Synthesis of Magnetic Molecularly Imprinted Polymer Nanowires Using a Nanoporous Alumina Template

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ABSTRACT: A convenient imprinting method for the preparation of magnetic molecularly imprinted nanowires within the pores of nanoporous alumina membrane is described. The template molecule (theophylline in this paper) was immobilized on the pore walls of a nanoporous alumina membrane. The nanopores were then filled with a prepolymerization mixture containing the superparamagnetic $MnFe_2O_4$ nanocrystallites. After polymerization, the alumina membrane was subsequently removed by chemical dissolution, leaving behind magnetic polymer nanowires that contain theophylline binding sites uniquely residing at the surface and have a saturated magnetization (M_S) of 1.97 emu/g. The resulting magnetic imprinted polymer nanowires were capable of binding theophylline more strongly than the nonimprinted nanowires.

Introduction

Molecular imprinting of synthetic polymers has been adopted for the generation of artificial biological macromolecular receptors in recent years.[1−3] The stability, ease of preparation, and low cost of these molecularly imprinted polymers (MIPs) make them particularly attractive. Although MIPs have already been used in some real applications, notably solid-phase extraction, for which they are commercialized,[4−10] a lot of work remains to upgrade their performance in order to make them into viable alternatives for biological macromolecules. One major area needing improvement is accessibility. In general, the imprinted sites created by conventional imprinting methods are not very accessible and rather inhomogeneous and thus having different binding affinities. These problems arise from the methods used in forming the polymer imprinting and providing access to the binding sites. By using the conventional imprinting process, the imprinted sites are completely encased within the polymer. To enable access to the binding sites, the polymer must be ground up, thereby exposing the sites. However, doing so causes the deformation of a large number of the binding sites and irreversibly alters the shape specificity and the complementary binding of the sites, thereby adversely affecting their selectivity.

Compared to conventional MIPs, MIPs with binding sites situated at the surface of the imprinting matrix have many advantages: the sites are more accessible, more homogeneous, and uniformly oriented.[11] Mosbach and co-workers have introduced a clever protocol for creating surface imprinting based on oriented immobilization of the imprint molecule on porous silica beads prior to polymerization.[12] The pores are then filled with the monomer mixture, and the polymerization is initiated. After the completion of polymerization reactions, the silica is removed by chemical dissolution, leaving behind a porous polymeric structure, which is the negative image of the original silica beads. This protocol has been further developed by Sellergren for the imprinting of amino acids and peptides.[13,14]

We recently reported the synthesis of conducting polymer nanowires with glutamic acid binding sites situated at the surface by immobilizing the imprint molecules within the pores of the nanoporous alumina membrane.[15] We decided to further investigate: (1) whether radical polymerization of a functional and cross-linking monomer with vinyl or acrylic groups, which have been used in synthesis of most MIPs at this time, could be used to prepare molecularly imprinted polymer nanowires in nanoporous alumina membrane; and (2) whether superparamagnetic nanocrystallites could be satisfactorily entrapped in these nanowires. Magnetic nanoparticles immobilized with biological receptors have been extensively studied and employed in biomedical and biotechnological applications.[16−18] Magnetic nanowires with artificial receptors would likewise find potential use in drug delivery, biochemical sensors, and concentration of trace amounts of specific targets.

In this paper, we describe the successful production of magnetic molecularly imprinted polymer nanowires and their application in binding studies with theophylline.

Experimental Section

Chemicals. The alumina membranes were purchased from Whatman that had nominally 200-nm-diameter pores. 8-Carboxypropyltheophylline, theophylline, theobromine, and caffeine were purchased from Sigma. Methacrylic acid (MAA) was purchased from Alfa and distilled to remove the polymerization inhibitor before use. Divinylbenzene (DVB) containing 20% ethylvinylbenzene was purchased from Fluka and treated with basic alumina immediately prior to use to remove the polymerization inhibitor. 3-Aminopropyltrimethoxysilane was purchased from Aldrich and used as received. Super-dry *N*,*N*′-dimethylformamide was purchased from Acros. *N*,*N*′-Diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) were purchased from GL Biochem (Shanghai) Ltd (China). Distilled, deionized water was used for preparation of all aqueous solutions.

Amino Modification of Alumina Membrane. This was accomplished by immersing the membrane into a solution prepared by mixing 0.5 mL of 3-aminopropyltrimethoxysilane with 3 mL of ethanol that containing 0.2 mL of sodium acetate buffer solution (50 mM, pH = 5.0).[19] The flask containing the above solution was vacuumized for 5 min to remove air from the pores of the

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membrane. The membrane remained in this solution for another 5 min under ambient pressure. The membrane was then cured by heating in a vacuum at 150 °C for 1 h. Note that the surfaces of the membrane are coated with a thin silica film, which is removed by a brief mechanical polish.

Immobilization of 8-Carboxypropyltheophylline. 8-Carboxypropyltheophylline (266 mg, 1 mmol), DIC (750 μL, 5 mmol), and HOBt (676 mg, 5 mmol) were dissolved in anhydrous DMF/DCM (1/1, 15 mL). Dry, freshly made aminomodified membrane was added, and the suspension was shaken on a rocking table for a minimum of 12 h at room temperature. The coupling reaction was allowed to continue until the Kaiser test was negative, which indicated that most amino groups had reacted. After washed with DMF, DCM, and methanol, the membrane was dried in a vacuum for 12 h.

Preparation of Magnetic Imprinted Polymer Nanowires. $MnFe_2O_4$ nanocrystallites were synthesized according to the reference through the formation of water-in-oil reverse micelles.[20] For the well dispersion of $MnFe_2O_4$ nanocrystallites in prepolymerization solution, the nanocrystallites were modified with 3-chloropropionic acid beforehand. Briefly, 10 mg of prepared superparamagnetic $MnFe_2O_4$ nanocrystallites were stirred overnight in 1.0 mol/L aqueous solution of 3-chloropropionic acid at room temperature. The pH of the solution was kept at 4 by adding HCl. The particles were collected with a magnet and washed several times to remove excess 3-chloropropionic acid. With this treatment, 3-chloropropionic acid was chemically attached onto the surface of $MnFe_2O_4$ nanocrystallites through the carboxyl group. The prepolymerization solution was prepared by mixing these modified $MnFe_2O_4$ nanocrystallites with DVB (4.28 mL, 24 mmol), MAA (1.02 mL, 12 mmol), and AIBN (10 mg).

The dried amino-modified membrane (control membrane) or 8-carboxypropyltheophylline-immobilized membrane (template membrane) was added to a flask and vacuumized for 60 min. Then the prepolymerization solution was added under vacuum, and the system was incubated at 30 °C for 2 h. After the system was incubated for 3 more hours at 60 °C with fast vibration, the membrane was thoroughly rinsed with methanol and dried overnight at 60 °C. After the dissolution of alumina membrane and silica with 0.1 M NaOH for 12 h and 5% HF for 12 h at 4 °C (Caution: Proper precautions should be exercised when handling HF. It is highly corrosive, toxic, and in extreme circumstances, can lead to skin grafts, amputation, or death.), the resulting nanowires were washed extensively with 20% acetone in deionized water and finally washed with methanol. The nanowires were then dried in an oven at 45 °C for 6 h and in a vacuum for another 6 h. Effective removal of silica was demonstrated by energy-dispersive X-ray (EDX) analysis. EDX data showed that only a small amount of silicon (0.4%) was detected after HF treatment (not shown).

Binding Assays Using Theophylline. Nanowires (different amounts) were incubated with 1 mL of 5×10^{-5} mol/L theophylline solution in acetonitrile. After incubation on a rocking table for 1 h, the nanowires were separated from the supernatant by centrifugation or by a magnet. The supernatant was withdrawn and analyzed with UV detection. Adsorption percentage was then calculated.

Results and Discussions

Immobilization of 8-Carboxypropyltheophylline on the Pore Walls of Alumina Membrane. Template synthesis within the pores of nanoporous alumina membrane is a common method for producing nanotubes and nanowires.[21,22] Commercial alumina membrane having pores with diameters of 200 nm was used for this study. A sol−gel template synthesis method was used to modify the nanopores of the alumina membranes (using 3-aminopropyltrimethoxysilane).[19] The terminal amino group was then reacted with the carboxy group of 8-carboxypropyltheophylline to attach theophylline to the inside walls of the nanopores.

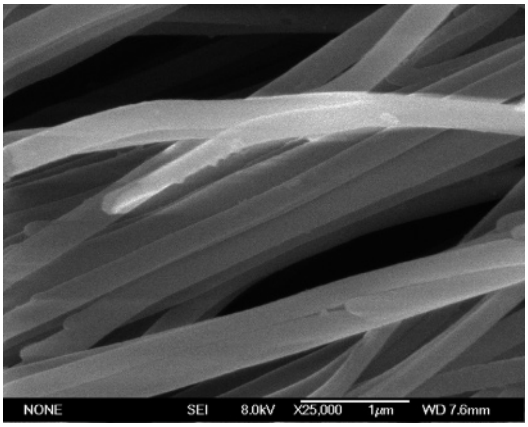

Figure 1. Scanning electron micrograph (SEM) image of magnetic imprinted nanowires.

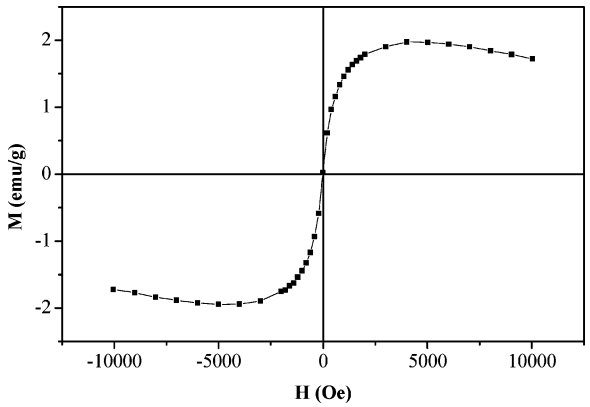

Figure 2. Magnetization vs applied field for the magnetic nanowires at 300 K.

Magnetic Imprinted Polymer Nanowires Structural Characterization. The SEM result (Figure 1) verifies the formation of polymer nanowires with controlled size in the alumina template membrane. The diameter of the nanowires was determined by the pore diameter of the alumina template membrane. The magnetic properties of the polymer nanowires were studied by a SQUID magnetometer. Magnetic measurements reveal superparamagnetic behavior of the nanowires at 300 K (Figure 2). This means that $MnFe_2O_4$ nanocrystallites were successfully entrapped in the polymer nanowires.

Binding Assays Using Theophylline. Binding assays were carried out in acetonitrile. Theophylline was used to evaluate the capacity of the magnetic nanowires imprinted with immobilized theophylline and the corresponding control magnetic nanowires (Figure 3). The imprinted nanowires have a higher capacity than those of control nanowires. At the nanowire concentration where the imprinted nanowires bind 50% of the theophylline, the control nanowires bind only 8.6%. To elucidate the effects of template molecule shape and functionality on imprinting, an additional type of nanowires was synthesized by using alumina membrane immobilized with 2-naphthyacetic acid as the template. Naphthalene is about as bulky as theophylline, but contains no functional groups. As can be seen in Figure 3, this kind of nanowires has a binding capacity that is only slightly higher than the control nanowires. This result indicates that the functional groups on the theophylline molecule are responsible for the observed imprinted effect.

Binding Selectivity to Theophylline and Its Structural Analogues. The binding selectivity toward theophylline against its two structural analogues, theobromine and caffeine, was compared between the imprinted nanowires and the control

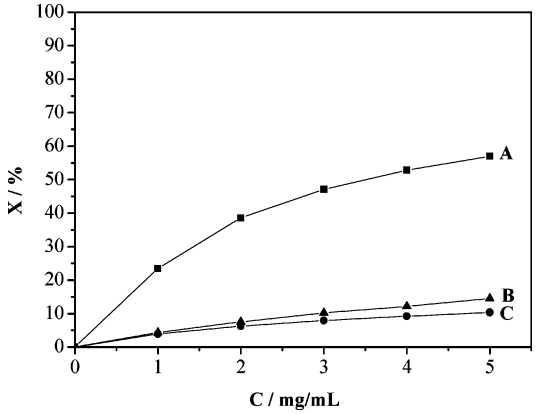

Figure 3. Binding profiles of theophylline as a function of the nanowires concentration. (A) Theophylline-imprinted nanowires, (B) 2-naphthyacetic acid-imprinted nanowires, and (C) control nanowires. The points represent mean values of three measurements.

Table 1. Absorption Selectivity toward Theophylline over Theobromine and Caffeine by the Imprinted Nanowires[a,b]

theophylline	theobromine	caffeine
23.5 ± 2.1 nmol	4.1 ± 0.6 nmol	4.7 ± 0.5 nmol

[a] Experiment was conducted by the addition of 2 mg of imprinted nanowires in 1×10^{-4} mol/L analyte solution in acetonitrile (1 mL) at room temperature. [b] $n = 5$.

nanowires. It can be seen from Table 1 that the imprinted nanowires showed a higher adsorption to theophylline to its structural analogues. This result is similar to that obtained with bulk polymers imprinted with the free template molecule.[22]

Conclusions

In conclusion, the results presented here demonstrate that radical polymerization of a functional and cross-linking monomer with vinyl or acrylic groups, which have been used in synthesis of most MIPs in recent years, can be used to prepare molecularly imprinted polymer nanowires in nanoporous alumina membrane. The use of theophylline-immobilized nanopores as the template resulted in imprinted nanowires having recognition sites on or close to the surface, making them more accessible for analytes to diffuse in. As a result, the commonly practiced porogen and polymer ground-up process used in making conventional molecularly imprinted nanowires is no longer needed. Furthermore, the size of these imprinted nanowires is in the nanometer range and can be well dispersed in solution, and their applications should therefore be compatible with procedures where biological antibodies might otherwise be used.

The results also demonstrate that superparamagnetic nanocrystallites can be entrapped in polymer nanowires by using alumina membrane as template. Magnetic nanoparticles immobilized with biological receptors have been extensively used in biomedical and biotechnological areas. Magnetic nanowires with artificial receptors such as those reported in this paper could have potential applications in drug delivery, as biochemical sensors, and for trace enrichment of specific targets. Work in this direction is already in progress and will be communicated in due course.

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