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Molecularly imprinted polymers with novel functional monomer for selective solid-phase extraction of gastrodin from the aqueous extract of *Gastrodia elata*

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ABSTRACT

Molecular imprinted polymers (MIPs) with high selectivity and affinity to gastrodin in water were designed using allyl 2,3,4,6-tetra-*O*-acetyl-glucopyranoside (TAGL) and 1,2,3,4,5-pentafluoro-6-vinylbenzene (PFVB) as novel functional monomers. Binding characterization of pre-polymerization complexes was researched by nuclear magnetic resonance (NMR) and the MIPs were characterized by scanning electron microscopy (SEM). The properties involving adsorption isotherm, adsorption kinetics and selective recognition capacity were evaluated. The MIPs/TAGL exhibited good site accessibility in which it only took 30 min to achieve adsorption equilibrium and highly selective recognition for the template. Furthermore, the performance of the MIPs/TAGL as solid phase extraction material was investigated in detail and hot water at 50 °C served as the eluting solvent. Pure gastrodin with the recovery of 76.6% was obtained from the aqueous extract of *Gastrodia elata* roots.

1. Introduction

Gastrodia elata Bl. (*G. elata*) is a traditional Chinese medicinal herbal drug, used for the treatment of convulsions and epilepsy [1]. Gastrodin (GAS) is the main bioactive component of *G. elata* and the content of GAS in *G. elata* is 1.97% [2,3]. GAS has sedative [4] and anticonvulsant actions [5], neuroprotective effect [6], facilitating memory consolidation and retrieval [7], and antioxidant and free radical scavenging activities [1]. Due to its biological activities, many researches on selective extraction and determination of GAS from natural sources have been explored using high-speed counter-current chromatography (HSCCC) [8] and high performance liquid chromatography (HPLC) [9,10]. However, these separation procedures are time-consuming owing to their inefficient affinity and selectivity. Separation procedures of GAS are environmental hazardous because they consume large quantities of chemicals and release toxic effluent. Thus it is necessary to develop an efficient method to separate and enrich GAS from natural sources.

Among the extraction techniques, solid phase extraction on molecularly imprinted polymers (MISPE) is an efficient approach for purification of analytes from complex matrices and the preconcentration of the samples, and it is gaining considerable interest in environmental, clinical, and food analysis [11–15]. Molecularly imprinted polymers (MIPs) are man-made porous materials with specific selectivity toward a given analyte or a group of structurally related species [16–29]. The widespread acceptance of MISPE means it has become the most frequently used method to separate and enrich sample from natural sources.

However, previously developed MIPs that target small organic molecules are normally only compatible with organic solvents, and they mostly fail to show specific template binding in aqueous solution [30]. To some extent, this is because inter-molecular reactions between the template and the functional monomer cannot be formed easily in aqueous solution. Moreover, due to the hydrophobic nature of the polymer, non-specific adsorption is often encountered under aqueous conditions [31]. Water-soluble compounds, such as GAS (the solubility of GAS was more than 300 mg mL^{-1} [32]), exhibit notoriously poor solubility in a variety of

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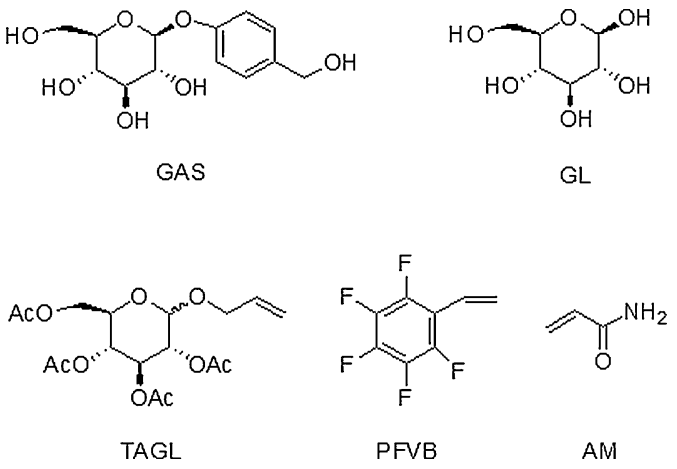

Fig. 1. Chemical structures: gastrodin (GAS), glucose (GL) and functional monomers.

organic solvents (including alcohol). So the design of MIPs directly capable of specifically recognizing the water-soluble compounds in aqueous conditions remains a formidable challenge owing to the complex nature of the sample matrices.

In a previous work, GAS-imprinted material has been prepared successfully by using the intermolecular forces of the hydrogen bonds between GAS and the grafted macromolecules [33]. In this study, GAS imprinted polymers were synthesized by employing 2,3,4,6-tetra-*O*-acetyl-glucopyranoside (TAGL) and 1,2,3,4,5-pentafluoro-6-vinylbenzene (PFVB) as novel functional monomers which were used firstly. After the evaluation of the imprinting efficiency in aqueous solution, a SPE protocol was optimized and applied for the selective extraction of GAS from *G. elata* roots.

2. Material and methods

2.1. Reagents and standards

G. elata roots are obtained from Shandong, China. 1,2,3,4,5-Pentafluoro-6-vinylbenzene (functional monomer), ethylene glycol dimethacrylate (EGDMA, cross linker), and 2,2-azoisobutyronitrile (AIBN, initiator) were purchased from Aladdin chemistry Co. Ltd (Shanghai, China). Gastrodin (≥98%, template) was supplied by Xi′an Guanyu Bio-tech Co., Ltd. All solvents were reagent grade or HPLC-grade and used without further purification. 2,3,4,6-Tetra-*O*-acetyl-glucopyranoside (functional monomer) was synthesized as described in the literature [34]. The chemical structures of compounds are shown in Fig. 1.

2.2. Synthesis of molecularly imprinted polymers

In 10 mL glass tube, repolymerization solutions with a molar ratio template: functional monomer: cross-linker 1:2:4 were prepared by dissolving 0.3 mmol of template in 2 mL dimethyl sulfoxide. Then, 0.6 mmol functional monomer, 1.2 mmol cross-linker and 5 mg AIBN were added to the mixture. The tube was sparged with nitrogen, sonicated for 15 min, thermo-polymerized under a nitrogen atmosphere for 24 h at 60 °C. The resultant bulk rigid polymers were grounded using a manual mortar and then wet-sieved through a 35–45 μm metal sieve. The sieved particles were washed with methanol/acetic acid (9:1, *v*/*v*) under Soxhlet extraction until no template was detected by HPLC in the extract and then washed with methanol till neutral. Fine particles were removed by suspended in acetone. The obtained polymer particles were dried under vacuum at 60 °C. For comparison, the nonimprinted polymer was also prepared using an identical procedure, but without the addition of GAS.

2.3. ^1H NMR studies

For this work, GAS and the functional monomer were in an equivolume mixture of D_2O and *d*-DMSO. The solution of GAS was mixed with the functional monomer in a molar ratio of 1:2.

2.4. SEM analysis

Scanning electron microscopy images of the surface morphology of imprinted and non-imprinted polymers were recorded on a SWPRATM55 microscope (Carl Zeiss, AG, Germany) on a gold-sputtered sample.

2.5. Selectivity study

The MIPs and NIPs were equilibrated with an aqueous mixture of 0.10 mmol L^{-1} of GAS and the structural analogue glucose (GL) for 6 h, respectively. The residual concentration of GAS and glucose was analyzed by HPLC after centrifugation.

2.6. Adsorption isotherm

To investigate the adsorption ability of MIPs for GAS, 10 mg of polymers were added to 2.5 mL of GAS working solution at different concentrations (0.05–0.5 mmol L^{-1}). The suspensions were placed on a SHZ-82 Vapour-bathing Constant Temperature shaker (Jintan, China) for 240 min at 25 °C and then centrifuged at 4000 rpm for 15 min. The supernatant was measured for free GAS by HPLC. A similar procedure was performed for NIPs particles. The adsorption amount (Q_e, mg g^{-1}) was calculated by the following formula:

$$Q_e = (C_i - C_e) \times v \times \frac{M}{m} \quad (1)$$

where C_i (mmol L^{-1}) is the initial concentration of GAS, C_e (mmol L^{-1}) is equilibrium concentration of GAS in solution, V (mL) is sample volume, m (mg) is the mass of the polymer and M (g mol^{-1}) is the molar mass of GAS.

The adsorption isotherms were described by the Langmuir equation and Freundlich equation [35]. The linearized forms of the two isotherms are:

$$\frac{C_e}{Q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad (2)$$

$$\ln Q_e = \frac{\ln C_e}{n} + \ln K_F \quad (3)$$

where C_e (mmol L^{-1})and Q_e (mg g^{-1}) are the equilibrium concentration and the amount of GAS adsorbed at equilibrium, respectively. q_m and K_L are theoretical maximum adsorption capacity and Langmuir equilibrium constant, respectively. K_F and n are the Freundlich constants, which are indicators of adsorption capacity and adsorption intensity. According to the Freundlich theory, n can be used to determine whether the adsorption is favorable. When $n > 1$, it is favorable adsorption; when $n = 1$, it is linear adsorption; when $n < 1$, it is unfavorable adsorption [36].

2.7. Adsorption kinetics

The kinetic study was performed with 0.1 mmol L^{-1} GAS standard solutions and 10 mg of MIPs for different periods of time (10–240 min). The mixture was shaken at 25 °C and the adsorption amount was determined by HPLC. The Lagergren's pseudo first order and pseudo second order models were used to describe the