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Molecularly imprinted stir bar sorptive extraction coupled with high performance liquid chromatography for trace analysis of sulfa drugs in complex samples

Zhigang Xu, Chaoyong Song, Yuling Hu, Gongke Li*

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China

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ABSTRACT

A novel sulfamethazine molecularly imprinted polymer (MIP)-coated stir bar for sorptive extraction of eight sulfa drugs from biological samples was prepared. The MIP-coating was about 20 μm thickness with the relative standard deviation (RSD) of 6.7% (n = 10). It was characterized by scanning electron microscope, infrared spectrum, thermogravimetric analysis, and solvent-resistant investigation, respectively. The non-imprinted polymer (NIP)-coating was used for comparison. The adsorptive capacity and selectivity of MIP-coating were evaluated in detail. The MIP-coating showed higher adsorption capability and selectivity than the NIP-coating. The saturated adsorption amount of the MIP-coating was 4.6 times over that of the NIP-coating in toluene. Sulfamethazine could be detected after the MIP-coated stir bar sorptive extraction even at a low concentration of 0.2 μg/L. The MIP-coating also exhibited selective adsorption ability to analogues of the template. A method for the determination of eight sulfa drugs in biological samples by MIP coated stir bar sorptive extraction coupled with high performance liquid chromatography (HPLC) was developed. The extraction conditions, including extraction solvent, extraction time, desorption solvent, desorption time and stirring speed, were optimized. The linear ranges were 1.0–100 μg/L and 2.0–100 μg/L for eight sulfonamides, respectively. The detection limits were within the range of 0.20–0.72 μg/L. The method was successfully applied to simultaneous multi-residue analysis of eight sulfonamides in spiked pork, liver and chicken samples with the satisfactory recoveries.

1. Introduction

Molecularly imprinted polymer (MIP) is a kind of synthetic material to generate the binding sites with a high affinity and selective recognition to the template molecule and its analogue compounds. Due to its advantages of high selectivity, easy preparation and low cost, it has been widely utilized as molecular recognition and separation materials in different fields, such as sensors [1], macromolecules and proteins recognition [2], chiral separation [3,4], drug delivery [5,6], sample pretreatment [7–9] and speciation analysis [10].

MIP is usually synthesized by covalent or non-covalent approaches. But the latter is the most common and flexible method since the template is easy to remove without chemical reaction [11,12]. In the application of MIP, it has been immobilized on different substrates for molecular recognition, such as magnetic beads [13,14], which can be separated easily by a magnet after extraction, and solid-phase microextraction (SPME) fiber [15–18], which could be coupled directly to high performance liquid chromatography (HPLC) for on-line analysis. But a method to further accelerate the adsorption equilibrium was necessary. Magnetic stirring is an efficient method to accelerate the adsorption equilibrium. But an additional stirrer may result in competitive adsorption. This problem can be solved by immobilizing the coating on a magneton. It was firstly proposed by Baltussen et al. to use a polydimethylsiloxane (PDMS) sorbent as the coating [19]. Some novel stir bars, such as "dumbbell-shaped" stir bar [20], rotating-disk [21] and stir rod [22] were developed. A MIP-coating coated on a commercial PDMS was also proposed for selective adsorption of monocrotophos [23]. To avoid the MIP-coating loss during stirring, improved extraction apparatus was also reported [24–26].

Sulfonamides, which belong to a group of antibacterial drugs, have been gained more and more concerns for the residues in food products and their potential carcinogenicity [27–29]. In some previous reports, molecularly imprinted polymers had been used as solid-phase extraction (SPE) for the selectively extraction of sulfonamides in various matrixes, such as milk [30,31], pork and chicken [32], pond water and fishes [33].

In this paper, a novel sulfamethazine molecularly imprinted polymer (MIP)-coated stir bar was prepared for the selective

* Corresponding author. Tel.: +86 20 84110922; fax: +86 20 84115107.
E-mail address: cesgkl@mail.sysu.edu.cn (G. Li).

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extraction of sulfonamides. The relationship between thickness and adsorption amounts was studied and the suitable thickness was selected. The MIP-coating was characterized and the extraction performance was investigated. The extraction conditions were optimized and a method for determination sulfonamides by MIP-coated stir bar sorptive extraction coupled with HPLC was developed. Spiked sample analysis was performed for the evaluation of MIP-coated stir bar sorptive extraction.

2. Experimental

2.1. Chemicals

Sulfamethazine, sulfachloropyridazine, sulfamethizole, sulfathiazole, sulfameter and sulfamethoxazole were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Sulfamerazine and sulfadiazine were purchased from Alfa Aesar (Lancaster, UK). Triazolone was obtained from Factory of Limin (Yancheng, China). Pyridine and N,N-dimethylaniline were obtained from Guanghua Chemical Plant (Shantou, China). Acetonitrile (HPLC grade) was obtained from LAB-SCAN (Bangkok, Thailand). Other chemicals were analytical pure. Glass capillary (1 mm diameter, 15 mm length) was purchased from West China University of Medical Sciences Instrument Plant. Water used for HPLC was doubly distilled and filtered through a 0.45 μm nylon filter.

2.2. Stir bar preparation

MIP-coated stir bar was prepared based on the method of our previous work [24,25]. The substrate should be silanized before polymerization. Subsequently, 185.0 mg sulfamethazine and 0.22 mL methacrylic acid (MAA) were dissolved in 10 mL methanol. The solution was mixed thoroughly and kept for 12 h at room temperature. Then 1.26 mL ethylene glycol dimethacrylate (EGDMA) and 75.0 mg azoisobutyronitrile (AIBN) were added. The mixture solution was degassed in an ultrasonic bath for 5 min. Then 1.5 mL solution was transferred into a test tube. Then a silylated glass capillary was inserted into the test tube and the polymerization was performed at 60 °C. The capillary was pulled out 2 h later. A suitable thickness can be got by repeating the procedure. A NIP-coated stir bar was prepared following the same procedures but without sulfamethazine in the synthesis. New coated stir bars were eluted by methanol–acetic acid (9:1, v/v) to remove the template until it could not be monitored by HPLC.

2.3. Coating characterization

The scanning electron micrography was obtained with an S-4300 scanning electron microscope (HITACHI, Japan). An AVATAR 330 Fourier transform infrared (FT-IR) spectrometer (Thermo Nicolet, USA) was used for the coating composition investigation. A thermal gravity (TG) analyzer (Netzsch-209, Bavaria, Germany) was used to evaluate the thermal stability of coatings. The solvent-resistant ability was also examined by immersing the MIP-coating in different polar solvents.

2.4. Stir bar sorptive extraction procedure

Extraction experiment was performed in a round bottom flask. The stirring speed was 500 rpm at room temperature. The extraction solution was 5 mL. After extraction, the stir bar was taken out and inserted in a 200 μL glass vial, desorbed with 150 μL methanol by ultrasonic bath for 10 min. Then 20 μL desorption liquid was injected for HPLC analysis.

2.5. Chromatographic conditions

Sulfonamides were determined by a LC-20AB (Shimadzu, Japan) with a UV detector at 270 nm and a C_{18} column (250 mm × 4.6 mm i.d., 5 μm packing, Dikma). The mobile phase was acetonitrile/1% (v/v) acetic acid (2:8, v/v) at the flow rate of 1.0 mL/min. The mixture of sulfonamides was separated by gradient elution at the flow rate of 1.2 mL/min. Acetonitrile phase was increased from 10 to 23% during 5 min, and held for 20 min.

2.6. Sample preparation

Pork, liver and chicken were selected for spiking sample analysis. 5 g of thinly sliced tissue of each sample was mixed with sulfonamides mixed standard solution. It was mixed thoroughly and kept for 0.5 h at room temperature. The spiking concentrations for each sulfonamide were set with three levels of 5.0, 10 and 25 μg/kg. The spiked sample was extracted by 10 mL dichloromethane in an ultrasonic bath for 10 min. The operation was repeated for another two times and the extraction solution was dried with the reduced pressure distillation. Then it was dissolved with 5 mL toluene for MIP-coated stir bar sorptive extraction. The NIP-coated stir bar sorptive extraction was used for comparison. The extracted solutions after reduced pressure distillation were also dissolved in 2 mL methanol by direct injection for comparison.

3. Results and discussion

3.1. Preparation of MIP-coated stir bar

The molecularly imprinted polymer was synthesized by co-polymerization. A suitable thickness of 20.1 μm with the relative standard deviations (RSDs) of 6.7% (n = 10) was obtained by reproducible method. Then a 1.7 cm magnetic core was inserted in a 2.3 cm glass capillary, which was coated with 2.0 cm coating. It was sealed by flame to generate a stir bar. The precision of stir bars was investigated to extract 20 μg/L sulfamethazine standard solution. The RSD was 3.3% (n = 4) in batch and 12.0% (n = 4) for inter-batch, respectively. The MIP-coating was eluted with methanol to remove absorbents. Both the MIP- and NIP-coatings could be used at least 40 times. It could be kept at least 6 months in a dryer.

3.2. Coating characterization

The morphological structure of sulfamethazine MIP-coating was investigated with the scanning electron micrographics. Fig. 1 shows the surface structure and pore structure of MIP- and NIP-coatings under the magnification of 2500 and 10,000. It was obvious that the MIP-coating surface was homogeneous and porous (Fig. 1(a) and (c)), whereas the NIP-coating appeared to consist of larger cluster units and less pores (Fig. 1(b) and (d)). The porous structure of MIP was beneficial to adsorb analytes.

The infrared spectra indicated that the NIP-coating (Fig. S1(a)) and MIP-coating (Fig. S1(b)) almost had the same absorption peaks. Fig. S1(b) and (c) shows the infrared spectra of MIP-coating after and before eluting template. There were no obvious differences except the absorption peak at 1597 cm^{-1}, which could be found in the infrared spectra of template (Fig. S1(d)) and corresponded to C=C stretching vibration in the benzene ring of sulfamethazine molecular. The results indicated that both MIP- and NIP-coatings had the same chemical constitution, and the template molecule only interacted with the functional monomer by hydrogen bond but did not take part in the polymerization.

The thermal stabilities of MIP- and NIP-coatings were investigated with the thermogravimetric analysis. The results indicated