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Magnetic molecularly imprinted polydopamine nanolayer on multi-walled carbon nanotubes surface for protein capture

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ABSTRACT

A novel, facile and low cost process for imprinting protein on the surface of magnetic multiwalled carbon nanotubes (MMWNTs) was developed using human serum albumin (HSA) as the template and dopamine as the functional monomer. The magnetic imprinted polymers were characterized with transmission electron microscope (TEM), scanning electron microscope (SEM), Fourier-transform infrared spectrometry (FT-IR), vibrating sample magnetometer (VSM) and thermogravimetric analysis (TGA) in detail. The maximum adsorption capacity of the magnetic imprinted polymers toward HSA was 66.23 mg g^{-1} and it took 20 min to achieve the adsorption equilibrium. The magnetic imprinted polymers exhibited the specific selective adsorption toward HSA. Coupled with high performance liquid chromatography (HPLC) analysis, the magnetic imprinted polymers were used to solid-phase extract and detect HSA in urine samples successfully with the recoveries of 91.95–97.8%.

1. Introduction

Molecularly imprinted polymers (MIPs) are a kind of polymer materials with the complementarities in shape, size and functional groups with the template molecule, which can selectively recognize the template molecule [1]. Owing to their special molecular recognition ability, chemical stability, durability, reusability and ease of preparation, the MIPs have been applied in various fields involving extraction, sensor, catalysis, and drug delivery [2]. Recently, the combination of MIPs and other sample preparation techniques such as solid-phase extraction, solid-phase microextraction and matrix solid-phase dispersion have been developed as novel trends for selective extraction and recognition of target molecules from the complex matrices [3]. Although the MIPs have a wide application prospect, the conventional MIPs preparation technique involving bulk polymerization and precipitation polymerization exhibited poor accessibility, low-affinity binding and high diffusion barrier because the recognition sites are embedded deeply in the polymers [4]. Fortunately, surface molecular imprinting technique provides an alternative way to overcome these drawbacks, which has built molecular recognition system on the supporting materials surface involving silica particles [5–7], magnetic nanoparticles [8,9], quantum dots (QDs) [10,11] and polystyrene nanoparticles [12].

Up to now, magnetic molecularly imprinted polymers (mag-MIPs) have captured considerable attention attributing to they not only exhibit specific selective binding toward the template molecule, but also have outstanding magnetism. Thus, the mag-MIPs can be isolated easily from these samples with an external magnet. Currently, magnetic imprinted solid-phase extraction (M-SPE) has been the most accepted sample pretreatment method for the extraction and preconcentration of analytes in field of environmental, food and biological samples [13]. In recent years,the preparation of magnetic protein imprinted polymers has also been implemented through the covalent or noncovalent interactions between the functional monomer and the template molecule[14–16]e Additionally, some novel functional monomers such as dopamine (DA) and 3-aminobenzeneboronicacid (APBA) which can auto-polymerize without cross-linking or initiator agent to quickly prepare the MIPs were captured researchers' attention [17–19]. For example, Ali Nematollahzadeh et al. reported a molecularly imprinted polydopamine nanolayer on porous particles surface for protein capture in HPLC column [20]; Zian Lin et al. reported a boronate-functionalized molecularly imprinted monolithic column with polydopamine coating for glycoprotein recognition and enrichment [21]; a facile synthesis of polydopamine-coated molecularly imprinted silica nanoparticles for protein recognition and separation was reported by Zhiwei Xiaet al [22]. However, at the best of our knowledge, there is no paper reported on the magnetic protein imprinted polymers based on MWNTs using DA as functional monomer.

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Herein, a simple and convenient method for preparation of human serum albumin (HSA) imprinted polymers was developed using DA as the functional monomer based on magnetic multi-walled carbon nanotubes (MWNTs). The magnetic imprinted polymers were evaluated by transmission electron microscope (TEM), Fourier transform infrared spectrometry (FT-IR), vibrating sample magnetometer (VSM) and thermogravimetric analysis (TGA) in detail. Combined with high performance liquid chromatography (HPLC), the magnetic imprinted polymers were used to separate and enrich HSA from real urine sample successfully.

2. Experimental

2.1. Materials

Multiwalled carbon nanotubes (MWNTs, diameters range of 20–40 nm) were obtained from Shenzhen Bill Corporation. Bovine serum albumin (BSA), HSA, lysozyme (Lyz) and dopamine (DA) were purchased from Sigma-Aldrich. Ferric chloride ($FeCl_3 \cdot 6H_2O$), sodium acetate (NaAc), ethylene glycol (EG), polyethylene glycol (PEG, Mw=4000) and alcohol were purchased from Changsha Chemical Reagent Company (Hunan, China). The remaining chemical reagents were of analytical grade. Ultra-pure water was used throughout the experiment.

2.2. Characterization

Fourier transform infrared (FT-IR) spectra were acquired on a Nicolet iS10 FTIR spectrometer (Thermoscientific, USA). The morphology of the Mag-MIPs was characterized by transmission electron microscopy (TEM, JEM1010, Japan) and scanning electron microscopy (SEM, JSM-6700F). The magnetic property of the Mag-MIPs was assessed with a vibrating sample magnetometer (VSM, M27407, Lake Shore Ltd.). The thermal stability of the Mag-MIPs was determined with thermogravimetric analysis (TGA, METTLER TOLEDO) in the temperature ranged from room temperature to 800 °C with a heating rate of 10 °C min^{-1} under nitrogen atmosphere. The adsorption property was determined through UV-2450 (SHIMADZU, Japan) and high performance liquid chromatography (HPLC) analysis with a LC2010AHT solution system (SHIMADZU, Japan). Chromatography analysis was achieved on a Spherigel C_{18} column (5 μm, 250 × 4.6 mm^2). The mobile phase is a 50 mM phosphate buffer solution at pH 6.0 with a flow rate of 0.5 mL min^{-1}. The injection sample volume is 10.0 μL and the wavelength for UV detector is 287 nm. All detected solutions were filtered through a 0.45 μm polytetrafluorethylene membrane (PTFE) before use.

2.3. Preparation of MWNTs@Fe_3O_4@MIPs

2.3.1. Pretreatment of multiwalled carbon nanotubes (MWNTs)

Five hundred milligrams of MWNTs were dispersed in 50 mL of nitric acid solution under sonication for 10 min. Then the mixture was stirred continuously at 80 °C for 24 h. Cooled to room temperature, the mixture was diluted tenfold with double-distilled water. The mixture was then filtered through a 0.45 μm PTFE membrane and rinsed with double-distilled water until the pH was neutral. Finally, the filtered solid was dried under vacuum at 80 °C for 24 h to obtain MWNTs-COOH.

2.3.2. Synthesis of MWNTs@Fe_3O_4

First, 1.6 g of $FeCl_3 \cdot 6H_2O$ was dissolved into 40 mL of EG to form an orange solution. Then 0.4 g of MWNTs-COOH was dispersed in this solution by sonication for 3 h. After that, 5.0 g of NaAc and 2.0 g of polyethylene glycol (PEG) were added under constant stirring for 30 min. The mixture was sealed in a Teflon-lined stainless steel autoclave and maintained at 200 °C for 8 h. Cooled to RT, the MWNTs@Fe_3O_4 composites were collected by an external magnet and rinsed six times with ethanol and purified water. Finally, the MWNTs@Fe_3O_4 composites were dried in a vacuum at 60 °C for 10 h.

2.3.3. Preparation of MWNTs@Fe_3O_4@MIPs

The preparation procedure of the MIPs based on MWNTs@Fe_3O_4 (MWNTs@Fe_3O_4@MIPs) was as follows. First, 0.2 g of MWNTs@Fe_3O_4 was dispersed in 200 mL of Tris buffer (pH 8.0) by sonication 10 min. Next, 0.05 g of HSA and 0.16 g of DA were added. The mixture was stirred for 8 h at RT. Finally, the resultant composite was washed with pure water and then with methanol to remove the template and finally dried under vacuum at 40 °C overnight.

Non-imprinted polymers based on MWNTs@Fe_3O_4 (MWNTs@Fe_3O_4@NIPs) were prepared in the same manner without the template molecule.

2.4. Elution of template protein

The MWNTs@Fe_3O_4@MIPs were washed several times with distilled water and separated by an external magnet to remove the unreacted monomer and the entrapped protein molecule. Next, they were rinsed repeatedly with 0.5 M NaCl to remove the entrapped HSA protein at RT. The complete removal of HSA from the MWNTs@Fe_3O_4@MIPs was confirmed by a UV-2450 (SHIMADZU, Japan) spectrophotometer at 287 nm.

2.5. Adsorption experiment

2.5.1. pH experiment

To study the adsorption performance of the MWNTs@Fe_3O_4@MIPs and MWNTs@Fe_3O_4@NIPs toward HSA under different pH values, 100 mg of the MWNTs@Fe_3O_4@MIPs or the MWNTs@Fe_3O_4@NIPs was dispersed in 10 mL of 0.4 mg mL^{-1} HSA solutions with different pH values (pH=4.0, 4.5, 5.0, 6.0, 7.0, and 8.0). After the mixture was stirred for 2 h at RT, the polymers were separated by an external magnet and the concentration of HAS in the supernatant was analyzed by UV at 278 nm.

2.5.2. Static adsorption experiment

All the static adsorption experiments were carried out as follows. First, 10 mg of the MWNTs@Fe_3O_4@MIPs or the MWNTs@Fe_3O_4@NIPs was suspended in 10 mL of HSA solutions with different concentrations ranged from 0.1 mg mL^{-1} to 0.8 mg mL^{-1}. After the sample was shaken on a rocking table for 20 min at RT, the MWNTs@Fe_3O_4@MIPs and the MWNTs@Fe_3O_4@NIPs were separated by an external magnet and the supernatant was collected. The concentration of free HSA in the supernatant was measured by UV at 278 nm. The amount of adsorbed protein by these polymers was calculated by the difference in concentration before and after the adsorption.

The adsorption capacity (Q) of the MWNTs@Fe_3O_4@MIPs for template protein was calculated by the following equation [23]:

$$Q\,(mg/g) = (C_0 - C)V/m$$

where C_0 is the initial concentration of HSA solution (mg mL^{-1}), C is the HSA concentration of the supernatant solution (mg mL^{-1}), V is the volume of the HSA solution (mL) and m is the mass of MWNTs@Fe_3O_4@ MIPs (mg).

To investigate the adsorption equilibrium of the MWNTs@Fe_3O_4@MIPs toward HSA, the saturated adsorption capacity was obtained according to Langmuir adsorption equation [24]: