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Novel dual functional monomers based molecularly imprinted polymers for selective extraction of myricetin from herbal medicines

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ABSTRACT

Herein, novel dual functional monomers based molecularly imprinted polymers (MIPs) were successfully prepared and used to extract myricetin from *Carthamus tinctorius* L., also named safflower (family, Compositae) and the flower of *Abelmoschus manihot* (Linn.) Medicus (family, Malvaceae). The polymers were prepared using myricetin as template, 4-vinylpyridine (4-VP) and glycidyl methacrylate (GMA) as dual functional monomers, ethylene glycol dimethyl acrylate (EGDMA) as cross-linker and methanol-acetonitrile (1:2, v/v) as solvent, respectively. Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were applied to characterize the polymers. Further, the adsorption and selectivity experiments of MIPs were evaluated. The results revealed that MIPs showed high adsorption ability and selectivity toward myricetin. Finally, MIPs were employed as adsorbents for solid phase extraction (SPE) of myricetin from safflower and the flowers of *A. manihot* (Linn.) Medicus. Further analysis was conducted by using high performance liquid chromatography-diode array detection (HPLC-DAD). The recovery of mrricetin in safflower and in the flowers of *A. manihot* ranged from 79.82% to 83.91%, 81.50% to 84.32%, respectively. These results indicated that MIPs can be applied to the extraction and separation of myricetin from various complex matrixes.

1. Introduction

In recent years, the pharmacological components of herbal medicines, such as flavonoids, alkaloids, terpenoids, have been widely used to cure diseases [1,2]. It is reported that these compounds are expected to become the potentially therapeutic constituents in the future [3,4]. Myricetin (3,3,4,5,5,7-hexahydroxyflavone), a dietary flavonoid found in many herbal medicines, is identified as active constituent in *Carthamus tinctorius* L. (also named safflower) [5] and *Abelmoschus manihot* (Linn.) Medicus [6]. Recently, myricetin has gained much attention due to its positive health effects. Emerging evidence clearly indicates that myricetin is an active constituent that can inhibit platelet aggregation [7]. Moreover, it is proved to be a natural class B GPCR agonist and a α-glucosidase inhibitor for the treatment of type 2 diabetes [8,9]. In addition, myricetin also has other activities such as anti-inflammatory and anti-cancer activities [10,11]. Considering that myricetin demonstrates so many pharmacological activities, it is the focus of many research activities to develop an effective method for the enrichment and purification of myricetin from complex herbal medicines.

However, myricetin usually exists with low concentration in herbal medicines and the constituents of herbal medicines are complex, it is difficult to separate and purify it. In recent years, many efforts have been made to separate myricetin from herbal medicines by using thin-layer chromatography [12], column chromatography [13] and high-speed counter-current chromatography [14]. In these methods, the most common used one is column chromatography. Although this method can effectively separate myricetin, it usually consumes large time and large quantities of chemical organic solvents for the repetitive separating processes [15]. What's more, the separating process is influenced by the complex sample matrix, resulting in the poor selectivity. Therefore, an effective method which consumes less chemical organic solvents, takes less time and has high selectivity for the separation of myricetin needs to be developed.

Molecularly imprinted polymers (MIPs) are porous materials which prepared by copolymerizing template, functional monomer and cross-linker [16]. Due to the special binding sites in polymers, MIPs demonstrate high selectivity toward target molecules [17]. In recent years, molecularly imprinted-solid phase extraction (MISPE) method has received much attention due to its high selectivity and high

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adsorption capacity toward target compounds [18,19]. Moreover, it possesses many other advantages such as simple extraction process and less solvent consumption [20]. Therefore, MISPE method has already been widely used to selectively extract target molecules from complex samples such as herbal medicines [21,22], food samples [23], environmental samples and biological samples [24]. Thus, MISPE method can be applied to the separation and extraction of myricetin from herbal medicines.

As we all knows, adsorbents play the most important role in the extraction of target compounds in MISPE. It is crucial for extraction performance that whether the MIPs demonstrate high selectivity or not. In previous studies, several methods have already used to prepare myricetin based MIPs. Zhong's group [25] has synthesized silica microspheres supported MIPs for the extraction of myricetin from *Ampelopsis grossedentata* extract. Although they have proposed an effective way to extract myricetin, the imprinted factor (IF) was only 2.10, demonstrated that the selectivity of MIPs was not particularly ideal for the extraction of myriceitn from complex matrix. Xiao and et al. [26] have also successfully prepared myricetin based MIPs by using silica microspheres as the support materials with IF value 2.00, even lower than the MIPs prepared by Zhong's group. In addition, it can be easily seen that the preparation procedures of the above mentioned MIPs are complex and need to consume much time. Therefore, it is necessary to develop a simple and convenient method to prepare myricetin based MIPs which own high selectivity and high adsorption capacity.

Precipitation polymerization is the most widely used technique for obtaining spherical imprinted particles, and the prepared particles are suitable adsorbents used in SPE [27,28]. Wang and et al. [29] synthesized podophyllotoxin based MIPs by using microwave heating initiated precipitation polymerization. The MIPs were successfully used as the adsorbents of SPE to extract of podophyllotox in three kinds of traditional Chinese medicines. In our previous studies, nano-sized luteolin based MIPs by precipitation polymerization were successfully synthesized for solid phase extraction of luteolin from *Chrysanthemum morifolium* Ramat [30]. To our best knowledge, in order to obtain high adsorption, high selectivity and stable spherical MIPs by using precipitation polymerization, synthetic parameters need to be optimized [31]. Functional monomer, one of the synthetic parameters which affect the binding properties and selectivity of MIPs, has been proven to be the bridge between template molecules and crosslinkers [32]. Suitable functional monomers can not only effectively interact with template molecules, but also can interact with crosslinkers, and result in stabilizing the spherical structure of the MIPs. In recent years, glycidyl methacrylate (GMA) has gained much attention. It is reported that GMA and ethylene glycol dimethyl acrylate (EGDMA) usually used together to prepare the stable spherical polymers [33–35]. The poly (GMA-*co*-EGDMA) also can interact with other compositions and stabilize the structure of the polymers [36]. Moreover, when the epoxy ring in GMA is opened, it can provide active groups such as hydroxyl, and the hydroxyl can interact with template molecules [37]. Thus, MIPs which prepared by using GMA as co-functional monomer, EGDMA as crosslinkers may demonstrate high capacity, high selectivity and stable spherical structure.

In present work, novel MIPs were successfully synthesized by precipitation polymerization using myricetin as template molecule, 4-VP and GMA as dual functional monomers, EGDMA as cross-linker and methanol-acetonitrile (1:2, v/v) as solvent, respectively. Furthermore, fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were employed to characterize the MIPs. The adsorption performances of MIPs, containing adsorption capacity and selectivity, were explored systematically. Finally, the myricetin based MIPs were applied as adsorbents of SPE. And the MISPE were successfully applied to enrich and extract myricetin from the extracts of safflower and flowers of *A. manihot*, respectively.

2. Materials and methods

2.1. Materials and regents

Myricetin and diosmetin (purity > 98%) were obtained from PUSH Biotechnology Co., Ltd. (Sichuan, China). Isorhamnetin and ombuin were self-separated and purified (purity > 98%, the preparation process was shown in supporting information). Safflower and the flowers of *A. manihot* were purchased from Lotus pond traditional Chinese medicine market in Chengdu (Sichuan, China), authenticated by professor Kailian Zhang (Southwest Medical University). Acrylamide (AM), 4-VP, methacrylic acid (MAA), EGDMA, GMA, 2, 2-azobisisobutyronitrile (AIBN), the chromatographic grade methanol and acetonitrile were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Instruments

The HPLC analysis was conducted on an Agilent 1260 Series liquid chromatograph system (Agilent Technologies, USA). And the Agilent SB-C18 column (250 × 4.6 mm, i.d., 5 μm) were used for the analysis. The morphological evaluation was analyzed by a JSM-7600F field emission scanning electron microscope (Japan Electronics Co. Ltd., Japan). IR affinity-1R fourier transform near infrared spectrometer (Shimadzu, Japan) was used to obtain FT-IR spectra.

2.3. Synthesis of the MIPs and NIPs

Myricetin based MIPs and non-molecularly imprinted polymers (NIPs) were synthesized by a precipitation polymerization method. Briefly, myricetin (0.2 mmol) and functional monomer (4-VP, 1.2 mmol) were firstly mixed in methanol-acetonitrile (1:2, v/v, 30 mL) at room temperature. The above mixed solution was continuously stirring for 5 h for pre-polymerization. After that, the cross-linker (EGDMA, 1.9 mL), co-functional monomer (GMA, 0.164 mL) and initiator (AIBN, 60 mg) were added. The solution was ultrasonic for 10 min and degassed with nitrogen for 10 min to remove oxygen. Then, the reaction mixture was conducted at 60 °C under N_2 protection for 24 h with a stirring speed of 200 rmp. Finally, the polymers were collected by centrifugation and dried under vacuum at 50 °C. For the elution of templates, methanol/acetic acid (8:2, v/v) solution was used to wash MIPs under soxhlet extraction until no myricetin was detected in the eluent. After extraction, the polymers were washed by methanol to remove acetic acid. Finally, the obtained polymers were dried under vacuum at 50 °C. The preparation process of MIPs was shown in Scheme 1a. The non-imprinted polymers (NIPs) were also synthetized using the same method but without the addition of myricetin.

2.4. Characterization of the MIPs and NIPs

Morphology of polymers was performed by SEM. Before the SEM experiments, all samples were coated with a thin layer of gold film under vacuum. The functional groups of the MIPs and NIPs were investigated by FT-IR, and the samples were ground with anhydrous KBr and the spectra recorded between 4000 and 500 cm^{-1}.

2.5. Adsorption experiments

In order to investigate the binding properties of the prepared MIPs, static and dynamic adsorption experiments were conducted. The static adsorption test were investigated by mixing MIPs or NIPs (15 mg) with 3 mL of methanol-acetonitrile (1:2, v/v) solution containing different concentrations (1–400 μg/mL) of myricetin. Then, the mixtures were shaken at 120 rmp for 5 h under ambient temperature. After that, the MIPs or NIPs were separated from solution by centrifugation at 11000 rpm for 10 min. The supernatants were measured using HPLC