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General Method for Producing Organic Nanoparticles Using Nanoporous Membranes

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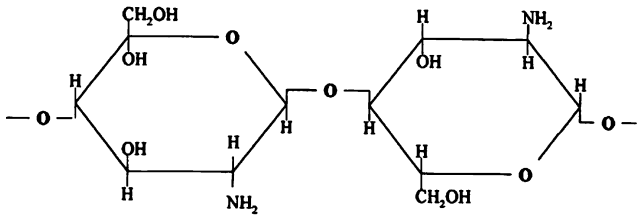
ABSTRACT Two liquids are separated by a nanoporous membrane and one liquid is made to flow into the other, causing nanoparticles to be formed at the exits of the nanopores. In particular, we report the generation of nanoparticles of the biodegradable polysaccharide polymer chitosan by placing the chitosan in a low pH aqueous solution that is flowed into a high pH aqueous solution. The size of the nanoparticles (5−20 nm) can be roughly controlled by choosing the size of the nanopores and the pumping rate. In addition, it is possible to load the chitosan nanoparticles with drug molecules, which is demonstrated by incorporation of up to 3.3% rhodamine 6G molecules in the chitosan nanoparticles.

KEYWORDS Nanopore, nanoparticle, chitosan, drug delivery

The spatial and temporal control of the release of pharmaceuticals at the site of where they act is a key requirement for the therapeutic use of a drug.[1−3] One method for realizing this objective is to create drug-loaded nanoparticles made out of biodegradable polymers.[4] Previous work in two laboratories, one at Stanford University, the other at the University of Florida, has featured the generation of such nanoparticles.[5−8] We present here an alternative strategy based on the use of a nanoporous membrane that separates the two liquids. By pumping one liquid into the other, through the membrane, we can generate nanoparticles at the exits of the membrane nanopores. We illustrate this technique for the low molecular weight biopolymer chitosan, which is a polysaccharide consisting of 13−17% units of monomeric *N*-acetyl-glucosamine and 83−87% glucosamine units:[9]



Low molecular weight chitosan (average MW 20 000 Da) is used as a model polymer in our work because it is a naturally biodegradable and biocompatible polysaccharide, which has broad applications in pharmaceutical and biomedical fields.[10−12] Chitosan is also known as a pH-response polymer, because at low pH, chitosan's amines are protonated and positively charged causing chitosan to be a water-soluble cationic polyelectrolyte. At high pH, these amines become deprotonated, and the polymer loses its charge and becomes insoluble.[13,14] Chitosan serves as a representative material for our process that can be adopted for the productions of other organic nanoparticles. In the case of chitosan, we use the precipitation caused by pH change, but other precipitation methods are applicable, such as temperature or antisolvent, or chemical reaction.

Droplet formation in liquid−liquid systems on the micrometer scale has been studied previously by Anna, Bontoux, and Stone.[15] Xu et al.[16] reported generating particles from microfluidic structures with sizes from 20 to 1000 μm. The closest paper involving particle generation on the nanoscale using nanopores appears to be the work of Powell et al.[17] who observed the transient formation and dissolution of nanoparticles in conical nanopores caused by the presence of permanent surface charges on the walls, whose electric field induces precipitation. The present work differs in that the nanoparticles are not formed inside the nanopores.

Procedure. The experimental device (Figure 1) is composed of a nanoporous membrane, which separates two solutions. The pH of the feed solution (left in Figure 1) is adjusted so that chitosan is soluble in this solution. The feed solution is forced under pressure through the pores of the membrane into the receiver solution (right in Figure 1). The pH of the receiver solution is adjusted such that chitosan is insoluble. When nanodroplets of the soluble chiotsan are injected through the membrane into the receiver solution nanoparticles of chitosan are formed at the exits of the nanopores.

For the preparation of nanoparticles with reduced sizes, membranes with uniform and well-defined nanopores are essential.[18−20] In our work, we use commercially available track-etched polycarbonate (PCTE, OSMONIC Inc.) and an-

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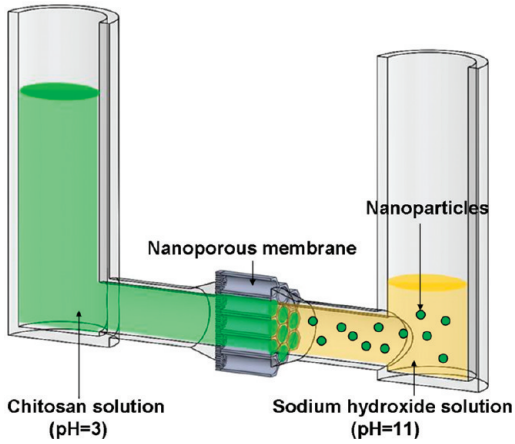

FIGURE 1. Method for producing chitosan nanoparticles by flow though a nanoporous membrane.

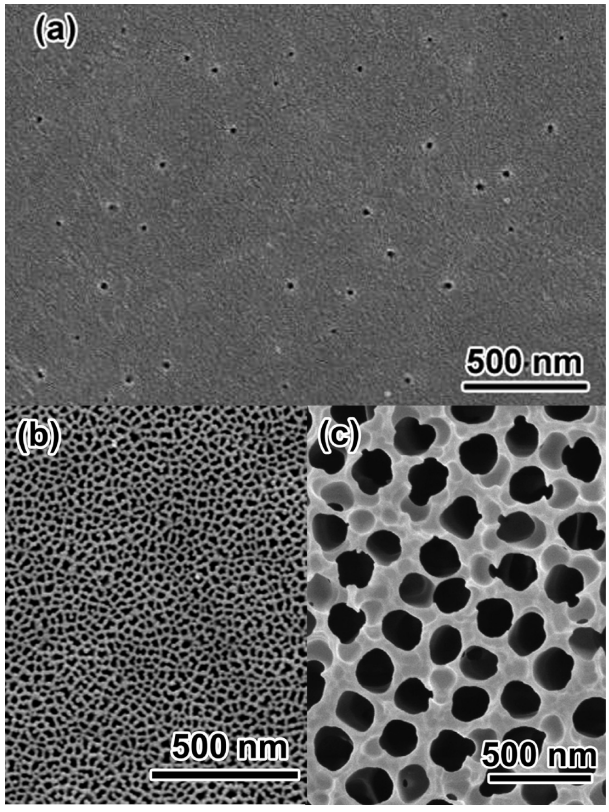

FIGURE 2. SEM images of nanoporous membranes. (a) Track-etched polycarbonate (PCTE) membrane with 10 nm pores; AAO membrane with (b) a 20 nm inlet and (c) a 200 nm outlet.

odized aluminum oxide (AAO, Whatman Inc.) nanoporous membranes. The PCTE membrane is 6 μm thick and contains track-etched cylindrical pores with a diameter of 10 nm and pore density of $6 \times 10^8/\text{cm}^2$ (Figure 2a). The AAO membrane is 60 μm thick and contains 20 nm cylindrical pores at the face of the membrane in contact with the feed solution. These pores run parallel to one another for approximately 2 μm and then feed much larger (200 nm in diameter) pores that run parallel to one another through the remaining thickness of the membrane. The pore density of the AAO membrane at the entrance (i.e., in contact with the feed solution) is around $6 \times 10^{14}/\text{cm}^2$ (Figures 2b,c).[21]

The feed solution contained 25 mg of chitosan in 20 mL of 10^{-3} M HCl (pH = 3.0). The receiver solution was 10 mL of 10^{-3} M NaOH (pH = 11). The area of membrane exposed to these solutions, either PCTE or AAO, was 2 cm^2. Gravity flow was achieved via a height difference between the two solutions, causing the low pH chitosan feed solution to flow into the high pH receiver solution. Nanodroplets are formed at the outlet of the PCTE nanoporous membrane

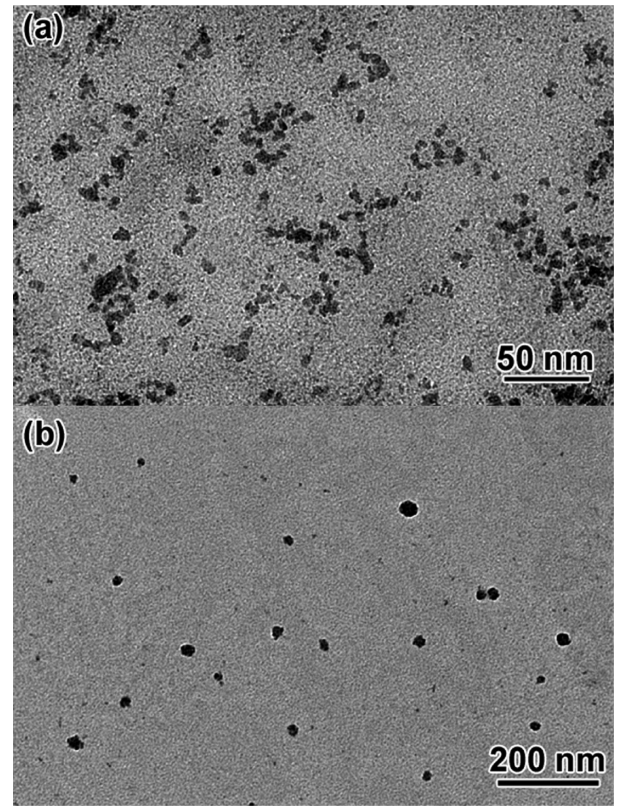

FIGURE 3. Typical TEM images of chitosan nanoparticles (CSNPs) prepared by using (a) the PCTE membrane and (b) the AAO membrane. In these TEM images, the black area represents the nanoparticle, and the gray area represents the background.

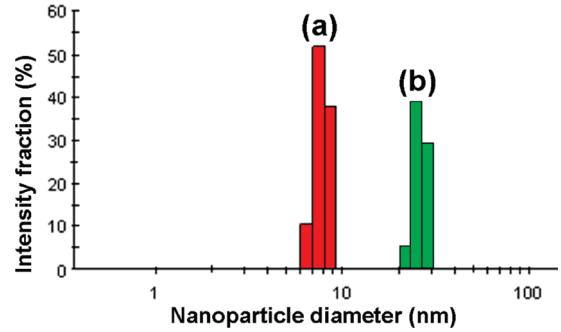

FIGURE 4. Comparison of size distributions of chitosan nanoparticles (CSNPs) prepared by using different nanoporous membranes determined by dynamic light scattering (a) size of CSNPs obtained by PCTE membrane and (b) size of CSNPs obtained by AAO membrane.

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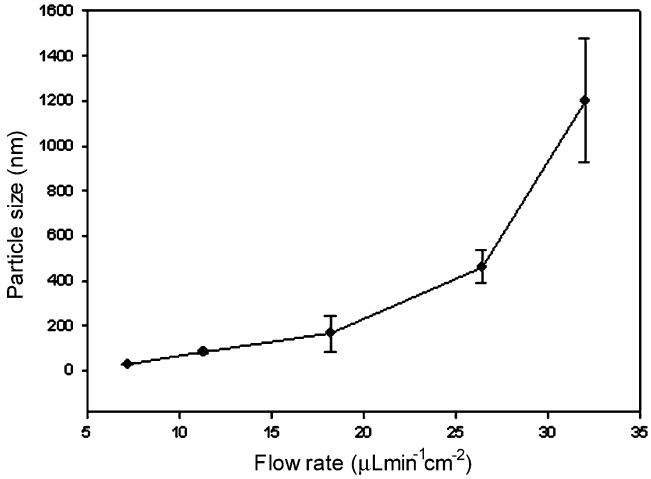

FIGURE 5. Effect of solution flow rate on the diameter of the chitosan nanoparticle.

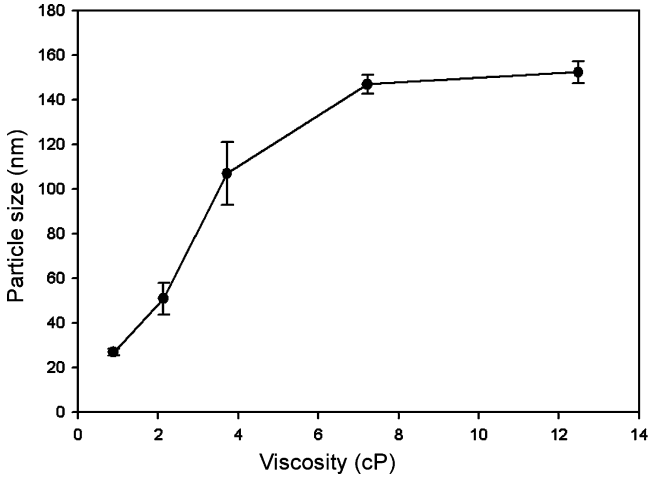

FIGURE 6. Effect of the viscosity of the chitosan feed solution on the diameter of the nanoparticles obtained.

in contact with the high pH solution, causing precipitation of the chitosan. In the case of the AAO membrane, the precipitation occurs at the exits of the 20 nm nanopores, which feed the 200 nm nanopores in this structure. The chitosan nanoparticles (CSNPs) are carried away from the membrane by the constant gravity flow. No instances of clogging or sticking were found. Nanoparticles were collected from the receiver solution by filtration, rinsed three times with deionized water, and dried in air at room temperature. We obtained 4.2 μg of nanoparticles per hour by PCTE, and 610 μg of nanoparticles per hour by AAO. These differing values are caused by the large pore density difference between the two kinds of nanoporous membranes. By replacing the gravity flow with pressure flow, we achieved in the AAO membrane the production rate of 36 mg/h but with an increase of the diameter of the nanoparticle to about 45 nm.

CSNPs were imaged using a TEM-1230 (JEOL) electron microscope, operated at 100 kV. Samples were deposited on carbon-coated copper grids and negatively stained with 1 % uranyl acetate. Figure 3a shows a typical TEM image of the CSNP obtained using the PCTE membrane having 10 nm nanopores. The nanoparticles were found to have a mean diameter of 5 nm. Figure 3b shows that CSNPs obtained using the AAO membrane. These nanoparticles have a mean diameter of 21 nm, which suggests that they are formed at the exit of the smaller nanopores (20 nm) in the AAO membrane.

Dynamic light scattering (DLS), measured with a Zetasizer Nano ZS (Malvern Instruments, Malvern, PA), was

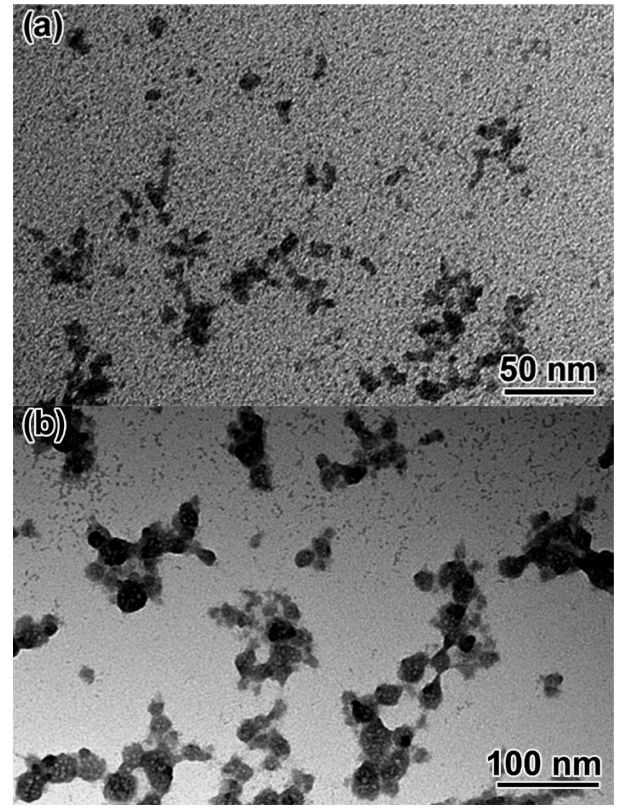

FIGURE 7. Typical TEM images of chitosan-rhodamine 6G nanoparticles prepared by using (a) the PCTE membrane and (b) the AAO membrane. In these TEM images, the black area represents the nanoparticle, and the gray area represents the background.

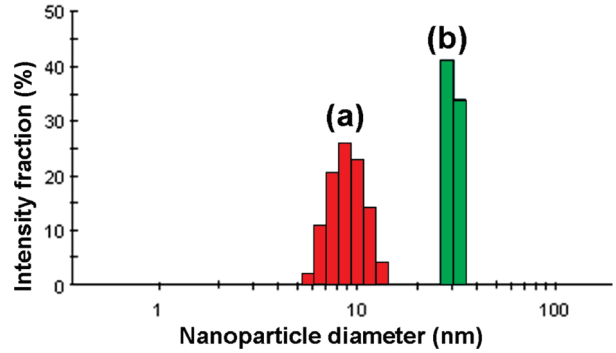

FIGURE 8. Comparison of size distributions of chitosan-rhodamine 6G nanoparticles prepared by using different nanoporous membranes determined by dynamic light scattering. (a) PCTE membrane and (b) AAO membrane.

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TABLE 1. Statistical Size and Encapsulation Efficiency Data for Chitosan (CS) and Chitosan-Rhodamine 6G (CS-R6G) Nanoparticles

membrane	nanoparticle	diameter TEM (nm)	diameter DLS (nm)	PDI	encapsulation ratio (%)
PCTE	CS	5 ± 2	8 ± 1	0.204	
PCTE	CS-R6G	5 ± 3	9 ± 2	0.108	2.7
AAO	CS	21 ± 5	26 ± 2	0.228	
AAO	CS-R6G	30 ± 8	30 ± 4	0.333	3.3

used to obtain hydrodynamic particle diameters. The hydrodynamic diameters of the particles obtained using the PCTE and AAO membranes were 8 and 26 nm, respectively (Figure 4). The particle size from DLS is slightly larger than the diameter estimated using electron microscopy because DLS measures the diameter of the particles while still in solution, whereas TEM provides the diameter of the particles after thorough drying.[22] That larger particles are obtained using the AAO membrane reflects the fact that the pore diameter in contact with the receiver solution is 20 nm for this membrane versus 10 nm for the PCTE membrane.

We also investigated the effect of flow rate of chitosan solution on the particle-formation process. CSNPs obtained using the AAO membrane were used in these studies. The flow rate of chitosan solution was varied from 7.2 to 32 μL $min^{-1}cm^{-2}$ by adjusting the height difference between the feed and receiver solutions. DLS measurements were used to obtain the particle diameters. Particle diameter was found to increase exponentially with flow rate, over the flow-rate range investigated (Figure 5). At higher flow rates hollow nanotubes and solid nanowires are formed as found from SEM images (not shown). It was also found that the narrowest particle size distribution was obtained at a flow rate of 7.2 μL min^{-1} cm^{-2}.

The viscosity of the chitosan feed solution also has a profound effect on nanoparticle-formation process. The viscosity of chitosan feed solution was varied by adding glycerol, while maintaining its pH at 3. Particle sizes initially increased with viscosity but leveled at higher viscosities (Figure 6). We suggest that this is caused by a change in the diffusion rate, which decreases rapidly as the viscosity increases, causing larger particles to be formed at slower diffusion rates. When the viscosity of chitosan solution achieves a certain point, particle size stops growing, perhaps owing to the gravity-induced detachment of the nanodroplets from the smaller nanopores in the membrane into the sodium hydroxide solution. The ultimate size is limited by the larger, 200 nm nanopores in the AAO structure.

For the drug loading and encapsulation study, we use rhodamine 6G (R6G) as a model system to mimic a drug molecule. The organic molecule R6G is one of the most often used fluorescent dyes with excitation and emission wavelengths at 525 and 555 nm, respectively.[23,24] Using such a fluorescent model compound provides us with a rapid method to evaluate the encapsulation data, which in turn allows us to optimize the process parameters.

In our experiment, 5.0 wt % R6G is premixed with the chitosan solution. Figure 7 shows the TEM images of R6G-loaded chitosan nanoparticles obtained using the PCTE and AAO membranes, respectively, and Figure 8 shows the corresponding results obtained using dynamic light scattering.

The amount of R6G encapsulated in the chitosan particle was determined by dissolving the dry particles in a phosphate/citrate buffer solution at pH = 3 followed by fluorescence measurements of the released R6G. When 5.0 wt % of R6G, referred to the weight of chitosan, was added to the feed solution and the PCTE membrane was used, the amount of R6G incorporated into the nanoparticles was 2.7 wt % (Table 1). The amount incorporated into the particles prepared using the AAO membrane was 3.3 wt %. Table 1 summarized these results and includes the polydispersity index (PDI) values.

Conclusion. The method of flowing liquid through a nanoporous membrane provides a general technique for incorporating guest molecules in the host chitosan nanoparticles. We believe that many other biodegradable polymer systems can be loaded with different organic compounds, which suggests the practical use of this technique in preparing pharmaceuticals in nanoparticle form for drug delivery.

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