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Hollow molecular imprinted polymers towards rapid, effective and selective extraction of caffeic acid from fruits

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ABSTRACT

Rapid and selective extraction and enrichment of trace bioactive analytes from complex matrices were of significant importance for efficient and accurate quantification. Here, novel hollow molecular imprinted polymers (HMIPs) were prepared using caffeic acid (CA) as template, 4-vinylpyridine (4-VP) as functional monomer, and Fe_3O_4@SiO_2 as sacrificial support. Fourier transform infrared spectrometer (FT-IR), transmission electron microscopy (TEM), nitrogen adsorption and thermo-gravimetric analysis (TGA) were used to verify the successful synthesis of HMIPs. Hollow structure with large surface area (325.8 m^2/g) made most recognition sites locate on the surface of HMIPs, resulting in high binding capacity (21.10 mg/g) and fast kinetic binding (35 min) in comparison with magnetic MIPs (MMIPs) and solid MIPs. The equilibrium data fitted well to Freundlich equation and the adsorption process could be described by pseudo-second order model. The selectivity performance of HMIPs was favorable. Finally, HMIPs were successfully used as adsorbent to rapidly and selectively extract and enrich CA from fruits with a relatively satisfactory recovery (85.6–103.5%). Coupling with high-performance liquid chromatography (HPLC), the content of CA in four kinds of fruits (kiwifruit, apple, papaya and waxberry) was determined as less than 1.0 μg/g fresh fruit. Results indicated the superiority of HMIPs in the selective extraction of target compound from complex matrices.

1. Introduction

Molecularly imprinted polymers (MIPs), containing specific recognition sites complementary in size, shape, and chemical functionality to the template, have attracted wide attentions and attained significant applications in selective extraction of target components from complex matrices (e.g. natural product, food, biological and environmental samples) [1–7]. However, MIPs prepared by traditional imprinting methods (e.g. bulk polymerization, suspension polymerization, precipitation polymerization) face 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 [3]. Hence, design and preparation of MIPs with high capacity and quick mass transfer rate are necessary.

MIPs can be imprinted on the surface of nano/micro solid supports (e.g. SiO_2, Fe_3O_4, carbon nanotube, polymer support) [8], which made highly dense recognition sites locate on the surface of solid support for remarkable facilitation of mass transfer, adsorption and desorption. Notably, magnetic MIPs (MMIPs) possess high supermagnetism, which could be easily and rapidly separated under external magnetic field [9]. Although surface MIPs contain remarkable advantages, the solid core without any recognition sites would decrease binding capacity per unit mass of MIPs [8,10]. Then, (single-hole) hollow MIPs (HMIPs) and hollow porous MIPs (HPMIPs) have attracted much interests and achieved great advances [8,11–13]. Xu et al. found that adsorption capacity of atrazine on multihole HMIPs was about 3.2 times that on solid MIPs [14]. Our previous report also indicated that adsorption capacity of protocatechuic acid (PCA) on HPMIPs was about 2.0 times that on surface MIPs because of the removal of solid core [8]. A soft core (polystyrene) or a hard core (SiO_2, mesoporous SiO_2, TiO_2, $K2Ti_4O_9$) was usually selected as sacrifice supports to synthesize HMIPs and HPMIPs [8,11–13,15]. A thinner imprinting shell with higher adsorption capacity and faster binding kinetics could be achieved by selection of a solid core [15]. Unsatisfactorily, solid MIPs by precipitation polymerization sometimes accompanied with surface HMIPs/HPMIPs using sacrifice supports with no magnetic capacity. Then, we supposed that pure HMIPs could be achieved by removal of magnetic core when MMIPs were separated from solid MIPs magnetically.

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Caffeic acid (CA), one of the well-known and representative phenolic acids, is commonly distributed in fruits, vegetables, beverages and herbs. It has gained enormous attention in the field of life and nutrition because of its multiple benefic effects, such as antioxidant, anti-inflammatory, antitumor, immunomodulatory, and neuroprotective effects [16–18]. Therefore, it is of great importance to determine CA in complex matrices. So far, some progress has been made to quantify CA by high-performance liquid chromatography (HPLC) [19], capillary gas chromatography [20], capillary electrophoresis [21], quartz crystal microbalance (QCM) [22], ultraviolet–visible spectroscopy (UV–vis) [23], electrochemical [24] and fluorometric techniques [25]. Although CA is usually found in fruits [26], it may be present at trace level. Moreover, it is usually found the existence of multi-interfering components. Therefore, selective extraction and enrichment processes can be urgently needed in the analytical procedures [19,27]. Most MIPs for CA have been prepared by conventional polymerization methods using 4-vinylpyridine (4-VP), methacrylic acid, allylurea, allylaniline or ionic liquids as functional monomer [28–32]. And surface MIPs on QCM resonator and electrode with incubation time about 25 min have been configured for effective and selective determination of CA [22,33]. However, to the best of our knowledge, no paper has reported the preparation of HMIPs for CA to rapidly and selectively extract CA from complex matrices.

Thus in the present work, we present the novel synthesis of HMIPs by using CA as dummy template, 4-VP as functional monomer, Fe_3O_4@SiO_2 microspheres as sacrificial support. The characterization, adsorption isotherms/kinetics and competitive adsorption of HMIPs were investigated, and their amenability for real sample analysis (i.e. kiwifruit, apple, papaya and waxberry) was explored. By comparison with MMIPs and solid MIPs, HMIPs exerted higher binding capacity and faster binding kinetics.

2. Experimental

2.1. Chemicals and reagents

Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), polyethylene glycol 6000 (PEG 6000), tetraethyl orthosilicate (TEOS), 2,2-azobis(isobutyronitrile) (AIBN) and HPLC grade acetonitrile were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ethylene glycol dimethacrylate (EGDMA), 4-VP and 3-(trimethoxysily) propyl methacrylate (MPS) were bought from Shaen Chemical Technology Co., Ltd (Shanghai, China). Anhydrous acetonitrile, methanol, ethanol, $NH_3{\cdot}H_2O$ (28 wt%), HF solution (40%) and ethylene glycol were obtained from Kemiou Chemical Reagent Co., Ltd (Tianjin, China). CA, *p*-coumaric acid (pCA), ferulic acid (FA), cinnamic acid (CMA), and PCA with purities over 99% were supplied by Xiya Reagent Co., Ltd. (Chengdu, China). Other reagents were of analytical grade.

2.2. Preparation of MMIPs/HMIPs/solid MIPs

The schematic rout for preparation of MMIPs and HMIPs was shown in Fig. 1. At first, Fe_3O_4@SiO_2 microspheres were synthesized according to our previous reports [10,34–36]. Then, Fe_3O_4@SiO_2 was modified by vinyl groups. Typically, Fe_3O_4@SiO_2 (250.0 mg) was dissolved in 40 ml of MPS solution (MPS: 10% acetic acid, v/v, 0.15: 40) and stirred at 60 °C for 5 h. After that, vinyl-modified Fe_3O_4@SiO_2 was magnetically collected. Subsequently, under nitrogen protection, CA (0.25 mmol) and 4-VP (1.0 mmol) were dissolved in acetonitrile/methanol (3/2, v/v, 20.0 ml) at 4 °C for 12 h to prepare preassembly solution. Then, vinyl-modified Fe_3O_4@SiO_2 (100 mg), EGDMA (5.0 mmol) and AIBN (100 mg) were added, and the reaction was allowed to proceed for 24 h at 60 °C

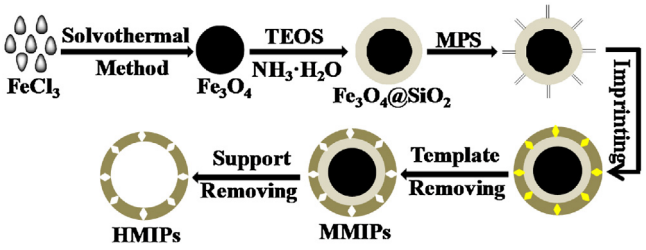

Fig. 1. The synthetic procedure for preparing MMIPs and HMIPs.

under constant stirring. After polymerization, MMIPs were collected magnetically, and then eluted with methanol–acetic acid (9/1, v/v) to remove CA absolutely.

HMIPs were obtained by removing Fe_3O_4@SiO_2 from MMIPs in 10% HF water solution for 12 h.

Solid MIPs were prepared with the same procedures with those for MMIPs without addition of vinyl-modified Fe_3O_4@SiO_2.

As a control, the corresponding nonmolecularly imprinted polymers (MNIPs/HNIPs/solid NIPs) were prepared with the same corresponding procedures in the absence of template CA.

2.3. Characterization of HMIPs

Fourier transform infrared spectrometer (FT-IR) (Nicolet 6700, Thermo Nicolet Co., Waltham, MA, USA) were used to collect infrared spectra (4000–400 cm^{-1}). Transmission electron microscopy (TEM) (JEM-2100F, JEOL, Japan) was used to observe the size, structure and morphology. The Brunauer-Emmett-Teller (BET) surface area was determined by a Micromeritics ASAP 2020 device (Micromeritics, Norcross GA, USA). Thermo-gravimetric analysis (TGA SDTQ600, TA, USA) was detected from room temperature to 800 °C with a heating rate of 10 °C/min.

2.4. HPLC analysis

HPLC analysis was operated on an Agilent 1260 HPLC system with a diode array detector at 323 nm. Separation was conducted on a SunFire™ C_{18} column (250 mm × 4.6 mm i.d., 5 μm, Waters, Milford, MA). Isocratic mobile phase consisted of 0.4% acetic acid and acetonitrile (85/15, v/v) with a flow rate of 0.8 ml/min was prepared for analysis of CA and fruit extract at 25 °C.

2.5. Adsorption experiments

For kinetic adsorption experiments, HMIPs/HNIPs or MMIPs/MNIPs (5.0 mg) were mixed with CA (0.5 mg/ml, 4.0 ml of acetonitrile/methanol (3/2, v/v) solution). The mixtures were continuously shaken at 25 °C and the temporal concentrations of CA at a certain intervals (5, 15, 25, 35, 40, 50 and 60 min) were detected. For solid MIPs/NIPs, the incubation times were set at 2, 4, 6, 8, 12, 16, 20 and 24 h. The adsorption capacity Q_t (mg/g) at different times t was calculated as:

$$Q_t = (C_0 - C_t)\cdot V/m \quad (1)$$

where C_t (mg/ml) is the CA concentration at different contact times, V (ml) is the volume of CA solution, and m means the mass of HMIPs/HNIPs/MMIPs/MNIPs/solid MIPs/solid NIPs (g).

For equilibrium adsorption experiments, HMIPs/HNIPs or MMIPs (5.0 mg) were suspended in CA solution (4.0 ml) with the concentrations from 0.24 to 0.70 mg/ml. While for solid MIPs, the concentrations of CA ranged from 0.06 to 0.7 mg/ml. After shaken