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# Superparamagnetic surface molecularly imprinted nanoparticles for water-soluble pefloxacin mesylate prepared via surface initiated atom transfer radical polymerization and its application in egg sample analysis

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ABSTRACT

The novel superparamagnetic surface molecularly imprinted $Fe_3O_4$@MIP nanoparticles for water-soluble pefloxacin mesylate (PEF-M) were prepared via surface initiated atom transfer radical polymerization (si-ATRP). The binary mixture of methanol and water was selected as the polar solvents for fabricating PEF-M imprinted MIPs. The $Fe_3O_4$@MIP exhibited high saturation magnetization of 41.4 emu/g leading to the fast separation. The adsorption behaviors indicated that the $Fe_3O_4$@MIP nanoparticles possessed specific recognition and high affinity towards template PEF-M in aqueous media. Moreover, $Fe_3O_4$@MIP nanoparticles were directly used to selectively enrich PEF-M from egg samples. By RP-HPLC analysis, the recoveries of PEF-M were obtained as 92.8–96.5% with relative standard division of 2.4–4.0%.



## 1. Introduction

Molecular imprinting technique is a promising and facile method to produce molecularly imprinted polymers (MIPs) with molecular-specific recognition sites to the target molecules [1–3]. MIPs possess desired selectivity, physical robustness and thermal stability, which have been widely applied in many fields, such as solid-phase extraction [4–6], chemical sensors [7,8], artificial antibodies [9] and drug delivery [10]. However, imprinting hydrophilic components especially highly water-soluble compounds is one of the greatest challenges in MIP preparation [11], owing to the poor solubility of highly water-soluble compounds in the common solvents of MIP preparation as well as the difficult formation of the hydrogen bonding interactions between templates and functional monomers in polar organic solvents.

Some efforts have been made to realize the preparation of MIPs for water-soluble templates. For instance, Tominaga et al. [12] prepared the MIPs with ion-pair complex as the template, which was formed with target molecule 4-(tributylammonium-methyl) benzyltributylammonium and an ion-pair reagent. Sellergren and co-workers [13] described the conversion of hydrophilic target riboflavin to hydrophobic riboflavin tetraester as the template for MIP fabrication. Moreover, Sellergren and co-workers [14] used the hydrophobic analogue trimethoprim as pseudo templates instead of hydrophilic target folic acid to prepare the MIPs. In addition, some hydrophilic compounds, which were dissolved in either aqueous solution or common solvents of MIP preparation, have also been effectively imprinted [15,16]. Even though, it is still necessary to develop novel imprinting methods for imprinting highly water-soluble small molecules in aqueous media.

Pefloxacin mesylate (PEF-M) is a synthetic broad-spectrum fluoroquinolone antibacterial agent and widely used to treat the infections of urinary tract, respiratory tract and tissue in human and animals [17,18]. As a widely used animal antibiotic, its persistence in animal products such as milk, meat and eggs must be monitored for avoiding the potential hazard to human health. European Union has established maximum residue limits for fluoroquinolones presented in edible tissues, such as 300 ng/g for difloxacin [19]. Owing to the complexity of matrix and the low concentration of PEF-M existed, it is necessary to develop the effective and selective method for PEF-M enrichment before further analysis.

Since PEF-M is a highly water-soluble small molecule, it is not dissolved in conventional aprotic and low polar organic solvents such as acetonitrile, chloroform and toluene. Therefore, it is difficult to prepare MIPs for PEF-M using routine methods. The atom transfer radical polymerization (ATRP) is a class of controlled radical polymerization [20]. Compared with the traditional radical polymerization, ATRP reactions are very robust in which high polar

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reagents such as dimethylformamide, water and methanol can be used as the solvents for MIP preparation [21]. So, ATRP is ideal for imprinting water-soluble small molecules such as PEF-M.

Surface imprinted polymers reveal high binding capacities, fast mass transfer and rapid binding kinetics due to the easy accessibility to recognition sites and the homogeneous distribution of binding sites [22,23]. Furthermore, the magnetic nanoparticles are superior for rapid separation since they can be easily collected and isolated by an external magnetic field. Fabrication of surface imprinted nano-scale MIPs with magnetic nanoparticles is benefit for highly selective and fast separation. The magnetic core–shell MIP nanoparticles have been successfully synthesized via si-ATRP using an ATRP agent functionalized $Fe_3O_4$ nanoparticles [24]. These magnetic MIP nanoparticles exhibited excellent properties for protein separation [25–27] and environmental contaminant enrichments [24,28]. However, to the best of our knowledge, there have been no reports about imprinting water-soluble small molecules using si-ATRP combined with magnetic nanoparticles.

In this study, the novel superparamagnetic surface molecularly imprinted $Fe_3O_4$@MIP nanoparticles were prepared via si-ATRP using water-soluble PEF-M as template. The binary mixture of methanol and water was used as the reaction solvent. The parameters in MIP preparation were optimized. The prepared $Fe_3O_4$@MIPs were characterized by Fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM) and vibrating sample magnetometry (VSM). The adsorption properties of MIPs to template PEF-M were evaluated in aqueous media. The $Fe_3O_4$@MIP was also used for selective enrichment of PEF-M from egg samples before further RP-HPLC analysis.

## 2. Experimental

### 2.1. Reagents and materials

Methacrylate acid (MAA, 98%) was obtained from Beijing Xin Guang Chemical Works (Beijing, China). Ethylene glycol dimethacrylate (EGDMA, 99%), 2-bromoisobutyryl bromide, 2-Hydroxyethyl methacrylate (HEMA, 99%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%) and hydrocortisone (HDCS) were supplied from Aldrich (St. Louis, MO, USA). Sodium citrate, sodium acetate anhydrous, ferric chloride, tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), dichloromethane, ethylene glycol, triethylamine (TEA), 4-di(methylamino)pyridine (DMAP), ethanol and CuBr were obtained from Beijing Chemical Works (Beijing, China). Methanol was obtained from Concord Technology Co. Ltd. (Tianjin, China). Pefloxacin mesylate (98%), norfloxacin (NOR) and gatifloxacin (GAT) were purchased from Taiyuan Kangruibao Veterinary Drug Manufacture Co. Ltd. (Taiyuan, Shanxi, China). Ultrapure water was obtained from MilliQ gradient ultrapure water system (Millipore Inc., Massachusetts, USA).

MAA, HEMA and EDMA were distilled under reduced pressure to remove inhibitors. PMDETA and 2-bromoisobutyryl bromide were dried by $CaH_2$ overnight and distilled under reduced pressure, respectively. All other reagents were of analytical grade.

### 2.2. Preparation of superparamagnetic $Fe_3O_4$@MIP nanoparticles

The overall preparation of nanoparticles is depicted in Fig. 1. $Fe_3O_4$ nanoparticles were synthesized via solvothermal method according to the literature [29]. The obtained $Fe_3O_4$ nanoparticles were dispersed in the mixture of ethanol (160 mL) and water (40 mL) by sonication for 15 min, followed by the addition of ammonium hydroxide (5 mL) and TEOS (0.7 mL). The mixture was reacted for 12 h at 40 °C under continuous mechanical stirring. The obtained $Fe_3O_4$@$SiO_2$ nanoparticles were collected by an external magnetic field, and then thoroughly washed with ethanol and water, respectively. The $Fe_3O_4$@$SiO_2$ (400 mg), 3-aminopropyltriethoxysilane (1 mL) and absolutely dry toluene (30 mL) were mixed in a flask under nitrogen atmosphere. The reaction was carried out at 90 °C for 48 h under continuous mechanical stirring. The resulting $Fe_3O_4$@$SiO_2$@$NH_2$ was washed with toluene and methanol thoroughly, and then dried in the vacuum. After the $Fe_3O_4$@$SiO_2$@$NH_2$ particles was dispersed in dichloromethane (40 mL) for 30 min in an ice bath, TEA (0.67 mL), 2-bromoisobutyryl bromide (0.53 mL) and DMAP (50 mg) were orderly added into the mixture. The mixture was kept at 0 °C for 1 h and then at room temperature for 12 h. The obtained ATRP initiator $Fe_3O_4$@$SiO_2$@Br was washed with dichloromethane, ethanol and ultrapure water, and then dried.

The superparamagnetic PEF-M surface-imprinted polymer $Fe_3O_4$@MIP was prepared as follows. The obtained $Fe_3O_4$@$SiO_2$@Br (250 mg) was used as an initiator and dispersed in the mixture of methanol and water, which consisted of PEF-M (116.5 mg), the functional monomer HEMA (124 μl) and MAA (88 μl), as well as the cross-linker EDMA (0.94 mL), respectively. And then PMDETA (100 μl) was added. The mixture was vacuumed and bubbled with nitrogen three times. CuBr (72.4 mg) was quickly added in the flask under nitrogen atmosphere. The polymerization proceeded at 60 °C for 24 h under continuous mechanical stirring. The final product $Fe_3O_4$@MIPs were collected from the mixture by a magnet and washed with methanol and ultrapure water three times to remove the unreacted components. Then, the $Fe_3O_4$@MIP nanoparticles were immersed into acetic acid–methanol (1:4, v/v) solution in an incubator shaker for 10 h to remove the templates thoroughly. After that, the $Fe_3O_4$@MIPs were washed with ultrapure water until neutral. The control $Fe_3O_4$@NIPs were synthesized using the same procedure mentioned above, except for the addition of template PEF-M.

### 2.3. Characterization

Morphological observation of the polymer particles was carried out on an H800 transmission electron microscope (Hitachi Ltd., Tokyo, Japan). Magnetic properties were determined with a 7410 vibrating sample magnetometer (VSM, Lake Shore, Cryotronics Inc., Ohio, USA). FT-IR characterization was performed with a Tensor-27 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany).

### 2.4. HPLC analysis

The HPLC analysis was performed on a Hitachi 7100 series HPLC system (Hitachi Ltd., Tokyo, Japan), which consisted of L-7100 pump, L-7420 UV–Vis detector, and L-7300 column oven. A BonChrom $C_{18}$ column (250 mm × 4.6 mm, 5 μm, Agela Technologies Inc, Tianjin, China) and a Dikma Diamonsil $C_{18}$ HPLC column (250 mm × 4.6 mm, 5 μm, Dikma Technologies Inc, Beijing, China) were employed for analysis. The mobile phase used for PEF-M, NOR, GAT analysis was acetonitrile:50 mmol/L phosphate buffer solution (19:81, v/v, pH = 2.4), the flow rate was 1.0 mL/min and UV detection was set at 280 nm. The mobile phase used for HDCS separation was methanol:water solution (70:30, v/v), the flow-rate was 1.0 mL/min and UV detection was set at 254 nm.

### 2.5. Adsorption capacity of the $Fe_3O_4$@MIP nanoparticles

The adsorption capacity was determined by adding 2 mg $Fe_3O_4$@MIP nanoparticles into 2 mL 5% methanol–water solution with concentrations of PEF-M varying from 0.05 to 0.8 mmol/L. The mixture was incubated in an incubator shaker for 1 h at room temperature. After incubation, the $Fe_3O_4$@MIP nanoparticles were separated by a magnet and the supernatant solution was