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Water-compatible dummy molecularly imprinted resin prepared in aqueous solution for green miniaturized solid-phase extraction of plant growth regulators

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ABSTRACT

A water-compatible dummy molecularly imprinted resin (MIR) was synthesized in water using melamine, urea, and formaldehyde as hydrophilic monomers of co-polycondensation. A triblock copolymer (PEO-PPO-PEO, P123) was used as porogen to dredge the network structure of MIR, and *N*-(1-naphthyl) ethylenediamine dihydrochloride, which has similar shape and size to the target analytes, was the dummy template of molecular imprinting. The obtained MIR was used as the adsorbent in a green miniaturized solid-phase extraction (MIR–mini-SPE) of plant growth regulators, and there was no organic solvent used in the entire MIR–mini-SPE procedure. The calibration linearity of MIR–mini-SPE–HPLC method was obtained in a range 5–250 ng mL^{-1} for IAA, IPA, IBA, and NAA with correlation coefficient (r) $\geq$0.9998. Recoveries at three spike levels are in the range of 87.6–100.0% for coconut juice with relative standard deviations $\leq$8.1%. The MIR–mini-SPE method possesses the advantages of environmental friendliness, simple operation, and high efficiency, so it is potential to apply the green pretreatment strategy to extraction of trace analytes in aqueous samples.

1. Introduction

Molecular recognition is a fundamental and important biological mechanism ubiquitous in nature (including antibody/antigen recognition in immune system, enzymatic catalysis, signal transduction, and nucleic acid interactions such as replication, transcription, and translation). The recognition mechanism relies on the complex between receptor and substrate, which Fischer first described as the "lock and key model" over a century ago [1,2]. However, not only these natural receptors are expensive and difficult to produce, but also their lifetime and applicability are limited. Molecular imprinting is a promising technique that was developed to overcome these limitations. Molecularly imprinted polymers (MIPs) are artificial receptors made by imprinting template molecules into polymer matrices followed by the removal of template to generate permanent template grooves [3]. With the merits of high affinity, good stability, and low cost, MIPs have been successfully applied to the areas of selective adsorbent [4,5], artificial enzyme [6,7], chemical sensor [8], and pharmacy [9]. Until now, most MIPs are made from fat-soluble reagents in organic solvents and show poor specific selectivity in aqueous solution [10–12]. However, water is the matrix of most environmental and biological samples, and the incompatibility with aqueous solution significantly limits MIPs' applications [13]. Besides, the large amount of organic solvents in the preparation of MIPs is quite harmful to environment and people's health [14,15]. So, the exploration of water-compatible MIPs and green preparation process is imperative.

The presence of hydrophobic groups on MIPs is considered to be the main reason for their water-incompatibility. To suppress hydrophobically-driven nonspecific interactions, the use of hydrophilic monomers and preparation strategies in aqueous solution are effective methods [16]. Hydrophilic resins are synthesized in water with different hydrophilic monomers, which can be introduced in molecular imprinting technique to produce molecularly imprinted resins (MIRs) [17]. Hydrogen bonding is the most common interaction between templates and functional monomers for non-covalent molecular imprinting. However, it is easily destroyed in aqueous media, because solvent molecules could compete with templates for functional monomers [18]. Ionic interaction is in theory stronger than hydrogen bonding, so it should be more resistant to the interference of water molecules in molecular

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imprinting and recognition processes [19]. It was reported that hydrophilic comonomers of melamine–urea–formaldehyde has plenty of amino groups, secondary amino groups, and tertiary amino groups [20], and these groups can be positively charged to perform ion exchange interaction with the molecules which have negative charges in a certain pH range. Thus, it can enhance the affinity between MIRs and target molecules, making molecular imprinting and recognition efficient.

Plant growth regulators, which manage almost all aspects of plant life, can exert plant physiological responses and serve as mediators of endogenous progress to regulate and optimize plant growth [21]. Besides, the use of plant growth regulators in agriculture has led to the residues in fruits and vegetables, which show potential toxicity to humans and animals [22]. So the extraction and separation strategies of these compounds have attracted interest recently. Solid-phase extraction (SPE) [23], QuEChERS extraction [24], dispersive liquid–liquid microextraction [25], matrix solid-phase dispersion [26], and liquid–liquid–liquid extraction [27] have been applied to separate plant growth regulators, but organic solvents, such as methanol, acetonitrile, chloroform, dichloromethane, acetone, and phenetole, which are harmful to environment and human beings [28], have been utilized in these methods. Miniaturized SPE has been used due to its ability to isolate and enrich trace level of analytes, and it has high versatility and low cost as a sample pretreatment step before instrumental analysis [29]. Although the amount of organic solvents could be reduced using miniaturized devices, the waste and emissions of organic solvents threaten people's health [30]. Therefore, the development of green sample preparation methods is desired to overcome the potential harms of organic solvents.

In the present work, a water-compatible dummy molecularly imprinted resin was prepared in water with hydrophilic comonomers (melamine, urea, and formaldehyde). It showed special molecular recognition to the structural analogues of template in aqueous matrices, and it eliminated the effect of template leakage on quantitative analysis by *N*-(1-naphthyl) ethylenediamine dihydrochloride used as the dummy template [31]. The MIR was applied as the adsorbent of green MIR–mini-SPE, in which none of organic solvents were employed. The green MIR–mini-SPE method was successfully applied for the selective extraction of plant growth regulators in fruit juice samples.

2. Experimental

2.1. Materials

Indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), and PEO-PPO-PEO (P123) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Melamine, *N*-(1-naphthyl) ethylenediamine dihydrochloride, and tetrabutylammonium bromide (TBAB) were purchased from Kermel Chemical Co., Ltd. (Tianjin, China). Urea, formaldehyde solution (37%), ethanol, and glacial acetic acid were ordered from Huadong Chemical Reagent Co., Ltd. (Tianjin, China). Methanol was obtained from Xingke Biochemistry Co., Ltd. (Shanghai, China). All water used was double-deionized and filtered with a 0.45-μm filter membrane.

2.2. Instrumentation and conditions

The morphological evaluation was performed by KYKY-2800B scanning electron microscopy (FEI, Hillsboro, USA). An FTIR-8400S Fourier transform infrared spectrometer (Shimadzu, Kyoto, Japan) was employed to obtain the infrared spectra of MIRs in a range of 400–4000 cm^{-1}. The BET surface areas and pore volumes of MIRs were tested using a Micromeritics Tristar II 3020 analyzer (Micromeritics, Norcross, GA, USA). High-performance liquid chromatography (HPLC) analysis was carried out by a Shimadzu HPLC system equipped with two LC-20AT solvent delivery units, an SUS-20A gradient controller, and an RF-20A fluorescence detector (Shimadzu, Kyoto, Japan). An LC solution workstation (Shimadzu, Kyoto, Japan) was used to control the system and process data. The C_{18} column (250 mm × 4.6 mm I.D., 5 μm) was obtained from Agela Technologies Co., Ltd. (Tianjin, China). The mobile phase was methanol–water (6:4, v/v, containing 0.5% acetic acid) with a flow rate of 1.0 mL min^{-1}. The excitation wavelength and emission wavelength of RF-20A detector were set at 254 nm and 338 nm, and the injection volume was 20 μL.

2.3. Synthesis of MIR adsorbent

Melamine (12.6 g), urea (18 g), formaldehyde solution (50 g, 37%), and P123 (1 g) were mixed in a flask. The mixture (pH 10) was kept stirring at 45 °C for 1 h, and then heated to 85 °C for 2.5 h. When the mixture cooled to 45 °C, ethanol (10 mL) was added dropwise to the reaction system at 45 °C for 1 h. After the mixture cooled to room temperature, it became a transparent hydrogel. The hydrogel (30 g) and *N*-(1-naphthyl) ethylenediamine dihydrochloride (0.15 g) were mixed in an acidic condition (pH 4) at 50 °C to form a solid resin, and the resultant solid was placed in an oven for 24 h at 60 °C to ensure complete dryness. The product was ground and sieved with a 0.054-mm aperture sieve, and the small particles were removed by flotation in water. In order to remove the template, the particles were washed with ethanol–acetic acid (9:1, v/v) and ethanol successively until no template was detected by HPLC, and then dried under vacuum. Non-imprinted resin (NIR) was prepared in a similar manner except the addition of *N*-(1-naphthyl) ethylenediamine dihydrochloride in the curing procedure of MIR. The schematic illustration of the synthesis of MIR is shown in Fig. 1.

2.4. Preparation of coconut juice samples

Different varieties of fresh coconuts were purchased from Hainan, China. Lead acetate solution (0.25 mL, 16%) was added into coconut juice (5 mL) to precipitate proteins, and the white suspension was centrifuged at 12000 rpm for 15 min, after which the supernatant was diluted with water (1:1, v/v) and adjusted to pH 4 by acetic acid for the MIR–mini-SPE procedure.

2.5. Procedure of MIR–mini-SPE

The MIR–mini-SPE device was constructed by an empty polypropylene cartridge (50 mm × 8 mm I.D.) and a 100-μL pipette tip with a syringe. The device and operating steps are shown in Fig. 2. MIR adsorbent (5 mg) was packed into the pipette tip, and degrease cotton was placed at both ends of MIR adsorbent to prevent the loss of MIR (7 mm in height). After the MIR–mini-SPE cartridge was preconditioned with water (1 mL, pH 4, adjusted by acetic acid), coconut sample solution (0.5 mL) was loaded into the cartridge, and then the analytes were eluted by TBAB aqueous solution (1.5 mol L^{-1}, 0.5 mL) at 35 °C. A syringe could be used to adjust the flow rate manually via drawing liquid from the thin end of pipette tip. The eluate was collected for HPLC analysis.

3. Results and discussion

3.1. Synthesis of MIR adsorbents

Four types of MIRs were prepared with hydrophilic monomers. These monomers were reacted to become a transparent hydrogel in an alkaline environment, and then the hydrogel could be solidified