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High-capacity magnetic hollow porous molecularly imprinted polymers for specific extraction of protocatechuic acid

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ABSTRACT

Magnetic hollow porous molecularly imprinted polymers (HPMIPs) with high binding capacity, fast mass transfer, and easy magnetic separation have been fabricated for the first time. In this method, HPMIPs was firstly synthesized using protocatechuic acid (PCA) as template, 4-vinylpyridine (4-VP) as functional monomer, glycidilmethacrylate (GMA) as co-monomer, and MCM-48 as sacrificial support. After that, epoxide ring of GMA was opened for chemisorbing Fe_3O_4 nanoparticles to prepare magnetic HPMIPs. The results of characterization indicated that magnetic HPMIPs exhibited large surface area (548 m^2/g) with hollow porous structure and magnetic sensitivity (magnetic saturation at 2.9 emu/g). The following adsorption characteristics investigation exhibited surprisingly higher adsorption capacity (37.7 mg/g), and faster kinetic binding (25 min) than any previously reported PCA imprinted MIPs by traditional or surface imprinting technology. The equilibrium data fitted well to Langmuir equation and the adsorption process could be described by pseudo-second order model. The selective recognition experiments also demonstrated the high selectivity of magnetic HPMIPs towards PCA over analogues. The results of the real sample analysis confirmed the superiority of the proposed magnetic HPMIPs for selective and efficient enrichment of trace PCA from complex matrices.

1. Introduction

Molecular imprinted polymers (MIPs) have attracted wide attentions and attained significant applications in selective extraction of target compounds from complex matrices (e.g. natural product, food, biological and environmental samples) [1–3]. However, MIPs prepared by traditional imprinting methods still faces challenges, such as low binding capacity, poor site accessibility and slow binding kinetics because of the deeply embedded recognition sites in highly cross-linked polymer network [4]. Therefore, extensive research of MIPs with high capacity and rapid binding kinetics is imperative.

Numerous studies have investigated MIPs with high densities of recognition sites at the surface of MIPs for remarkable facilitation of target compounds adsorption and mass transfer by novel imprinting strategies (e.g. surface molecular imprinting [2,5], and hollow porous molecular imprinting [6–9]). Up to now, various forms of surface imprinted polymers, imprinting MIPs on the surface of nano/micro solid supports (e.g. silica [10,11], carbon nanotube [12], polymer supports [13] and metal particles [14]), have been successfully synthesized with obviously increased binding capacity and shortened equilibrium time. In our previous reports [15], the maximum adsorption capacity of protocatechuic acid (PCA) on $Fe_3O_4@SiO_2@MIPs$ and $Fe_3O_4@mSiO_2@MIPs$ can reach 7.5 and 17.2 mg/g, respectively, largely higher than traditional MIPs (34.7 μg/g and 0.16 mg/g) [10,15–17], and the equilibrium time was shortened to 50–140 min from 12 to 24 h in traditional MIPs. Notably, MIPs over magnetic solid supports possess high supermagnetism, which could be easily and rapidly separated under external magnetic field without additional column-packing procedure or centrifugation/filtration procedure. Although magnetic MIPs contain remarkable advantages, the solid cores without any recognition sites possess most mass of MIPs (about 80%) [10,15], which then decreases the binding capacity per unit mass of MIPs. Then novel hollow porous MIPs (HPMIPs) has increasingly attracted more interests and achieved great advances. Especially when mesoporous silica spheres are used as sacrificial support, HPMIPs have pores on the surface and hollow structure in the core for favoring mass transfer, and the highly dense recognition sites spread at the internal and external surface of the HPMIPs can be fully used for high capacity [9,18–21]. Xu and coworkers have testified that adsorption capacity of atrazine on multihole hollow MIPs is about 3.2 times that on porous solid MIPs [6]. However, difficult separation (e.g. filtration, centrifugation) comes to HPMIPs.

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Surprisedly, magnetic HPMIPs have rarely been reported since previous reported HPMIPs contain no groups for Fe_3O_4 nanoparticles immobilization.

Glycidilmethacrylate (GMA) has an epoxide ring, offering fewer potential hydrogen bonds compared with a free hydroxyl group, which then has less interfere in MIPs formation [22]. Furthermore, the epoxide ring can be opened to from 1,2-diol groups. Therefore, GMA is always and only used as pro-hydrophilic co-monomer for preparation of hydrophilic MIPs [23–25]. Interestingly, Wan and coworkers have reported that poly(glyceryl monomethacrylate) (PGMMA) can be chemisorbed onto Fe_3O_4 nanoparticles surface through their 1,2-diol groups by forming five-membered chelate rings with the Fe atoms [26]. Based on this, Zhao and co-workers successfully prepare magnetic MIPs by introducing PGMMA on the surface of MIPs, and the binding capacity has no obvious decrease because of the less mass content of Fe_3O_4 (about 4%) [27]. Accordingly, we speculate that magnetic HPMIPs could be designed by using GMA as co-monomer to immobilize Fe_3O_4. However, there has no relevant report.

Thus, in this study we are presenting a novel preparation method for the synthesis of magnetic HPMIPs. PCA (a phenolic compound, widely distributed in commonly consumed foods, fruits, juices and traditional Chinese medicine) is chosen as model compound in this proof of concept study. Firstly, HPMIPs has been prepared using MCM-48 spheres as a sacrificial support, PCA as template, 4-vinylpyridine (4-VP) as functional monomer and GMA as co-monomer. Secondly, epoxide ring of GMA is opened with perchloric acid. Lastly, magnetic Fe_3O_4 nanoparticles are attached *via* a simple coprecipitation process. The characterization, adsorption isotherms/kinetics and competitive adsorption of novel magnetic HPMIPs were investigated, and their amenability for real sample enrichment (PCA from *Syzygium aromaticum*) was explored. The results suggested that prepared magnetic HPMIPs exerted higher binding capacity, faster binding kinetics and more excellent selectivity than previously reported MIPs for PCA. This finding may open the door for preparation of MIPs with outstanding performance.

2. Experimental

2.1. Chemicals and reagents

Cetyltrimethyl ammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), 2,2-azobis(isobutyronitrile) (AIBN), Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), Iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) and HPLC grade acetonitrile were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ethylene glycol dimethacrylate (EGDMA), 4-VP, and GMA were acquired from Shaen Chemical Technology Co., Ltd (Shanghai, China). Anhydrous acetonitrile, ethanol, $NH_3{\cdot}H_2O$ (28 wt%), and HF solution (40%) were purchased from Kemiou Chemical Reagent Co., Ltd (Tianjin, China). 4-Hydroxybenzoic acid (4-HBA), salicylic acid (SA), caffeic acid (CA), gallic acid (GA), and PCA with purities over 99% were supplied by Xiya Reagent Co., Ltd. (Chengdu, China). Ultrapure water (18.2 MΩ), collected from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used to prepare all aqueous solutions. The other reagents were of analytical grade.

2.2. Instruments

Transmission electron microscopy (TEM) (JEM-2100F, JEOL, Japan) was used to observe the size, structure and morphology of microspheres. The infrared spectra (4000–400 cm^{-1}) using KBr pellets of the solid samples were performed on a Fourier transform infrared spectrometer (FT-IR) (Nicolet 6700, Thermo Nicolet Co., Waltham, MA, USA). The identification of crystalline phase was performed using powder X-ray diffractometer (Rigaku RINT 2500, Rigaku Corporation, Japan) with Cu/Kα radiation at 200 mA, 40 kV, and the diffractogram was recorded in the 2θ range of 1–10° and 10–80°. The encapsulation efficiency of microspheres was carried out by thermo-gravimetric analysis (TGA SDTQ600, TA, USA) from room temperature to 800 °C with a heating rate of 10 °C/min. The Brunauer–Emmett–Teller (BET) surface area was detected by a Micromeritics ASAP 2020 device (Micromeritics, Norcross GA, USA). Magnetization was measured at room temperature in a vibration sample magnetometer (VSM7307, Lake Shore, USA).

2.3. HPLC analysis

All chromatographic measurements were performed on an analytical SunFire^{TM} C_{18} column (250 mm × 4.6 mm i.d., 5 μm, Waters, Milford, MA) using an Agilent 1260 HPLC system with a diode array detector. The mobile phase was acetonitrile/water (20/80, v/v) mixture containing 0.4% acetic acid at a flow rate of 0.8 ml/min while the temperature was controlled at 25 °C. Spectra were acquired at 260 nm.

2.4. Preparation of magnetic HPMIPs

The schematic rout for the preparation of magnetic HPMIPs is shown in Fig. 1, which is composed of the following steps: synthesis of MCM-48 spheres; preparation of HPMIPs; epoxide ring opening; immobilization of magnetic Fe_3O_4 on HPMIPs.

At first, MCM-48 spheres were synthesized according to previous report [9]. Typically, CTAB (2.6 g) was dissolved in ultrapure water (120.0 ml), and then TEOS/ethanol (1/20, v/v) solution (84 ml) was added to the surfactant solution and stirred for 30 min at room temperature. Subsequently, aqueous ammonia (12 ml) was added, and the mixtures were stirred for 6 h at room temperature. The MCM-48/CTAB spheres were collected by filtration and then redispersed in ethanol for refluxing at 80 °C twice of 24 h each to remove CTAB. Finally, MCM-48 spheres were filtered, washed by ultrapure water and ethanol, and then dried in vacuum at 50 °C for 12 h.

After that, HPMIPs was obtained by a two-step procedure: a self-assembly polymerization of MIPs on the internal surface of MCM-48 spheres, and then the removal of MCM-48 spheres and templates. In the first stage, PCA (0.5 mmol) and 4-VP (2.0 mmol) were dissolved in anhydrous acetonitrile (40.0 ml), purged with N_2, and then stored in refrigerator at 4 °C for 12 h to prepare preassembly solution. Then, GMA (2.0 mmol) was added to the preassembly solution and the mixtures were stirred for 10 min. Subsequently, MCM-48 (500.0 mg), EGDMA (10 mmol) were dissolved in anhydrous acetonitrile (40.0 ml) and added into the above solution, purged with N_2 on ice, and then AIBN (0.15 g) was added and the reaction was allowed to proceed for 24 h at 60 °C under constant stirring. After polymerization, MCM-48/MIPs were collected by filtration, rinsed with ethanol until the supernatant was clear. In the second stage, MCM-48/MIPs was immersed in ethanol solution of HF and continuously stirred for 12 h to remove MCM-48 matrix and PCA absolutely. Finally, HPMIPs were filtered, washed with ethanol to neutral pH and dried overnight under vacuum at 50 °C.

Then, above HPMIPs (400 mg) were added into perchloric acid water solution (10%, v/v) (25 ml), and the mixtures were stirred at room temperature for 24 h. After epoxide ring was opened, the HPMIPs with 1,2-diol groups was achieved by filtration, washed with ethanol and water to neutral pH, and then dried overnight under vacuum at 50 °C.

At last, magnetic HPMIPs were synthesized by chemical coprecipitation of Fe^{2+} and Fe^{3+} (with a molar ratio of 1:2) on the surface of HPMIPs with 1,2-diol groups according to previous literatures with minor modification [26,27]. Briefly, HPMIPs with 1,2-diol groups (200 mg) and iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$,