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Molecularly Imprinted Sol−Gel Nanotubes Membrane for Biochemical Separations

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Molecular imprinting has become a powerful method for the preparation of robust materials that have the ability to recognize a specific chemical species.[1] The stability, ease of preparation, and low cost of these materials have led to their assessment as substitutes for antibodies or enzymes in chemical sensors, catalysis, and separations.[2] Presently, techniques used to prepare molecularly imprinted materials most often result in materials exhibiting high affinity and selectivity but low capacity and poor site accessibility to the target molecules. To advance this technique to the application with preparative-scale or high-efficiency separations, new morphologies and manufacturing techniques need to be developed.[3]

Recently, Martin's group have pioneered a technology, called template synthesis, for preparing monodisperse nanotubes of nearly any size and composed of nearly any material.[4] These nanotubes offer some interesting advantages for biochemical application. However, the present application of these nanotubes is not common.[5] In this study, we report a simple procedure for applying a molecular imprinting technique to imprint functional groups into the walls of the template-synthesized sol−gel nanotubes (Figure 1A, 3A) for biochemical separation of estrone. Silica nanotubes are ideal vehicles for such proof-of-concept experiments because they are easy to make,[6] have cross-linked structure, and are highly suitable for the formation of a delicate recognition site.[7]

Estrone is one of several naturally occurring estrogens, which influence the normal development and maturation of the female. In this study, we used a thermally reversible bond for the preparation of the silica monomer−imprinted molecule complex,[7b,c] which allowed us to remove the imprinted estrone by a simple thermal reaction and to simultaneously introduce functional groups into the cavity. The monomer−imprinted molecule complex was prepared according to the Chang's method (Figure 2).[7c] The reaction occurred between the isocyanate group of 3-(triethoxysilyl)propyl isocyanate and a phenol moiety of estrone, forming a thermally cleavable urethane bond, which is stable at room temperature and undergoes reversible cleavage at elevated temperature.

The silica nanotubes were synthesized within the pores of nanopore alumina template membranes (Figure 1B) using the sol−gel method. A template with pore diameters of 100 nm was made by us, using an electrochemical method, and was used for these studies. The TEM and SEM results (Figure 1 and Figure 3) prove the formation of silica nanotubes with controlled size in the alumina template membrane.

It is very difficult to remove imprinted molecules located in current molecular imprinted materials because the highly cross-linked structure does not allow these molecules to move freely. However, due to the nanometer wall thickness of the silica

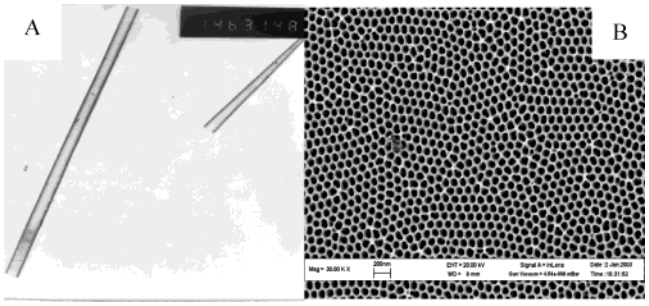

Figure 1. (A) Transmission electron micrograph (TEM) of 100-nm diameter silica nanotubes after the removal of alumina template membranes by phosphoric acid. (B) Scanning electron micrograph (SEM) of the surface of a typical alumina template membrane.

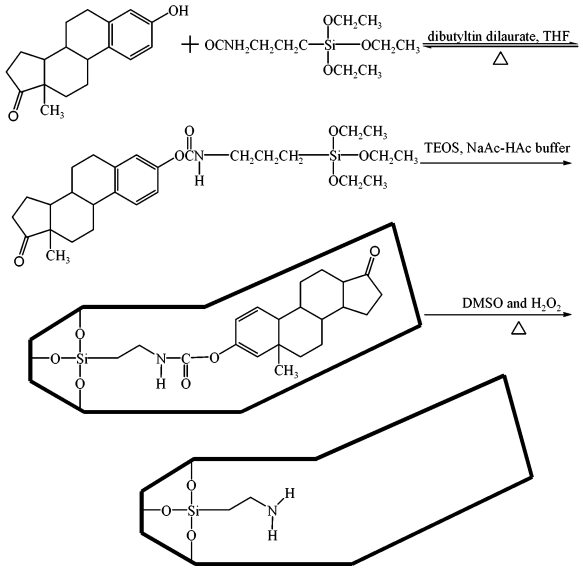

Figure 2. Schematic diagram of the molecular imprinting technique used in this study.

nanotubes used in this communication, the imprinted estrone was very easy to remove from the imprinted location in the nanotubes. To extract the imprinted estrone molecules from the silica nanotubes, the imprinted membrane was heated at 180 °C in a mixture of DMSO and water, and the dissociated isocyanato group in silica nanotubes was converted to an amino group by its reaction with H_2O. After this step, the nanotube membrane was placed into a spring filter holder. Then DMSO and water mixture solution was passed through this device to fully elute the cleaved residual estrone. The removal of imprinted estrone and the presence of the molecule-imprinted surface were confirmed by means of IR spectroscopy

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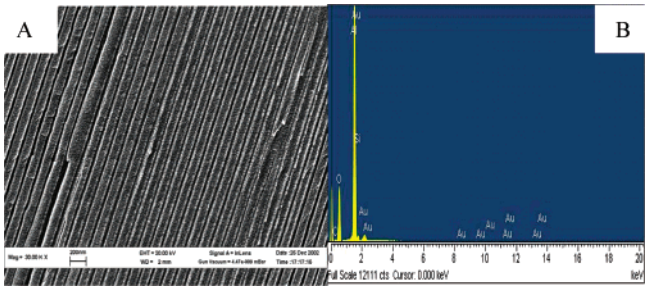

Figure 3. (A) SEM cross-sectional view of alumina template deposited with SiO_2. (B) EDS result of alumina template deposited with SiO_2.

measurements taken before and after removal of imprinted estrone. In the IR spectrum taken after the washing process, the carbonyl peak of the urethane group of monomer−imprinted molecule complex at 1736 cm^{-1} is significantly reduced, and the amino group at 3455 cm^{-1} appeared. The content of the imprinted estrone which cannot be cleaved and remained in the imprinted nanotubes is only ~1.2%.

The estrone recognition ability of the imprinted nanotubes was investigated by a steady-state binding method. The nanotube membrane assembly described above was placed in a circulating flow system, and the test solutions of estrone (at various concentrations) in 20 mL of chloroform were passed through the nanotube membrane by a peristaltic pump for 12 h. The filtrate was concentrated by the evaporation. The amount of estrone adsorbed by imprinted nanotubes was determined by measuring the residual estrone in filtrate by competitive indirect enzyme-linked immunoassay. As shown in Figure 4, imprinted silica nanotubes had much higher recognition ability than the control silica nanotubes formed under almost the same conditions except the replacement of estrone by phenol. We also investigated the specific recognition ability of the imprinted silica nanotubes for testosterone propionate under the same conditions. Testosterone propionate is a structural analogue of estrone. The imprinted silica nanotubes showed much higher specific recognition ability for estrone than testosterone propionate (Figure 4).

$$K = \frac{\dfrac{\text{moles of test estrone bound to silica nanotubes}}{\text{mass of silica nanotubes}}}{\dfrac{\text{moles of test estrone remaining in solution}}{\text{mass of solution}}} \quad (1)$$

The success of the molecular imprinting was further assessed by measuring the affinity of estrone to imprinted silica nanotubes versus that of control silica nanotubes. Knowing the volume of solution used and the mass of the silica nanotubes (calculated from the mass of silica nanotubes after the removal of the alumina template membrane), a partition coefficient, *K*, could be calculated. A comparison between the molecularly imprinted and control nanotubes was accomplished by calculating a ratio of the partition coefficients.

The experimental results show that the molecularly imprinted silica nanotubes have a *K* value of 864 ± 137 ($n = 3$) and a high selectivity ratio over control nanotubes of 6.94.

The general kinetic profile of binding estrone to molecularly imprinted silica nanotubes was also investigated (see Supporting Information). It can be seen that the silica naotubes show a very fast uptake profile, with significant specific binding after only 10 min and saturation time within 3 h. This means that the porosity of the silica matrix and nanometer wall size of the nanotubes has a favorable effect on the diffusion times of the target molecule into the recognition sites.

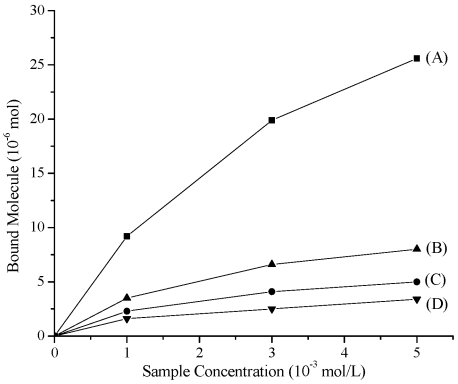

Figure 4. Amount of bound estrone by (A) the molecularly imprinted nanotubes and (C) the control silica nanotubes. Amount of bound testosterone propionate by (B) the molecularly imprinted nanotubes, and (D) the control silica nanotubes

In conclusion, we explored the use of a silica nanotube membrane for molecular imprinting. The use of nanotubes as molecularly imprinted materials has the advantage of high affinity, selectivity, capacity, and site accessibility of the target molecules. It is important to emphasize that the template route can be used to prepare molecularly imprinted nanotubes of nearly any material. Such molecularly imprinted nanotubes should also prove useful for recognition of chiral compounds and large-molecular weight proteins.

Supporting Information Available: Experimental procedures of silica monomer−estrone complex and silica nanotube membrane (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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