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Preparation of molecular imprinted microspheres based on inorganic–organic co-functional monomer for miniaturized solid-phase extraction of fluoroquinolones in milk

Hui Wang [a,*], Ruiling Wang [b], Yehong Han [c]

[a] Department of Pharmacology, Xingtai Medical College, Xingtai 054000, China
[b] Department of Pharmacy, Shijiazhuang Maternal and Child Health Hospital, Shijiazhuang 050080, China
[c] College of Pharmaceutical Sciences, Hebei University, Baoding 071002, China

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ABSTRACT

An inorganic–organic co-functional monomer, methacrylic acid-vinyltriethoxysilan (MAA-VTES) was designed for the synthesis of molecularly imprinted microspheres (MIMs). By virtue of the aqueous suspension polymerization and dummy template (pazufloxacin), the obtained MAA-VTES based MIMs exhibited good recognition and selectivity to fluoroquinolones (FQs), and were successfully applied as selective sorbents of a miniaturized home-made solid phase extraction device for the determination of ofloxacin (OFL), lomefloxacin (LOM) and ciprofloxacin (CIP) in milk samples. Under the optimum conditions of the miniaturized molecularly imprinted solid phase extraction (mini-MISPE) coupled with liquid chromatography-ultraviolet detector (LC-UV), good linearities were obtained for three FQs in a range of 0.2–20.0 $\mu g\,mL^{-1}$ and the average recoveries at three spiked levels were ranged from 87.2% to 106.1% with the relative standard deviation (RSD) less than 5.4%. The presented co-functional monomer based mini-MISPE-LC-UV protocol introduced the rigidity and flexibility of inorganic silicon materials, exhibited excellent extraction performance towards targets, and could be potentially applied to the determination of FQs in milk samples.

1. Introduction

Fluoroquinolones (FQs) are broad-spectrum synthetic antibiotics, commonly used for the prevention of diseases caused by several bacterial agents in both human and veterinary field [1,2]. However, the abuse or misuse of these medicines may be responsible for increased concerns on public health, such as allergic reactions and antibiotic resistance. Especially the drug residues existed in edible animal products resulting from the inappropriate use of FQs, which may be a potential hazard for humans. Therefore, FQs are banned from use as growth promoting agents in many countries. Meanwhile, to protect the health of consumers and minimize the potential harm, the European Union (EU) has established specific permitted maximum residue limits (MRLs) for FQs in animal products entering in human food chain. In particular for milk, MRLs have been fixed at 100 $\mu g\,kg^{-1}$ for the sum of enrofloxacin and ciprofloxacin [3]. Therefore, monitoring these drugs at trace levels in a sensitive and selective manner is necessary to ensure that food is completely free from antibiotic residues.

At present, many analytical methods for FQs determination in food using different extraction procedures, clean-up conditions and detection principles are available. The commonly employed ones are based on liquid chromatography with different detection techniques; mainly ultra-violet (UV) [4,5], fluorescence [6], or mass spectrometry (MS) [7,8]. Due to the complexity of food and biological samples, a sample pretreatment step is required prior to instrumental analysis, aiming to clean up the matrices, isolate the analytes, and render them in a form that is compatible with analytical systems. Currently, several processes, such as liquid–liquid extraction (LLE) [9,10], solid phase extraction (SPE) [11,12], supercritical fluid extraction (SFE) [13], dispersive liquid–liquid microextraction (DLLME) [14], stir bar sorptive extraction (SBSE) [15], microwave-assisted extraction (MAE) [16], diphasic dialysis [17] and even combination of the basic ones [18] have been suggested. Among them, SPE is one of the most important and frequently used methods because of its matured technology and the good adaptability towards multiple matrix samples, but also restricted by defective purifying effect caused by those conventional sorbents.

As a solution, molecularly imprinted polymers (MIPs) are desired to be new selective sorbents and the so-called molecularly imprinted solid-phase extraction (MISPE) has been applied

* Corresponding author. Tel.: +86 319 2233385.
E-mail address: hzlx87@163.com (H. Wang).

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in sample pretreatment. In contrast to classical sorbents used for clean-up procedures, MIPs exhibit high selectivity and specific recognition towards target analytes, allowing them to be eluted from the SPE cartridge almost free of co-extracted compounds. The superiority of MISPE has been extensively demonstrated by plenty of literature [19–23].

Most molecularly imprinted systems are organic MIPs synthesized by free-radical polymerization of vinylic or acrylic functional monomers. These materials are normally prepared in relatively non-polar organic solvents (such as chloroform or toluene), and therefore always exhibit poor recognition properties to templates in polar environment. What's more, their development is limited by a handful of optional monomers and cross-linking agents [24,25]. For that, one response is the synthesis of MIPs based on silicon materials by surface imprinting or sol–gel process, in which silicon materials could act as carriers, monomers or even cross-linking agents, simultaneously make MIPs capable of recognition in polar solvent (mainly in aqueous system), and further enhance the rigidity of recognition sites as well as the materials to be adapted to multiple applications [26–28]. However, neither surface imprinting nor sol–gel process methodology could be totally free of tedious modification processes, or get rid of poor permeability resulting from the too close structure of silicon materials.

In this work, an inorganic–organic co-functional monomer, methacrylic acid-vinyltriethoxysilan (MAA-VTES) was designed to offer more binding sites with template and continuous rigid-structure. To the best of our knowledge, this was also the first time to introduce a silane coupling agent into mix functional monomers. By virtue of suspension polymerization and dummy template (pazufloxacin), the inorganic–organic co-functional monomer based molecularly imprinted microspheres (MIMs) were synthesized, showing high affinity to ofloxacin (OFL), lomefloxacin (LOM) and ciprofloxacin (CIP), which was further proved by the low consumption of such sorbents based on the miniaturized home-made SPE device. Various factors affecting the separation and extraction of the analytes were discussed in detail and the applicability of this method was also evaluated.

2. Experimental

2.1. Chemicals

Ofloxacin (OFL), lomefloxacin (LOM), ciprofloxacin (CIP) and pazufloxacin were obtained from Sigma (St. Louis, MO, USA). Vinyltriethoxysilan (VTES), polyvinylpyrrolidone (PVP), chloroform, dichloromethane, tetrabutylammonium bromide (TBAB), trifluoroacetic acid (TFA), isopropanol, lead acetate and ammonia water were obtained from Huaxin Chemical Co. (Baoding, China). Methacrylic acid (MAA), methanol, acetonitrile, acetone, acetic acid, and 2,2-azobisisobutyronitrile (AIBN) were purchased from Kermel Chemical Co., Ltd. (Tianjin, China). Ethylene glycoldimethacrylate (EGDMA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with 0.45 μm filter membrane before use.

2.2. Instrumentation and conditions

Chromatographic analysis was carried out on a Shimadzu HPLC system equipped with two LC-20AT Solvent Delivery Units, a SUS20A gradient controller, and a SPD-20A UV–VIS Detector (Shimadzu, Kyoto, Japan). An N-2000 data workstation (Zheda Zhineng Co., Ltd., Hangzhou, China) was used as the data acquisition system. The analytical column was a YMC-Pack Pro C18 column (4.6 mm × 250 mm, 5 μm) from YMC Company (Shimogyo-ku, Kyoto, Japan). The mobile phase was a mixture of acetonitrile–water (1:9, v/v, containing 0.63% TFA and 0.018 mol/L of TBAB) with a flow rate of 1.0 mL/min. The injection volume was 10 μL for all the solutions and the UV detector was performed at the wavelength of 280 nm.

2.3. Synthesis of the MIMs based on inorganic–organic co-functional monomer

The imprinted microspheres were prepared by aqueous suspension polymerization as follows. (I) 6 mmol VTES and 8 mmol MAA were mixed thoroughly in a 20 mL glass bottle immersing into a water bath (60 °C) for 24 h. (II) 3.0 g of PVP was dissolved into 120 mL of water. The solution was poured into a 250 mL flanged reactor flask in a water bath (60 °C) and then was stirred at 600 rpm under a nitrogen stream. (III) The co-functional monomer (1 mL, obtained from step I), pazufloxacin (0.637 g), EGDMA (9.4 mL) and AIBN (0.250 g) were dissolved in 20 mL chloroform and sonicated for 5.0 min to maintain homogeneity. (IV) Adding the solution (III) dropwise to solution (II), then the flask with all the reagents still maintained in the water bath with stir for 24 h for polymerization. The produced suspension was filtered and the MIMs were washed with methanol–acetic acid (9:1, v/v) and methanol successively to remove the template and unreacted monomers. Non-imprinted microspheres (NIMs) were synthesized by the similar procedure in the absence of template molecules.

2.4. Extraction and co-functional monomer based MISPE procedure

The milk samples were purchased from the local supermarkets. 10.0 g samples or spiked milks were pretreated by 2 mL of lead acetate solution (16%) to precipitate the protein. Subsequently, the mixed solution was centrifuged at 4000 rpm for 15 min and the obtained supernatant solution was centrifuged twice in the same manner. Finally, 1 mL of the clarification solution was used for further SPE purification.

Home-made miniaturized MISPE procedure: As shown in Fig. 1, a SPE device was mainly constructed by an empty polypropylene cartridge (5.0 cm × 8.0 mm I.D.) and a 100 μL-pipette. Concretely, cotton was pre-placed into the tip section of the 100 μL-pipette and packed with 40 mg of co-functional monomer based materials. Then the opposite section was connected tightly with the cartridge by a section of plastic straw. The assembled SPE cartridge was pretreated with 1.0 mL of methanol and 3.0 mL of water, respectively, followed by loading 1.0 mL of sample solution. Then the cartridge was washed with 1.0 mL of water and eluted by 3.0 mL of methanol–acetic acid (19:1, v/v). The eluents were evaporated to dryness under vacuum and then re-dissolved in 1.0 mL of mobile phase for further HPLC analysis.

3. Results and discussion

3.1. Preparation and characteristics of the co-functional monomer based MIMs

To avoid the effect of templates leakage, pazufloxacin was selected as dummy template molecule to synthesize MIMs towards OFL, LOM and CIP using aqueous suspension polymerization. Other parameters involved in the production of MIMs including functional monomer, cross-linker, and porogen were also under consideration.

Rational selection of the functional monomer can produce better extraction performance, because this will determine, on one hand, the stability of the complex formed before and during the polymerization process and, on the other hand, the subsequent