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The Use of a Thermally Reversible Bond for Molecular Imprinting of Silica Spheres

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The study of mechanically stable materials having molecular recognition ability is a subject of great interest due to the potential use of these materials in applications such as chemical sensors, stationary phases for high-performance chromatography, catalysts, and membranes for separating toxic chemicals.[1] Molecular imprinting is a process that has been widely used for the preparation of polymeric materials with specific binding properties.[2] To accomplish this, a template is complexed with a monomer either covalently or noncovalently, and this is then followed by polymerization in the presence of a cross-linking agent. The removal of the template from the matrix generates cavities with a recognition ability, which is greatly influenced by the rigidity of the matrix and the nature of the bond used for the template−monomer complexation.

In this study, we prepared molecularly imprinted spherical silica particles with controlled sizes. Since silica materials have cross-linked rigid structures, they are highly suitable for the formation of a delicate recognition site.[3] We used a thermally reversible bond for the preparation of the silica monomer−template complex, which allowed us to remove the template by simple thermal reaction and to simultaneously introduce various functional groups into the cavity. The monomer−template complex (EstSi) was prepared by the reaction of 3-(triethoxysilyl)propyl isocyanate with estrone in the presence of dibutyltin dilaurate (Scheme 1). The reaction occurred between an isocyanato group of the monomer and a phenol moiety of estrone, forming a thermally cleavable urethane bond.[4] It is known that the urethane bond formed between an isocyanate and a phenol is stable at room temperature, but reversible cleavage occurs at elevated temperatures.[5] Estrone is one of several naturally occurring estrogens, which influence the normal development and maturation of the female. Estrogens have been suspected of having carcinogenic properties and adverse environmental effects, and extensive quantitative analysis has been done concerning these molecules.[6]

The thermal cleavage of the urethane bond was investigated by ^{1}H NMR spectroscopy. EstSi was dissolved in DMSO-d_6, and the ^{1}H NMR spectra were measured at 25 and 140 °C (Figure 1). The aromatic ring proton peaks appeared at 7.25, 6.81, and 6.78 ppm, and the NH peak, at 7.67 ppm at room temperature. After increasing the sample temperature to 140 °C, the NH peak was shifted to 7.1 ppm, and new peaks appeared at around 6.5 and 7.0 ppm, corresponding to the aromatic protons from the dissociated estrone, showing that the urethane bond had been thermally cleaved.

Silica particles with controlled sizes were prepared by means of the gelation of EstSi with tetraethoxysilane (TEOS) in *n*-decyl alcohol in the presence of hydroxypropyl cellulose, sorbitan monooleate, and aqueous NH_4OH. The FE-SEM images in Figure 2 show that spherical silica particles with sizes of 1.5−3 μm were formed from EstSi and TEOS (the mole ratio was 1:50). Control silica particles that had the same sizes and shapes were prepared with 3-aminopropyl triethoxysilane and TEOS in the absence of a template molecule.

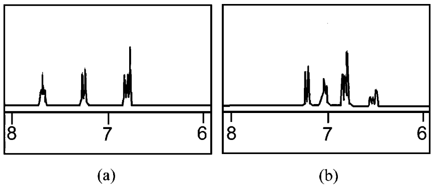


Figure 1. ^{1}H NMR spectra of EstSi (a) at 25 °C and (b) at 140 °C.

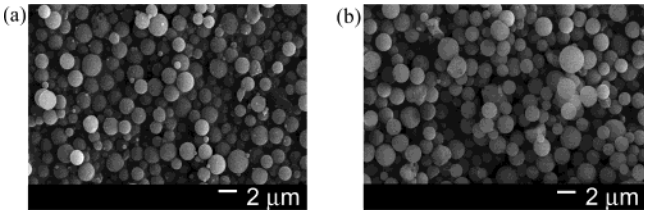
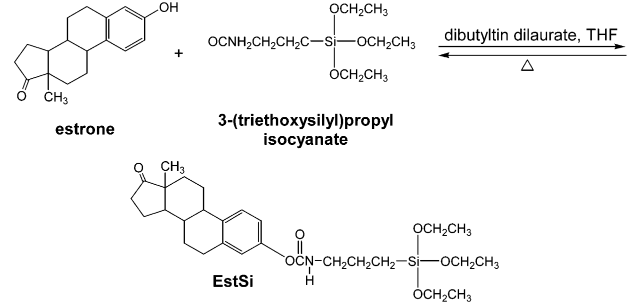


Figure 2. FE-SEM images of (a) estrone-imprinted silica particles and (b) control silica particles.

Scheme 1. Synthesis of EstSi



It is difficult to remove template molecules located in the center of the silica particles because the highly cross-linked rigid structure does not allow these molecules to move freely.[7] To overcome this problem, TEOS was first added to the reaction mixture, and the EstSi was added later. By means of this sequential addition, the particle nuclei were formed mainly from the reaction of TEOS, while EstSi was condensed mainly onto the outer shell of these particles. The presence of this EstSi-rich surface was confirmed by means of IR spectroscopy measurements taken before and after the removal of the outer shell by mechanical grinding. In the IR spectrum taken after grinding and filtering through a filtering paper (diameter of cavity: 1.2 μm), the carbonyl peak for the urethane

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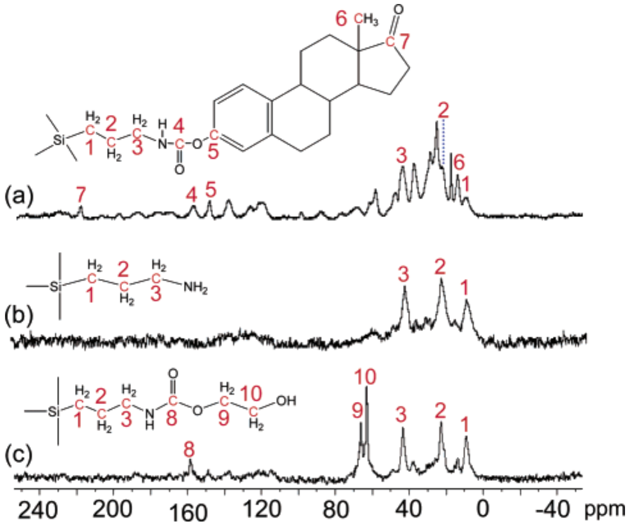
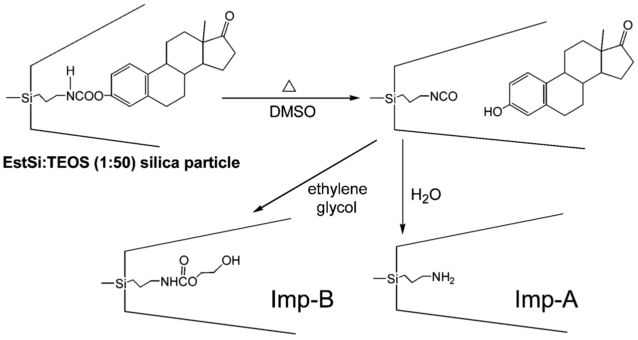


Figure 3. Solid-state ^{13}C NMR spectra of (a) EstSi:TEOS (1:50) silica particles before removal of the template molecules, (b) Imp-A, and (c) Imp-B.

Scheme 2. Extraction of a Template Molecule and Generation of a Recognition Site



group of EstSi at 1736 cm^{-1} almost disappeared. To extract the template molecules from the silica matrix, the EstSi:TEOS silica particles were heated at 180 °C in DMSO. In the presence of water, the dissociated isocyanato group was converted to an amino group by its reaction with H_2O. By adding ethylene glycol instead of H_2O, it was possible to introduce hydroxy groups into the binding cavities (Scheme 2).

The extraction of the template and the consequent generation of the recognition site were confirmed by solid-state ^{13}C NMR spectroscopy (Figure 3). Although quantitative analysis was difficult, the disappearance of the peaks corresponding to the template indicated that most template molecules were removed from the silica particles. In the spectrum of Imp-B, new peaks corresponding to an ethylene glycol unit appeared, showing its successful introduction into the cavities.

The estrone recognition ability of the imprinted silica particles was investigated. Imp-A (0.1 g) was added to solutions of estrone in chloroform (50 mL) at various concentrations. After incubating for 24 h, the silica particles were isolated by filtration, and the filtrate was concentrated to dryness by the evaporation of the solvent. The amount of estrone absorbed by Imp-A was determined by measuring the residual estrone in the filtrate by HPLC. The recognition abilities of Imp-B and of the control were measured in the same manner. As shown in Figure 4, Imp-A had a much higher recognition ability than the control silica particles. Interestingly, Imp-B showed better interactions with estrone than Imp-A. We also investigated the specific recognition ability of the silica particles for testosterone propionate under the same conditions. Testosterone propionate is the structural analogue of estrone. Both Imp-A and Imp-B showed higher specific recognition ability for estrone than testosterone propionate (Figure 4).

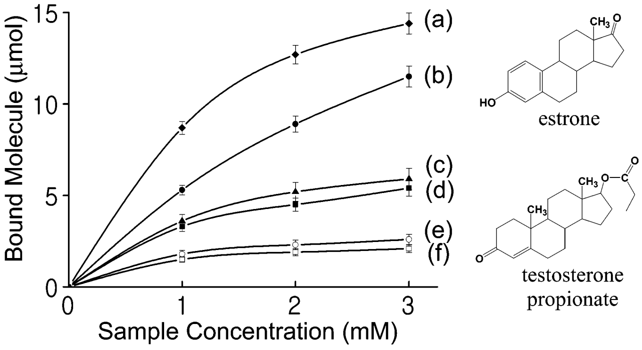


Figure 4. Amount of bound estrone by (a) Imp-B, (b) Imp-A, and (e) the control silica particle; amount of bound testosterone propionate by (c) Imp-B, (d) Imp-A, and (f) the control silica particle. In all experiments, 0.1 g of the silica particles were added to 50 mL of the sample solutions in chloroform.

In conclusion, we explored the use of a thermally reversible bond in the process of template−monomer complexation for use in molecular imprinting. Estrone-bound silica particles were prepared through the intermediary of a urethane bond. The removal of the template by means of a simple thermal reaction in the presence of a nucleophile generated estrone recognition sites in the silica particles. This method has the advantage of being able to introduce various functional groups into the cavities by using different nucleophiles during the removal of the template.

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Supporting Information Available: Experimental procedures of EstSi, Imp-A, and Imp-B (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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