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CHARACTERIZATION OF PVA-BASED MAGNETIC AFFINITY SUPPORT FOR PROTEIN ADSORPTION

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CHARACTERIZATION OF PVA-BASED MAGNETIC AFFINITY SUPPORT FOR PROTEIN ADSORPTION

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ABSTRACT

A magnetic fluid was prepared by an oxidization-precipitation with $FeCl_2$ and H_2O_2 in polyvinyl alcohol (PVA) solution. Cross-linked directly with glutaraldehyde, a magnetic support with magnetic particles entrapped by cross-linked PVA gel was produced. Cibacron blue 3GA (CB) was immobilized to the magnetic support to prepare the magnetic affinity support (MAS). The MAS was characterized by transmission electron microscopy (TEM) and infrared spectrum analysis. The TEM showed that the MAS ranged from 1 to 10 μm and consisted of nanometer-sized colloidal magnetite particles. It was determined that the MAS had a saturation magnetization of 1.16×10^3 A/m and showed no hysteresis in an external magnetic field of up to 5.6×10^5 A/m. The adsorption kinetics and equilibrium of bovine serum albumin (BSA) confirmed rapid adsorption and large capacity of the MAS. BSA adsorption reached equilibrium in 5 min. At a CB coupling density of 23 μmol/g, the adsorption capacity of the MAS was 35 mg/g at an aqueous phase concentration of 0.1 mg/mL. The possibility of

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repetitive uses of the MAS was also demonstrated, indicating the stability of the magnetic support for protein adsorption.

Key Words: Magnetic affinity support; Polyvinyl alcohol; Cibacron blue; Protein adsorption

INTRODUCTION

Use of magnetic separation has remained an attractive research subject in the field of biotechnology. Based on the magnetic features of solid materials, many techniques, such as magnetically stabilized fluidized bed (1), high-gradient magnetic filtration (2), magnetic, aqueous two-phase extraction (3), and magnetophoresis (4), have been developed. Magnetic supports can be used for enzyme-catalyzed bioreactors (5), cell separation (4), and protein fractionation (6). Through the use of magnetism for separation, these techniques show advantages in terms of rapidity, high efficiency, cost-effectiveness, and lack of negative influence on biological activity.

The availability of magnetic supports is one of the main factors limiting the large-scale application of the magnetic techniques. Therefore, many researches have contributed to the development of magnetic materials (7–11). The magnetic supports are usually composed of one or more ferromagnetic cores, which ensure a strong magnetic response, and a polymeric shell, which provides favorable biocompatibility and functional groups for covalently coupling ligands for selective binding. The most commonly used ferromagnetic materials in the synthesis of magnetic supports are γ-Fe_2O_3 and Fe_3O_4, which are formed by either coprecipitation of Fe(II) and Fe(III) or oxidation-precipitation of Fe(II) and an oxidizer in a basic environment. The choice of shell polymers is extensive: polysaccharides, such as agarose, cellulose, and dextran (7); polyvinyl alcohol (PVA) (8); polystyrene (9); polyacrylamide (10); and proteins (11) have been utilized for the purpose.

PVA is one of the most hydrophilic synthetic polymers available and has been used for the surface coating of hydrophobic matrices (12). In addition, matrices made of cross-linked PVA have been utilized for cell (13) and enzyme (14) immobilization. PVA-based magnetic microspheres are usually synthesized by a 2-phase suspension–cross-linking procedure (8,15). However, to remove the bound oil phase from the microspheres, this procedure is invariably followed by a wash step that is usually time consuming and needs a large amount of organic solvents.

In the present work, we develop a simple procedure to prepare a PVA-based magnetic solid support. The procedure involves an oxidization-precipitation of $FeCl_2$ and H_2O_2 in PVA solution and a subsequent aqueous phase cross-linking of PVA to form a stable PVA shell around magnetic cores. The magnetic properties

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of the magnetic support have been identified. After coupling a reactive dye, Cibacron blue 3GA (CB), to the magnetic support, the affinity adsorption of bovine serum albumin (BSA) to the support has been extensively investigated.

MATERIALS AND METHODS

Materials

PVA (M_r = 7.48 × 10^4–7.92 × 10^4, 88% hydrolyzed) was obtained from Beijing Organic Chemicals Co (Beijing, China). Antifoamer 7010, poly(oxyethylene-oxypropylene)glycerol ether, was purchased from Tianjin Tianzhu Fine Chemicals, Ltd (Tianjin, China). CB and BSA (fraction V, minimum 98%) were received from Sigma. All other reagents were of analytical grade and used as received. A neodymium-iron-boron permanent magnet (maximum field strength 0.4 T) was obtained from the Research Institute of Rare Earth Elements (Baotou, China).

Preparation of Magnetic Support

Magnetic fluid stabilized by PVA was prepared by a modified method reported by Qiu, Zhang, and Sun (16) in which polyethylene glycol was used as a stabilizer. In a typical preparation, 30 mL of 0.18 mol/L $FeCl_2$ was mixed with 40 mL of 50 g/L PVA at 50°C under a nitrogen atmosphere. Three to 6 drops of antifoamer 7010 was introduced to prevent foam formation. Under vigorous agitation, 10 mL of 0.18 mol/L H_2O_2 and 20 mL of 3.0 mol/L NaOH were slowly and separately added to the mixture. In this process, the color of the reaction mixture gradually changed from light green to nacarat, dark green, and finally black. The black mixture was aged at 50°C for 2 h and then cooled to ambient temperature. The magnetic fluid thus obtained was dialyzed against water for 24 h and collected in a ground-glass, stoppered flask prior to the cross-linking treatment.

Twenty-five milliliters of magnetic fluid was diluted twice and treated by ultrasonication for 15 min to ensure homogeneous dispersion of magnetic particles. It was then titrated to pH 4 by additions of 1.0 mol/L hydrochloric acid and was then kept at 70°C. Fifty milliliters of 1% (w/w) glutaraldehyde were added drop wise under vigorous agitation. This initiated the cross-linking reaction between the hydroxyl groups on PVA chains and the formyl groups of glutaraldehyde. After 60 min, the cross-linking reaction was terminated through the introduction of 2 mL of 3.0 mol/L NaOH, and the mixture was cooled to room temperature. The magnetic particles were recovered by magnetic sedimentation with the permanent magnet from the mixture and washed extensively with deion-

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ized water to remove physically adsorbed glutaraldehyde. Formyl groups remaining on the particles were reduced to hydroxyl groups by suspending the solid phase to 100 mL of 0.1 mol/L $NaBH_4$ solution and shaking the suspension overnight in a shaking incubator (160 rpm) at room temperature. The magnetic support thus obtained was repeatedly washed with deionized water prior to the covalent coupling of CB to the solid phase.

CB Coupling to Magnetic Support

Because of the abundance of hydroxyl groups on the magnetic support, CB was directly coupled to it without the aid of any activating agent. Typically, about 3.0 g of wet magnetic affinity support (MAS) was dispersed in 100 mL of 2 mmol/L CB solution (pH 12), and the mixture was placed in an incubator at 25°C and shaken at 160 rpm for 24 h. The CB-modified magnetic support was routinely washed with deionized water, 25% (v/v) ethyl alcohol, and finally with Tris-HCl buffer (0.01 mol/L, pH 7.6) to remove unbound CB. The coupling density of CB to the support was determined by mass balance. Different coupling densities of CB were achieved by changing the initial concentration of CB in the reaction system. The CB-modified magnetic support is referred to as magnetic affinity support (MAS) hereafter.

Protein Adsorption

BSA adsorption to the MAS was carried out by the batch adsorption method (17). All experiments were performed in 0.01 mol/L Tris-HCl buffer containing NaCl (pH 7.6) at 25°C. Each batch of BSA/MAS mixture consisted of approximately 20 mg of wet MAS in 5 mL, while BSA concentration ranged from 0 to 2 mg/mL. For adsorption kinetics studies, up to 10 batches of BSA/MAS mixture were prepared and incubated for different periods before magnetic separation to remove the MAS from each protein solution. The supernatant was subjected to protein measurement. To determine the adsorption isotherms, a 15-min incubation was performed, which enabled the adsorption to reach equilibrium. The adsorbed BSA density in terms of milligrams per gram of the MAS (dry weight) was calculated by mass balance (18).

Analysis and Measurements

The morphology of the MAS was observed with a transmission electron microscope (JEM-100CXII, JEOL). The infrared spectrum (IR) of the MAS was measured with an IR spectrometer (5DX, Nicolet). A small fraction of the MAS

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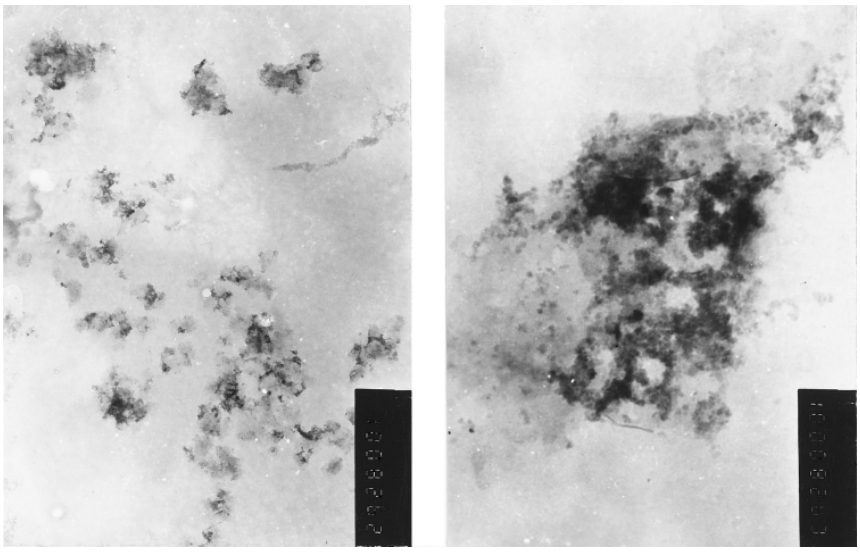
was ground with potassium bromide in an agate mortar and the powder was pressed into a disc prior to the measurement. The packing density of wet MAS and the density of dried MAS were measured using a 25-ml pycnometer. The magnetization of the MAS was determined with a Faraday magnetic balance (19) developed by the Department of Catalysis Science and Engineering, Tianjin University, and ammonium ferrous sulfate (Mohr's salt) was used as a standard.

Protein and CB concentrations were measured with a VIS-UV spectrophotometer (752C, Shanghai, China) at 280 nm and 620 nm, respectively. The extinction coefficients determined by preliminary experiments were 0.633 L/g·cm for BSA and 12.35 $(\text{mmol/L})^{-1}\cdot\text{cm}^{-1}$ for CB. These values are in agreement with those reported previously (20,21).

RESULTS AND DISCUSSION

TEM and Infrared Spectrum of MAS

Transmission electron microscopy (TEM) revealed the morphology and structure of the MAS (Fig. 1). The MAS was irregular in shape, with dimensions of 1 to 10 μm (Fig. 1a). A single MAS is shown in Fig. 1b at a larger magnification. The MAS is an assembly of many fine particles of nanometer magnitude



(a) (b)

Figure 1. TEM photographs of the MAS. The morphology (a) and the internal structure (b) of the MAS were observed under magnifications of 10k (a) and 100k (b).

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(black dots in Fig. 1b). The fine particles are considered to be crystallites of both Fe_3O_4 and γ-Fe_2O_3. The crystallites were formed during the oxidation-precipitation as expressed in the following reaction formula:

$$3Fe^{2+} + H_2O_2 + 6OH^- \rightarrow Fe_3O_4\downarrow + 4H_2O \quad (1)$$

These crystallites would partially transform into maghemite (γ-Fe_2O_3) in the weakly acidic medium (22) during the cross-linking procedure. However, this transformation does not significantly influence the magnetic response of the MAS because magnetite and maghemite both exhibit ferromagnetism.

Some important functional groups of the MAS can be identified from the infrared spectrum. Figure 2 shows the peak representing hydroxyl groups at 3400 cm^{-1}, indicating the presence of PVA in the MAS. The other peaks, at 1059 cm^{-1}, 1163 cm^{-1}, and 1271 cm^{-1} represent symmetric and asymmetric stretching of —S═O on the CB molecule. No absorbance is observed between 1800–1700 cm^{-1}, indicating the lack of —C═O, which had been purposefully eliminated in the reduction procedure.

The packing density of wet MAS, $\rho_{packing}$, and the density of dried MAS, ρ_{dry}, were determined to be 1.034 and 1.657 g/cm^3, respectively. The voidage of settled particles is generally in the range of 0.26 to 0.4 (20,23). Within this range, the variation of the voidage of settled MAS will lead to, at maximum, 2% deviation of the calculated wet density and porosity of the MAS. So the voidage value in this research was chosen as 0.3 instead of measured experimentally. The wet

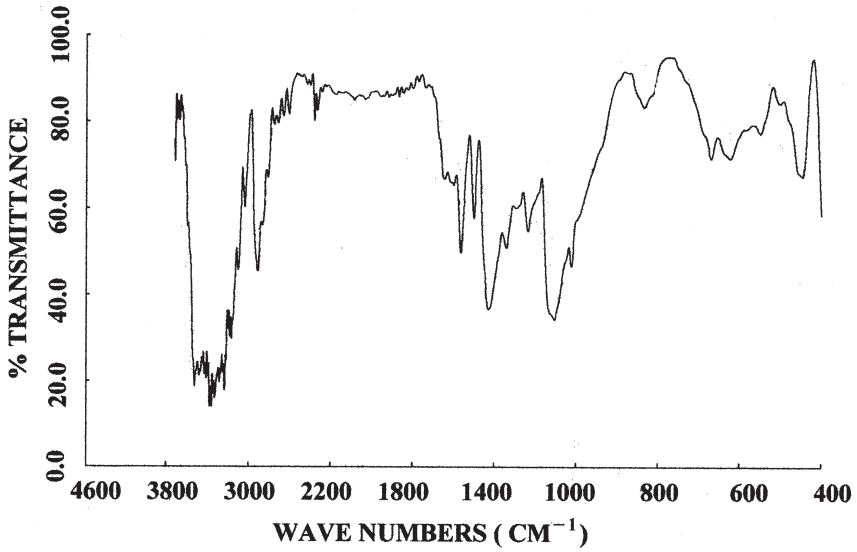

Figure 2. Infrared spectrum of the MAS. CB coupling density was 23 μmol/g dry MAS.

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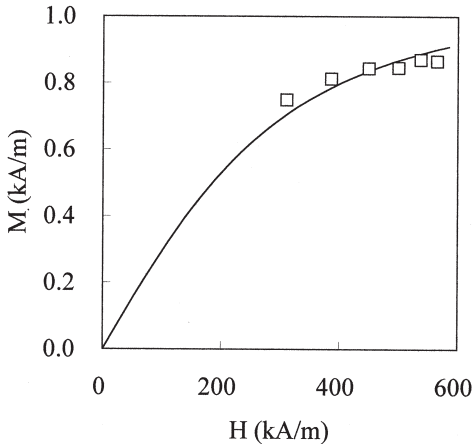

Figure 3. Magnetization of the MAS. The solid line was calculated from Langevin's equation.

density, ρ_{wet}, and the porosity, ε_p, of the MAS were then estimated to be 1.05 g/cm^3 and 0.92, respectively. Then, 1.0 g of dried MAS was found to correspond to 7.5 mL of wet MAS from the following equation:

$$V_{eq} = \frac{W_{\mathrm{MAS}}}{(1 - \varepsilon_p)\,\rho_{\mathrm{dry}}} \tag{2}$$

Where V_{eq} is the equivalent volume and W_{MAS} is the mass of dry MAS.

Magnetic Properties of MAS

In the magnetization measurements, the external magnetic field ranged from 0 to 5.6×10^5 A/m. It rose and declined 3 times, and no hysteresis loop was observed in the procedure. Figure 3 shows the magnetization curve of the MAS. The solid line in the figure was calculated from Langevin's theory (24):

$$M = fM_s\left[\coth\left(\frac{\mu_0 M_s V H}{kT}\right) - \frac{kT}{\mu_0 M_s V H}\right] \tag{3}$$

Where f is the volumetric fraction of colloidal magnetite in the MAS, M_s the saturation magnetization of the colloidal magnetite, μ_0 the permeability in vacuum, V the volume of the colloidal magnetite, H the external magnetic field strength, k the Boltzmann's constant, and T the absolute temperature. In the calculation, the diameter of the magnetite particles in the MAS was fixed at 8.0 nm according to the TEM observation (Fig. 1b). Using the density of wet MAS (1.05 g/cm^3); the

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density of PVA gel, ρ_{gel}, which is assumed to be 1.0 g/cm^3; and the density of magnetite, ρ_{mag} (5.18 g/cm^3) (25), the volumetric fraction of the colloidal magnetite in the MAS was calculated to be 0.012 using the following equation:

$$f = \frac{\rho_{wet} - \rho_{gel}}{\rho_{mag} - \rho_{gel}} \quad (4)$$

Thus, least-square fitting of Eq. (3) to the experimental data gives the M_s at 9.7×10^4 A/m. This M_s value is about one-fifth that of the bulk magnetite, and results because the colloidal magnetite is smaller than 25 nm, the critical size for ferromagnetism of magnetite (26). The saturation magnetization of the whole MAS particle, 1.16×10^3 A/m, was thus calculated from fM_s. Because the Faraday magnetic balance was not sensitive enough to give precise results at low magnetic field strength, the magnetization data were only available at the magnetic field strength higher than 300 kA/m.

We also tested the magnetic response of the MAS by measuring its sedimentation in a magnetic field. The MAS suspension of 5-cm height in a test tube could settle down completely within approximately 5 min in the magnetic field generated by the NdFeB permanent magnet with a maximum field strength of 0.4 T and an average field strength gradient of 5.7 T/m. However, without the magnetic field, the sedimentation of the MAS was 1000 times slower. The results indicate a strong magnetic response of the MAS. This feature is crucial to the potential applications of the MAS in fast magnetic separation processes.

Adsorption Kinetics

The BSA uptake rate by the MAS is described by the adsorbed density q as a function of incubation time and is shown in Fig. 4. BSA adsorption to the MAS reached equilibrium within 5 min. This is comparable with the dynamic adsorption behavior of the nanometer-sized nonporous magnetic polymer latex (MPL) (27) and commercial magnetic agarose beads (6). Therefore, the equilibrium binding experiments were carried out for 15 min to determine BSA adsorption isotherms.

Adsorption Equilibrium

The adsorption isotherms of BSA to the MAS with different CB coupling densities are provided in Fig. 5. Linear adsorption equilibrium of BSA was observed in the CB coupling density range, and the equilibrium data can be well fitted with Henry's law:

$$q = kc \quad (5)$$

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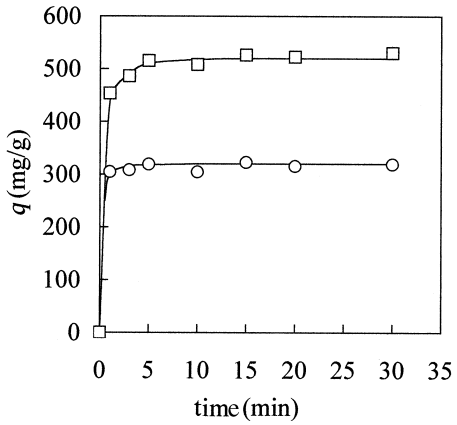

Figure 4. Adsorption kinetics of BSA to the MAS. Initial BSA concentrations were (in mg/mL) 1.286 (□) and 0.429 (○). CB coupling density was 17.7 μmol/g dry MAS. NaCl was not added.

A linear relation exists between the *k* values and the CB coupling densities (Fig. 6). The *k* value at the CB coupling density of 23 μmol/g was 348, which means up to 35 mg of BSA were adsorbed by the MAS when the equilibrium BSA concentration at aqueous phase reached 0.1 mg/mL. This capacity is similar to that of the MPL (27).

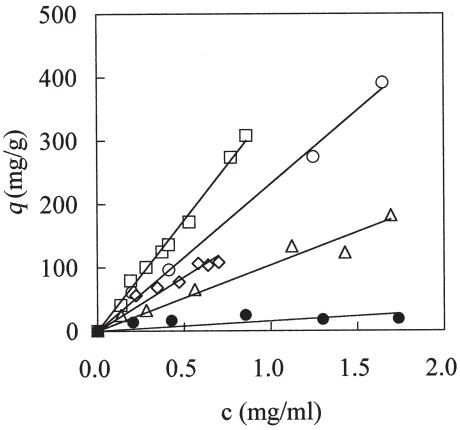

Figure 5. Effects of CB coupling density and aqueous-phase ionic strength on BSA adsorption equilibrium to the MAS. CB coupling densities were (in μmol/g dry MAS) 6.7 (△), 11.3 (◇), 17.7 (○, ●), and 23 (□). NaCl concentrations (in mol/L) were 0.01 (△, ◇, ○, □) and 1.0 (●).

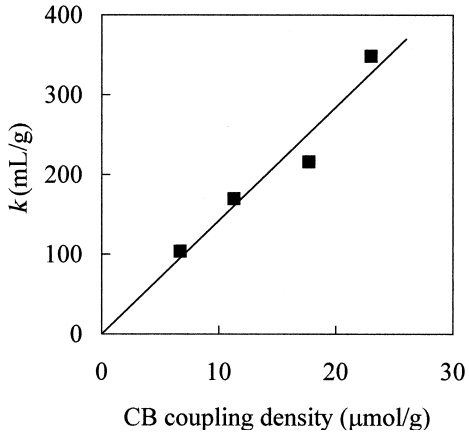

Figure 6. Linear relationship between the *k* value and CB coupling density.

It is widely known that the capacity of protein adsorption to dye-ligand adsorbents decreases with increased aqueous-phase ionic strength (18,20). Hence, the effect of ionic strength on BSA adsorption to the MAS was also investigated (Fig. 5). As expected, the adsorption ability of the MAS is suppressed at higher ionic strength. The decrease of BSA adsorption with increasing ionic strength has been explained as the result of hydrophobic interactions between CB molecules and the matrix surface, which decreases the number of ligands accessible to protein binding (28).

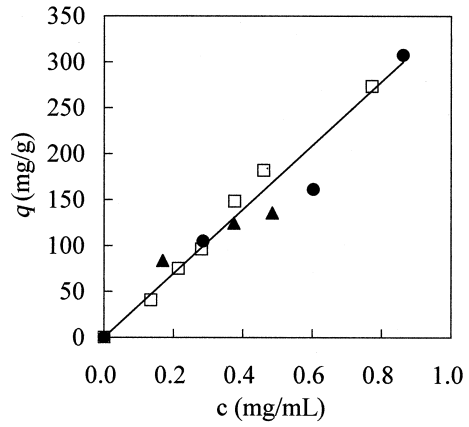

Figure 7. Recycled MAS. Cycle 1 (▲), Cycle 2 (□), Cycle 3 (●). CB coupling density was 23 μmol/g dry MAS. NaCl concentration was 0.01 mol/L.

Recycled use of the MAS for BSA adsorption was also investigated to test the stability of the MAS. NaOH solution (0.1 mol/L) was used to regenerate the MAS after each cycle. Figure 7 shows that BSA adsorption equilibrium is unchanged in 3 cycles. Moreover, no obvious changes of the morphology, size, and magnetic response of the MAS were found in the recycling process.

CONCLUSIONS

A micron-sized PVA-based magnetic affinity support has been synthesized by an aqueous phase suspension cross-linking procedure. The method was fast, simple, and environmentally benign because organic solvents were not involved in the process. The saturation magnetization of the MAS was determined to be 1.16×10^3 A/m. Hysteresis loop was not observed in a magnetic field up to 5.6×10^5 A/m. These properties ensure a strong magnetic response and easy redispersion of the MAS in aqueous phase. The adsorption kinetics and equilibrium of BSA adsorption data confirmed rapid adsorption and large capacity of the MAS. BSA adsorption reached equilibrium in 5 min, and the adsorption capacity of the MAS at a CB coupling density of 23 μmol/g was as high as 35 mg/g at aqueous phase concentration of 0.1 mg/mL. Moreover, recycled use of the MAS was possible, indicating the stability of the MAS for protein adsorption.

ACKNOWLEDGMENT

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