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Template Synthesized Molecularly Imprinted Polymer Nanotube Membranes for Chemical Separations

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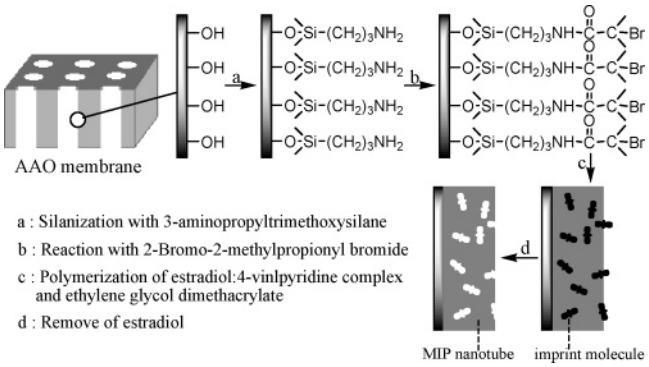
Martin and co-works have pioneered a method, called template synthesis, for preparing nanotubes. This method has been used to prepare nanotubes composed of many types of materials, including silica, polymers, metals, and biological macromolecules.[1] Nanotubes produced from this method promise to have a broad range of important applications. Free-standing nanotubes have been used for applications in biosensors, biocatalysis, biorecognition, and drug delivery.[2] Nanotube membranes readily formed from template synthesis have been used for bioseparations in an amazingly diverse field of biochemical species, from simple ions and drug molecules to DNA and proteins.[3] The selectivity of these nanotube membranes is governed by the inherent properties of the nanotube themselves or the tailored selectivities of the additional immobilized selective complexing agents.

The technique of molecular imprinting allows the formation of specific recognition sites in synthetic polymers through the use of imprint molecules.[4] These recognition sites mimic the binding sites of antibodies and other biological receptor molecules. Molecularly imprinted polymers (MIPs) can therefore be used in applications relying on specific molecular binding events.

We have been investigating the selective separation using template-synthesized nanotube membranes on the basis of molecular imprinting.[5] In this report, we further describe the synthesis of the MIP nanotube membrane using a porous anodic alumina oxide (AAO) membrane by surface-initiated atom transfer radical polymerization (ATRP). ATRP is a new class of controlled living radical polymerization.[6] ATRP is based on the transfer of a halogen atom from the initiator to the monomer and the successive transfer to the growing polymer chain catalyzed by a transition-metal complex that mediates the propagation. ATRP has been a popular means to graft polymer brushes,[6c−e] including MIP brushes,[6f] on a solid support because of the broad selection of monomers, its good control over the product molecular weight and dispersity, and its good tolerance for functional groups.

The AAO membrane with the immobilized ATRP initiators was prepared by a two-step method. The AAO membrane with a thickness of 60 μm and a pore diameter of 100 nm was first modified with 3-aminopropyltrimethoxysilane. The ATRP initiator, 2-bromo-2-methylpropionyl bromide, was then grafted onto the silanized AAO membrane.[6e] This AAO membrane was used as a macroinitiator in the subsequent molecular imprinting process (Scheme 1). The imprint molecule, functional monomer, and cross-linking agent used in this study are β-estradiol, 4-vinlpyridine (4-VP), and ethylene glycol dimethacrylate (EGDMA), respectively. β-Estradiol (0.125 mmol), 4-VP (1 mmol) and EGDMA (8 mmol) were dissolved in 8 mL of acetonitrile. This mixture solution was stirred for 2 h at room temperature for the formation of a complex of imprint molecule and monomers. This mixture was then purged with N_2 for 15 min and transferred to a flask containing ATRP initiator immobilized AAO membrane and organometallic catalyst—CuBr (2 μmol) and 1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam) (4 μmol). This reaction system was incubated at 70 °C under the protection of nitrogen for 24 h. The AAO membrane was flowing washed (using a peristaltic pump and a syringe filter) with acetonitrile, methanol−acetic acid (9:1, v/v), and methanol in sequence before drying under vacuum. The TEM result and SEM result (Figure 1) verify the formation of MIP nanotubes with controlled size in the AAO membrane.

Scheme 1



The β-estradiol recognition ability of the MIP nanotube membrane was investigated by the steady-state binding method. The MIP nanotube membrane described above was placed in a circulative flow system, and the test solutions of β-estradiol (at various concentrations) in 5 mL acetonitrile were pumped through the nanotube membrane using a peristaltic pump for 2 h. The filtrate was concentrated by N_2 blow down evaporation. The amount of β-estradiol adsorbed by the MIP nanotube membrane was determined by measuring the residual β-estradiol in filtrate by UV adsorption. As shown in Figure 2, the MIP nanotube membrane indeed exhibits a higher capacity for β-estradiol than the control nanotube membrane formed in the absence of imprinted β-estradiol. The data from the binding experiment were further processed with the Scatchard equation to evaluate the binding properties of the MIP nanotubes. Two straight lines fitting the Scatchard equation, $B/F = (B_{max} - B)/K_d$, can be drawn (see the Supporting Information), and these give two typical dissociation constants. A dissociation constant (7.25 μmol/L) and a maximum number of binding sites (12.38 μmol/g) were calculated for high-affinity binding sites. When compared to traditionally formed bulk MIPs for β-estradiol,[7] the MIP nanotubes have 11-fold higher binding capacity and 13-fold better imprinting effect.

The binding of the MIP nanotube membrane for β-estradiol was compared to the other two naturally occurring, related phenolic steroid compounds, estrone and cholesterol, which have the same general structure as β-estradiol but differ only in their functionalities

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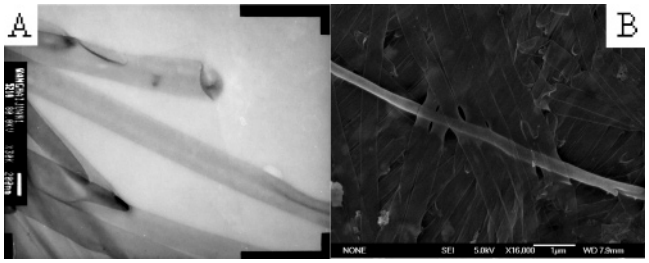

Figure 1. Transmission electron micrograph (A) and scanning electron micrograph (B) of the MIP nanotubes after the removal of the alumina template membranes by 1 mol/L NaOH solution.

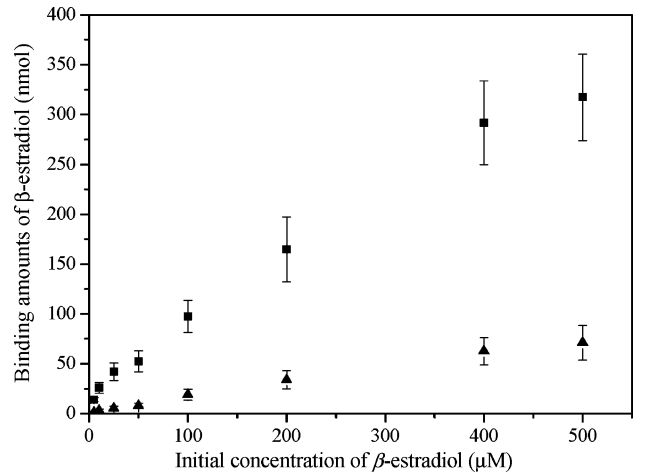

Figure 2. Binding isotherm of the β-estradiol imprinted nanotube membrane (■) and the control nanotube membrane (▲) for β-estradiol from 5 mL of β-estradiol solution. The points represent mean values of three measurements.

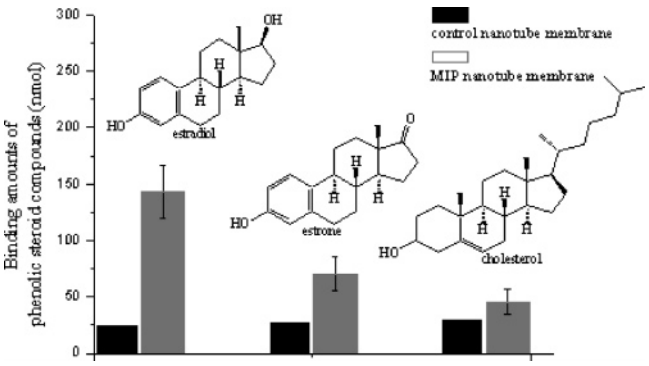

Figure 3. Cross binding reactivity of the MIP nanotube membrane: phenolic steroid compound (nmol) adsorbed by MIP nanotube membrane from 5 mL of 200 μM solution of phenolic steroid compound in acetonitrile in 2 h.

in the 17 position. As shown in Figure 3, besides binding β-estradiol, the MIP nanotube membrane also adsorbed estrone and cholesterol. A comparison of the MIP nanotube membrane to the control nanotube membrane suggests that estrone and cholesterol binding is partially specific to the binding sites created by β-estradiol. The binding capacities toward estrone and cholesterol are at less two times lower than that of β-estradiol. While the cross binding reactivity of the MIP nanotube membrane may be undesirable for the application of the MIP nanotube membrane for sensors, this could actually be an advantage in sample treatment because different kinds of phenolic steroid compounds can also be removed or enriched efficiently.

In summary, this report demonstrates the advantages of high affinity and selectivity of the MIP nanotube membrane in chemical separation. Furthermore, because the molecular imprinting technique can be applied to different kinds of target molecules, ranging from small organic molecules (e.g., pharmaceuticals, pesticides, amino acids, nucleotide bases, steroids and sugars) to peptides and proteins, such MIP nanotube membranes will broaden considerably the application of nanotube membranes in chemical separations and sensors.

This report also shows that the ATRP route is an efficient procedure for the preparation of molecularly imprinted polymers. Furthermore, the ATRP route works well in its formation of MIP nanotubes within a porous AAO membrane. The controllable nature of ATRP allows the growth of MIP nanotubes with uniform pores and adjustable thickness (see the Supporting Information). Thus, using the same route, it is possible to tailor the synthesis of MIP nanotube membranes with either thicker MIP nanotubes for capacity improvement or thinner nanotubes for efficiency improvement.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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