA. Peaks: 1, cytidine; 2, uridine; 3, inosine; 4, guanosine; 5, adenosine

obtained with correlation coefficients of $R^2 \geq 0.995$. The limits of detection (LODs) were in the range of 1–4 μg L^{-1}, which were calculated based on the 3σ/m criterion (where σ is the standard deviation of the blank and m is the slope of the calibration plot). In table S5, the LODs in this work are comparable with them except Li's work, which have the lowest LOD. In Li's work, extra ionic liquid was used during enrichment, and the material was powder. In the present work, bulk material was used, which is easy to recover from the solution. The higher sensitivity can be achieved when using mass spectrum (MS) as the detector. Recoveries at spiking levels of 100, 500 and 2000 μg·L^{-1} are in the range of 85.8–101% and the relative standard deviation (RSD) are less than 9.9% for all analytes (Table S6).

To illustrate the potential application of this method in the analysis of real complicate samples, the hybrid material was used to extract 1.0×10^5 HepG2 cells. It can be seen from Fig. 3 that, there exist obvious matrix interferents with untreated cells. However, after extraction with the synthesized materials, major interfering matrix were eliminated and the peaks of five nucleosides are easy to be observed. These results further proved the selectivity and potential application in the analysis of complex samples.

Furthermore, to study the influence of AuNPs on the metabolism of cells. The presented method was used to analyze the nucleosides in HepG2 cells which were incubated with different concentrations of AuNPs (0, 5, 100 μM) for 6 h and 24 h, respectively. As shown in Fig. 4, the content of nucleosides became higher with the increase of amounts of AuNPs. With the same concentration of AuNPs, the content of nucleosides were also higher with the increase of incubated time. It means that AuNPs can affect the metabolism of nucleosides in HepG2 cells. According to previous research, the toxicity of AuNPs was caused by oxidative stress, and further caused cells cycle changes. So AuNPs may also have effect on the metabolism of small molecules in HepG2 cells [25].

These results also support the proposal that nanoparticles can induce cytotoxicity.

Reusability

Reusability can decrease the cost of analysis obviously, so we survey the reuse ability of our absorbent by comparing the change of enrichment capacities. From Fig. S4, this presented material showed good stability and reproduced performance, because it can keep 93.1% of the maximum enrichment capacity after 6 recyclings. We think the good elasticity and mechanical capacity of the material make the major contribution to this performance. It means the modification with GO do not change the mechanical capacity of MMS and surface. GO is also very stable during multiple suction filtrations and presses.

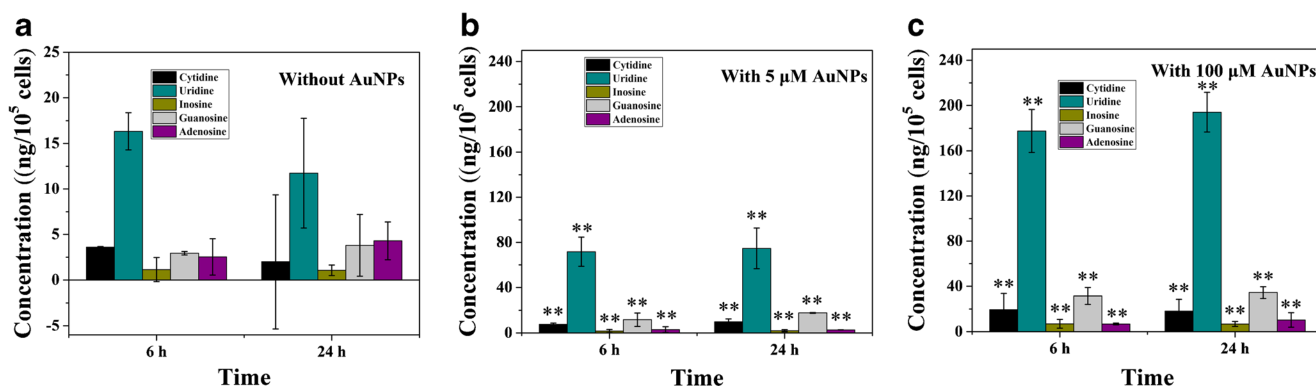

Fig. 4 Concentration levels (μg L^{-1}) of five nucleosides determined in the 1.0×10^5 HepG2 cells by the standard addition method. **a** Blank HepG2 cells, **b** HepG2 cells incubated with 5 μM AuNPs, **c** HepG2 cells incubated with 100 μM AuNPs. Data are presented as the average ± standard deviation ($n = 3$, $0.01 < {*p} \leq 0.05$, ${**p} \leq 0.01$, *p and **p stand for significant difference between 0 μM and 5 μM in **b**, *p and **p stand for significant difference between 5 μM and 100 μM in **c**)

Conclusion

In conclusion, an easily accessed and well-performed boron affinity absorbent based on MMS was synthesized and its enrichment of nucleosides was studied. The deposition of GO on the surface of MMS can increase the specific surface area and enlarge the grafting amount of affinity groups. The absorbent can be filled in solid phase extraction column, then the enrichment can be realized semi-automatically with the help of injection pump. Owing to the excellent stability of this material, it can be reused for 6 times without obvious decrease of performance. The method also have good selectivity and enrichment capacity owing to the specific reaction of boric acid group. At last, this absorbent was applied in practical samples to analyze nucleosides from HepG2 cells. It is still need to improve the adsorption capacity of these kind of materials for the analysis in the more complicated biological samples.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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