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pH-Controlled Permeability of Layered Hydrogen-Bonded Polymer Capsules

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Introduction

Hollow capsules formed by layer-by-layer (LbL) self-assembly of polymers have been recently intensively studied for their potential applications in medicine and biotechnology. One of the attractive properties of such multilayer polymer shells is the possibility to control capsule wall permeability to low-molecular-weight compounds or macromolecules. For the case of electrostatically assembled containers, such as poly(styrenesulfonate)/poly(allylamine hydrocloride) (PSS/PAH) capsules, semipermeability of the multilayer wall has been demonstrated in several studies. Specifically, small molecules of dyes and ions could permeate through the PSS/PAH capsule wall while high-molecular-weight compounds (with a typical molecular weight cutoff of ∼2K) were excluded.[1,2] There have been suggested several ways to decrease permeability of electrostatically assembled LbL capsules to small and oligomeric molecules, including the use of the increased number of deposited polyelectrolyte layers,[3] capsule resealing,[4] and additional coating of the capsule wall with lipid layers,[5] photo-[6] or thermal cross-linking,[7] or chemical cross-linking of the capsule wall.[8,9] Capsules with pH- or ionic-strength-responsive permeability were also produced through electrostatic self-assembly.[10−14]

Our focus has recently been on LbL hydrogen-bonded films and capsules which are produced by self-assembly of a weak polyacid and a neutral polymer in aqueous environment. Such LbL assemblies are produced at low pH and, unless cross-linked, dissolve at neutral and basic pHs. Cross-linking of hydrogen-bonded multilayer capsules was introduced to stabilize capsules constructed from weak polyelectrolytes against pH changes.[15,16] We have shown recently that covalently cross-linked one-component poly(methacrylic acid) (PMAA) hydrogel capsules can be produced[17] and that their permeability to fluorescein isothiocyanate (FITC) labeled dextrans can be controlled via variation of the cross-linking density of the capsule wall.[18] However, permeability properties of the capsule wall composed of two hydrogen-bonded polymers remained largely unexplored. Here we report on hollow capsules whose wall contains LbL-deposited and chemically cross-linked hydrogen-bonded polymers. Specifically, we contrast the capsule swelling and permeability in a wide pH range, when such capsules transit from hydrogen-bonded cross-linked multilayers to highly swollen hydrogels with no intermolecular association, and compare their properties with those found for hollow capsules composed of PMAA hydrogel.

Experimental Section

Materials. Poly(methacrylic acid) (PMAA; M_w 150 kDa), hydrochloric acid, sodium hydroxide, sodium chloride, dibasic and monobasic sodium phosphate, 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (NSS), and FITC-labeled dextrans of various molecular weights were purchased from Sigma-Aldrich and used as received. Cascade Blue (CB) dye was acquired from Molecular Probes. The SiO_2 template particles of 4.0 ± 0.2 μm diameter were purchased from Polysciences Inc. as 10% dispersions in water. Dialysis of capsules was performed in 100 μL Micro DispoDialysers (SpectrumLabs, Canada). Millipore (Milli-Q system) filtered water with a resistivity 18.2 MΩ was used in all experiments.

Preparation of PVPON-NH$_2$-20/PMAA Films and Capsules. Copolymer of *N*-vinylpyrrolidone and glycidyl methacrylate was synthesized by free-radical solution polymerization in dioxane using AIBN as initiator as described previously.[19,20] The produced copolymer, poly(*N*-vinylpyrrolidone-*co*-GMA-20) (20% of GMA, M_w 19 000), was reacted with 100-fold excess of ethylenediamine (with respect to GMA content in the copolymer) at room temperature for 24 h. The produced amino-containing copolymer is denoted as PVPON-NH$_2$-20, where NH$_2$-20 denotes aminated groups on the copolymer.

Hydrogen-bonded films of PVPON-NH$_2$-20/PMAA were deposited on the precursor-treated silicon wafers. Deposition of precursors to enhance surface adhesion of the subsequently grown multilayer was performed as described elsewhere,[17] except that poly(ethyleneimine) was used as a polycation.

Deposition of PVPON-NH$_2$-20/PMAA multilayers on silica particles was performed as described previously[17] using 0.2 mg/mL polymer solutions at pH 2. For cross-linking, carboxylic groups of the capsule wall were activated by exposing the capsules to 5 mg/mL solution of EDC and NSS at pH 5 for 14 or 18 h with continuous agitation. After several washings of the particle suspension in 0.01 M phosphate buffer at pH 4.6, the cores were dissolved by shaking the particle dispersion for 4 h in 8% aqueous HF solution. The dispersion of hollow capsules was dialyzed for 48 h in a 0.01 M phosphate buffer at pH 8.0 to ensure a removal of activation agents and finally for 4 h against aqueous solution at pH 2.

Measurements. SEM analysis was performed using a LEO 982 DSM instrument with a field-emission gun at an operation voltage of 1 or 5 kV. Samples were prepared by putting a drop of a capsule suspension on a precleaned silicon wafer and allowing it to dry for 2 h.

Thickness measurements of dry LbL non-cross-linked and cross-linked films were done using a homemade phase-modulated ellipsometer.

Confocal images of capsules were obtained with an LSM 5 PASCAL laser scanning microscope (Zeiss, Germany) equipped with C-Apochromat 63x/1.2 water immersion objective. Capsules were visualized through deposition of the FITC-labeled PMAA within the last three bilayers of PVPON-NH$_2$-20/PMAA or PMAA capsules.[17] The excitation wavelength was 488 nm. To observe pH-dependent size changes, a drop of a dispersion of hollow capsules was added to several Lab-Tek chambers, which were then filled with buffer solutions at a certain pH. Capsules were allowed to settle down and then analyzed. To investigate the pH effects on capsule permeability to FITC-dextrans, a drop of a dispersion of hollow capsules was added to several Lab-Tek chambers, which were then half-filled with buffer solutions at a certain pH and then mixed with FITC-dextrans (1 mg/mL) with the same pH values adjusted with 0.1 N NaOH. CLSM images of the capsules were taken after 20 min.

Results and Discussion

PVPON-NH$_2$-20/PMAA Films. Deposition of non-cross-linked PVPON-NH$_2$-20/PMAA films was robust, producing uniform coatings with a bilayer thickness of 5.7 ± 0.5 nm up to 10 bilayers and a higher incremental growth of 7 ± 2 nm for thicker films. As-prepared non-cross-linked PVPON-NH$_2$-20/PMAA films were stable at low pH and dissolved under neutral or basic conditions. Figure 1 shows that the thickness of 10-

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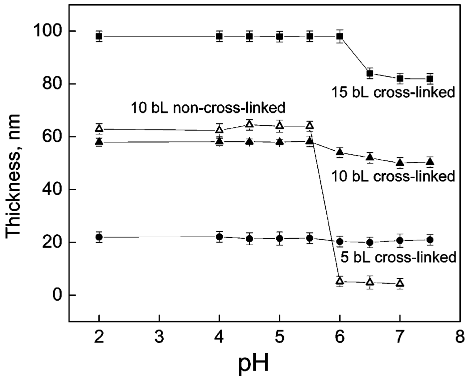

Figure 1. Thickness of surface-deposited (PVPON-NH_2-20/PMAA) non-cross-linked (open triangles) and cross-linked (filled circles, 5 bilayers; filled triangles, 10 bilayers; filled squares, 15 bilayers) films after exposure to different pH values. Prior to ellipsometry measurements, films were exposed to a buffer solution with a certain pH value for 15 min, then to buffer solution at pH 4.6, and finally dried with a stream of air. The refractive index used for ellipsometry measurements was 1.5.[24] The pH values were supported by 0.01 M phosphate buffers.

bilayer non-cross-linked surface-attached films decreased drastically after exposure to pH 6 due to disruption of hydrogen-bonding interactions between PVPON-NH_2-20 and PMAA layers caused by increased ionization of the polyacid. However, chemical cross-linking via introduction of carbodiimide-assisted amide cross-links between amino groups of the copolymer and carboxylic groups of the polyacid afforded film stability at basic pH values. The degree of stabilization, however, was dependent on the total film thickness. While thinner 5-bilayer cross-linked films did not show any mass loss, thicker films of 10 and 15 bilayers slightly decreased in thickness at pH > 6. This is likely to be a result of the increased amount of polymers deposited within an individual layer for thicker films (average bilayer thickness of 5.4 nm for a 5-bilayer film versus 6.7 nm for a 15-bilayer film), which decreases the probability of cross-linking of carboxylic and amino groups due to larger interlayer spacing.

PVPON-NH_2-20/PMAA Capsules: pH-Dependent Swelling. The developed cross-linking strategy was used to produce cross-linked (PVPON-NH_2-20/PMAA)$_7$ multilayers on silica microspheres. Figure 2, curve A, shows size evolution of (PVPON-NH_2-20/PMAA)$_7$ hollow cross-linked capsules as a function of pH. A sharp increase in the capsule diameter is observed at pH 6, the same pH value at which non-cross-linked PVPON-NH_2-20/PMAA films disintegrate when deposited on silicon wafers (see Figure 1). At pH > 6, the capsule wall presents a hydrogel composed of covalently cross-linked non-

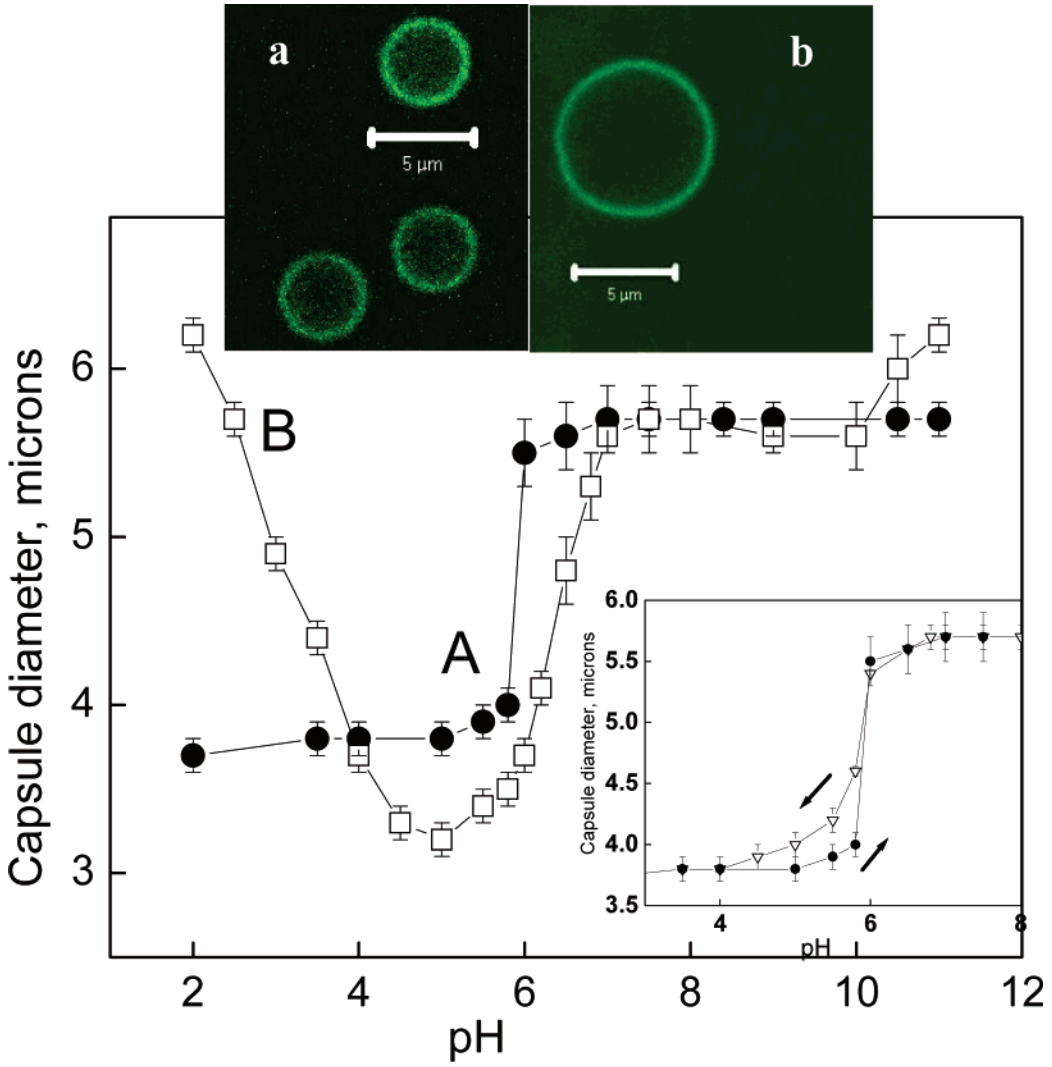

Figure 2. CLSM images of (PVPON-NH_2-20/PMAA)$_7$ (a) and (PMAA)$_7$ (b) capsules at pH 2. pH dependence of the diameter of the (PVPON-NH_2-20/PMAA)$_7$ capsules (curve A, filled circles) and (PMAA)$_7$ capsules (curve B, open squares) cross-linked for 18 h. The inset shows hysteresis of (PVPON-NH_2-20/PMAA)$_7$ capsule size upon increasing (filled circles) and decreasing (open triangles) pH. The pH values were supported by 0.01 M phosphate buffer.

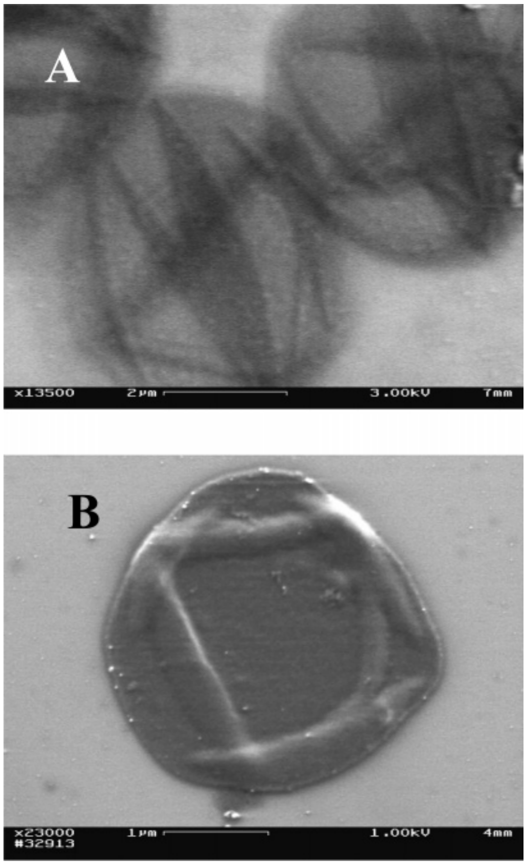

Figure 3. SEM images of the hydrogen-bonded (PVPON-NH_2-20/PMAA)$_7$ (A) and (PMAA)$_7$ (B) cross-linked capsules dried from deionized water after dialysis at pH = 8.

associated PVPON and PMAA chain segments. The capsule size changes saturated at pH > 7 when PMAA segments became fully ionized, according to the reported pK_a values for PMAA of ∼6−7.[21] Importantly, unlike one-component PMAA-*cross*-linked capsules earlier studied in our group[18] (shown in Figure 2, curve B), (PVPON-NH_2-20/PMAA)$_7$ capsules showed no changes in swelling at pH < 4.5 or pH > 10. This is because the cross-linking of PVPON-NH_2-20/PMAA did not require the additional use of amino-containing cross-linker such as ethylenediamine, used in cross-linking of PVPON/PMAA multilayers. The amino groups were supplied by PVPON-NH_2-20 chains. Interestingly, 14 and 18 h cross-linked (PVPON-NH_2-20/PMAA)$_7$ capsules showed similar swelling ratios, suggesting saturated conversion of PVPON-NH_2-20 amino groups and introduction of amide linkages through every 5th unit of PVPON-NH_2-20 chains. When cross-linking of 5-bilayer flat films was performed for shorter times of 1, 3, and 6 h, the initial film thickness retained was 89%, 93%, and 96% (±4%), respectively. This result suggests that at least one cross-link per macromolecule is formed during the first hour of cross-linking.

Swelling/deswelling of (PVPON-NH_2-20/PMAA)$_7$ cross-linked capsules was reversible but showed distinct hysteresis at pH values from 4 to 6 (see inset in Figure 2). Specifically, a larger capsule size was detected in this region when pH was lowered, compared to that when pH was increased. Indeed, hydrogen bonding between PVPON and PMAA chains at low pH values strongly suppresses PMAA ionization, and dissociation of hydrogen bonds occurs when a critical ionization of PMAA reached.[22] When approaching the same pH region from the high pH values, in the absence of interchain association, ionization of the −COOH groups is higher than that in preassembled film. Therefore, it is necessary to bring the hydrogel network to a lower pH value to induce formation of hydrogen bonds. The latter appears as the capsule size hysteresis between swelling and deswelling curves in the inset in Figure 2.

The pH-induced transition from interaction to dissociation of the two polymer chains in the capsule wall was also evident from the contrasting of morphology of (PVPON-NH_2-20/PMAA)$_7$ and PMAA-*cross*-linked capsules using SEM. Figure 3 shows SEM images of cross-linked hollow (PVPON-NH_2-20/PMAA)$_7$ capsules dried from deionized water (A) as well as an image of a one-component (PMAA)$_7$-*cross*-linked capsule exposed to the same conditions (B). As we have shown earlier,[17] dried PMAA-*cross*-linked capsules have fluffy hydrogel-like morphology when dried from the pH of distilled water of 5.5. In sharp contrast, (PVPON-NH_2-20/PMAA)$_7$ capsules have distinct wrinkles and folds which indicate a stiffer capsule wall resulting from hydrogen bonding between PMAA and PVPON chains at this pH.

PVPON-NH_2-20/PMAA Capsules: pH-Dependent Permeability. In our previous work[15,18] we demonstrated that the permeability of PMAA-cross-linked capsules can be controlled by changing pH and switching from the open (capsules are highly swollen) to the closed (capsules are deswollen) states of the one-component capsules. Apart from changing cross-linking density of PMAA-cross-linked capsules, here we show that retaining the neutral component (PVPON) within the capsule wall can be another efficient way to control permeability of the cross-linked hydrogel capsules. The capsule permeability was monitored by CLSM using a CB dye or FITC-dextrans as a fluorescent probe. Capsules were considered impermeable for the probe if the capsule interiors remained dark for at least 20 min.[8−10]

Table 1 compares permeability of non-cross-linked two-component capsules and cross-linked one- and two-component capsules to CB dye molecules and FITC-dextrans of various molecular weights at pH 4.6 and 8. It is seen from Table 1 that (PVPON-NH_2-20/PMAA)$_7$ capsules, similar to the (PVPON/PMAA)$_7$ capsules, excluded all tested FITC-dextrans at pH 4.6 due to hydrogen-bonded interactions within the wall. For comparison, (PVPON/PMAA)$_7$ capsules were impermeable to all tested dextrans and a low-molecular-weight CB dye. In

Table 1. Permeability of Cross-Linked (PVPON-NH_2-20/PMAA)$_7$ and (PMAA)$_7$ and Non-Cross-Linked (PVPON/PMAA)$_7$ Capsules to FITC-Labeled Dextrans with Different Molecular Weights and CB Dye[23] at pH 4.6 and 8[a]

	CB dye		FITC-labeled dextrans							
	M_w 596 Da		M_w 4 kDa		M_w 70 kDa		M_w 150 kDa		M_w 500 kDa	
	pH 4.6	pH 8	pH 4.6	pH 8	pH 4.6	pH 8	pH 4.6	pH 8	pH 4.6	pH 8
(PVPON-NH_2 /PMAA)$_7$	O	O	X	O	X	X	X	X	X	X
(PVPON /PMAA)$_7$	X	–[b]	X	–	X	–	X	–	X	–
(PMAA)$_7$ 5 h	O	O	O	O	O	O	O	O	X	O
(PMAA)$_7$ 18 h	O	O	O	O	X	O	X	O	X	X

[a] Symbols X and O indicate capsule impermeability and permeability, respectively, to a specific permeant molecule, after 20 min of observation. [b] Note that the permeability of hydrogen-bonded non-cross-linked (PVPON/PMAA)$_7$ capsules cannot be measured at pH 8 due to their dissolution at pH = 6.

addition, in its hydrogel form at high pH, (PVPON-NH_2-20/PMAA)$_7$ capsules were able to selectively control permeability of macromolecules, exclusively allowing permeation of FITC-dextran with M_w 4 kDa and rejecting higher molecular weight permeants.

It is relevant to compare the permeability of (PVPON-NH_2-20/PMAA)$_7$ capsules with a different type of hydrogel capsules earlier reported by us,[17] in which hydrogen bonds could not be formed because of complete removal of PVPON chains after cross-linking. The data on permeability of (PMAA)$_7$ capsules are also shown in Table 1 and demonstrate high permeability of the capsule wall to macromolecules, which can be efficiently controlled via a decrease in polymer network mesh size achieved through increased cross-linking density demonstrated by us recently.[18] An increased cross-linking density of one-component hydrogel PMAA capsules, however, does not allow controlled permeation of oligomeric molecules with $M_w \sim 4$ kDa (see Table 1). In sharp contrast, the (PVPON-NH_2-20/PMAA)$_7$ system allows permeability control for (a) oligomeric compounds at low pH through hydrogen-bonding and (b) larger macromolecules at high pH through dissociation of hydrogen-bonded segments of the cross-linked polymer network.

Conclusion

We have demonstrated that pH-responsive two-component PVPON-NH_2-20/PMAA capsules can be produced through carbodiimide-assisted cross-linking of hydrogen-bonded multilayers. These capsules reversibly respond to changes in environmental pH by variations in capsule diameter and permeability and demonstrate the transition from highly associated to completely dissociated capsule wall as a function of pH. Importantly, retention of hydrogen-bonded components in the capsule wall was demonstrated to be an efficient means to reduce permeability of cross-linked capsules to oligomeric molecules. The pH-responsive (PVPON-NH_2-20/PMAA)$_7$ hydrogel containers show promise in applications for controlled delivery of functional molecules which can be useful in biomedicine and biotechnology.

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