Sensors and Actuators B 196 (2014) 265–271

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Effect of carboxyl density at the core–shell interface of surface-imprinted magnetic trilayer microspheres on recognition properties of proteins

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A R T I C L E I N F O

Article history:
Received 23 October 2013
Received in revised form 20 January 2014
Accepted 29 January 2014
Available online 6 February 2014

Keywords:
Surface imprinting
Carboxyl density
Protein recognition
Magnetic microspheres

A B S T R A C T

A novel protein surface-imprinted magnetic trilayer composite microsphere (Fe_3O_4@HEA@protein-MIPs) was designed and prepared. The objective of this study was to study the effect of carboxyl density at the core–shell interface on recognition performance. The imprinting process was carried out on the surface of Fe_3O_4@hydroxyethyl acrylate (Fe_3O_4@HEA) microsphere modified by maleic anhydride (MAH) in the presence of functional monomer acrylamide (AM) and cross-linker N,N′-methylenebisacrylamide (MBA). The results revealed that the adsorption rate, adsorption capacity and recognition capacity of surface-imprinted magnetic microsphere increased with increasing carboxyl density. Meanwhile, the increase of carboxyl density was conducive to improve the imprinting efficiency of the proteins with high isoelectric point (pI > 7). Furthermore, the effect of carboxyl at the core–shell interface on imprinting efficiency was restricted by the thickness of imprinted layer, and the critical thickness which related to the size of template protein was obtained by experiments.

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Introduction

Over the past decades, molecularly imprinted polymers (MIPs) have attracted intense attention because of their potential applications in chromatographic separation, solid-phase extraction, drug delivery, medical diagnostics and biosensors [1–4]. High separation efficiency and selectivity of MIPs can be attributed to their recognition sites, which are complementary with template molecules in shape, size and functional groups.

At present, most of the published reports are concerned with small template molecules. However, there are a few reports about macromolecules imprinting, especially protein imprinting [5–7]. The challenge lies in two aspects: (1) huge molecular size of protein renders it difficult to transfer and (2) sensitivity of biomacromolecule and complex structure of protein make the imprinting process less selective. In response to these limitations, several strategies have been addressed including surface imprinting [8–13] and epitope imprinting [14,15]. Among them, surface imprinting is a promising strategy due to its recognition sites existing on the surface of suitable support material, which makes the target molecules easier to elute and rebind.

So far, influences of several parameters, such as total monomer concentration, crosslinking density, pH of phosphate buffer solution (PBS), salt concentration and introduction of biocompatible molecular chains, on protein surface imprinting efficiency have been investigated. For example, Lin and co-workers [2] studied the effects of crosslinking density, total monomer concentration, pH of buffer solution and buffer concentration on adsorption amount and imprinting factor of imprinted microspheres for lysozyme. They found that the imprinting factor varied inversely with the degree of crosslinking, and it was first increased and then decreased along with the increase of total monomer concentration. Moreover, the optimal pH of buffer solution and buffer concentration were 7 and 0.02 mol L^{-1}, respectively. In addition, the effects of salt concentration on the shrinking and swelling properties of the MIPs hydrogel, which were closely related to the binding of Fe_3O_4@SiO_2@MIPs with template proteins, were reported in the literature [16]. Jing pointed out that the adsorption capacities of Fe_3O_4@SiO_2@MIPs to template proteins were decreased with the increase of salt concentration. Besides, the biocompatible maleic anhydride molecular chains and acryloyl-β-cyclodextrin were introduced in the preparation of MIPs to improve the affinity between imprinted sites and template proteins [17,18].

Generally speaking, the features of imprinted polymers are determined by their structure. For surface imprinted materials, functional groups on the surface may play more important role in adsorption and recognition of protein. Based on this

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http://dx.doi.org/10.1016/j.snb.2014.01.109

consideration, a series of model surface-imprinted magnetic trilayer microspheres, Fe_3O_4@HEA@protein-MIP, with different carboxyl densities at the core–shell interface and different shell thicknesses, were designed and synthesized in this work. The imprinted shell was composed of AM and MBA. AM was the functional monomer on account of the affinity of amide groups toward carboxyl groups of protein. MBA was used as the cross-linker. Among the trilayer structure of Fe_3O_4@HEA@protein-MIP, the carboxyl density could be adjusted through changing the HEA content in the middle polymer shell. Furthermore, the adsorption capacity and selectivity of Fe_3O_4@HEA@protein-MIP were discussed through adsorption kinetics and adsorption selectivity experiments. Finally, a series of experiments were carried out to study the influence of carboxyl density at the core–shell interface on imprinting efficiency and the effect of the thickness of imprinted layer on the function of carboxyl.

Experimental

Materials

Ferric chloride ($FeCl_3{\cdot}6H_2O$) and trisodium citrate were supplied by Shanghai Chemical Reagents Company (China). 2-Hydroxyethyl acrylate (HEA), N,N-methylenebisacrylamide (MBA), maleic anhydride (MAH), ammonium persulfate (APS) and N,N,N,N-tetramethylenebis(acrylamide) (TEMED) were obtained from Sigma–Aldrich (Tokyo, Japan). Azodiisobutyronitrile (AIBN), acetonitrile, triethylamine (TEA) and toluene were purchased from Tianjin Chemical Reagents Company (China). Acrylamide (AM) was provided by Acros Organics (Morris Plains, NJ, USA). Bovine serum albumin (BSA), Ribonuclease A (RNase A) and Lysozyme (Lyz) were supplied by Amresco (Solon, OH, USA).

Characterization

Fourier Transform Infrared (FTIR) spectra were acquired on a TENSOR27 FTIR spectrometer (Bruker). The samples were prepared by mixing the products with KBr and pressing into a compact pellet. The morphology of the resultant microspheres was determined by transmission electron microscopy (TEM) using a Tecnai G2 20-S-TWIN microscope. Thermogravimetric analysis (TGA) data were obtained with a heating rate of 10 K min^{-1} under nitrogen atmosphere using a TA TGA-2950 apparatus.

Synthesis of Fe_3O_4@HEA core–shell microspheres

The Fe_3O_4 core was synthesized according to the reported solvothermal method [19]. The detailed steps were described in the supporting information. A typical procedure for the synthesis of Fe_3O_4@HEA core–shell microspheres by precipitation polymerization (Fig. 1) was as follows: 0.5 g of Fe_3O_4 microspheres were suspended in 80 mL acetonitrile in a 250 mL three-neck flask. Then, HEA (0.75 g), MBA (0.25 g) and AIBN (0.01 g) were dissolved in the above suspension. The suspension solution was heated to boiling state within 30 min, and the reaction was ended after 40 mL acetonitrile being distilled from the reaction system within 2 h. After the polymerization, the resultant Fe_3O_4@HEA microspheres were purified by repeated centrifugation, decantation, and resuspension in water three times and freeze-dried for subsequent use. Note that, this sample was marked Fe_3O_4@HEA(1). Using the same method, addition amounts of HEA and MBA were changed to prepare Fe_3O_4@HEA(2) (0.5 g, 0.5 g) and Fe_3O_4@HEA(3) (0.25 g, 0.75 g).

Synthesis of Fe_3O_4@HEA@protein-MIPs(NIPs) microspheres

Fe_3O_4@HEA@protein-MIPs microspheres were synthesized by surface grafting copolymerization. As shown in Fig. 1, Fe_3O_4@HEA was modified by MAH first. Briefly, 0.3 g MAH was dissolved in 40 mL acetone/toluene (15 mL/25 mL) solution. Subsequently, 0.3 g Fe_3O_4@HEA was dispersed in the above solution. Then, 1.5 mL triethylamine was added. The solution was stirred for 8 h at 80 °C. The obtained Fe_3O_4@HEA—COO—C=C—COOH microspheres were purified completely for further processing. Here, carboxyl contents of Fe_3O_4@HEA—COO—C=C—COOH microspheres were measured by titration. After modification with carboxyl groups, the obtained Fe_3O_4@HEA—COO—C=C—COOH was dispersed in 10 mL phosphate buffer (0.2 mol L^{-1}, pH = 7.0) by ultrasonic vibration. Meanwhile, 71 mg AM, 77 mg MBA, and 20 mg protein were also dissolved in 10 mL phosphate buffer (0.2 mol L^{-1}, pH = 7.0) and then the mixture was added into the above solution. When the temperature was raised to 35 °C, 10 mL APS phosphate buffer (0.1%, w/w) and 10 μL TEMED were added into the mixture by injecting. The polymerization was initiated and continued under slight stirring at 35°C for 24 h. After the reaction, the resultant Fe_3O_4@HEA@protein-MIPs microspheres were washed repeatedly with the solution containing SDS (10%, w/v) and acetic acid (10%, v/v) to remove adsorbed oligomers, unreacted monomers, the entrapped and the embedded template proteins until no template protein in the supernatant was detected using a UV-2550 (Shimadzu) spectrophotometer at maximum absorption wavelength. Finally, the microspheres were washed several times with deionized water to remove the remaining SDS and acetic acid, and then dried by lyophilization for subsequent use. The Fe_3O_4@HEA@protein-NIPs were prepared using the same procedure but without template protein.

Adsorption experiments

The adsorption tests were performed as described below. 8 mg Fe_3O_4@HEA@protein-MIPs(NIPs) microspheres were incubated with 5.00 mL template protein solution at 0.8 mg mL^{-1} concentration for 3 h. Then the microspheres were centrifuged at 10,000 rpm for 8 min and the concentration of template protein in the supernatant solution was measured by a UV-2550 (Shimadzu) spectrophotometer at maximum absorption wavelength.

The amounts (Q) of template protein adsorbed by Fe_3O_4@HEA@protein-MIPs(NIPs) microspheres were calculated according to formula (1):

$$Q\,(\mathrm{mg\,g^{-1}}) = (C_0 - C_1)\frac{V}{m} \tag{1}$$

where C_0 is the initial concentration of template protein solution (mg mL^{-1}), C_1 is the template protein concentration of the supernatant solution (mg mL^{-1}), V is the volume of the template protein solution (mL) and m is the mass of Fe_3O_4@HEA@protein-MIPs(NIPs) (g).

The recognition ability of the Fe_3O_4@HEA@protein-MIPs(NIPs) was evaluated by imprinting factor (IF) and selectivity factor (β), which were defined as formulas (2) and (3), respectively:

$$\mathrm{IF} = \frac{Q_{\mathrm{MIPs}}}{Q_{\mathrm{NIPs}}} \tag{2}$$

$$\beta = \frac{\mathrm{IF_{TEM}}}{\mathrm{IFN_{TEM}}} \tag{3}$$

In formula (2), Q_{MIPs} and Q_{NIPs} represent the adsorption capacity of the template or the non-template on MIPs and NIPs, respectively. In formula (3), $\mathrm{IF_{TEM}}$ and $\mathrm{IF_{NTEM}}$ are the imprinting factors for template protein and non-template protein, respectively.

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