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Preparation of magnetic molecularly imprinted polymer for selective recognition of resveratrol in wine

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ABSTRACT

The magnetic molecularly imprinted polymers (MMIPs) for resveratrol were prepared by using surface molecular imprinting technique with a super paramagnetic core–shell nanoparticle as a supporter. Rhapontigenin, which is the analogues of resveratrol, was selected as dummy template molecules to avoid the leakage of trace amount of resveratrol. Acrylamide and ethylene glycol dimethacrylate were chosen as functional monomers and cross-linker, respectively. The obtained MMIPs were characterized by using scanning electron microscopy, Fourier transform infrared spectrum, X-ray diffraction and vibrating sample magnetometer. High performance liquid chromatography was used to analyze the target analytes. The resulting MMIPs exhibited high saturation magnetization of 53.14 emu g^{-1} leading to the fast separation. The adsorption test showed that the MMIPs had high adsorption capacity for resveratrol and contained homogeneous binding sites. The MMIPs were employed as adsorbent of solid phase extraction for determination of resveratrol in real wine samples, and the recoveries of spiked samples ranged from 79.3% to 90.6% with the limit of detection of 4.42 ng mL^{-1}. The prepared MMIPs could be employed to selectively pre-concentrate and determine resveratrol from wine samples.

1. Introduction

Resveratrol is a polyphenol compound which has beneficial effects on human health, such as antioxidative [1], anticancer [2], anti-inflammatory [3], antiestrogenic [4], and platelet aggregation inhibitory effects [5]. The concentrations of resveratrol contained in wine vary from one kind to another, according to the variety [6], origin [7], and growing conditions of the grapes [8]. In view of the increasing interest in resveratrol, various analytical methods were developed for the measurement of resveratrol in wine [9–11]. However, in some case, quantitation for resveratrol is influenced by the complex nature of the sample matrix. Therefore, it is necessary to develop a selective and practicable enrichment material for separation and determination of such an important bioactive compound.

Molecular imprinted polymers (MIPs) are functional porous materials with molecular-specific recognition sites to a particular target molecule [12,13]. They have been widely applied in the fields of catalysis [14], environmental analysis [15], pharmaceutical analysis [16], food analysis [17], chromatography [18], chemical sensors [19], and solid-phase extraction (SPE) etc. [12,13,20]. MIPs as SPE sorbents have attracted considerable attention for being able to selectively recognize the target molecules from a mixture of chemical species. With the development of molecular imprinted-SPE (MISPE), a technique that is based on magnetic polymer has received increasing attention. The magnetic MIPs (MMIPs) can be dispersed into the solution directly and then easily separated from the matrix using an external magnetic field without additional centrifugation or filtration. The MMIPs, which have already been prepared in some works [21–23], also display higher adsorption ability and excellent recognition selectivity.

The potential risk for leakage of the residual template molecules during the adsorption process is a common issue for the MIPs including MMIPs in SPE. To completely avoid the interference from residual template, the structural analogue of the target molecules as dummy template was used to prepare MIPs [24]. In the preparation process, the interaction of template molecules with functional monomer, and the conformation effects between template molecules and rebinding sites may affect the properties of

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Abbreviations: MIPs, molecularly imprinted polymers; MISPE, molecularly imprinted solid-phase extraction; MMIPs, magnetic MIPs; MNIPs, magnetic non imprinted polymers; RH, rhapontigenin.

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the MIPs [25]. Therefore, the screening of the dummy template is important to obtain proper affinity and sufficient recovery of MIPs. The dummy template of MIPs should not interfere with the analysis of target molecule, either.

MISPE was applied to selectively extract and determine resveratrol in previous work, in which resveratrol was used as the template molecule [26–30]. In this study, it was the first attempt to synthesize the dummy template MMIPs for the recognition of resveratrol in wine sample. Especially, rhapontigenin (RH) was used as the dummy template for the preparation of MMIPs using surface molecular imprinting technique with a super paramagnetic core–shell nanoparticle as the supporter. The characterization, adsorption capacity and selectivity of MMIPs and magnetic non imprinted polymers (MNIPs) were investigated. The resveratrol recognition in real wine samples was realized by using MMIPs as SPE sorbents with high selectivity and good recovery.

2. Experimental

2.1. Reagents and apparatus

Rhapontigenin (RH), desoxyrhapontigenin, desoxyrhaponticin, and rhaponticin were provided by our lab [31]. Resveratrol and protocatechuic acid were provided by the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Ethylene glycol dimethacrylamide (EGDMA), 2,2′-azobisissobutyronitrile (AIBN), 3-methacryloxypropyltrimethoxysilane (MPS), acrylamide (AM), and methacrylic acid were obtained from Alfa Aesar (Tianjin, China). Ferric chloride ($FeCl_3·6H_2O$) and ferrous chloride ($FeCl_2·4H_2O$) were purchased from Beijing Chemicals Corporation (Beijing, China). Anhydrous toluene and ethanol were purchased from Lianlong Bohua Pharmaceutical Chemical Co., Ltd. (Tianjin, China). Chromatographic grade methanol and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Tetraethoxysilane (TEOS), isopropanol, acetic acid, ammonium hydroxide and the other chemicals were supplied from Tianjin Chemical Reagent Co. (Tianjin, China). Deionized water (18 MΩ cm) was prepared with a water purification system (Shanghai, China). The following wines were obtained from a local wine shop (Gansu, China): Qilian ice white wine (Qianlian, Gansu), Mogao dry red wine (Wuwei, Gansu), red wine (Yantai, Shandong). All the solutions used for HPLC were filtered through a 0.45 μm filter before use.

Scanning electron microscopy (SEM) images were obtained via JEM-5600LV scanning electron microscope (Tokyo, Japan). The Fourier transform infrared (FT-IR) spectra were obtained via a Nicolet Nexus-670 FT-IR spectrometer. The wave numbers of FT-IR measurement range were controlled from 500 cm^{-1} to 4000 cm^{-1}. The magnetic properties were measured using Lake Shore 7304 vibrating sample magnetometer (VSM) (Lakeshore, USA). X-ray diffraction (XRD) pattern were carried out by an X-ray diffraction using Cu-Ka1 radiation (PANalytical X'Pert, Holland). Sample analysis was performed by using liquid chromatographic system equipped with Agilent 1200 HPLC system and diode array detection (DAD) system. The analytical column was a 250 mm × 4.6 mm, 5 μm C_{18} column (Agilent, USA). The mobile phase consisted of acetonitrile and water (65:35, v/v) with a flow rate at 1.0 mL min^{-1}. DAD monitoring was at 321 nm for resveratrol [29].

2.2. Preparation of MMIPs

The preparation protocol is shown in Fig. 1. At first $Fe_3O_4@SiO_2$–MPS nanoparticles were prepared. The Fe_3O_4 particles were prepared using chemical co-precipitation according to Zhang et al. with small modifications [32]: 15 mmol $FeCl_3·6H_2O$ and 10 mmol $FeCl_2·4H_2O$ were dissolved in 80 mL of deoxygenated water stirring at 300 rpm under nitrogen gas. Ammonium hydroxide solution (28%, weight percent) of 50 mL was dropwise added into the clear yellow solution. With the addition of ammonium hydroxide solution, the solution turned black. The reaction was maintained at 80 °C for 30 min. The black precipitates obtained (Fe_3O_4 nanoparticles) were collected and washed repeatedly with deionized water until the pH of the washings became neutral and finally dried under vacuum at 60 °C for 24 h. Then the Fe_3O_4 particles were modified with SiO_2 according to the work of Zeng et al. [21]. The obtained $Fe_3O_4@SiO_2$ nanoparticles were dried under vacuum at 60 °C, and then modified with MPS-introduced polymerizable double bonds [33]. Briefly, 1 g of $Fe_3O_4@SiO_2$ nanoparticles were dispersed in 50 mL of anhydrous toluene containing 5 mL of MPS, and the mixture was reacted with reflux at 90 °C for 24 h under dry nitrogen. The products were collected and washed with toluene and ethanol for several times. Finally, surface-modified magnetic particles ($Fe_3O_4@ SiO_2$–MPS nanoparticles) were dried under vacuum at 60 °C.

The MMIPs were prepared via surface-imprinted polymerization method as follows: RH (0.1 mmol) as the dummy template and AM (0.5 mmol) as the functional monomer were dissolved in 20 mL acetonitrile and stored in dark for 12 h at room temperature. Then, 100 mg of $Fe_3O_4@SiO_2$–MPS nanoparticles was added into the mixture, and stirred for 2 h. Subsequently, cross-linker EGDMA (3.0 mmol) and initiator AIBN (20 mg) were added into the system and the mixture was degassed in an ultrasonic bath for 15 min. After filled with nitrogen gas for 10 min to remove oxygen, the polymerization was performed at 60 °C with nitrogen protection for 24 h. The MMIPs were collected magnetically, and washed by a mixture of methanol/acetic acid (9:1, v/v) to remove the templates and then washed by methanol until no RH absorption was detected by HPLC. Finally, the particles were dried in vacuum. The MNIPs were prepared by the same method as MMIPs without the addition of template.

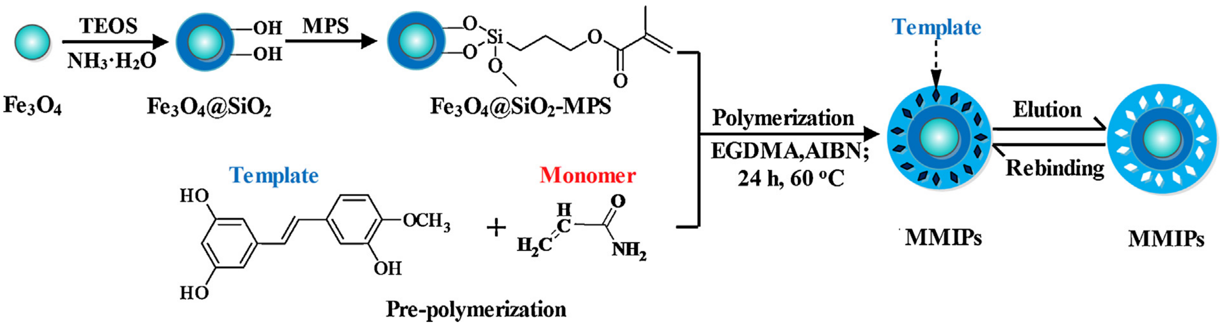

Fig. 1. Schematic representation of preparation for MMIPs.