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Development of novel magnetic solid phase extraction materials based on Fe_3O_4/SiO_2/poly(acrylamide-*N*,*N*′-methylene bisacrylamide)-Pluronic L64 composite microspheres and their application to the enrichment of natamycin

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ABSTRACT

Novel magnetic adsorbents based on Fe_3O_4/SiO_2/poly(acrylamide-*N*,*N*′-methylene bisacrylamide) magnetic microspheres modified with non-ionic triblock copolymer surfactant were successfully prepared as a magnetic solid phase extraction adsorbent for the determination of trace natamycin in jam samples. The adsorbent was characterized by scanning electron microscopy, transmission electron microscopy, Fourier transformed infrared spectroscopy, vibrating sample magnetometer, and X-ray diffractometer analysis, confirming that Pluronic L64 was effectively functionalized on the magnetic materials. Various experimental parameters affecting the extraction capacity were investigated including adsorbent amount, extraction time, desorption time, sample pH, and ionic strength. For recovery evaluations, the jam samples were spiked at two concentration levels of 100 and 200 $\mu g\,kg^{-1}$ of natamycin and the recovery values were in the range of 78.8–93.4%. The relative standard deviations (RSD) for the recoveries were less than 3.5%. The novel magnetic solid phase extraction method provided several advantages, such as simplicity, low environmental impact, convenient extraction procedure, and short analysis time when used for natamycin analysis.

1. Introduction

The synthesis of materials with novel structures has been a fundamental focus of the chemical research, which promotes the advancement in both academic and industrial fields. Nowadays, functionalized magnetic materials have received considerable attention in a variety of research fields, including separation science [1], catalysis [2], environment [3,4], enzyme immobilization [5], and drug delivery [6]. Magnetic solid phase extraction (MSPE), as a new alternative mode of solid phase extraction (SPE) technique, has been developed based on the application of magnetic nanoparticles because of its high extraction yield, short extraction time, and easy operation [7]. For effective extraction of the targets, magnetic nanoparticles usually need to be modified or coated with special polymer, surfactant, or ligand to provide functional groups, abundant adsorption sites, and high pH stability. In our preceding work, we synthesized two novel magnetic microspheres, Fe_3O_4@TiO_2/graphene oxide [8] and Fe_3O_4/pillararenes [9], which were successfully applied to the extraction of organic pollutants from real samples.

Porous materials with special structures and compositions, high surface area, and large pore volume have attracted much attention because of the potential applications in separation and preconcentration fields [10]. Recently, Pluronics, non-ionic triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) with different molecular weights, have been used in the procedure of synthesizing porous materials. The PO/EO ratio values play important roles in the Pluronics structure. For instance, Pluronics F127 ($EO_{106}PO_{70}EO_{106}$), F68 ($EO_{80}PO_{30}EO_{80}$), and L64 ($EO_{13}PO_{30}EO_{13}$) have different

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molecular weights (F127 > F68 > L64) with the PO/EO ratios of 0.66, 0.38, and 2.31, respectively. When PO/EO value is lower than 1, it suggests that the hydrophilicity is higher than hydrophobicity. This interesting feature of Pluronics makes them extremely useful in various applications as emulsifiers and stabilizers [11]. They have been extensively applied as nonionic surfactants and pharmaceutical agents because of their solubilizing and controlled release properties. Yao et al. firstly utilized Pluronic F127 to prepare a series of glycidyl methacrylate-based polymer porous monolithic materials and used for the fast and highthroughput separation of proteins [10]. Excellent permeability, good mechanical and thermal stability were achieved by using the modified monolithic material.

Natamycin is a type of biological preservative belonging to the group of polyene macrolides produced by actinomycetes streptomyces natalensis [12]. It is extensively used as additive to prevent the microbial deterioration in food. The food additives are limited by the National Food Safety Standards in China. The maximum level of natamycin used in most food products should not exceed 0.3 g kg^{-1} [13]. Therefore, it is necessary to develop a simple, fast and selective analytical method for detecting various food additives including natamycin. So far, there have been some reports about the determination of natamycin in cheeses, sausages, and wines, etc. However, few papers have been focused on the determination of natamycin in jams, a kind of popular fruit based products, in which natamycin is commonly used as the preservative [14].

In the present study, we developed triblock copolymer modified monolithic materials-based MSPE adsorbents, Fe_3O_4/SiO_2/poly(acrylamide-*N*,*N*'-methylene bisacrylamide)-Pluronic L64 (FSP-L64), and applied them to the preconcentration of natamycin. A rapid, efficient, and highly sensitive analytical method for the determination of natamycin in jam samples was developed by combining the MSPE strategy with HPLC detection.

2. Experimental

2.1. Chemicals and materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ethylene glycol (EG), polyethylene glycol (PEG, *M*w = 2000), sodium acetate (NaAc), isopropanol, and tetraethyl-orthosilicate (TEOS) were purchased from Sinopharm Chemical Reagent (Shanghai, China). 3-Methacryloxypropyl trimethoxysilane (MPS) was obtained from Sigma–Aldrich (USA). Azobisisobutyronitrile (AIBN) was supplied by Tianjin Chemical Plant (Tianjin, China). Acrylamide (AA) and methylene diacrylamide (MBAAm) were purchased from Aladdin Reagent (Shanghai, China) and Sigma–Aldrich (USA), respectively. AA and MBAAm were purified by distillation under reduced pressure prior to use. AIBN was purified by recrystallization from *n*-hexane and ethanol (Beijing Chemical Works, Beijing, China), followed by being dried at room temperature in vacuo.

HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were obtained from Fisher Scientic (New Jersey, USA). Natamycin was obtained from Aladdin Reagent (Shanghai, China). Stock solutions of natamycin (1000 $\mu g\,mL^{-1}$) were prepared in MeOH. The working solutions were stored at 4 °C and daily prepared by appropriate dilution from the stock solutions to obtain the required concentrations. Phosphate buffer (0.02 $mol\,L^{-1}$) was prepared with NaH_2PO_4, and pH was adjusted using NaOH or H_3PO_4 solutions. NaH_2PO_4, NaOH, H_3PO_4, and ammonia ($NH_3{\cdot}H_2O$) were all supplied by Beijing Chemical Works (Beijing, China). All other reagents were of analytical reagent grade chemicals and used without further purification.

All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.22 μm). NdFeB magnets were purchased from Yingke (Beijing, China) with a cuboid shape of 15 mm × 10 mm × 3 mm and a surface magnetic field of 3000 G.

2.2. Instrumentation

The HPLC system was equipped with a Waters 1525 binary HPLC pump and degasser, a heated column compartment, and a Waters 2489 UV-Visible detector. A breeze workstation was utilized to control the system for the acquisition and analysis of the chromatographic data. A reverse phase Agilent Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm) was employed for the chromatographic separation.

A PB-10 pH meter (Sartorius Scientific Instruments Co., Ltd., Beijing, China) was used for pH measurements. A Milli-Q SP system (Millipore, Milford, MA, USA) was used for preparing ultrapure water. An LD5-2A centrifuge (Beijing Jingli centrifuge Co., Ltd., China) and a 79-1 magnetic stirrer (Changzhou Guohua Instruments Co., Ltd., China) were used for centrifuging and stirring, respectively. A ZK-82BB electric vacuum drying oven (Shanghai Experimental Instrument Co., Ltd., China) was utilized. Magnetic properties of the materials were characterized by an MPMS3 vibrating sample magnetometer (Quantom, USA). Scanning electron microscope (SEM, JSM 6700-F, JEOL Company, Japan) and transmission electron microscope (TEM, JSM 2000-F, JEOL Company, Japan) were used to characterize the surface morphology. The Fourier transformed infrared spectra (FT-IR) were obtained using a Thermo Nicolet 670 FT-IR instrument (Thermo, USA). Phase identification was conducted by the X-ray diffractometer (XRD) (R-AXIS RAPID-F, Rigaku Corporation, Japan).

2.3. HPLC conditions

The optimized mobile phases consisted of 60% water and 40% MeCN containing 0.1% trifluoroacetic acid (TFA, *v*/*v*). The flow rate was controlled at 0.8 $mL\,min^{-1}$ throughout the process. The preferential detection wavelength was 304 nm and the column temperature was set at 30 °C.

2.4. Preparation of Fe_3O_4/SiO_2/poly(AA-MBAAm) composites

The procedure to prepare Fe_3O_4/SiO_2/poly(AA-MBAAm) (FSP) composites was depicted in Fig. 1. Firstly, Fe_3O_4 was synthesized with the well-known solvothermal method. $FeCl_3{\cdot}6H_2O$ (1.5 g) was dissolved in EG (40 mL) to form an orange solution, followed by the addition of NaAc (3.6 g) and PEG-2000 (1.0 g). The mixture was stirred vigorously for 30 min, and then sealed in a teflon-lined stainless steel autoclave (100 mL). The autoclave was maintained at 200 °C for 8 h before cooled to room temperature. The obtained Fe_3O_4 powder was washed with ethanol for several times and dried in a vacuum at 60 °C for 6 h. Then, the $Fe_3O_4@SiO_2$ (FS) microspheres were prepared using a sol–gel method. The as-prepared Fe_3O_4 powder was treated with 2 $mol\,L^{-1}$ HCl and re-dispersed in a mixture of ethanol, water, and aqueous ammonia. Subsequently, 0.05 mL TEOS was quickly added to the dispersion with vigorous stirring and the reaction was allowed to proceed for 12 h. Finally, ethanol was added to disperse the obtained FS microspheres.

The surface of FS was modified with MPS for further synthesis according to previous work [15]. Briefly, $Fe_3O_4@SiO_2$ (1.0 g) was ultrasonically dispersed in 50 mL water for 10 min. pH was adjusted to 3.0–4.0 using acetic acid (5 wt%) and then the solution was stirred for 1 h. After that, 0.2 mL MPS dispersed in 50 mL ethanol was added to the above solution and the mixture was stirred at 80 °C for 5 h. The modified MPS-modified FS was washed with water and ethanol successivly and dried at 80 °C for 24 h. The preparation of FSP composites was referred to a previous report [16] with some modifications. FSP particles were prepared with the mixture of MPS-modified FS (1.0 g), AA (0.5 g), MBAAm (0.25 g), and AIBN (0.01 g) under the condition of water bath and boiling state.