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Preparation and adsorption of bovine serum albumin-imprinted polyacrylamide hydrogel membrane grafted on non-woven polypropylene

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ABSTRACT

Bovine serum albumin (BSA) imprinted polypropylene (PP) fiber-grafted polyacrylamide (PAM) hydrogel membrane (PP-g-PAM MIP) was prepared using non-woven PP fiber as matrix, BSA as template molecule, and acrylamide (AM) as functional monomer via UV radiation-reduced polymerization in an aqueous phase. SEM, FT-IR, DSC and TG were used to characterize the PP grafted PAM hydrogel. Influence factors on the adsorption capacity of PP-g-PAM MIP were investigated, such as monomer concentration, cross-linker concentration, template molecule amount and pH values in BSA solution. The adsorption and recognition properties of PP-g-PAM MIP were evaluated and the results showed that the PP-g-PAM MIP exhibited an obvious improvement in terms of adsorption capacity for BSA as compared with non-imprinted ones. PP-g-PAM MIPs could recognize the template protein using Lys, Ova, BHB, and Glo as control proteins, and the selectivity factor (β) was above 2.0. The imprinting efficiency of PP-g-PAM MIP tended to be stable after three cycles and maintained 76% of the initial value of the imprinting efficiency even after five repetitions, which was more excellent than that of PAM microsphere. The PP-g-PAM MIP is low cost and easy to be prepared, which would show its potential applications in the fields of extracting and testing required proteins from cells or particulate samples.

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1. Introduction

Molecular imprinting is a promising and evolving technology to synthesize tailor-made materials by means of co-polymerizing functional monomers and cross-linkers in the presence of desired template molecules [1]. Upon removal of the template molecules, pits or cavities are memorized and created in the molecularly imprinted polymer (MIP) matrix to complement to the template molecules sterically and chemically [2]. Compared with natural antibodies, MIPs exhibit lower selectivity. However, they have more advantages, such as chemical stability, excellent heat resistance, organic solvent resistance, low cost, and ease of mass preparation. Since 2000, great progress has been achieved in the research on molecular imprinting. Potential applications in chromatographic separations, solid-phase extraction, enzyme-like catalysis, bio-sensors, drug delivery, and other areas have been

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identified [3–10]. To date, the imprinting of low-molecular weight compounds (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars) has now been well established [11–14].

However, several challenges remain in bio-macromolecule imprinting, such as those involving proteins, DNAs, and even whole cells and viruses. Many inherent problems of bio-macromolecules hinder the advancement of their imprinting, such as large molecular size, structural complexity, environmental sensitivity, and flexible conformation [15]. Given these obstacles, the fabrication of bio-macromolecule MIPs in the applications of diagnostics, bio-sensors, and bio-separation is still carried out [16–19]. An increasing number of researchers have focused on alternative strategies to overcome the aforementioned barriers from different viewpoints. One strategy is based on the surface imprinting technique to prepare MIP matrices on the surface, where cavities and pits are exposed [20–23]. The cavities and pits of the polymer matrix are accessible to protein molecules. Another method involves the use of hydrogel with soft and macroporous structure to manufacture an artificial antibody [24–31], which helps diffuse and elute the template. Given that the preparation conditions are beneficial in maintaining the native protein conformation, either synthetic or natural hydrogel materials are often selected as the hydrogel matrix.

Biocompatible polyacrylamide (PAM) hydrogel possesses soft and wet macroporous structure that can allow the diffusion of large proteins. PAM chains contain many amide functional groups capable of forming strong interactions with peptide bonds in the protein even in polar solvents [32]. Hjerten et al. synthesized protein-imprinted hydrogels with a low degree of cross-linking, which demonstrated highly selective recognition ability for template molecules. Guo et al. [33] prepared bovine hemoglobin-imprinted chitosan microspheres by trapping selective soft PAM gel in the pores of the cross-linked chitosan beads. Pang et al. [34] reported bovine serum albumin (BSA)-imprinted PAM gel beads via inverse-phase seed suspension polymerization using high-density cross-linked gel beads as the core and low-density cross-linked PAM gel as the imprinting shell. Lu et al. [35] fabricated BSA and lysozyme surface-imprinted magnetic gel microspheres using magnetic composite gel microspheres as seeds via inverse-phase seed suspension polymerization. Qin et al. [36] fabricated lysozyme imprinted polymer beads using chloromethylated polystyrene beads as supports via surface-initiated living radical polymerization in aqueous media.

In previous studies, almost all protein-imprinted PAM hydrogels are particles or microspheres, whose shapes are difficult to maintain during recycling in aqueous solution. Microsphere preparation often uses organic solvent as a dispersion medium, which inevitably causes protein denaturation. Film materials have more advantages than microspheres or granules in several applications, such as extraction of required proteins from cells or particulate samples. However, obtaining a thin film of PAM hydrogel is difficult. Materials with neat form, good thermal stability, and good mechanical strength can be obtained via surface-grafting imprinting technology on inorganic or organic carrier. In recent years, several studies have reported on small molecule imprinting fibrous materials [37], which exhibit good flexibility and mechanical strength.

This paper presents a simple method of preparing protein-imprinted PAM hydrogel membrane using non-woven polypropylene (PP) fiber as matrix, BSA as template molecule, acrylamide (AM) as functional monomer, and *N,N'*-methylenebisacrylamide (MBA) as cross-linker via UV radiation-reduced polymerization. Factors that influence the adsorption capacity of MIPs were investigated, such as monomer concentration, cross-linker concentration, template molecule amount, and pH values in BSA solution. The rebinding and recognition properties of the PP-grafted imprinting PAM hydrogel membrane were evaluated.

2. Experimental

2.1. Materials

Non-woven polypropylene (PP) fiber (22 g/m²) were purchased from Xianghehuaxin Non-woven Co., Ltd (Langfang, China). Acrylamide (AM) and *N,N'*-methylenebisacrylamide (MBA) were purchased from Chemistry Reagent Factory of Tianjin (Tianjin, China). Ammonium persulfate (APS), glacial acetic acid (HAc) and sodium dodecyl sulfate (SDS) were obtained from the Institute of Tianjin Guangfu Fine Chemicals (Tianjin, China). Bovine serum albumin (BSA, *M_w* 67 kDa, *pI* 4.9), lysozyme (Lys, *M_w* 14.4 kDa, *pI* 11), ovalbumin (Ova, *M_w* 43 kDa, *pI* 4.7), bovine hemoglobin (BHb, *M_w* 64.0 kDa, *pI* 6.9) and bovine γ -globulin (Glo, *M_w* 43 kDa, *pI* 7.1) were purchased from Lanji of Shanghai Science and Technology Development Company (Shanghai, China). All other chemicals were of analytical grade and used as received.

2.2. Preparation of non-woven PP-grafted BSA-imprinted PAM

Non-woven PP fiber (640 mg) was immersed in 33.6 mL of deionized (DI) water containing BSA (45 mg), AM (6 g), APS (60 mg),

and MBA (60 mg). This mixture was incubated for 1 h at room temperature to allow the pre-assembly between the template molecules and the functional monomers. The non-woven PP was then placed on the quartz glass sheet, purged with nitrogen for 8 min, and sealed. Subsequent grafting polymerization was conducted for 1 h with ultraviolet (UV) irradiation at room temperature to produce the polymer hydrogel. The non-woven PP-grafted hydrogel was then repeatedly rinsed to remove the unreacted monomer and cross-linker with distilled water, and then, the template was eluted with acetic acid solution (10%, v/v) containing SDS (10%, w/v) until no BSA in the supernatant was detected by measuring the ultraviolet absorbance at 280 nm. Ultimately, the non-woven PP-grafted hydrogel was extensively washed with deionized water to remove remnant SDS and acetic acid. The non-woven PP-grafted BSA-imprinted PAM was prepared and labeled as PP-g-PAM MIP. The non-woven PP-grafted non-imprinted PAM was also prepared, which corresponds to PP-g-PAM MIP but without the template and was labeled as PP-g-PAM NIP.

2.3. Characterization

Fourier transform infrared (FT-IR) spectra of PP, PAM and PP-g-PAM were recorded with an Avatar 360 instrument (Nicolet, Waltham, MA, USA). The morphologies of PP and PP-g-PAM MIP were observed using a scanning electron microscope (FESEM; S-4800, HITACHI, Japan). PP and PP-g-PAM were dried at 55 °C under vacuum to constant weight. DSC and TG measurements were carried out for these dry samples using a NETZSCH STA 409 PC/PG (NETZSCH, Germany) analyzer at a heating rate of 10 °C/min from 20 °C to 200 °C under continuous flow of dry nitrogen.

2.4. Grafting rate of PAM

PP-g-PAM was dried at 50–70 °C in vacuum for several hours to remove water, and the grafting rate *G* was calculated using the following equation:

$$G(\%) = [(W_1 - W_0)/W_0] \times 100\% \quad (1)$$

where *W*₀ and *W*₁ were defined as the weight of the fiber before and after the grafting process, respectively.

2.5. Adsorption experiments

Adsorption experiments were carried out using a batch-wise adsorption method [23,27,33]. Wet PP-g-PAM MIPs or NIPs (1.0 g) were placed in each glass bottle containing 10 mL of 1.36 mg/mL BSA solutions to evaluate the imprinting efficiency and dynamic adsorption or various concentrations (0–3.0 mg/mL) and to determine the adsorption isotherms. BSA concentrations were measured using a UV-vis spectrophotometer at specific time intervals. The equilibrium adsorption capacity *Q_e* (mg/g) of the protein on the polymers was determined according to the following formula:

$$Q_e = (C_0 - C_e)V/W \quad (2)$$

where *Q_e* (mg/mL) is the equilibrium absorption, *C*₀ (mg/mL) is the initial BSA concentration, *C_e* (mg/mL) is the final concentration of BSA at equilibrium, *V* (mL) is the volume of BSA solution, and *W* (g) is the weight of MIPs or NIPs. The imprinting efficiency (IE) of MIPs was defined as follows:

$$IE = Q_{MIP}/Q_{NIP} \quad (3)$$

where *Q_{MIP}* and *Q_{NIP}* are the *Q_e* of MIPs and the corresponding NIPs, respectively.

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