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Jacket-free stir bar sorptive extraction with bio-inspired polydopamine-functionalized immobilization of cross-linked polymer on stainless steel wire

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ABSTRACT

Stainless steel wire (SSW) is a good substrate for stir bar sorptive extraction (SBSE). However, it is still a challenge to immobilize commonly used cross-linked polymers onto SSW. In this work, we present a new approach for immobilization of the cross-linked organic polymer onto SSW for jacket-free SBSE. A dopamine derivative was firstly synthesized; by introducing a mussel-inspired polydopamine process, a stable coating layer was finally generated on the surface of SSW. Secondly, the cross-linked polymer was synthesized on the polydopamine-modified SSW by using acetonitrile as the porogen, acrylamide (AA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker and 2,2′-azobis (2-methylpropionitrile) as the initiator. A diluted pre-polymerization solution was carefully prepared to generate a thin layer of the polymer. The prepared poly(EGDMA-AA)-modified stir bar showed high stability and good tolerance toward stirring, ultrasonication, organic solvents, and strong acidic and basic conditions. Morphology and structure characterization of coatings were performed by scanning electron microscopy and Fourier transform infrared spectra, respectively. The prepared poly(EGDMA-AA)-modified stir bar showed great extraction efficiency toward protoberberines, with enrichment factors of 19–42. An SBSE-HPLC method was also developed for quantitative analysis of protoberberines. The method showed low limits of detection (0.06–0.15 ng mL^{-1}), wide linear range (0.5–400 ng mL^{-1}), good linearity ($R \geq 0.9980$) and good reproducibility (RSD ≤ 3.60% for intra-day, RSD ≤ 4.73% for inter-day). The developed method has been successfully applied to determine protoberberines in herb and rat plasma samples, with recoveries of 88.53–114.61%.

1. Introduction

Sample pretreatment techniques are greatly useful for analysis of different kinds of compounds in complex matrices [1–3]. In recent years, polar compounds have been paid more and more attention for their wide presence in pharmaceutical, medical and biological samples [4,5]; however, the development of sample pretreatment methods for polar compouns are far behind of that for non-polar compouds.

Sorbent is one of the most important parts of a sample pretreatment technique, which usually determines the nature of the extraction process. Several materials have been developed as sorbents to extract polar compounds in complex matrices, such as organic polymers [6,7], metal oxides [8], ion exchange resins [9,10] and natural macromolecules [11]. Cross-linked organic polymers are used widely in separation science; the polymer can be synthesized conveniently by co-polymerization of functional monomers and cross-linkers in the presence of porogens. The availability of functional monomers and cross-linkers with variable properties makes the cross-linked organic polymers applicable to different kinds of compounds [12,13]. In addition, both polar and nonpolar compounds can be extracted by polymers [14,15]. Cross-linked organic polymers have been applied in sample pretreatment methods such as miniaturized solid-phase extraction [14], solid-phase microextraction (SPME) [7] and stir-bar sorptive extraction (SBSE) [13].

SBSE is regarded as a solventless sample preparation method, which shares similar principles of solid-phase microextraction (SPME) but shows better extraction capability [16]. It is important

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to develop new substrates and new sorbents for SBSE since limited types of stir-bars are commercially available [17]. Several new materials have been introduced into SBSE, such as graphene [18] and hydrophilic polymer [19]. Stir bars with glass jacket are widely used, but may not be stable enough because the glass jacket is relatively fragile. Meanwhile, stainless steel wire (SSW) is of easy accessibility, low cost, high strength, and can stir spontaneously under magnetic stirring apparatus, which is ideal for SBSE. In the previous work, we fabricated a jacket-free stir bar by modifying sorbents directly onto the SSW [18]. The fabricated graphene-SSW showed good stability and great extraction efficiency toward polycyclic aromatic hydrocarbons. However, other materials cannot be immobilized by the same method, and only hydrophobic compounds can be extracted by graphene-SSW. Therefore, new modification procedures are needed in order to use jacket-free SBSE for polar compounds such as polar drugs.

In this work, we introduced the cross-linked polymer onto the SSW to prepare a new kind of stir bar, and applied it as jacket-free SBSE. Mussel-inspired polydopamine method was used to functionalize the stainless steel surface, where dopamine was derivatized with an acryloxy group to facilitate the binding with polymer. Three protoberberines were employed as the analytes. The cross-linked polymer was made of ethylene glycol dimethacrylate (EGDMA) and acrylamide (AA) according to our previous work [20]. The prepared stir bar was characterized by SEM and FT-IR. Extraction efficiency of the cross-linked polymer-modified SSW stir bar was investigated and an SBSE-HPLC method has been developed for sensitive determination of protoberberines in herb and rat plasma samples.

2. Experimental

2.1. Chemicals and materials

Jatrorrhizine, palmatine, berberine and methyl acrylic acid propyl trimethoxy silane (MAPS) were obtained from Aladdin (Shanghai, China). Dopamine hydrochloride was provided by Sigma-Aldrich (MO, USA). EGDMA was obtained from Alfa-Aesar (Lancashire, UK). AA and 2,2′-azobis (2-methylpropionitrile) (AIBN) were purchased from Shanghai Reagent Factory (Shanghai, China). Acetonitrile (Tedia, OH, USA) was HPLC grade and water used was purified by Milli-Q system (MA, USA). Stainless steel wires were obtained from a local store.

2.2. Instrumentation

A Shimadzu 20A HPLC system (Tokyo, Japan), which consisted of two 20AD pumps, a six-port valve, a sample loop of 20 μL and a 20A UA detector was used for chromatographic analysis. A C-18 column (250 mm × 4.6 mm i.d.) with 5 μm particle size from GL Science (Tokyo, Japan) was used for separation. Mobile phase consists of acetonitrile and 10 mM KH_2PO_4 (32/48, v/v; 0.8 mL min^{-1}). The wavelength of UA detector was set at 345 nm and column temperature for HPLC separation was set at 30 °C. Data collection and handling was performed on a shimadzu LC Solution software.

For comparison, an HPLC-MS method was used. Agilent 1100 HPLC system was coupled on-line to a LC/MSD Trap SL Plus spectrometer (Agilent Corp, Waldbronn, Germany) equipped with an ESI source. The Auto MS operation parameters are presented as follows: positive ion mode; nitrogen drying gas, 8 L min^{-1}; nebulizer, 30 psi; gas temperature, 350 °C; mass range, *m*/*z* 100–1000. Selected-ion monitoring (SIM) mode was employed. Because protoberberine molecules are positively charged, the ions monitored were $[M]^+$. Detections were performed in SIM mode at *m*/*z* 338 for jatrorrhizine, *m*/*z* 352 for palmatine and *m*/*z* 336 for berberine.

Characterization was performed by a Thermo Nexus 470 FT-IR system (MA, UAS) and a Quanta 200 scanning electron microscope (SEM, FEI, Holand).

2.3. Preparation of poly(EGDMA-AA)-MAPS-PD-SSW

SSW (3 cm) was rinsed successively with deionized water, ethanol and deionized water under ultrasonication for 10 min. Dopamine hydrochloride (4 mg) and MAPS (50 μL) were dissolved in alcohol (2 mL). The solution was agitated with a vortex mixer for mixing and put into a water bath for reaction (80 °C, 6 h). Tris was added to adjust the pH of the solution to 8.5, then the SSW was immersed into the solution [18], the reaction was performed at room temperature with continuous stirring for 4 h. The obtained MAPS-PD-SSW was cleaned with alcohol (2 mL) under ultrasonication for 5 min and then was dried in the oven (60 °C, 2 h). For poly(EGDMA-AA) modification [20], the mixture of AA (36 mg), EGDMA (94 μL), AIBN (3 mg) and acetonitrile (1200 μL) was added to the MAPS-PD-SSW, which was put in a screw-cap vial. The screw-cap vial was placed under nitrogen for 10 min to remove dissolved oxygen. The vial was sealed and then put into a water bath for reaction (60 °C, 2 h). After washing with acetonitrile (2 mL) under ultrasonication for 5 min and drying in the oven (60 °C, 2 h), the poly(EGDMA-AA)-MAPS-PD-SSW was obtained.

2.4. SBSE procedures

The prepared poly(EGDMA-AA)-MAPS-PD-SSW was applied to extract three protoberberines in aqueous solutions. SBSE consisted of two steps. In the extraction step, analytes were extracted from the water samples. Sample solution of 20 mL (containing 10 mM Na_2HPO_4, pH 8) was added into a 50 mL beaker. The poly(EGDMA-AA)-MAPS-PD-SSW was put into the beaker, stirred for 2 h under magnetic stirring apparatus, at the rate of 600 revolutions per minute (rpm). In the desorption step, the poly(EGDMA-AA)-MAPS-PD-SSW was taken out of the beaker and put into a small tube with 200 μL acetonitrile; the tube was ultrasonicated for 2 min to desorb the analytes for HPLC analysis.

2.5. Sample preparation

Cortex phellodendri and rat plasma samples served as real samples. *Cortex phellodendri* (0.2 g) was cut into small pieces and put into a tube with 5 mL H_2O. After ultrasonication for 40 min for extraction, the mixture was centrifuged (10,000 rmp, 8 min) to get supernatant for future treatment. The supernatant was diluted 10 times with water. 50 μL of the diluted mixture was diluted to 20 mL with 10 mM Na_2HPO_4 (pH 8) and loaded for SBSE. For comparison, the same sample solution was injected directly into the HPLC system for analysis.

Rat plasma samples were obtained from the healthy female Sprague-Dawley (SD) rat (150-200 g) from the Experimental Animal Center of Wuhan University. *Cortex phellodendri* (0.5 g) powder was put into a tube with 4 mL H_2O and ultrasonicated for 40 min for extraction, and the extraction solutions was orally administrated to different rats, respectively. After 0.5 h, the eyeball blood was collected from one rat as the rat plasma sample (a). After 1 h, the eyeball blood was collected from another rat as the rat plasma sample (b). Blood samples were transferred to a heparinized eppendorf tube and centrifuged for 10 min at 10,000 rpm. After deproteinization and pH adjusting, the rat plasma samples were prepared for further studies. The sample of 1 mL was diluted to 20 mL with