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Silica – Boronate affinity material for quick enrichment of intracellular nucleosides

Shuxia Wang, Huihui Li, Xiujuan Guan, Ting Cheng, Haixia Zhang*

Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, PR China

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ABSTRACT

Boronic acid modified materials have been widely used to adsorb nucleosides, but their adsorption capacities require further improvement. Most *cis*-diol containing biomolecules are in very low abundance along with interfering components in real samples, and need to be enriched specially. In this study, we synthesize a kind of silica absorbent modified with boronic acid derivative, using amorphous silica as raw material and obtaining high adsorption capacity for adenosine. In addition, the adsorption equilibrium can be completed within 10 s and 1 min for the desorption. Finally, the material was successfully applied to enrich nucleosides from cells and the spiked recoveries were found between 82.21% and 118.9%. The results showed that the prepared adsorbent has potential to effectively enrich cis-diol substances from cell samples.

1. Introduction

Boronic acid modified material is widely used to extract *cis*-diol compounds including saccharides [1,2], glycoproteins [3,4], nucleosides [5,6] etc. Nucleosides play crucial roles in many biological processes [7], for example, their intracellular concentrations provide information for understanding cellular energy metabolism, and their contents in plasma can be used to assess oxidative stress [8]. In real samples, nucleosides are in microscale accompanying many interferences, which made it difficult to determine them in matrix, so it is necessary to develop the methods to specifically capture nucleosides before instrumental analysis. Recently, various materials have been developed for the selective capture of *cis*-diols, including titanium dioxide [9,10], mesoporous materials [11], molecularly imprinted polymers [12], nanoparticles [13], and temperature-responsive materials [14]. Boronic acid modified materials have attracted much attention [15] and can be applied to any nucleoside, based on boronic acids forming five or six cyclic esters by covalent bond with *cis*-diols under weak basic condition. The covalent bond can then be dissociated under acidic condition. In sample pretreatment procedure, both extraction efficiency and specificity are important parameters. To improve extraction efficiency and specificity, dendrimer-assisted boronate affinity magnetic nanoparticles [16], boronic acid-functionalized attapulgite connecting self-assembly ionic liquid layer [6] and metal organic framework [17] owning large specific surface area, were introduced as supporting materials. Polymers with many reaction sites can be easily functionalized. Polyethyleneimine-grafted boronate affinity materials [18] was used to the enrichment of *cis*-diol-containing compounds. Two different kinds of materials were introduced [19] synergistically, one is boronic-acid-functionalized Fe_3O_4 nanoparticle to capture target molecules and the other is poly(methyl methacrylate) nanobead to adsorb disturbing molecules.

Increasing boronic sites on the material surface is one way for obtaining large adsorption capacity. However, effective utilization of boronic sites are more green and economic. In this present work, a kind of boronic acid-functionalized silica was prepared, which possessed two advantages including maximal adsorption capacity (46.30 mg g^{-1} for adenosine) and short equilibrium time (10 s), both are prior to the analogous materials. It was successfully used to capture intracellular nucleosides.

2. Experimental

2.1. Materials and chemicals

Silica (average particle size 25 μm) was purchased from Haiyang factory of Qingdao (Shandong, China), and activated with 3.0 mol L^{-1} hydrochloric acid before used; 3-Aminopropyltrimethoxysilane (APTMS), cytidine, uridine, guanosine, inosine, adenosine and deoxyadenosine were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China); cyanuric chloride was obtained from J & K Chemical Reagent Co. Ltd (Beijing, China); 3-aminophenylboronic

* Corresponding author.
E-mail address: zhanghx@lzu.edu.cn (H. Zhang).

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acid (3-APBA) was purchased from Energy Chemical (Shanghai, China); ammonium hydroxide (NH_4OH, 25% ammonia), acetic acid, toluene, and tetrahydrofuran (THF), N,N-Dimethylformamide (DMF) were received from Tianjin Chemical Co. (Tianjin, China); acetonitrile (ACN) of HPLC grade was from Dikma Technology (VA, USA). The water used throughout the experiments was supplied by Milli-Q system (Millipore, Bedford, MA, USA). All the other chemical reagents were of analytical grade. The store solution of 0.1 g L^{-1} for each analyte was prepared in water and stored at 4 °C in the refrigerator.

2.2. Instruments

The obtained materials were characterized by Fourier transform infrared (FT-IR) spectroscopy (VEREEX 70V FTIR, Bruker, USA), Elemental analysis (Vario EL, Elementar, Germany), Inductive coupled plasma emission spectrometer (ICP) (Agilent 700, USA), High resolution mass spectra (HRMS) using a spectrometer APEX II 47e FT-ICR with ESI or APCI positive ion mode (Bruker Daltonics, America). NMR spectra were measured using a 400 MHz instrument (JEOL, Japan). Analyses were performed on a Varian 210 high performance liquid chromatography system (CA, USA) equipped with two high pressure gradient pumps, a 325 UV–vis detector and a Varian Star Chromatographic workstation. All separations were carried out on a C18 column (Dikma Technologies, 250×4.6 mm, 5 μm) at room temperature. The UV–vis detector was operated at 259 nm and the flow-rate of the mobile phase was maintained at 0.8 mL min^{-1}. Separation of five nucleosides (cytidine, uridine, inosine, guanosine and adenosine) was accomplished using a gradient elution: mobile phase A: 12.5 mM ammonium formate in water; B: ACN / water =1/1 (v/v); 0–2 min, A: 94%; 2–8 min, A: 86%; 8–20 min, A: 30%. Isocratic elution using water/ acetonnitrile (9/1, v/v) was used to separate adenosine and 2-deoxyadenosine.

2.3. Synthesis of SiO_2@NH_2@PBA materials

SiO_2@NH_2 was synthesized under same condition described in literature [20].

The synthesis and characterization of triazine-APBA were illustrated in Supporting materials.

SiO_2@NH_2 (2.5 g) was dispersed in 70 mL of redistilled THF, 0.75 g triazine-APBA and 0.6 mL N,N-Diisopropylethylamine (DIPEA) were added under stirring. The system was kept at 45 ℃ for 12 h, then the resulting mixture was washed with DMF and ethanol several times to remove the unreacted triazine-APBA and DIPEA. At last the obtained product SiO_2@NH_2@PBA was dried at 60 ℃ under vacuum.

2.4. Solid phase extraction procedure

SiO_2@NH_2@PBA (10 mg) was dispersed in 2.0 mL of nucleoside aqueous sample (containing 1 mM ammonium hydroxide), the mixture was ultrasound for 10 s and then centrifuge at 12,000 rpm for 5 min to collect the absorbent. Subsequently, 2×0.4 mL of formic acid (20 mmol L^{-1}, pH=3.0) were used to elute the analytes for 1 min. Then the eluate was collected and evaporated at 50 °C under a gentle N_2 stream with a Termovap sample concentrator (HP5016SY, Shanghai, China). The residue was dissolved in 100 μL water, filtered, and 20 μL was injected into the chromatographic system.

2.5. Evaluation of extraction and desorption conditions

The extraction procedure was optimized by adjusting extraction time and pH. SiO_2@NH_2@PBA (10 mg) and 2.0 mL of nucleoside sample (2.0 mg L^{-1}) was ultrasound for different time (10 , 30 , 50 , 90 , 180 , 300 s), while the extraction pH (from 4 to 9) was adjusted through formic acid and ammonia, the supernate was analyzed to evaluate the equilibrium time and optimum pH. Similarly, desorption times and time were both optimized by analyzing the eluant.

2.6. Evaluation of selectivity

To evaluate the specificity of SiO_2@NH_2@PBA, adenosine (*cis*-diol) and 2′-deoxyadenosine (non *cis*-diol) were mixed with different mass ratio (1:1, 1:10, 1:100). The extraction was carried out with the optimal procedure.

2.7. Evaluation of binding capacity

In order to evaluate the maximum binding capacity of the absorbent, the concentration of adenosine was prepared from 0 to 2.0 g L^{-1}. The equilibrium absorption amount was calculated through this formula: $Q = (C_。 - C_e)V/m$, while Q (mg g^{-1}) is the maximum binding capacity of the absorbent, $C_。$ and C_e (mg L^{-1}) are the original and final concentration of the sample before and after extraction, respectively, V (L) is the volume of the extraction system, m (mg) is the mass of the absorbent.

2.8. Application to cell samples

SMMC-7721 cells are incubated with 10% (v/v) inactivated calf serum dulbecco's modified eagle medium, containing 5% CO_2 saturated humidity incubator at 37 °C. The cells in logarithmic phase are collected for subsequent tests. Cells ($6×10^6$) are added 0.2 mL 1 mM ammonium hydroxide for disruption, then centrifuged to remove cytomembrane residue. The supernatant was diluted 40 times as final cell samples. Spiked samples were prepared by addition 20, 50 and 100 μg L^{-1} nucleoside standards in cell samples, and the final volume was 2.0 mL. The extraction was carried out using the optimal conditions described in Solid phase extraction procedure.

3. Results and discussion

3.1. Characterization

The synthesis of SiO_2@NH_2@PBA was described in Fig. 1.

The elemental analysis results were used to judge the successful synthesis of SiO_2@NH_2@PBA, as shown in Table 1. The increasing of N % from 0 to 2.87 indicates the successful amination, while the final product containing 4.63% N proved the triazine-APBA modified on the surface of SiO_2@NH_2.

The FT-IR spectra of SiO_2 (a), SiO_2@NH_2 (b) and SiO_2@NH_2@PBA (c) are obtained and shown in Fig. 2. The difference is not obvious with the spectra of SiO_2@NH_2 and SiO_2, because the peak from stretching vibration of N–H is overlapped with that from O–H broad bonds at 3436 cm^{-1}. 1550 cm^{-1} on the (b) is from -NH_2 group. The overlapping peaks between 1335 cm^{-1} and 1635 cm^{-1} can be attributed to B-O, N-H, C=N and phenyl on the spectrum of SiO_2@NH_2@PBA.

SEM technique was used to observe the morphology change of silica after modification. The raw material silica has anomalous morphology that was unchanged after modification, except for the smaller particles resulting from the stirring in synthesis (Fig. 3). Additionally, nitrogen adsorption-desorption measurement at 77 K showed that the BET surface area of SiO_2@NH_2@PBA was 169.2 m^2 g^{-1}, less than that of SiO_2 (265.2 m^2 g^{-1}). The modification led to the decreased surface area owing to some pores stopped by the triazine-APBA groups.

3.2. Optimization of extraction and desorption conditions

The extraction equilibrium was so fast that the whole extraction could be finished within 10 s (Fig. 4A). The quick extraction procedure can be attributed to the anomalous morphology, which improved the mass transfer rate. Fig. 4(B) showed that pH 9.0 was the best

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