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Short Communication

Rapid preparation of molecularly imprinted polymers by microwave-assisted emulsion polymerization for the extraction of florfenicol in milk

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ABSTRACT

In this study, we proposed a rapid and simple method for the preparation of molecularly imprinted polymers (MIPs) by emulsion polymerization. The polymerization process was accelerated by microwave heating, and the reaction time was greatly shortened. The obtained MIPs were spherical in shape and exhibited a uniform morphology. The MIPs with selectivity and high affinity to florfenicol were successfully applied as solid-phase extraction materials to extract and clean up the florfenicol in milk, followed by liquid chromatography–tandem mass spectrometry (LC–MS) analysis. The parameters affecting the performance of extraction and LC–MS analysis were evaluated. The detection limit of the method was 4.1 ng mL^{-1}. The relative standard deviations of intra- and inter-day were in the range of 3.5–4.7% and 3.9–7.5%, respectively.

1. Introduction

Florfenicol (FF), a fluorinated derivative of thiamphenicol, is a new generation of fenicol drugs [1,2]. Nowadays, due to the ban of the use of chloramphenicol in animals, FF is widely used as chloramphenicol alternative to prevent and treat bacterial diseases in pigs [3], bovine [4], poultry [5] and animal edible tissues [6]. Although normal dose of FF has no adverse reactions to animals, the presence of residues in tissues and the increased emergence of resistance of pathogenic bacteria may lead to potential health risks to humans [7]. At present, no maximum residue limits (MRLs) has been set for FF in milk, but it is of vital importance to develop a reliable method for FF analysis in milk to avoid potentially harmful to consumers [8].

Various analytical techniques have been reported for the analysis of FF, including high performance liquid chromatography (HPLC) [1], gas chromatography (GC) [9], liquid chromatography–tandem mass spectrometry (LC–MS) [2], and gas chromatography–mass spectrometry (GC–MS) [5,10]. But, due to the complexity of sample matrices, pretreatment procedures are usually needed prior to the technical analysis. To date, some different pretreatment methods, such as liquid–liquid extraction (LLE) [11], solid phase extraction (SPE) [12,13] and supercritical fluid extraction (SFE) [14,15] have been applied for the pretreatment of milk. LLE usually needs large amounts of organic solvent and complex consequent operations. SFE, a separation technique with special advantages, is particularly suitable for extracting heat-sensitive or easily oxidized substances. SPE is a more efficient and solvent-saving method, but the traditional SPE method is still not satisfactory when it is applied to extract target compounds at trace level in complex sample matrices due to their poor selectivity and weak anti-interference ability [16].

In recent years, a lot of the SPE methods based on molecularly imprinted polymers (MIPs) have been extensively developed for extracting of antibiotic residues from milk samples [17–20]. Mohamed et al. have compared commercial MIPs-SPE sorbents with classical SPE with Oasis HLB for the extraction of chloramphenicol in milk-based matrixes [21]. Their results showed that the selectivity of the method, the recovery of chloramphenicol and the cleanliness of the final extract have been improved obviously because of the use of highly selective MIPs. Several commonly used methods have been reported for the preparation of MIPs, such as bulk polymerization [17,19], suspension polymerization [20], precipitation polymerization [22] and emulsion polymerization [23]. The conventional trigger mode of these polymerization methods was heating or UV light. However, the polymerization process

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induced by heating or UV light was usually time-consuming. In 1994, Murray et al. first proposed that microwave heating could accelerate the polymerization process and simultaneously achieve satisfactory morphology [24]. Then, the applications of microwave heating in the synthesis of polymers were successively reported. Li and co-workers had synthesized the magnetic MIPs using a suspension polymerization method through microwave heating and the polymerization time was dramatically shortened as compared to that by conventional heating from 24 h to 1 h. The obtained MIPs were successfully applied for selective extraction of triazines [25], sterols [26], β-agonists [27] and gibberellin acid 3 [28] in complicated samples, respectively.

In this work, a fast and simple method based on microwave-assisted emulsion polymerization was proposed for the preparation of MIPs using FF as the template. To the best of our knowledge, there are a few reports on MIPs using FF as the template for solid-phase extraction of FF [29,30] and the polymers prepared by bulk polymerization were irregular. Our proposed method was desirable to prepare monodispersed and spherical polymers with good selectivity and high adsorption capacity. In addition, the polymerization time was greatly shortened and the consumption of organic solvent was significantly decreased. The obtained MIPs were successfully applied as solid-phase extraction materials to extract and clean up the FF in milk, followed by the LC–MS/MS analysis.

2. Experimental

2.1. Chemicals and materials

Florfenicol (97%), chloramphenicol (99%), cefadroxil (97%), roxithromycin (97%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acrylamide (AM), azobisisobutyronitrile (AIBN), dimethyl sulfoxide (DMSO), styrene (St), sodium dodecyl sulfate (SDS) were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile (ACN), trifluoroacetic acid (TFA), ammonium acetate, sodium hydroxide and acetic acid were sourced from Beijing Chemical (Beijing, China). Methanol of chromatographic grade was obtained from Fisher (Pittsburgh, PA). All other reagents were of analytical grade. High purity water with a resistivity of 18.2 MΩ cm^{-1} was obtained from a Milli-Q water system (Millipore, Billerica, MA).

Stock standard solution (500 μg mL^{-1}) of FF was prepared in methanol and stored under dark conditions at −18 °C. Working standard solutions were prepared by diluting the stock solutions with pure water every day.

The milk samples were randomly purchased from the local market in Changchun (China). One milk sample was checked to be free of any FF and was used as blank milk sample. The spiked milk samples were obtained by adding appropriate amounts of standard solutions to the blank milk samples.

The Oasis HLB cartridges were purchased from Waters (Milford, MA, USA).

2.2. Preparation of MIPs

First, functional monomer (AM, 0.284 g) and template molecule (FF, 0.358 g) were dissolved in 10 mL of DMSO and stirred in dark for 1 h to form the preassembly solution. Then, cross linker (EGDMA, 4 mL), polymer monomer (St, 1 mL) and initiator (AIBN, 0.1 g) were added into the preassembly solution and the solution was stirred for 30 min in dark. Next, the above solution was dispersed in 50 mL of water containing 150 mg of SDS by stirring at 600 rpm for 25 min to form the emulsion. The emulsion was subsequently transferred to high pressure microwave vessel and the polymerization was carried out under microwave heating for 1 h at 70 °C. The whole preparation process was performed under a stream of nitrogen. The nonimprinted polymers (NIPs) were synthesized in the same method, except for the absence of the template molecule FF. The obtained polymers were washed repeatedly with methanol/acetic acid (8:2, v/v) under ultrasound until no template molecule could be detected by LC–MS/MS. Finally, the polymers were washed with water and dried at 60 °C.

2.3. Characterizations of MIPs

The morphology of the MIPs were observed using SEM with an SSX-550 scanning electron microscope (Shimadzu Corporation, Japan) at 1 kV. The composition and structure of the MIPs were investigated using a Nicolet Fourier-transform infrared Spectrometer (FT-IR 360, Nicolet, Madison, WI, USA) from 4000 to 500 cm^{-1}. For each spectrum, 16 scans were averaged with a resolution of 4 cm^{-1}.

2.4. Binding experiment

Twenty milligrams of MIPs or NIPs were added to 2 mL of FF standard solution with sequentially concentrations varying from 0.05 to 2.0 mmol L^{-1}. After incubated at room temperature for 6 h, the suspension was separated and analyzed by HPLC. The amount of FF adsorbed on the polymers was calculated by subtracting the free concentration from the initial concentration.

2.5. Selectivity of MIPs

The selectivity of the MIPs or NIPs was investigated by using chloramphenicol as the structural analogs, cefadroxil and roxithromycin as the reference compound. The experiment was carried out by adding 20 mg of MIPs or NIPs to 2.0 mL of each standard solution at the concentration of 400 mg L^{-1}. The solutions were incubated for 6 h at room temperature, and subsequently the suspension was separated, followed by HPLC analysis.

2.6. Sample preparation

To remove the protein which might interfere with the analysis, the milk sample was deproteinized as follows. First, 2 mL of 15% trichloroacetic acid aqueous solution was added to 5 mL of milk sample which was diluted with 13 mL of distilled water previously. After being shaken for 30 s, the sample was centrifuged at 4500 × *g* for 5 min. Then, the supernatant was transferred to a new tube and the pH was adjusted to 6 with 5% sodium hydroxide solution for further use.

2.7. SPE for milk sample

The MISPE cartridge was prepared by packing a 100 mg amount of MIPs into a 2.5 mL syringe. Two sieve plates were placed at the bottom end and the top end of the syringe, respectively. Prior to sample loading, the MISPE cartridge was consecutively conditioned with 3 mL of methanol and 3 mL of water. Next, the supernatant prepared in Section 2.6 was passed through the cartridge at a flow rate of 0.75 mL min^{-1}. After the cartridge was washed with 2 mL of 50% acetonitrile aqueous solution, the analyte adsorbed on the cartridge was eluted with 4 mL of methanol solution containing 4% acetic acid. The eluate was filtered through microfilters with a pore size of 0.22 μm and evaporated to dryness under nitrogen gas at 40 °C. The residue was reconstituted with 1.0 mL of 50% methanol aqueous solution for subsequent LC–MS/MS analysis.