

Preparation of Biodegradable Hollow Nanocapsules by Silica Template Method

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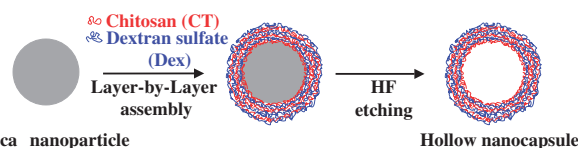
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Biodegradable hollow nanocapsules were successfully prepared by a combination of layer-by-layer assembly and the silica template method. Ultrathin polymer films were constructed on the surface of the silica nanoparticles by layer-by-layer assembly of oppositely charged biodegradable polyelectrolytes, chitosan and dextran sulfate. The silica templates were easily removed from the ultrathin polymer film-coated nanoparticles by treatment with hydrofluoric acid solution to create hollow nanocapsules. The hollow shape of these biodegradable nanocapsules was confirmed by SEM observation.

In recent years, the development of self-assembling systems for drug delivery, such as polymeric micelles and polymeric nanospheres, has been actively pursued.¹ In particular, hollow capsules prepared by layer-by-layer (LbL) assembly have attracted a great deal of attention because their shell permeability can be controlled by changes in environmental conditions such as temperature, pH, and the presence of organic solvents.² These properties of the hollow capsules permit the efficient encapsulation of substances into them as well as the controlled release of the encapsulated substances.

In general, these hollow capsules have been prepared by the deposition of ultrathin polymer films onto the surface of colloidal core templates via LbL assembly of oppositely charged polyelectrolytes,³ and the subsequent removal of the templates by the appropriate chemical treatment.⁴ Here, the choice of colloidal core template is very important in producing efficient hollow capsules, because the properties of the templates, such as their size, shape, and ease of removal from the ultrathin polymer film-coated ones, govern the properties of the resultant hollow capsules. In most of the previous studies, melamine formaldehyde (MF) and polystyrene (PS) particles have been used as colloidal templates to prepare hollow capsules. These particles, however, have a serious drawback that they cannot be completely removed from the ultrathin polymer film-coated particles. The oligomers generated from the MF particles during the dissolution process and the polymers with a high molecular mass from the PS particles are difficult to pass through the capsule shell.^{5,6} Thus, these particles are not suitable for the preparation of biodegradable hollow capsules.

Silica particles with sizes ranging from nanometers to micrometers are commercially available, and these are easily decomposed into silyl tetrafluoride by a hydrofluoric acid (HF) solution. Although silica particles are promising as colloidal templates, there have been only a few reports on the preparation of hollow capsules using silica templates.^{6,7} In particular, to the best of our knowledge, there have been no reports on biodegradable hollow capsules prepared from silica templates. In this letter, we report the preparation of biodegradable hollow nanocapsules by a combination of alternate LbL assembly of cationic



Scheme 1. Preparation of biodegradable hollow nanocapsules.

chitosan (CT) and anionic dextran sulfate (Dex), and the silica template method. The particle size of the hollow capsules thus prepared can be easily controlled from a nanometer to micrometer range, depending on the size of the silica template.

First, ultrathin polymer films composed of CT (M_w 650000) and Dex (M_w 500000) were constructed on a quartz-crystal microbalance (QCM) to monitor their growth from the frequency shift of the QCM. The preparation of these ultrathin polymer films was carried out in the presence of high concentrations of NaCl (1 M) to construct dense ultrathin films.⁸ The salt reduces the electrostatic repulsion of the polyelectrolytes and releases the hydrated water, and consequently the polyelectrolytes form globule structures, resulting in dense ultrathin polymer films. The cleaned QCM was immersed into an aqueous CT solution (1 mg/mL, 1 M NaCl) containing 25% formic acid for 15 min at ambient temperature, rinsed thoroughly with pure water, and then dried under N_2 gas. After the measurement of the frequency shift, the QCM was immersed into an aqueous Dex solution (1 mg/mL, 1 M NaCl) for 15 min, and the same procedure was repeated four times. The weight of the (CT/Dex)₅ films calculated from the frequency shift was 1.6 μ g (Figure 1a). The assembly of sequential films was confirmed by their FT-IR/ATR spectra (Figure 1b). When the outermost surface was CT, a peak of C–N stretching vibration (CT) at 1060 cm^{-1} was stronger than that of a C–O–S stretching vibration (Dex) at 980 cm^{-1} . In the case of a Dex-outermost surface, a peak of C–O–S stretching vibration (Dex) was observed stronger than that of the C–N stretching vibration (CT).

Next, on the basis of those results, LbL assembly was carried out on the surface of silica nanoparticles. In this study, we chose silica nanoparticles of 330 nm diameter as cores in order to demonstrate the preparation of nanosized stable hollow capsules. A 0.1-mL suspension of the silica nanoparticles (3.5 wt %) was alternatively incubated with 0.5-mL aqueous polyelectrolyte solution (1 mg/mL, 1 M NaCl) for 15 min under gentle shaking. The silica nanoparticles were then rinsed three times in pure water. The assembly of the ultrathin polymer films on the silica nanoparticles was confirmed by microelectrophoresis (pH 5.6, Figure 1d). The silica nanoparticles coated with ultrathin polymer films of CT and Dex yielded an alternating ζ -potential of –45 mV (silica surface), ca. +40 mV (CT-outermost surface), and ca. –30 to –40 mV (Dex-outermost surface). The alternating ζ -potential data suggested that sequential CT–Dex films

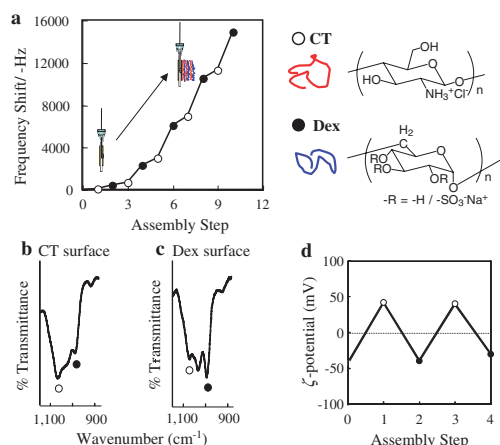


Figure 1. (a) Frequency shifts of CT (open symbol)–Dex (closed symbol) in ultrathin polymer films on QCM. FT-IR/ATR spectra of (b) (CT/Dex)₄/CT films (CT outermost surface) and (c) (CT/Dex)₅ films (Dex outermost surface): C–N (open symbol) and C–O–S (closed symbol) stretching vibrations. (d) Relationship between the ζ-potential of the outermost surface and the assembly step.

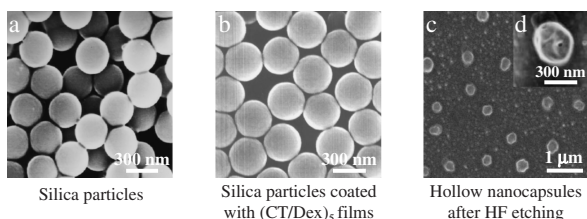


Figure 2. SEM images of (a) silica particles, (b) silica particles coated with (CT/Dex)₅ films and (c, d) hollow nanocapsules. The inset (d) shows a higher magnification.

were formed on the silica nanoparticles. The silica nanoparticles coated with ultrathin polymer films were then treated with 46% aqueous HF (25 °C, 12 h) in order to remove the silica cores. Inductively coupled plasma (ICP) emission analysis indicated that more than 99.9% of Si atom was removed from the silica particles coated with (CT/Dex)₅ films by the HF etching.

Figure 2 shows scanning electron microscopy (SEM) images of silica nanoparticles, silica nanoparticles coated with (CT/Dex)₅ films, and hollow nanocapsules. The shell thickness of the (CT/Dex)₅ films was estimated to be about 50 nm from the sizes of the silica particles coated with (CT/Dex)₅ films. On the other hand, the shell thickness of the (CT/Dex)₅ films was calculated to be about 60 nm by dynamic light scattering (data not shown). The difference in the estimated shell thickness between those two methods may be due to the dryness of the shells. Although several hollow nanocapsules observed on the SEM images had the characteristics of typical hollow capsules, including folds, crevasses, and strain forms which were possibly caused by the drying process, all other hollow nanocapsules maintained their spherical shapes without puncturing. This result suggests that dense and stable (CT/Dex)₅ films were constructed by the LbL assembly of CT and Dex in the presence of a high concentration of NaCl, and that these films were not damaged by the HF etching. The obtained hollow nanocapsules were also found to be stable enough to be redispersed in water after freeze-drying.

In conclusion, we demonstrated an effective method for the preparation of biodegradable hollow nanocapsules. The shell thickness of these hollow nanocapsules can be controlled by changing the LbL assembly steps. It is expected that hollow nanocapsules with various diameters can be easily prepared by changing the diameter of the silica nanoparticles. The release of drugs loaded into the hollow nanocapsules^{2c,9} can be controlled by altering the shell thickness and capsule diameter. Studies on the properties of the biodegradable hollow nanocapsules such as the incorporation of drugs into those capsules and the stability of encapsulated drugs are now in progress.

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