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A templating route to nanoporous chitosan materials

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Abstract—Nanoporous chitosan materials in both membrane and bulk form with pore diameter ranges of 10–50 nm were prepared by a novel emulsion-mediated templating method. Considering the biocompatibility and versatility of chitosan, the nanoporous chitosan material is expected to have a wide variety of applications in the biotechnological and biomaterials areas.

Keywords: Chitosan; Polymers; Microstructure; Nanomaterials; Biomembranes

New material systems can be created not only by arranging functional groups on molecular scale via chemical bonds, but also by manipulating the microstructures of existing materials on the nanometer to micrometer scale. A representative example of this is the construction of nanoporous materials from existing polymers. When an existing polymer is made into a nanoporous form, many properties such as permeability, mechanical strength, transparency, and adsorption ability of the polymer will be greatly changed. Therefore, the construction of nanoporous materials from existing polymers is an attractive way to produce new functional polymer systems.[1] This construction is also of great importance in both fundamental research and practical application. Nanoporous structures abound in Nature, both in biological systems and in natural minerals. Nanoporous materials can be conveniently divided into bulk forms and membranes. These membranes are very important structures in biological systems. They are usually found in organ tissue and help to provide the organ with its biological function. The design and fabrication of porous materials with nanosized pore diameters and highly ordered structures have attracted a great deal of attention in recent years.[2] Nanoporous polymeric materials are versatile structures with a broad range of potential and realized applications.[3] Some of these applications include having these materials serve as templates for both the synthesis of nanoobjects[4–10] and for confined crystallization,[11] utilizing them as membranes for selective transport,[12,13] and employing them as high-surface-area supports for catalysis application.[14–16] Although a number of methods have been developed for generating porous materials, it still remains as a great challenge to fabricate 3D porous membranes that have precisely controlled pore size, the surface fully exposed, completely interconnected pores (in the bulk), and highly ordered periodic structures.

Up until now, numerous inorganic and organic materials have been made into nanoporous forms using a templating method. However, chitosan, which is an abundant resource of biomaterials, has not been reported. Herein, we present the construction of chitosan nanoporous materials using a novel templating method. More significantly, because of the versatility and biocompatibility of chitosan, the nanoporous chitosan material could be used for a wide variety of applications in biomaterial construction and many other areas of science as well.

Scheme 1 shows the principles of the emulsion templating method. Chitosan is a natural polysaccharide that is positively charged and has a p$K_a \sim 6.3$–7. There are no large hydrophobic groups, but it has

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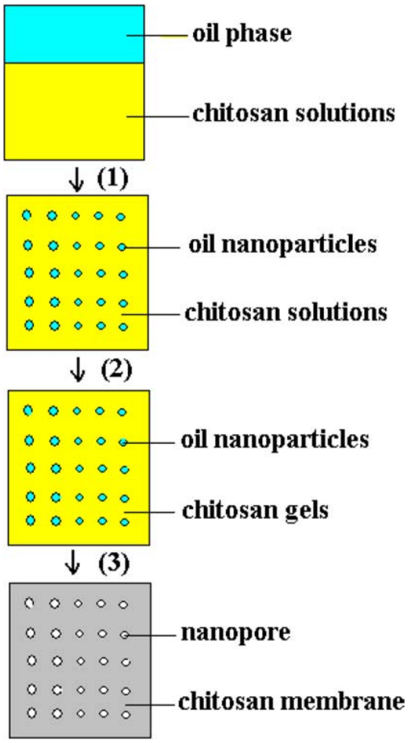
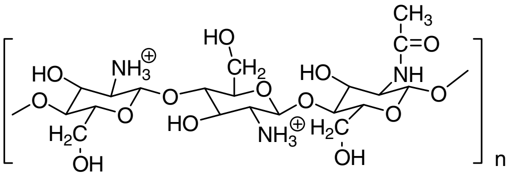


Scheme 1. Schematic representation of the emulsion templating method: (1) preparation of the inert-oil/chitosan solution emulsion system; (2) crosslinking and gelling process of chitosan solution phase containing inert-oil nanobeads; and (3) rinsing away the inert-oil nanobeads and forming the nanoporous chitosan materials.

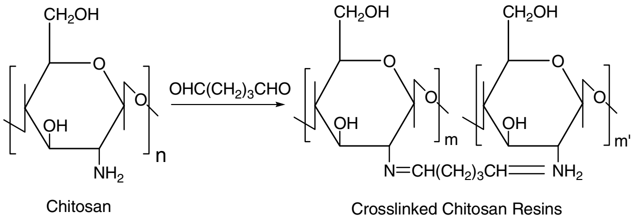
cationic-NH_3^+ and alcoholic-OH groups that are extensively distributed along its glucosamine ring unit structures (Scheme 2). Therefore, it can be potentially used as a polymer emulsifier, which has been explored by many researchers.[17] Here, chitosan was first employed as a cationic polymer emulsifier. As shown in Scheme 1, when inert corn oil was introduced to the chitosan solutions with violent stirring, the oil would be dispersed as fine nanoparticles suspended in the emulsion system. In this emulsion system, the fine inert particles are the dispersed phase, whereas, the chitosan solution is the continuous phase. Chitosan is not a pure compound, and it is probably composed of a mixture of molecules with different degrees of deacetylation. The emulsifying capability of chitosan is too limited to produce a stable emulsion system in which the size of the oil drops is in the nanometer range. In order to obtain nanosized oil particles, an assistant emulsifier (e.g., OP-10) was also introduced into the emulsion system. After the stable emulsion system had been formed, it was heated to 60 °C for the crosslinking of chitosan with glutaraldehyde. After crosslinking with glutaraldehyde, the continuous chitosan solution phase was transformed into a continuous gel phase. The mechanism of crosslinking chitosan with glutaraldehyde is presented in Scheme 3. After this step, the emulsion system has become a chitosan gel system containing inert oil nanoparticles (Scheme 1, step 2). During the process, some oil nanoparticles would move and coalesce, and as a result, some would combine to form bigger oil particles. This is the reason that the nanoporous membrane possesses different pore sizes after the oil phase was washed away (as marked in Fig. 1a). In addition, some of the oil particles combined with each other in large globules. After the oil particles were washed away, these cavities formed completely interconnected (in the bulk) pores (as shown in Fig. 1b and c).

The general morphology of the nanoporous products were investigated using SEM imaging. Figure 1 shows a typical SEM image of the chitosan membrane nanostructure obtained from the emulsion-mediated template method. Figure 1a shows the surface morphology of the nanoporous products. The figure indicates that the pore size has a range from 10 to 50 nm, and these nanopores are fully exposed on the face. Figure 1b was obtained from the fragment cut off from the bulk nanoporous material. It shows the morphology inside the bulk of the nanoporous materials. From Figure 1b we also find that some of the nanopores are completely interconnected (as marked by line in red). Figure 1c shows the SEM image of an ultra-thin nanoporous membrane.

The size of the nanopores can be adjusted via the introduction of assistant emulsifiers. With the increased level of assistant emulsifiers, the particle size of the inert oil is reduced (Fig. 1d). Therefore, as a result, the pore size would be reduced after the oil is washed away. In addition, the pore size can be adjusted by changing the concentration of the inert oil or by changing the conditions of the emulsifing process. Figure 2a shows the fracture section of the nanoporous membrane and Figure 2b shows a low amplification section of the membrane. Obviously, these nanopores are highly ordered in the chitosan bulk.



Scheme 2. Segment of chitosan molecule in acidic aqueous solutions.



Scheme 3. Crosslinking chitosan with glutaraldehyde.

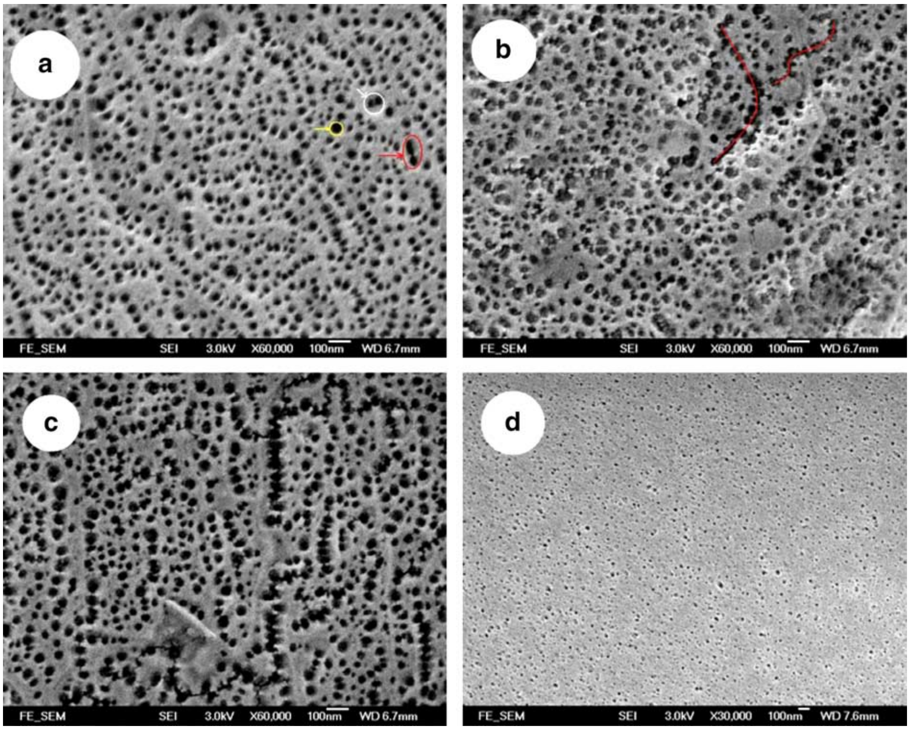


Figure 1. SEM images of the nanoporous membrane prepared from the emulsion templating method: (a) on the surface; (b) in the bulk; (c) ultra-thin membrane; and (d) on surface. (Assistant emulsifier concentration in a, b, and c: 0.5%; in d: 1.0%.)

1. Experimental

1.1. Fabrication of nanoporous chitosan materials

Chitosan (from Aldrich Chemical Co.) with different molecular weights and degrees of deacetylation have been used in this study. Stock solutions containing 0.5–2% (w/v) chitosan were prepared as follows: As a typical procedure, 1 g of chitosan was charged with 100 mL of 1% (v/v) aq HOAc, which was then kept stirring overnight. The chitosan solution was then filtrated with a commercial nylon stocking and was subsequently purified using a medium porous filter. The chitosan concentration in the solution was then measured by drying 1.0 g of the solution. 0.1 g of glutaraldehyde (Aldrich) was introduced into a 100 mL chitosan solution (0.98 g chitosan) and stirred for 20 min. A 5.0-g sample of inert oil (e.g., commercial corn oil or phenylmethylsiloxane) was dropped into the chitosan solution, and the mixture was then ultrasonically emulsified (JY92-2D, Ultrasonic crasher, Ningbo, China, 500 W, 20 min) to obtain a stable emulsion. In order to obtain a stable emulsion with minimal emulsion particle size, the assistant-emulsifier OP-10 (*p*-octylpolyethylene glycol phenyl ether, nonionic emulsifier) (~0.1 g) was also introduced to adjust the particle size of the oil droplets. After ultrasonic emulsification, the emulsion mixture was applied to a silicon wafer in order to form a film by the spin-coating method. Afterwards, the wafer with the chitosan film was subjected to ammonia gas and then heated to 60 °C for 4 h for crosslinking. After crosslinking, this wafer was then sequentially rinsed with acetone, water, and EtOH. As a result, the corn-oil nanobeads, as well as the adsorbed nonionic emulsifiers, were washed away, thus forming the chitosan nanoporous membrane. The principles of this method are presented in Scheme 1. The nanostructures of the chitosan nanoporous membranes were characterized using field-emission scanning electron microscopy (FE-SEM), on a JEOL JSM-6335F instrument.

1.2. Decontamination of Cr(III) from a wastewater sample

The applications of chitosan for wastewater treatment have been proposed and well discussed.[18] To explore the potential applications for nanoporous chitosan material in wastewater treatment, we investigated the elimination of chromium from a sample of wastewater. The elimination of chromium using chitosan resins as absorbents have been reported by many researchers.[19] Because effluents containing chromium are mostly acidic, the adsorption process is carried out at pH 4. The adsorption test is carried out using a modified chromatographic column shown in Figure 3. In a typical procedure, the previously produced nanoporous chitosan material was crushed using a plant crasher (FZ102, Hangzhou, China), resulting in a particle size

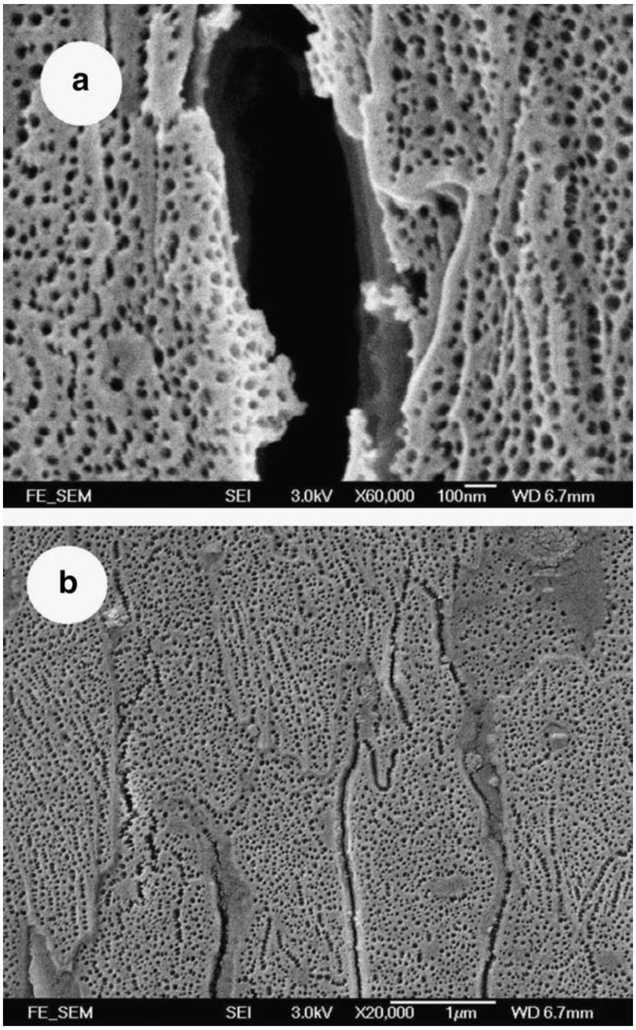


Figure 2. (a) Fracture section of the chitosan nanoporous membrane and (b) low amplification section of the chitosan nanoporous membrane.

powder of about 0.5–1 mm. Chitosan powder (2.0 g) was placed in a chromatographic column, and then 10 mL of water was poured into the column to wet the chitosan fillers. Wastewater samples were prepared by dissolving $CrCl_3{\cdot}6H_2O$ in deionized water. This wastewater solution (50 mL) was then placed into the column, filtered, and decontaminated by chitosan. The concentration of Cr(III) before and after decontamination was measured according to a literature method.[20] The adsorption capacity ($A\%$) of the nanoporous chitosan material is determined using the following equation: $A(\%) = (C_0 - C)/C_0 \times 100$, where C_0 represents the concentration of Cr(III) of the original sample and C is the concentration of Cr(III) of the treated sample. The adsorption capacity ($A\%$) of pure chitosan powder and glutaraldehyde crosslinked chitosan powder were also investigated under the same conditions. The adsorption results are presented in Table 1. From Table 1 we find that the adsorption capacity of chitosan to Cr(III) is greatly enhanced after it was made into nanoporous forms. Although the adsorption capacity of pure chitosan to Cr(III) is considerably high (52%), the pure chitosan fillers will become too slimy to be further used. After crosslinking with glutaraldehyde, a part of the amino groups on chitosan molecules was consumed; thus the adsorption capacity of chitosan is greatly reduced.

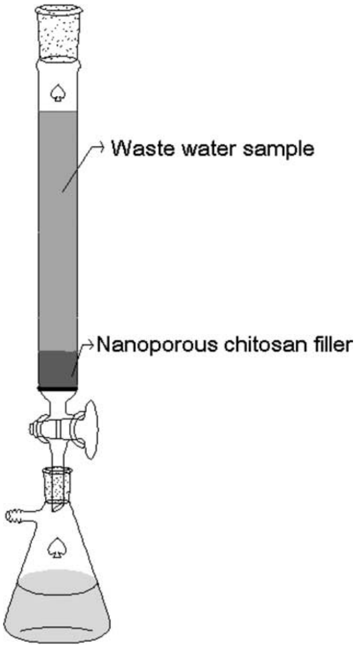


Figure 3. Apparatus for the adsorption of Cr(III) from wastewater sample.

Table 1. Results of Cr(III) adsorption with chitosan-filled chromatographic column[a]

A (%)	Fillers
52	Pure chitosan powder
34	Glutaraldehyde crosslinked chitosan powder
68	Nanoporous chitosan powder synthesized in this work

[a] Conditions: Cr(III) concentration of original sample 500 mg/L, $T = 27\,°C$, pH 4.

Nanoporous membrane is very important and has potential applications in many areas, especially in the biomedical or biomaterial sciences. With improvements in both structure and properties, nanoporous membrane design can directly benefit the fields of chemical separation, drug delivery, and wastewater remediation. In those applications, a biocompatible material is desirable and preferable. To date, most of the studies related to this subject are focused on synthetic polymer materials.[3] Chitosan (CS) is an N-deacetylated product of chitin that is one of the most abundant polysaccharides in nature and has good physical, biological, and biodegradable properties. It is readily processed into membranes from aqueous acidic solutions. The membranes obtained by chitosan and its derivatives have been reported to be suitable for various applications, such as the controlled release of drugs,[21,22] wound dressings,[23,24] chromatographic media,[24–26] and wastewater treatment.[27,28] As chitosan can be easily modified so that a wide range of functionalized derivatives are obtained, different types of nanoporous chitosan materials can be produced by further chemical modifications.

In summary, by combining the emulsifing property of chitosan, the emulsion particles templating method, the available crosslinking reaction of chitosan, and selective etching protocol, we have demonstrated that nanoporous chitosan membrane or bulk material can be prepared with a controlled nanoporous microstructure. Considering the versatility and biocompatibility of chitosan, this nanoporous chitosan material could have a variety applications in many areas of science.

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