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Amphoteric Hydrogel Capsules: Multiple Encapsulation and Release Routes

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ABSTRACT: We show that chemical cross-linking of hydrogen-bonded poly(methacrylic acid)/poly(vinylpyrrolidone) (PMAA/PVPON) capsules deposited onto silica core with ethylenediamine (EDA) followed by core dissolution and pH-triggered removal of PVPON produces amphoteric hydrogel hollow capsules, whose walls are composed of cross-linked PMAA and contain amino groups provided by the cross-linker. The capsules exhibit highly reversible swelling at acidic and basic pH values due to excess positive and negative charges in the capsule wall and are of minimum size at pH 5.5. Apart from covalent cross-linking, the hydrogel capsule wall is also stitched through ionic pairing in a wide interval of pH. At pH 5−6, where carboxylic acid groups are protonated, the capsule wall expands (and hydrogel capsules increase in size) in salt solutions as a result of dissociation of ionic cross-links. In contrast, at pH $>$ 6 and pH $<$ 5, where hydrogel wall acquires charge, salt results in the hydrogel contraction and in a decrease of capsule size. In addition to pH and ionic strength, capsule swelling can be controlled by the number of cross-links achieved through variation of cross-linking time. We also show that these soft hydrogel capsules easily flatten when in contact with a solid surface and that the degree of flattening is governed by the surface and capsule charge and the degree of cross-linking in the capsule wall. Finally, we demonstrate that this pH- and ionic-strength-dependent swelling of hydrogel capsules affords multiple routes for encapsulation and controlled release of macromolecules.

Introduction

Polymeric capsules produced with a layer-by-layer (LbL) deposition technique have recently attracted a great deal of research attention. The attractive features of such capsules include (1) the ease of control of the capsule layer thickness by simple variation of a number of deposition cycles, (2) the possibility of including macromolecules of various chemical nature within the capsule wall, and (3) the availability of internal volume within such capsules which can be solid or filled with solvent and serve as a depot for delivery of chemicals or as a microcompartment for chemical reactions or inclusion of catalysts.[1] Earlier work on layered polymer capsules has been using an advantage of electrostatic self-assembly in aqueous environment[1−3] or hydrogen-bonding-driven polymeric self-assembly in nonaqueous media.[4−7] Our group has introduced capsules that are based on hydrogen-bonded self-assembly in water.[8] As-produced, such capsules disintegrate upon an increase in environmental pH, affording a useful pH-triggering mechanism for controlled delivery applications. However, other potential biomedical or biosensing applications of these materials require their stability in a wide pH range. Cross-linking of hydrogen-bonded capsules through carbodiimine chemistry has been earlier demonstrated by our group.[9,10] Produced hollow capsules with hydrogel-like cross-linked walls undergo reversible pH-triggered changes in size and allow pH-controlled encapsulation and release of macromolecules through well-defined changes in mesh size of the hydrogel capsule wall.

There exist several other ways to produce environmentally responsive polymer-based nano- and/or microshells. The presence of anionic (such as carboxylic) or cationic (amino) groups in the polymer chain is responsible for pH sensitivity of the produced capsules. pH-responsive hollow spheres were produced, for example, based on a block copolymer approach[11] or synthesized by vesicular or emulsion polymerization using core−shell latexes.[12] Recently, a “block copolymer free” approach has been used to prepare hollow capsules by shell cross-linking and core dissolution of homopolymer/homopolymer micelles, which were originally produced from kinetic trapping of noncovalently associating polymer chains into core−shell structures. With poly(acrylic acid) as a shell component, produced hollow spheres exhibited pH- and ionic-strength-triggered size changes.[13] In another recent work, surface-initiated atom-transfer radical polymerization using gold nanocrystals followed by selective chemical cross-linking of a polymer shell was used to produce pH-sensitive nanocapsules in a range from 40 to 300 nm.[14] In the latter case, the authors have performed homopolymerization of 2-(dimethylamino)ethyl methacrylate or its copolymerization with poly(ethylene glycol) methyl ether methacrylate to yield capsules that swell at acidic pH values.

The LbL approach potentially affords a larger degree of control over functional properties of polyelectrolyte containers, such as their permeability to low molecular weight compounds or macromolecules. For example, Sukhorukov et al. and Antipov et al. have demonstrated that permeability of LbL electrostatically assembled capsules to low molecular weight compounds/dyes can be modulated by changing the chemical composition or the number of polymer layers deposited in the wall.[15,16] Using electrostatic self-assembly that involves such weak polyelectrolytes as poly(allylamine) hydrochloride (PAH) and poly(styrenesulfonate) (PSS), Antipov et al. described pH-responsive permeability of LbL capsules.[17] While capsule size remained invariable in the pH range from 3 to 10, reversible pH-triggered permeability changes of the capsule wall were observed. Specifically, the capsule wall was in an “open state” at pH $<$ 6 and in a “closed state” at pH $>$ 8.[18] The “open state” in the capsule permeability correlated with the formation of defects in the capsule wall which resulted from accumulation of excess of charges upon pH variations. The reversible nature of permeability was explained by healing of the capsule defects when pH was reversed. Still reversible size change of electro-

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statically assembled LbL capsules could be observed at extreme pH values (close to capsule dissolution), when intermolecular bindings between the capsule wall components were drastically weakened. Specifically, PSS/PAH and PMAA/PAH capsules significantly expanded when exposed to extreme pH conditions of 11.2 and 2.8, respectively.[19,20] The latter extreme pH values impose certain limitations on the use of the capsule expansion for encapsulation and release. An interesting approach toward pH-controlled release using LbL capsules was recently described by Lvov and co-workers.[21] These authors performed self-assembly of tannic acid with polycations and produced capsules with pH-dependent permeability of molecules (lowest permeability at pH 5−7) controlled by pH-triggered dissolution of the capsule wall.

LbL-deposited films can be stabilized to variations of pH and solvent composition through cross-linking of polymer components. Photo-cross-linking has been applied to electrostatically assembled LbL capsules to reduce permeability of the capsule wall.[22,23] In PSS/PAH systems, chemical cross-linking of the PAH component of the film with glutaraldehyde significantly improved mechanical stability of LbL capsules as well as lowered permeability of the PSS/PAH film to oligomeric dextran.[24] In the case of hydrogen-bonded LbL films involving poly(carboxylic acid)s, cross-linking results in hydrogel-like films which are stabilized at neutral and basic pH exclusively by covalent cross-links which can be introduced within the film via chemical reaction,[7,25] exposure to high temperature, or electromagnetic radiation.[26] We have applied carbodiimide chemistry to covalently cross-link PMAA within PMAA/PVPON or PMAA/poly(ethylene oxide) (PEO) hydrogen-bonded films or capsules,[8−10]producing either one-component PMAA hydrogels or two-component films.[10] In the latter case, PEO or PVPON was end-functionalized to allow chemical cross-linking. Caruso and co-workers have recently reported that when disulfide links are used for cross-linking of the PMAA/PVPON system, a chemical trigger—a disulfide exchange reagent—can be used to trigger release of a model drug.[27] Chemically cross-linked two-component poly(acrylamide)/poly(acrylic acid) hydrogen-bonded films and capsules have shown much promise for biomedical applications as they can be used as a medium for synthesis of silver nanoparticles[28]and have been demonstrated to exhibit resistance to cell adhesion.[29]

In this paper, we show that cross-linking of PMAA-containing hydrogen-bonded capsules with ethylenediamine using carbodiimide as an activating agent produces amphoteric pH-responsive hydrogel capsules with minimal size at pH 5.5 which show large swelling at both lower and higher pH values. The pH- and ionic-strength-induced capsule size changes are highly reversible and can be used as a robust route to entrap and release macromolecules at pH values close to neutral through several routes of pH and/or ionic strength variation. Finally, we show that these soft capsules can be flattened at a solid surface in response to capsule and surface charge.

Experimental Section

Materials. Poly(methacrylic acid) (PMAA; M_w 150 kDa), poly(*N*-vinylpyrrolidone) (PVPON; M_w 55 kDa), 1 M hydrochloric acid (HCl), 0.1 M sodium hydroxide (NaOH), sodium chloride (NaCl), dibasic and monobasic sodium phosphate, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), and *N*-hydroxysulfosuccinimide sodium salt (NSS), ethylenediamine (EDA), and fluorescein isothiocyanate (FITC) labeled dextrans with molecular weight of 500, 77, and 4.4 kDa as well as poly(allylamine) hydrochloride (PAH) were purchased from Sigma-Aldrich. All chemicals were used without any further purification. KBr powder of spectroscopic grade was obtained from International Crystal Labs. The SiO_2 template particles were purchased from Polysciences Inc. as 10% dispersions in water and were of 4.0 ± 0.2 μm diameter. 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.

Confocal Laser Scanning Microscopy (CLSM). Confocal images of capsules were obtained with an LSM 5 PASCAL laser scanning microscope (Zeiss, Germany) equipped with C-Apochromat 63×/1.2 water immersion objective. Capsules were visualized through deposition of the FITC-labeled PMAA within the last three bilayers of PVPON/PMAA capsules. 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 or ionic strength 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 ionic strength and then mixed with FITC−dextrans (1 mg/mL) at the same pH values adjusted with 0.1 M NaOH. CLSM images of the capsules were taken after 20 min. In pH- and salt-size-dependent measurements each value was averaged from 20 individual capsules. CLSM settings during entrapment and release measurements were kept the same.

ζ-Potential Measurements. The ζ-potentials of non-cross-linked hydrogen-bonded $(PVPON/PMAA)_6$ and 16 h cross-linked $(PMAA)_7$ capsules were measured at different pHs by Zetasizer Nano-ZS equipment (Malvern). Each value was obtained by averaging three parallel measurements.

Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR spectra were recorded using PARAGON 1000 PC FT-IR spectrometer (Perkin-Elmer). For FT-IR analysis of PAH, KBr pellets containing the polycation were prepared. Cross-linked (PMAA) capsules were freeze-dried from deionized water whose pH was maintained at 10 or 4.4 using 0.1 M NaOH or 1 M HCl. The freeze-dried capsules were then mixed with KBr, and their spectra were collected.

Fluorescently Tagged PMAA. The labeling of PMAA was performed as described elsewhere.[9] Specifically, PMAA (M_w 150 000) was dissolved in 0.1 M phosphate buffer solution, and the solution pH was adjusted to 5.0 with 0.1 M NaOH solution. The polymer solution was mixed with EDC and NSS solutions at the same pH, 5 mg/mL each, and stirred for 30 min. The activation step was followed by adding of fluoresceinyl−ethylenediamine solution at pH = 6 with constant stirring for 10 h in darkness. Fluoresceinyl−ethylenediamine was synthesized as described previously.[30] The labeled PMAA was exhaustively dialyzed against phosphate buffer (pH 7, 0.1 M) and then pure deionized water for at least 7 days. The molecular weight cutoff of dialysis tubing was 25 000. The dialysis was interrupted after no traces of fluorescence could be detected in the dialysis water. The dialyzed polymer solution was lyophilized, and 0.2 mg/mL solutions of labeled PMAA at pH = 2 were prepared and used for capsule preparation.

Preparation of (PMAA) Hydrogel Capsules. Deposition of hydrogen-bonded multilayers of PVPON/PMAA on particulate substrates has been described previously.[9] Briefly, 0.2 mg/mL polymer solutions were used with a typical deposition time of 15 min. Hydrogen-bonded multilayers were deposited directly onto silica microparticles at pH 2 starting from PVPON. Each deposition cycle was followed by washing three times with a water solution with pH adjusted to 2. Suspensions were settled down by centrifugation at 1200 rpm for 1 min to remove the supernatant. Deposition, washing, and redispersion steps were performed in a shaker (Fisher Scientific) at 1600 rpm. To make the capsules visible in CLSM, the labeled PMAA was deposited in the last three bilayers. When a desired number of layers was deposited, cross-linking was performed as described earlier,[9] where the detailed procedure included activation of the carboxylic groups with 5 mg/mL solution of EDC and NSS at pH 5 followed by reaction with

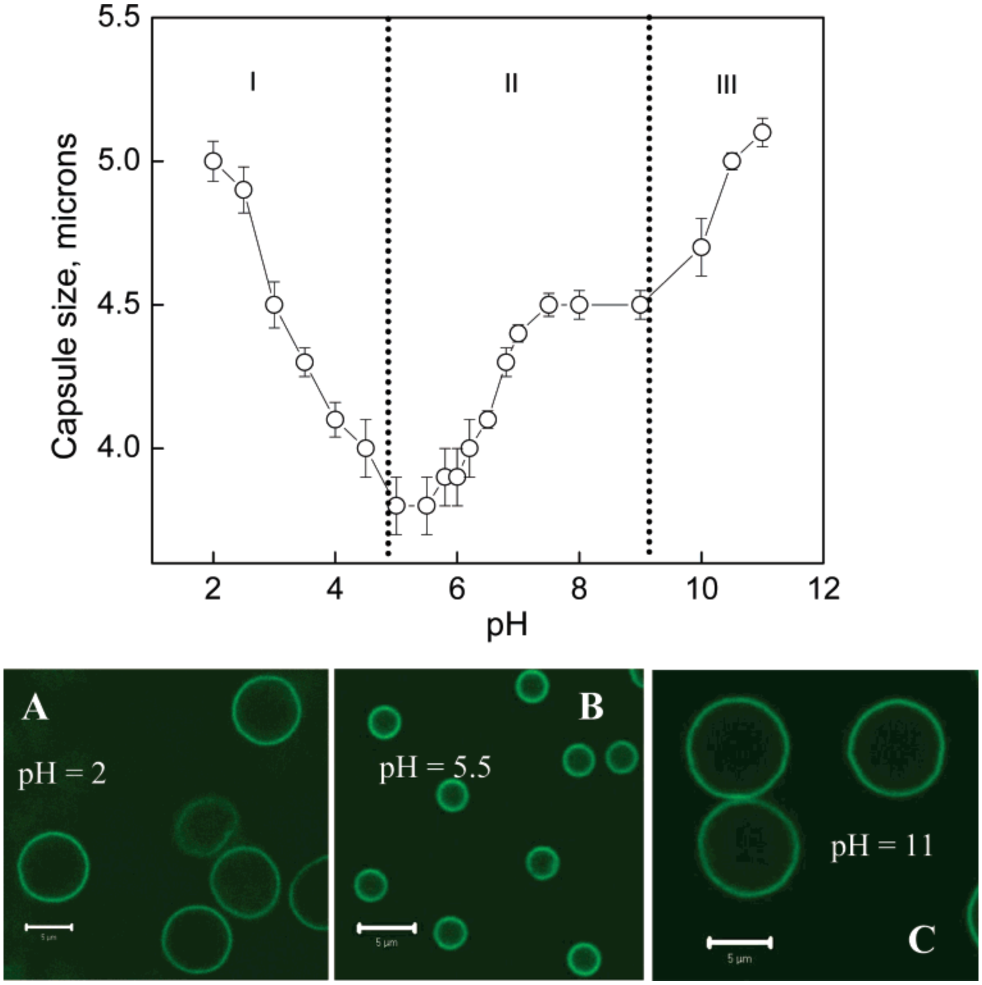

Figure 1. pH dependence of the diameter of the (PMAA)$_7$ capsules cross-linked for 22 h. CLSM images show the (PMAA)$_7$ capsules at pH = 2 (A), pH = 5.5 (B), and pH = 11 (C). These pH values were established by 0.01 M phosphate buffers. The scale bar is 5 μm for all images.

0.5 mg/mL solution of EDA at pH 5.8 for different periods of time. After several washings of the particle suspension in phosphate buffer at pH = 4.6, the cores were dissolved by shaking the particle dispersion for 4 h in 8% aqueous HF solution, yielding hollow capsules. After that, the dispersion of capsules with cross-linked PMAA walls was dialyzed in a buffer at pH 8.0 to ensure removal of PVPON. Capsules were then transferred to pH 4.6 by dialysis against the phosphate buffer solution for 4 h.

Preparation of (PMAA) Capsules for CLSM. For CLSM studies of cross-linked (PMAA) capsules, capsules were fixed at the surface of Lab-Tek chambered coverglass either bare or PEI-treated. The procedure included the addition of a drop of a dispersion of hollow (PMAA)$_7$ capsules to the chamber surface. The chambers of the Lab-Tek cover-glass were then sequentially filled with buffer solutions at a certain pH and ionic strength. Capsules were allowed to settle down for 5 h and then analyzed.

Results and Discussion

Amphoteric (PMAA) Hydrogel Capsules: Low Ionic Strength Studies. In our previous paper,[9] we demonstrated that 95% of PVPON with M_w 55 000 was already released from the (PVPON/PMAA)$_7$ capsule walls during cross-linking, and complete removal of PVPON was observed after exposure of cross-linked multilayers to basic pH values. Importantly, even for strongly cross-linked films (produced by 24 h cross-linking), the neutral component was able to completely leave cross-linked polymer network after the films were exposed to pH = 8.[9] We also observed that after (PMAA)$_7$ capsules were returned to pH 4.6 their size decreased from the PVPON/PMAA capsule and silica core template diameter of 4 μm to lower values. The decrease is explained by the entropically favorable coiling of PMAA chains between cross-link points.[9] The size of recently reported one-component chitosan cross-linked capsules did not differ significantly from initial chitosan/PAA capsules.[35] This difference can be rationalized by considering the intrinsically high flexibility of PMAA chains compared to that of chitosan which contain rigid glucose rings along the polymer backbone. We also showed that PMAA hollow hydrogel capsules reversibly increased their size when exposed to pH from 4.6 to 8.

Figure 1 illustrates (PMAA)$_7$ capsule swelling in a wider pH region from 2 to 12, which was not covered in our previous studies. Three distinct regions in swelling/deswelling behavior of capsules are clearly seen. Swelling in region II (from pH 5 to 8) has been previously reported and is explained by increased ionization of PMAA carboxylic groups (reported pK_a of PMAA range from 6 to 7[31]). However, unusual swelling behavior is observed in regions I ($pH < 4.5$) and III ($pH > 9$). These results can be rationalized through the presence of basic groups supplied from the EDA cross-linker used for preparation of the hydrogel capsules. Specifically, cross-linking with EDA occurs in the multilayer environment where mobility of polymer chains is significantly restricted due to extensive interchain association. As a result of such restriction, after one amino group of EDA reacted with a PMAA carboxylic group, covalent attachment of the second amino group to activated carboxylic groups within multilayers might be sterically restricted. However, after completion of cross-linking reaction and release of PVPON,

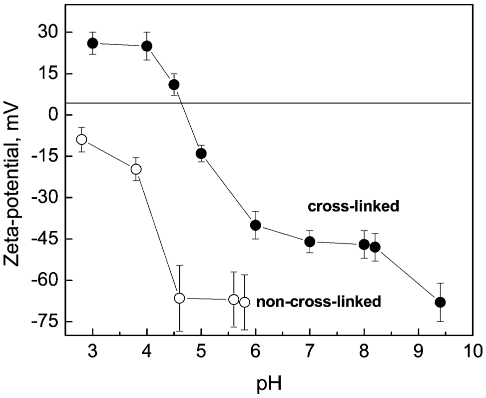

Figure 2. ζ-potentials of non-cross-linked hydrogen-bonded (PVPON/PMAA)$_6$ (open circles) and 16 h cross-linked (PMAA)$_7$ capsules (filled circles) as a function of pH. The pH values of aqueous capsule suspensions were adjusted using HCl or NaOH solutions.

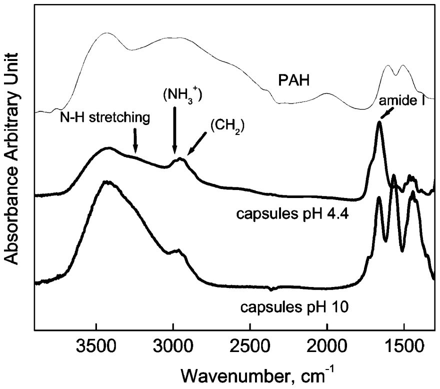

Figure 3. FT-IR spectra of cross-linked (PMAA) capsules freeze-dried from aqueous solutions at pH 4.4 and 10 and pressed within KBr pellets. The PAH spectrum is shown for comparison. Spectra are overlaid with arbitrary offset for clarity.

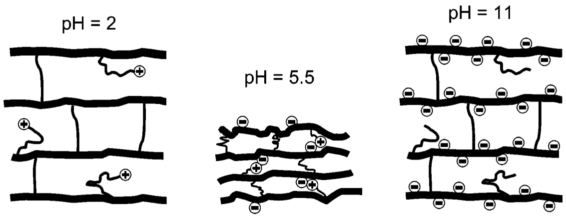

Figure 4. Schematic representation of the hydrogel capsule wall in different pH regions.

ionic pairs can be later formed within (PMAA) hydrogel through interaction of amino groups with carboxylic groups.

To prove the presence of positive charges provided by primary amino groups within (PMAA) hydrogel capsule wall, the ζ-potential of both cross-linked and non-cross-linked capsules was measured. The ζ-potential of non-cross-linked hydrogen-bonded (PVPON/PMAA) and (PMAA) capsules derived from the former ones by their cross-linking with EDA was investigated at different pH. Figure 2 shows that for non-cross-linked capsules the ζ-potential is negative over the pH range from 2.8 to 5.8. Previously, it has been reported that for hydrogen-bonded layers of poly(acrylic acid)/(poly(acrylamide),[25] or (PVPON/PMAA) and (PEO/PMAA)[32] deposited on colloidal particles, the ζ-potential remained negative in a wide pH range and did not show charge reversal, since surface charge in these systems is provided exclusively by self-assembled polyacids. In drastic contrast, with cross-linked hydrogel (PMAA) capsules described here, a decrease in pH results in switching of capsule charge from negative to positive. Note that the amide groups supplied by covalent cross-linking of EDA with carboxylic groups have low basicity (pK_b ~ -0.5) and do not carry positive charge in the studied pH range. Instead, positive charge originating from primary amino groups of the one-end-reacted cross-linker molecules is responsible for the observed pH-induced charge reversal. Insterestingly, at pH $>$ 9, where additonal capsule expansion occurred (Figure 1), the ζ-potential is also acquired more negative values (Figure 2). These facts are consistent with deprotonation of primary amino groups and resultant dissociation of ionic cross-links in this pH range.

Figure 3 displays the FT-IR spectra of (PMAA) cross-linked capsules freeze-dried from pH 4.4 and 10 and pressed within KBr pellets. In both spectra, there is a distinct peak centered at 1660 cm $^{-1}$ which is associated with amide I vibrations. Another peak characteristic for N−H stretching in amides is centered at 3250 cm $^{-1}$ and is present in spectra of the cross-linked capsules dried at both pH values. In the alkaline regime at pH 10, all the carboxylic groups are ionized as represented by the COO$^-$ peak at 1565 cm $^{-1}$. With decreasing solution pH, carboxylic groups are transferred into the protonated form. This results in a drastic decrease in intensity of the COO$^-$ peak and appearance of a broad peak centered at ~1700 cm^{-1} assigned to COOH groups which largely overlapped with the amide I band. A small peak at 1723 cm $^{-1}$ present in the spectra of capsules at both pH values is attributed to the C═O stretching of the ester groups remaining after the activation step of PMAA.[9] Since primary amine groups have pK_b of ~10, NH_3^+ vibrational bands are present at both pH of 4.4 and 10. Specifically, the absorption peak at 3000 cm^{-1} (located next to the asymmetric CH_2 stretching band at 2920 cm^{-1}) is attributed to NH_3^+ asymmetric stretching vibrations.[33]

The existence of the ionic cross-links was also confirmed by the fact that the addition of sodium chloride results in expansion of capsules at pH 5.5 where carboxylic groups of PMAA network do not carry charge (this point will be discussed below in the section "Effect of Ionic Strength on Capsule Size").

Suggested hydrogel capsule wall structure in different pH regions is depicted in Figure 4. The cross-linked PMAA multilayers contain both two- and one-end-reacted cross-linker molecules. The two-end-reacted EDA molecules provide permanent covalent cross-links with the hydrogel wall. The one-end-attached EDA molecules afford additional ionic cross-linking of the hydrogel through electrostatic pairing between $-COO^-$ groups of PMAA network and $-NH_3^+$ groups of one-end-attached EDA molecules. The most compact structure corresponds to the minimum capsule size observed at pH 5.5. The lowest swelling degree of capsule walls occurred at pH ~ 5.5 (see Figures 1 and 4) where both types of cross-links were formed (region II in Figure 1). Under these conditions, the overall number of cross-links, covalent and ionic, can be estimated[9] through swelling of surface-attached hydrogels using the Brannon−Peppas−Peppas equation. Reversible swelling/deswelling of capsules in this pH range is due to available carboxylic groups which do not participate in ionic cross-linking with NH_3^+ groups. Additional swelling at acidic pH (region I) is a consequence of disruption of ionic cross-links due to increased protonation of carboxylate ions included in $-COO^-$ NH_3^+ cross-links. Considering that pK_b value for protonation of primary amino groups is ~10[34], such a process results in accumulation of excess positive charges within the polymer network, which additionally favors expansion of the capsule wall. Respectively, deprotonation of $-NH_3^+$ groups at basic values of pH additionally results in dissociation of ionic cross-

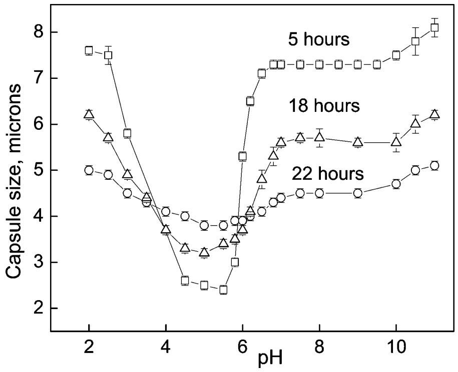

Figure 5. pH dependence of the diameter of the $(PMAA)_7$ capsules cross-linked for 5, 18, and 22 h (squares, triangles, and circles, respectively).

links and gel expansion (region III in Figure 1). Interestingly, because of significant difference in pK_a of carboxylic groups and pK_b of primary amino groups, ionic swelling of hydrogel capsules in region II and further loosening of the capsule structure at pH > 9 due to dissociation of ionic cross-links are well separated on the pH scale.

Figure 5 demonstrates that that capsules cross-linked for 5, 18, and 22 h display similar swelling regimes, while the degree of swelling drastically decreases with increasing cross-linking time, indicating increased number of cross-links within the capsule wall. This behavior is typical to slab hydrogels. In the case of hollow hydrogel capsules derived from electrostatically assembled multilayers, Zhang et al. have also recently demonstrated similar effect of cross-linking density on capsule size.[35] Interestingly, sharpness of ionic swelling at pH ~ 5.8−6 is also strongly affected by cross-linking time. After 14 h of cross-linking time, capsules are lightly cross-linked and contain ~11 monomer units between cross-links.[9] Swelling of such capsules is highly cooperative and occurs in a very narrow pH range from 5.8 to 6.0. At longer cross-linking time, when the length of polymer chains between cross-links became shorter, cooperativity of the swelling transition deteriorated, and the pH range of the transition was significantly widened (see Figure 5).

Effect of Ionic Strength on Capsule Size. Further evidence of the existence of the two types of cross-links within the hydrogel wall was obtained in the experiments in which the (PMAA) capsule size was studied as a function of salt concentration. The results of such experiments performed in three different capsule swelling regimes (pH 2, 5.5, and 8 for regimes I, II, and III, respectively) are shown in Figure 6. As one can expect, capsule diameter decreased in salt solutions at pH 8 and 2 due to screening of electrostatic charges within the hydrogel. The charge is provided to the hydrogel network by $-COO^-$ groups of PMAA at pH 8 and by $-NH_3{}^+$ groups of the hydrogel-attached nonreacted ends of EDA at pH 2. Interestingly, the addition of salt to the capsules at pH 5.5 resulted in the opposite effect, namely, in an increase of capsule size. Sharp capsule expansion occurred at relatively low salt concentration of 0.04 M NaCl, and further increase in size was small. This behavior reflects disruption of $-COO^-/-NH_3{}^+$ ionic cross-links within the hydrogel as a result of competition with salt ions. Figure 6 shows that at higher salt concentrations (>1 M), where ionic interactions are strongly screened, capsule size reaches ~4.7 μm at pH 5.5 and 8 and ~4.1 μm at pH 2. At each pH value, hydrogels exposed to concentrated salt solutions do not contain ionic cross-links but are exclusively stabilized through permanent covalent cross-linking.

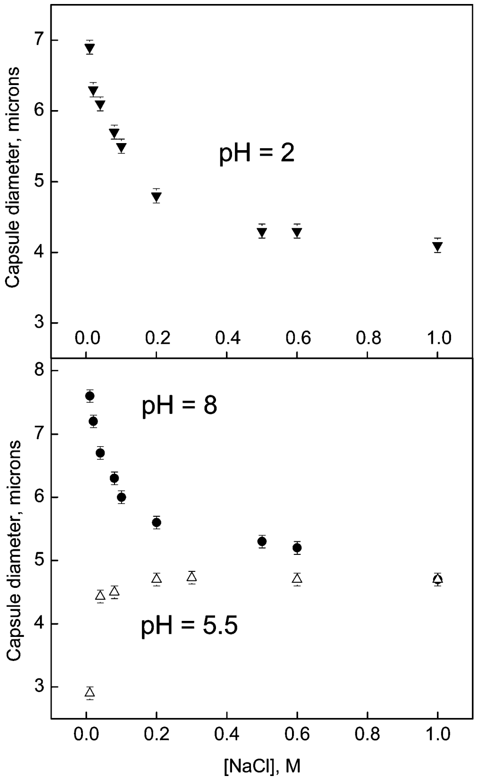

Figure 6. Evolution of $(PMAA)_7$ capsule size with increased concentration of sodium chloride at pH = 2 (top panel, filled triangles) and at pH = 8 and pH = 5.5 (bottom panel, filled circles and open triangles, respectively). Salt concentration of 10^{-3} M was established by phosphate buffer. Higher concentrations were achieved through addition of NaCl. Cross-linking time was 16 h.

The effect of salts on hydrogel-like capsule walls is drastically different from that observed for electrostatically assembled capsules whose walls contain oppositely charged polyelectrolyte chains stabilized through sequences of multiple ionic interactions. In the case of PSS/PAH capsules, softening of the capsule wall at high concentrations of NaCl (up to 3 M NaCl) was observed due to decrease in the number of polymer/polymer ionic contacts due to competition with salt,[36,37] which was accompanied by slight swelling of the multilayer film.[37] At even higher salt concentrations, competition with salt resulted in "melting" of initially highly rigid glassy multilayer wall and shrinking of PSS/PAH capsules.[38]

Capsules in Contact with a Solid Surface: pH and Ionic Strength Effects on Capsule Flattening. All hydrogel capsules remained spherically shaped unless they were in immediate contact with a solid surface (cleaned glass coverslip of the Lab-Tek chamber used in CLSM studies). When hydrogel capsules contacted a solid surface, they could either flatten, acquiring a pancake shape, or remain round. Specifically, we have observed that highly cross-linked $(PMAA)_7$ capsules retained their spherical shape at pH = 5.5 (image not shown) but were completely flattened at pH 2 when contacting bare glass (Figure 7, panel A). Panel B in Figure 7 shows that capsule flattening at this pH value was completely reversed when the glass surface was precoated with a polycation, PEI. It is important to note that flattening was never observed for capsules which were not in direct contact with a surface, for example, those which precipitated on top of the first "layer" of surface-adhered capsules. This fact suggests that this flattening is strongly induced by interaction of the capsule walls with the surface.

Flattening of capsules at pH 2 can be rationalized through combination of electrostatic attraction between positive charges

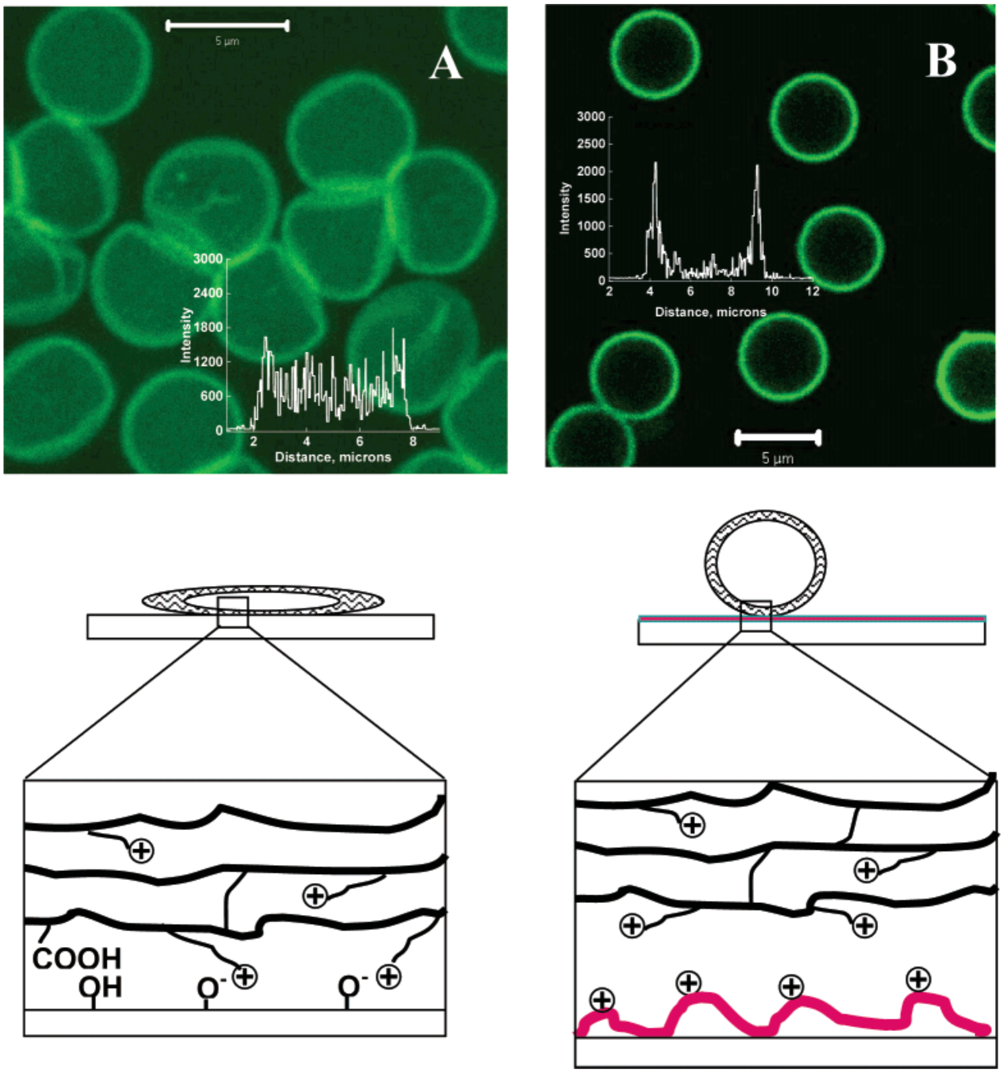

Figure 7. CLSM images of 22 h cross-linked $(PMAA)_7$ capsules at pH = 2 adsorbed on bare glass (A) and on PEI-coated glass (B) surfaces. The scale bar on both images is 5 μm. Below are schematics showing interaction of surface and capsule wall at pH = 2.

in the capsule wall and slightly negatively charged silica surface as well as hydrogen-bonding interaction of the protonated carboxylic groups in the capsule wall with hydroxyl groups at the bare glass surface (pK_0 and pK_a of surface silanol groups are 2−3 and 9.1−9.4, respectively[39]). Priming the surface with positively charged polymer introduces repulsive interactions between the surface and the capsule wall positively charged at pH 2 and completely suppressed flattening. Similar modulation of capsule adhesion at the surface was observed at basic pH values. Figure 8 contrasts CLSM images of the $(PMAA)_7$ capsules cross-linked for 22 h and allowed to precipitate on bare or PEI-treated glass surfaces at pH = 10. Capsules adsorbed on the bare glass surface keep their spherical shape due to mutual repulsions between the negatively charged glass surfaces and negatively charged capsule wall. However, pancake-shaped capsules were observed at the surface at this pH value in the case when glass surfaces were pretreated with PEI. Regulation of surface adhesion of capsules by switching between electrostatic attraction/repulsion of the surface and the capsule wall has been previously reported for electrostatically assembled rigid capsules.[40,41] It has been found that anionic PAH/PSS-terminated capsules do not adhere to bare glass but strongly bind to a PEI-coated surface.[42] However, because of much higher rigidity of PAH/PSS capsules, switching to adhesion never resulted in complete flattening of PAH/PSS capsules at surfaces. However, PAH/PSS capsules slightly deformed in contact with the surface to form an adhesion area. Flattening of hydrogel capsules in contact with adhesive surfaces is equivalent to formation of a very large adhesion area which equals the capsule diameter. The high propensity of (PMAA) capsules to such large deformations results from low rigidity of the hydrogel capsule wall.

It should be noted that ionic strength could also be used to modulate capsule adhesion to the surface. At low ionic strength (0.01 M NaCl), PMAA capsules remained spherical in contact with bare glass surface at pH 5.5 and 8 but were flattened at pH 2 when the capsule wall and the surface were of opposite charge. However, an increase of salt concentration to 0.04 M NaCl triggered flattening of surface-residing capsules at pH 5.5 and 8. Under these conditions, the adhesion argument cannot be used because of the same sign of charge in the capsule wall and the surface. Instead, salt-assisted surface deformation of capsules at pH 5.5 and 8 indicated softening of the hydrogel walls due to disruption of ionic cross-links within the hydrogel as well as decreased rigidity of the hydrogel network due to screening of hydrogel charges at pH 8.

Encapsulation and Release of Macromolecules: Multiple pH and Ionic Strength Paths. The permeability of the hydrogel capsule wall is determined by cross-linking degree and, consequently, by mesh size of polymer networks. In our previous

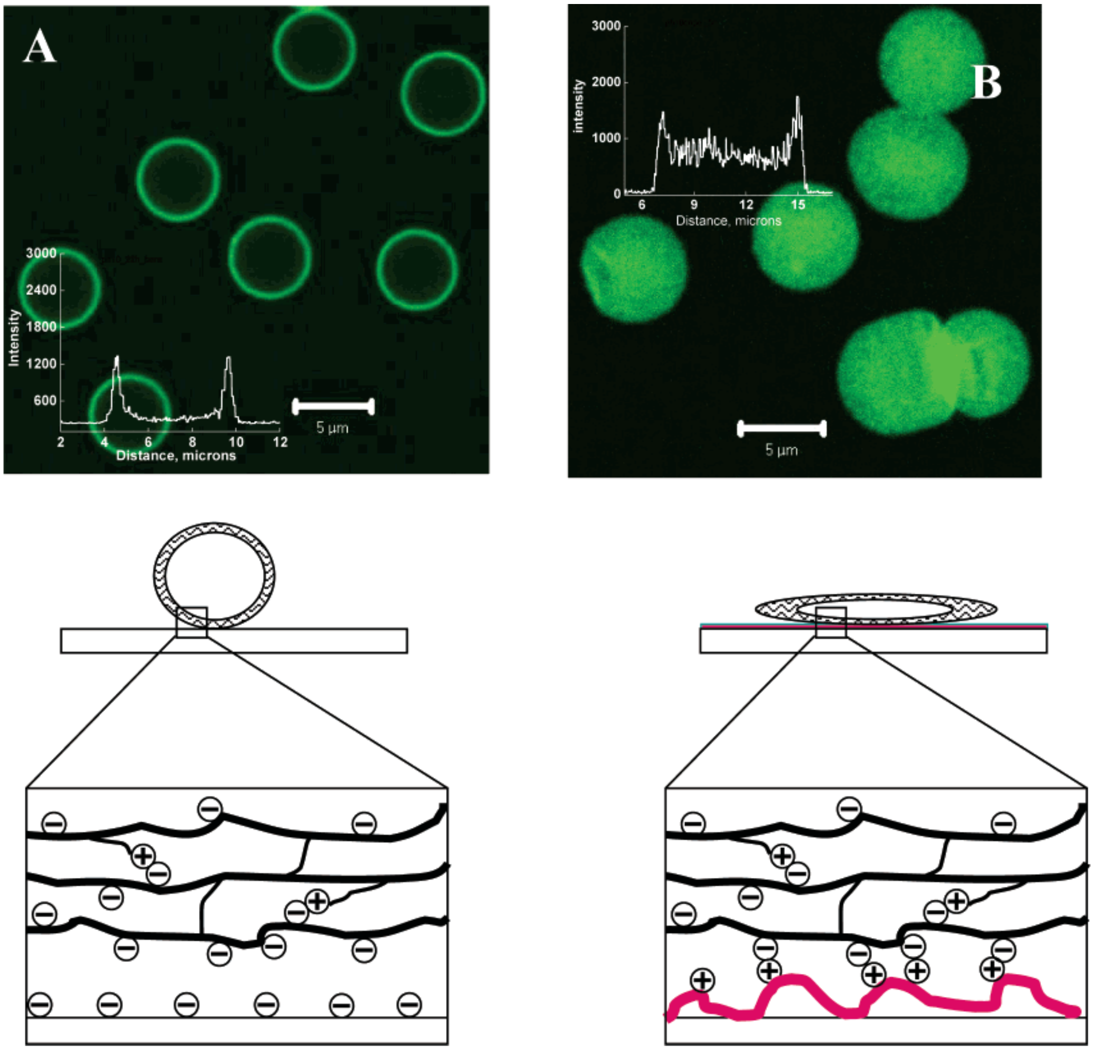

Figure 8. CLSM images of 22 h cross-linked $(PMAA)_7$ capsules at pH = 10 adsorbed on bare glass (A) and on PEI-coated glass (B) surfaces. The scale bar on both images is 5 μm. Below are schematics showing interactions of surface and capsule wall at pH = 10.

work, we found that (PMAA) capsules were highly permeable for macromolecules at pH higher than 7 when the hydrogel wall was highly swollen.[9] Here, we show that loading of macromolecules within hydrogel hollow capsules can be promoted in both high (pH > 7) and low (pH < 4) pH ranges and that ionic strength can be used as an effective means to release encapsulated molecules at neutral pH values. First, the effect of cross-linking density on capsule permeability was evident. At pH ~ 8 in 0.01 M buffer solutions, the 5 h cross-linked (PMAA) capsules were readily permeable to FITC−dextrans of all studied molecular weights (4, 70, and 500 kDa). However, an increase in cross-linking density afforded selective permeability of macromolecules with various molecular weights through the hydrogel wall. Specifically, the 16 h cross-linked capsules remained permeable to FITC−dextrans with M_w 70 kDa (Figure 9, panel A) at pH 8 but remained impermeable for FITC−dextran with M_w of 500 kDa at both pH 8 (Figure 9, panel B) and pH 2 (not shown). Such selective permeability suggests that cross-linking of the capsule wall for 16 h resulted in a decrease of the mesh size from that observed with 5 h cross-linked capsules (110 Å at pH 8 and low ionic strength[9]) so that the capsule wall could efficiently retain 500 kDa FITC−dextran whose hydrodynamic radius (R_h) is 160 Å.[43] At the same time, the mesh size remained large enough to allow 70 kDa FITC−dextran molecule with R_h ~ 80 Å[44] to permeate through the capsule wall.

Importantly, 16 h cross-linked capsules remained in the "open state" for the 70 kDa FITC−dextran in its highly swollen state at both high and low pH values (pH 8 and 2) but turned impermeable to this macromolecule at pH 5.5 where capsule wall expansion and capsule size were the smallest. The pH-controlled selective permeability could be used for loading of macromolecules within hollow capsules. Specifically, when the $(PMAA)_7$ capsules were exposed to 1 mg/mL 70 kDa FITC−dextran solution at pH 8 or 2, loading of dextran molecules within the hollow capsule core occurred after 30 and 15 min, respectively, and loaded materials remained "trapped" within the capsule after capsules were transferred to 0.01 M buffer at pH 5.5 As seen from the CLSM images in Figure 10A, the capsules are impermeable for the FITC−dextran molecules. The capsule interior remained dark at least for 24 h, suggesting that permeability of the capsule to 70 kDa FITC−dextran became negligible under these conditions.

Though loading of capsules with macromolecules could be achieved at both basic and acidic pH, loading capacity was ~2-fold larger at pH 2. This comparison was based on CLSM fluorescence intensities of capsules loaded at acidic or basic pH values after they were brought to the same pH = 5.5 and 0.01 M salt. This difference is explained by the fact that FITC−dextran acquires slight negative charges at high pH due to negative FITC moieties along dextran chains but remains neutral at low pH values.[45,45] The negative charges within the (PMAA)

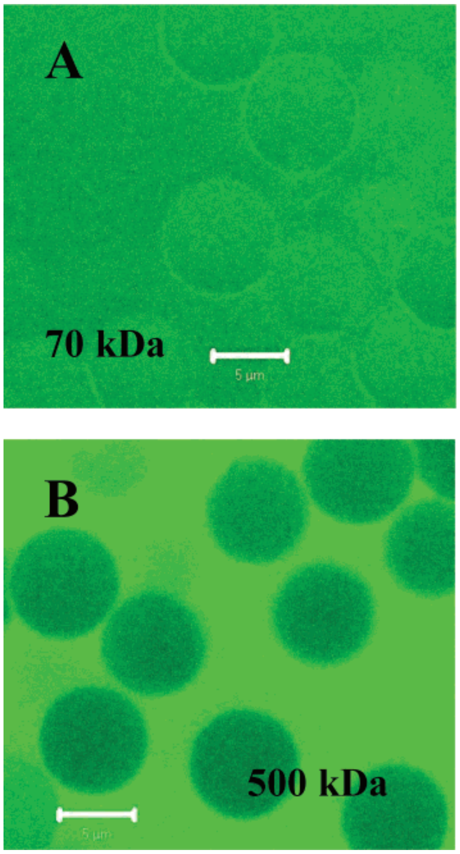

Figure 9. CLSM images of $(PMAA)_7$ capsules cross-linked for 16 h and incubated in 1 mg/mL FITC−dextran solutions of different molecular weights at pH = 8 (0.01 M phosphate buffer): 70 kDa for 3 min (A) or 500 kDa for 30 min (B). Scale bar on both images is 5 μm.

hydrogel wall limit permeability of FITC−dextran molecules at pH 8 due to repulsive interactions between the $-COO^-$ groups and negative FITC moieties along dextran chains at this pH. In contrast, permeability of neutral dextran molecules through the capsule wall at pH 2 is not restricted electrostatically and affords high capsule loading capacity. Similar charge-controlled loading was shown for PSS/PAH capsules containing free PSS chains inside the capsules.[46] Though such capsules rejected negatively charged FITC−dextran of 70 kDa and even small FITC molecules at pH 7 and low salt, they attracted positively charged species such as albumin at pH 2 when the protein was positively charged.

Encapsulated macromolecules could be released from the capsules at the same pH value of 5.5 in response to increased salt concentration. Specifically, addition of 0.2 M NaCl solution to 0.01 M phosphate buffer at pH 5.5 efficiently expands and “unlocks” the capsule wall via ionic-strength-triggered disruption of ionic cross-links. The CLSM image of Figure 10B shows that 70 kDa FITC−dextran was able to permeate through the capsule wall after the capsules were exposed to 0.2 M salt solution at the same pH 5.5. Permeation correlated with salt-assisted dissociation of ($-COO^-$ NH_3^+) pairing which resulted in capsule size increase from 3.5 to 4.7 μm. The latter value is consistent with ~4.7 μm—the capsule size observed for unloaded $(PMAA)_7$ capsules exposed to 0.2 M NaCl solution at pH 5.5 (see Figure 6).

Conclusions

We have demonstrated that amphoteric pH- and ionic-strength-responsive hydrogel capsules of a weak polyacid can be produced through cross-linking of hydrogen-bonded multilayers with ethylenediamine. These capsules respond to changes in environmental pH and ionic strength in a highly reversible way by large variations in capsule size. The hydrogel capsules are soft and easily flatten in contact with a solid surface, especially when there are electrostatic or hydrogen-bonding interactions between the capsule wall and the surface. The capsules are useful for entrapping and storing of macromolecules by pH-controlled loading of the macromolecules at basic or acidic pH with subsequent capsule “closing” at pH 5.5. The release of the encapsulated macromolecules was achieved at the same pH using high salt concentrations. The produced capsule containers allow selective control of permeation of macromolecules based on their molecular weight and show exceptional stability in a wide pH range and highly reversible swelling and permeability transitions as a function of pH and ionic strength. The possibility to adjust the mesh size of hydrogel capsule walls in a wide range by variation of cross-linking density will allow production of hydrogel containers which exhibit selective trapping and release of macromolecules based on their molecular size. The pH- and ionic-strength-responsive (PMAA) hydrogel containers show promise in applications for controlled delivery of functional macromolecules which might find use in biomedicine and biotechnology.

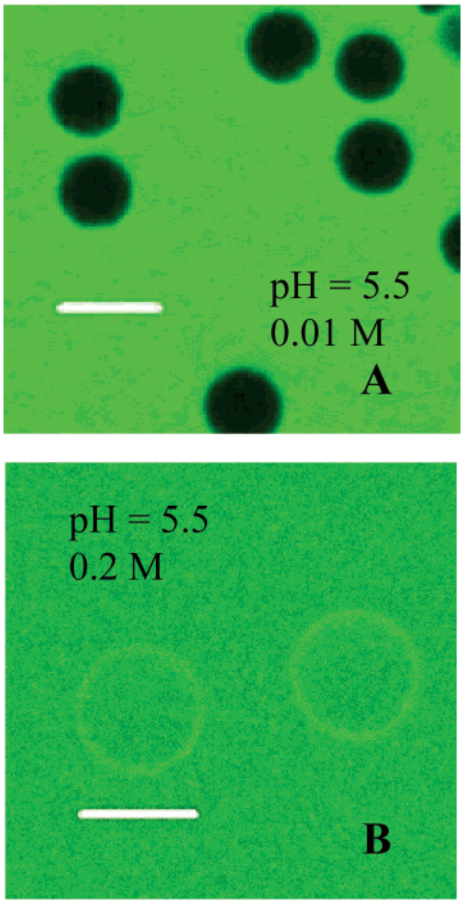

Figure 10. (A) CLSM images of 16 h cross-linked $(PMAA)_7$ capsules exposed to 1 mg/mL FITC−dextran 70 kDa solution for 15 min at pH 5.5 (0.01 M). (B) Capsules shown in panel A after exposure to 1 mg/mL FITC−dextran 70 kDa solution at pH = 5.5 with 0.2 M salt for 15 min. Scale bar on both images is 5 μm.

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