Hydrogen-Bonded Polymer Capsules Formed by Layer-by-Layer Self-Assembly

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Layer-by-layer sequential adsorption of polymers on solid substrates has proven to be a versatile technique to produce ultrathin polymer films using a variety of synthetic and natural molecules as building blocks.[1,2] Although multilayer films have mainly been deposited onto flat substrates, coating nano- and micrometer-sized solid particles with polyelectrolyte multilayers is attracting growing interest[3−4,5] as an effective means to encapsulate a large number of materials ranging from dyes[6,7] and low molecular weight organic molecules[8] to enzymes,[9] biological macromolecules,[10] and living cells.[11]

After the multilayer is deposited on a particulate substrate, the substrate can be dissolved to produce hollow polyelectrolyte capsules. Multilayer walls can be made which include a variety of different components such as synthetic polymers, inorganic nanoparticles,[12] dendrimers,[13] multivalent ions,[14] and lipids.[15] For many future applications of such capsules, including controlled delivery applications, it is desirable to regulate the multilayer wall in response to various external stimuli. One such stimulus is the pH of the external solution. Möhwald et al. were first to report the pH-dependent permeation of chemicals through multilayered capsules; "opening up" and "closing up" of their multilayered membranes occurred when the pH changed from below 6.5 to above 8.[16]

In this work, we introduce a new polymer chemistry that we believe will enable a tailored response of the capsule wall to pH. In particular, we focus on hydrogen-bonded multilayers. In contrast to electrostatic self-assembly that has been commonly used to produce the multilayer capsules, hydrogen-bonding interactions represent an alternative driving force for the LBL growth of multilayers.[17] We have earlier demonstrated that hydrogen-bonded multilayers are highly pH sensitive and can be completely dissolved at higher pH values.[18] More recently, Rubner and Yang reported stabilization of hydrogen-bonded layers at neutral and basic pH values via thermal or photoinduced cross-linking.[19] While all prior work on hydrogen-bonded multilayers was performed in a flat geometry, our work here is the first example where hydrogen-bonded self-assembly is used to build multilayer capsules.

In hydrogen-bonding self-assembly, unlike electrostatic self-assembly, the polymers are virtually uncharged. Concerns have been expressed that reducing a polyelectrolyte's linear charge density might destabilize particle dispersion. In particular, Caruso and coworkers have recently reported that irreversible aggregation of the particles occurred when the pH of a poly(acrylic acid) solution was lowered below 5.0.[20] Our results suggest, however, that robust multilayer capsules can be produced using uncharged polymer. We also show that unless they are covalently cross-linked, the capsules show a pH response similar to that found for the same hydrogen-bonded films on a flat substrate; i.e., the capsules disintegrate when the pH exceeds a certain critical value. However, hydrogen-bonded multilayers can be easily stabilized at neutral and basic pH values by covalently cross-linking the carboxylic groups using known carbodiimide chemistry.

To produce capsules, we used poly(*N*-vinylpyrrolidone) (PVPON; M_w 55 000), poly(methacrylic) acid (PMAA; M_w 150 000), and poly(ethylene oxide) (PEO; M_w 200 000). Other polymer pairs capable of adhering via hydrogen bonding should work by the same principle, e.g., poly(*N*-acrylamide), poly(*N*-isopropylacrylamide), poly(*N*-vinylcaprolactam) and poly(carboxylic acid). The template material was cadmium carbonate. Cadmium carbonate particles were synthesized by mixing equal amounts of 1 M cadmium nitrate solution and 2 M urea solution followed by heating the mixture for 16 h at 90 °C.[21] The resulting crystals were rhombohedral and ranged in size from 0.1 to 10 μm. The PVPON/PMAA or PEO/PMAA multilayers were then prepared using the layer-by-layer technique with a centrifugation setup as described in the literature.[3−6] Polymers were deposited from 0.2 mg/mL solutions. The solution pHs during the deposition and washing steps were controlled using 0.01 M phosphate buffer whose pH was adjusted with hydrochloric acid to produce acidic solutions. To increase film adhesion, the $CdCO_3$ particles were pretreated with a 0.2 mg/mL solution of PMAA at $pH = 7.0$, followed by washing with buffer at $pH = 3.5$. The deposition of PVPON/PMAA or PEO/PMAA layers was then continued at $pH = 3.5$, starting from the PVPON or PEO layer. After every polymer deposition cycle, excess polymer was removed by (a) centrifugation of the particle dispersion, (b) redispersing particles into a polymer-free buffer solution, and (c) repeating this washing procedure at least twice. Starting from the second layer, a buffer at $pH = 3.5$ was used at every washing step. In neither system did we observe severe particle aggregation. Gently shaking the precipitate was usually sufficient to redisperse the particles after centrifugation. On occasions, the precipitate was sonicated for 1 min to reverse aggregation. In a typical experiment, 10 polymer layers were deposited, with PVPON or PEO as the outermost layer. The $CdCO_3$ core of the polymer-covered particles was then dissolved by exposing the particles to buffer solution at $pH = 1.1$. The time allowed to completely remove the core was 30 min. Note that hydrogen-bonded multilayers show remarkable stability at such low pH, whereas electrostatically adhering polymers, specifically those composed of a weak polyacid, dissociate in such extremely acidic conditions.[22]

To determine the amounts of polymers deposited into capsule walls, we used two strategies. First, using in situ ATR−FTIR we followed the multilayer growth in a model system where polymers were deposited onto a flat surface of oxidized Si. The oxidation of the surface, priming with the first layer, multilayer deposition, as well as calculation of the amount adsorbed were done as described in our previous work.[18] Here, however, multilayers were deposited at $pH = 3.5$ instead of $pH = 2$ as studied earlier.[18] The result of these ATR−FTIR

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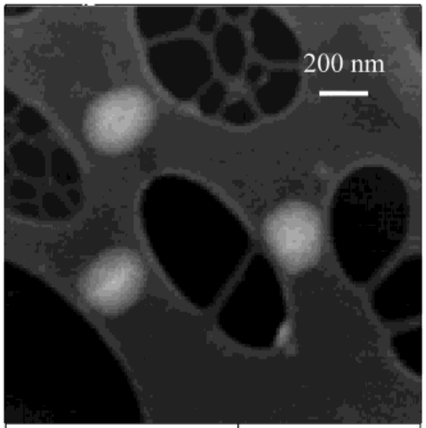

Polymer System	Thickness (nm)
PEO/PMAA	16 ± 3
PVPON/PMAA	18 ± 4

Figure 1. High-angle annular-dark-field STEM image of PEO/PMAA capsules (bright contrast) on a lacy-carbon TEM support film. The darkest areas represent pores in the support film. The table below summarizes the average multilayer film thicknesses, determined from at least 12 different capsules per specimen. PEELS spectra were collected at 20 nm intervals along line scans that transected individual multilayer capsules. The average capsule wall thickness was obtained by averaging 10 measurements from a given capsule and at least 12 capsules in each of the two specimens (PEO/PMAA and PVPON/PMAA) studied.

studies gave the total amounts adsorbed as 56 and 36 mg/m^2 for 10-layer PEO/PMAA and PVPON/PMAA systems, respectively.

Second, we have used electron energy loss spectrometry (EELS) to determine the thickness of capsule walls after the core dissolution. Figure 1 shows a STEM image of PEO/PMAA capsules. The capsules were deposited from buffer solution at pH = 3.5 onto a lacy-carbon TEM support film, dried, cooled to −165 °C, and analyzed using a 200 keV Philips CM20 field emission, scanning-transmission electron microscope (FEG-STEM) with a Gatan 776 Enfina PEELS spectrometer. The capsule wall thickness was derived using the relation: $2t/\lambda = \ln(I_t/I_0)$, where t is thickness, λ is the mean free path (MFP) for total inelastic electron scattering of the polymer, and I_t and I_0 are the total and the zero-loss spectral intensities,[23] respectively; we used $\lambda = 260$ nm based on our independent measurements of inelastic electron scattering in polystyrene. One can see that a 10-layer thickness was significantly smaller when deposited onto a $CdCO_3$ template, as compared to the model Si surface (16 nm vs 56 nm for the PEO/PMAA system and 18 nm vs 36 nm for the PVPON/PMAA system, assuming a density of 1 g/cm^3). We suggest that the differences in surface charge of the Si and $CdCO_3$ substrates as well as different procedures used in the deposition of a primer layer may be responsible for the smaller amount of polymer adsorbed within each layer of the film in the case of $CdCO_3$ substrate. The effect of the substrate on the amount of polymers adsorbed within a layer, specifically for a small number of deposition cycles, was reported earlier.[24]

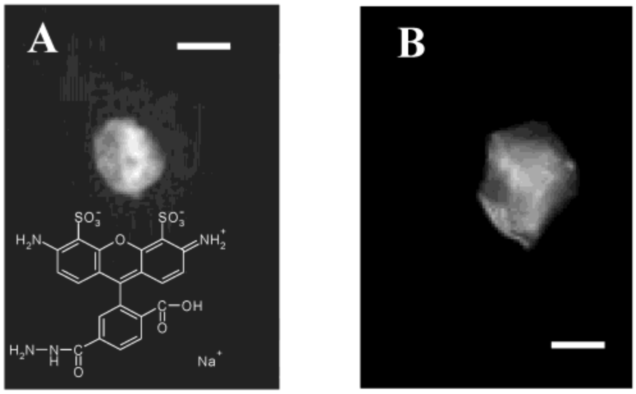

Figure 2. Fluorescence images of 10-layer PEO/PMAA (panel A) and PVPON/PMAA capsules (panel B) at pH = 2.0. The inset shows the chemical structure of the Alexa Fluor 488 hydrazide fluorophore. 0.02 M Alexa Fluor 488 hydrazide sodium salt solution has been used for staining capsules. The bar corresponds to 4 μm.

The formation of multilayer capsules was followed by fluorescence optical microscopy using a Nikon Eclipse E1000 microscope with a 40×LU Plan objective lens. For this imaging, capsule specimens were stained with a high-quantum yield photostable Alexa 488 Fluor hydrazide fluorophore (Molecular Probes Inc.) The structural formula of this label is shown in Figure 2. This label noncovalently attaches to the functional groups of the capsule wall over a wide interval of pH, due to electrostatic and hydrogen-bonding as well as van der Waals interactions, allowing us to fluorescently image hydrogen-bonding capsules. However, the staining is more efficient at acidic pH values, when the label is probably able to form hydrogen bonds with un-ionized carboxylic groups.

Figure 2 shows fluorescence images of the 10-layer PVPON/PMAA and PEO/PMAA capsules at pH = 2.0. One can see that robust capsules are produced. On the basis of our earlier studies of the PVPON/PMAA and PEO/PMAA multilayers on a flat substrate, the hydrogen-bonded capsules should fall apart at higher pH values due to internal ionization of the self-assembled polyacid. The disintegration threshold expected for PEO/PMAA multilayer is pH = 4.6, and for the PVPON/PMAA system, pH = 6.9.[18] In the case of capsules, these critical pHs for layer disintegration of capsules were in good agreement with those obtained for the films on flat substrates.

A greater degree of stability can be imparted to hydrogen-bonded multilayer capsules by introducing covalent cross-links within the multilayer wall. The cross-linking procedure we used is based on carbodiimide chemistry. Recently, Akashi et al. reported the cross-linking of charged polymers during their layer-by-layer deposition onto a flat substrate.[25] In the present work, we performed cross-linking of hydrogen-bonding polymers deposited onto a particulate substrate by adding carbodiimide after the multilayer self-assembly. The specific procedure included activation of the carboxylic groups with 5 mg/mL of 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDC) solution at pH 5.0 (for PVPON/PMAA multilayers) or pH 4.0 (for PEO/PMAA multilayers), followed by reacting with 0.01 mg/mL of ethylenediamine at pH 5.8 or 4.0 for PVPON/PMAA or PEO/PMAA multilayers, respectively. The cadmium carbonate core was removed after cross-linking by exposing the modified particles to pH = 1.1, leaving behind cross-linked capsule walls.

Figure 3 shows that after the cross-linking treatment PVPON/PMAA capsules became stable at pH = 10.

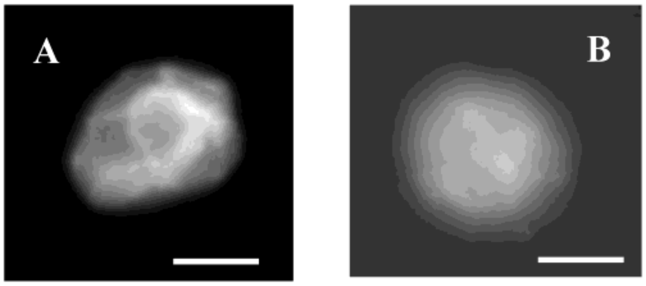

Figure 3. Fluorescence images of cross-linked 10-layer PVPON/PMAA capsules at pH = 2 (panel A) and after exposure for 2 h to pH = 10 (panel B). The bar corresponds to 4 μm.

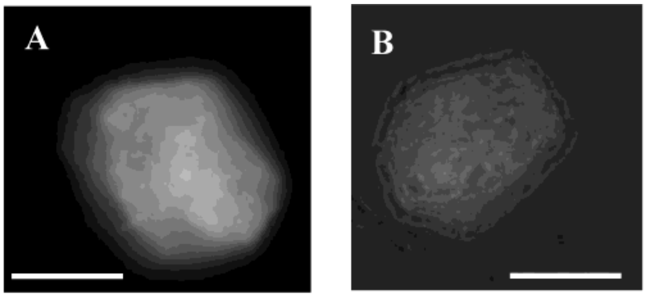

Figure 4. Fluorescence images of cross-linked 10-layer PEO/PMAA capsules at pH = 2 (panel A) and after exposure for 2 h to pH = 7 (panel B). The bar corresponds to 4 μm.

Cross-linked capsules stored for several months at pH = 10 also did not show any signs of disintegration. The lower contrast in the image taken at high pH is probably due to the swelling of the PVPON/PMAA capsules and weaker binding of Alexa 488 fluorophore to the multilayer wall at high pH values. The pH-stability of PEO/PMAA capsules was also greatly improved (see Figure 4). Capsules could be observed for at least 6 days when exposed to pH = 7. In a control experiment using in situ ATR−FTIR we have determined that in the PVPON/PMAA system, all PMAA remained in the cross-linked film after the film was exposed to pH = 10. However, in the PEO/PMAA system, 50% of PMAA was lost from the film within 2 days of exposure at pH = 7, despite the fact that the remaining film was stable for at least several weeks. Our work to further improve the stability of the PEO/PMAA capsule wall by more efficient cross-linking is ongoing.

The hydrogen bonded capsules produced here present a new type of multilayer capsule with novel attractive features. First, in their as-deposited state, these capsules disintegrate at a critical pH that can be tuned by choosing different combinations of polymers. Then, after cross-linking, the multilayer wall acquires a new feature at high pH values where there is no intermolecular adhesion between components of the wall and the wall resembles a cross-linked ultrathin gel whose thickness can be conveniently controlled by the number of polymer layers initially deposited onto a solid core. These features can all be exploited in biomaterials and biotechnology capsule applications.

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