Disulfide-Stabilized Poly(methacrylic acid) Capsules: Formation, Cross-Linking, and Degradation Behavior

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We report the preparation of monodisperse, single-component degradable polymer capsules for potential applications in encapsulation, catalysis, and controlled drug delivery. The synthesized capsules, composed entirely of poly(methacrylic acid) (PMA), are obtained by the sequential deposition of thiolated poly(methacrylic acid) (PMA_{SH}) and poly(vinylpyrrolidone) (PVPON) onto silica particles, controlled oxidation of thiol groups into bridging disulfide linkages in the PMA_{SH}, removal of the silica particles, and finally, release of PVPON by altering the solution pH to disrupt hydrogen bonding between PMA_{SH} and PVPON. The PMA capsules are held together solely through biodegradable disulfide linkages. We demonstrate that the capsules undergo reversible swelling in response to changes in external pH, and degrade in the presence of a physiological concentration of a natural thiol-containing peptide, glutathione. These capsules are of interest for in vivo applications, where degradation of the capsules, through cleavage of the disulfide bonds, can be facilitated by the reducing environment within cells.

Introduction

Encapsulation of reagents and drug molecules is desired for a multitude of applications, ranging from catalysis to drug delivery.[1] In catalysis, spatial separation of multiple components in solution can allow several reactions to occur simultaneously in the same vessel.[2] This is of particular interest for reactions that would contaminate each other or result in a different product when performed without spatial separation. In directed evolution studies,[3] replication of DNA sequences individually confined and separated from each other makes it possible to select and amplify one of the components in a mixture of multiple nucleic acid sequences. In drug delivery, encapsulation of drugs is desired to prolong the circulation time of the drugs[4] and for controlled release[5] of therapeutics into the blood stream or surrounding tissue. In many of these applications, the assembly and loading processes necessitate all-aqueous conditions when the drugs are susceptible to degradation in organic solvents.

Polyelectrolyte capsules, obtained via the layer-by-layer (LbL) deposition of polymers on colloidal template particles and subsequent removal of the core particles, are attractive candidates for encapsulation of a diverse range of low molecular weight species and macromolecules.[6–8] An advantage of the LbL technique is the ability to prepare monodisperse capsules with control over the capsule wall thickness, permeability, stability, and degradation characteristics. For the preparation of degradable multilayered polymer films, the use of biodegradable polymers such as polypeptides,[9] polysaccharides,[10,11] and biodegradable synthetic polymers[12] have been reported.

Recently, we introduced a method to create degradable capsules based on poly(methacrylic acid) cross-linked via disulfide linkages.[13] The capsules are obtained by the sequential deposition of thiol-modified polymethacrylic acid (PMA_{SH}) and poly(vinylpyrrolidone) (PVPON), a pair of polymers that form hydrogen bonds at moderately acidic solutions. At pH above the pK_a of PMA, 6.5, hydrogen bonding becomes inefficient, and in the absence of other forces stabilizing the layers that comprise the capsules, the capsules spontaneously disintegrate. Conversion of thiol groups into inter- and intralayer disulfide linkages within the formed multilayers endows the capsules with stability above this threshold pH and makes them stable at pH values corresponding to those in the bloodstream.[13] The disulfide linkages that maintain the integrity of the capsules render the capsules (bio)degradable.[14] We have demonstrated that these capsules can effectively retain proteins,[13] oligonucleotides,[15] and double-stranded linear and plasmid DNA[16]

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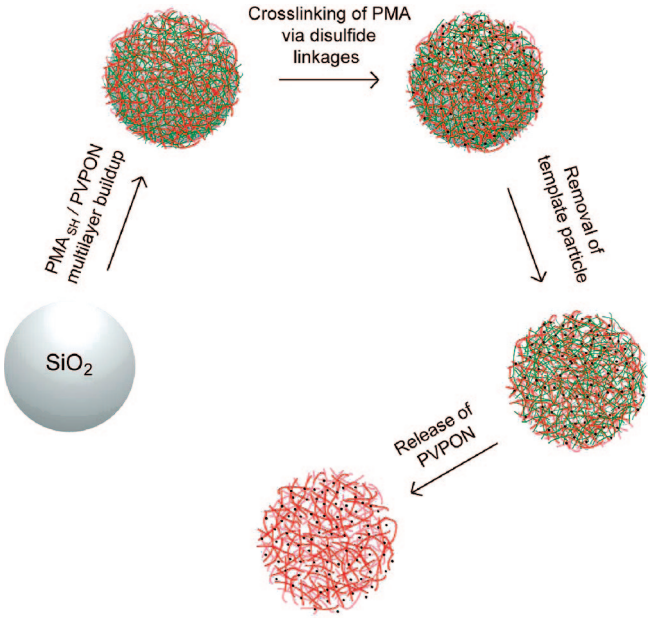
Scheme 1. Preparation of Disulfide-Cross-Linked PMA Capsules Involves Assembling PMA_{SH}/PVPON Multilayers on the Surface of Template Particles, Conversion of the Thiol Groups into Bridging Disulfide Linkages, Removal of the Core Particle, and Release of PVPON



within the capsule interior and release them in a reducing environment.

In this article, we examine in detail the different steps involved in the preparation of PMA capsules (Scheme 1). We first investigate the construction of PMA_{SH}/PVPON multilayers on silica particles and study the effect of PVPON molecular weight on layer buildup. The influence of different oxidizing agents on the extent of cross-linking of the thiol groups in PMA_{SH} into the bridging disulfide linkages is examined, as this determines the ultimate stability of the capsules and is expected to play a critical role in governing the release characteristics of encapsulated materials from the capsules. We also present data that confirm removal of the PVPON from the multilayer films upon altering the pH, which leads to the formation of single-component PMA capsules. Finally, we study the degradation of the capsules under reducing conditions that mimic the intracellular environment, which cleaves the disulfide bonds between PMA_{SH} layers, causing the capsules to disassemble. An understanding of the factors that influence each of the above steps is essential in designing and assembling PMA capsules with tailored properties for therapeutic and diagnostic applications.

Experimental Section

Materials. One- and three-micrometer-diameter SiO_2 particles were purchased from MicroParticles GmbH as a 5 wt % suspension and were used as received. Poly(methacrylic acid, sodium salt) (PMA), M_W 15 kDa, was purchased from Polysciences (USA), and poly(vinylpyrrolidone) (PVPON), M_W 10, 40, and 55 kDa, dithiothreitol (DTT), chloramine T (CaT), cystamine dihydrochloride, cysteamine hydrochloride, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 3-aminopropyltrimethoxysilane (APS), tris(hydroxymethyl)amidomethane (TRIS), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich and used as received. Alexa Fluor 488 (AF488) and Alexa Fluor 633 (AF633) maleimides were obtained from Invitrogen. High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin system.

Methods. Flow cytometry was performed on a Partec CyFlow Space using an excitation wavelength of 488 and 633 nm. Fluorescence spectroscopy was performed using a Fluorolog Horiba fluorescence spectrophotometer. Particles were imaged on an Olympus IX71 Digital Wide field fluorescence microscope equipped with a FITC filter cube, and with a Leica time-correlated single photon counting confocal laser scanning fluorescence microscope (CLSM).

Preparation of Thiolated Poly(methacrylic acid) (PMA_{SH}). Poly(methacrylic acid) with 12 mol % thiol groups was synthesized from PMA and cystamine dihydrochloride via carbodiimide coupling as described previously.[13] The thiol content in the resulting polymer was characterized by using 5,5′-dithiobis-2-nitrobenzoic acid (Ellman's reagent) using a standard curve obtained with the use of cysteamine. For sequential deposition, PMA_{SH} was incubated in a 1 M, pH 8 solution of DTT for at least 12 h and the PMA_{SH} solution was diluted with 10 mM sodium acetate buffer (pH 4) to the desired concentration.

Fluorescent labeling of PMA_{SH} was carried out using AF dye maleimide (typically 10 μg) and a solution of PMA_{SH} in pH 7.2 phosphate buffer (typically ~1−10 mg of PMA_{SH} in 10−100 μL). The reaction was allowed to proceed overnight, after which time the polymer was purified via gel filtration and isolated via freeze-drying.

Preparation of PVPON via RAFT Polymerization. A sample of PVPON (M_n 12 KDa, PDI 1.2) was synthesized via reversible addition fragmentation chain transfer (RAFT) polymerization using *O*-ethyl *S*-(phthalimidylmethyl) xanthate as the chain transfer reagent (CTA), as described previously.[17] Cleavage of the terminal thiocarbonylthio group was achieved using sodium borohydride. The resulting thiol-containing polymer was reacted with excess Ellman's reagent, purified by column chromatography using a NAP-5 column (GE Healthcare), and recovered by freeze-drying.[17] Fluorescent labeling of PVPON was performed in a 10 g L^{-1} solution of PVPON in pH 7.5 TRIS/EDTA buffer in the presence of 1 mM TCEP and 0.1 g L^{-1} of AF488 maleimide. The reaction was allowed to proceed overnight, after which the polymer was purified on a NAP-5 column and recovered by freeze-drying.

Assembly of PMA_{SH}/PVPON Capsules. A suspension of the SiO_2 particles (0.25 wt%) was washed with pH 4 buffer via several water centrifugation/redispersion cycles. The resulting suspension was combined with an equal volume of 2 g L^{-1} solution of PVPON in 10 mM sodium acetate buffer, pH 4, and adsorption of the PVPON was allowed to proceed for 15 min with constant shaking. After this, the particles were washed with fresh pH 4 buffer (three times), redispersed and combined with a solution of reduced PMA_{SH} in 10 mM sodium acetate buffer, pH 4 to a final concentration of PMA_{SH} of 1 g L^{-1}. PMA_{SH} adsorption was allowed to proceed for 15 min, after which the particles were washed with fresh pH 4, 10 mM acetate buffer. This procedure describes the assembly of a

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single bilayer, and the process was repeated until the desired number of bilayers was assembled. Unless stated otherwise, the multilayers contained 5 bilayers of PMA_{SH}/PVPON and had a PVPON outermost layer.

Quantification of Thiol Groups and PMA_{SH} Cross-Linking. To quantify the thiol groups within the polymer multilayers, an aliquot of the particles was transferred into a 10 mM Ellman's reagent solution in 1 M phosphate buffer, pH 7.5. After 10 min, the particles were separated via centrifugation, and the absorbance of the supernatant at 412 nm was quantified by UV–vis spectrophotometry. The concentration of the thiol groups was then calculated using a calibration curve obtained under the same conditions using standard solutions of reduced glutathione (GSH). To quantify the extent of thiol conversion, we first incubated the particles with assembled multilayers with a solution of CaT in 10 mM 2-morpholinoethane sulfonic acid (MES) buffer, pH 6, for 1 min and then quantified the remaining thiol groups as described above.

For comparison of multilayers cross-linked with CaT and hydrogen peroxide, the thiol oxidation was performed using a 2 mM CaT solution in 10 mM MES buffer at pH 6 for 1 min or a 10 mM solution of hydrogen peroxide (H_2O_2) in 10 mM sodium acetate buffer at pH 4 for 15 min. The resulting particles were washed with 10 mM pH 6.5 phosphate buffer, combined with a 0.1 g L^{-1} solution of AF488 maleimide, and incubated for 2 h. The particles were extensively washed with fresh buffer, and the fluorescence of the particles was quantified using flow cytometry.

Hollow Capsules. Capsules were formed via dissolution of the template silica core by treatment with 2 M HF/8 M NH_4F solution (pH 5) at 20 °C for 5 min, followed by multiple centrifugation (4500*g* for 5 min)/buffer washing cycles.[18] The washing cycles were repeated until the pH of the capsule suspension was identical to the pH of the fresh washing buffer.

To quantify the swelling of the PMA capsules, we incubated aliquots of capsules in pH 7.2 phosphate buffer overnight (to ensure release of PVPON); they were then isolated via centrifugation, transferred into buffers with varied pH, and allowed to swell for at least 1 h. The capsules were then visualized using fluorescence microscopy, and the sizes of the capsules were estimated using ImagePro software.

Capsule Stability and Degradation. The stability and degradation of capsules dispersed in pH 7.2 phosphate buffered saline (PBS) were monitored in the presence or absence of 5 mM GSH at 37 °C. At specified times, the fluorescence of the capsules was quantified using flow cytometry and the size of the polymer capsules was estimated using fluorescence microscopy.

Results and Discussion

Assembly of PMA_{SH}/PVPON Multilayers. Studies of hydrogen bonding between PMA and PVPON date back decades.[19] Within the field of polymer multilayers, this system has been pioneered and extensively studied by Sukhishvili's group[20–22] and Xu and co-workers[23] both on planar and colloidal substrates. A main feature of PMA/PVPON multilayers, as well as other hydrogen bonded multilayers with a polyacid as a hydrogen donor, is their inherent instability above a threshold pH value, at which ionization of the carboxylic acid groups exceeds a certain value.[24] The pH threshold depends on the molecular weight of the polymers and the ionic strength of solution. For PMA/PVPON, this typically occurs at pH 6−7, at the pK_a of PMA. To stabilize the hydrogen bonded multilayers against degradation above their respective threshold pH, several routes of cross-linking, which involve one or both of the polymers have been proposed. These include photochemical reactions,[25] thermally induced formation of amide bonds,[26] and carbodiimide-mediated coupling.[20] Our efforts concentrate on creating polymer capsules that remain stable at physiological pH, and which are amenable to deconstruction in response to a chemical stimulus that mimics that within biological cells, namely, the formation of multilayers cross-linked through disulfide linkages, as the reducing intracellular environment can be used to cleave disulfide bonds. To this end, we investigated the assembly of the multilayers using PVPON and a thiol-modified PMA (PMA_{SH}) on colloidal particles and capsules derived from these precursor particles. The thiol groups on PMA_{SH} make it possible to use facile thiol-maleimide chemistry. In this work, PMA_{SH} of M_w 15 kDa with 12 mol % thiol groups was labeled with AF488 maleimide. Flow cytometry (high throughput quantification of fluorescence of individual particles) and confocal laser scanning microscopy (visualization technique)[27] were used to follow the multilayer buildup.

The sequential adsorption of the polymers on the surface of the silica particles was initiated by deposition of a PVPON layer. This polymer adsorbs onto silica and facilitates adsorption of the next polymer layer, PMA_{SH}. Interaction of the two polymers occurs through hydrogen bonding. The subsequent alternate deposition of the polymers results in linear growth of the PMA_{SH}/PVPON multilayers over at least seven PMA_{SH}/PVPON bilayers, as evidenced by the linear increase in fluorescence of the particles by flow cytometry (Figure 1). This indicates regular buildup of the multilayers on the particle surface, in agreement with our previous study.[13]

In biomedical applications, the use of polymers with low molecular weights facilitates their removal from the body via normal renal secretion.[28] However, using low molecular weight polymers in the multilayer assembly can result in a decreased amount of polymer(s) adsorbed within each deposited layer and a reduced overall multilayer thickness, both for electrostatically stabilized[29] and hydrogen bonded systems.[30] In this work, we used PMA_{SH} with a M_w of 15 kDa and PVPON with M_w of 10, 40, and 55 kDa. For each PMA_{SH}/PVPON pair, the observed increase in particle fluorescence as a result of PMA_{SH} adsorption was comparable, indicating a similar amount of PMA_{SH} was adsorbed,

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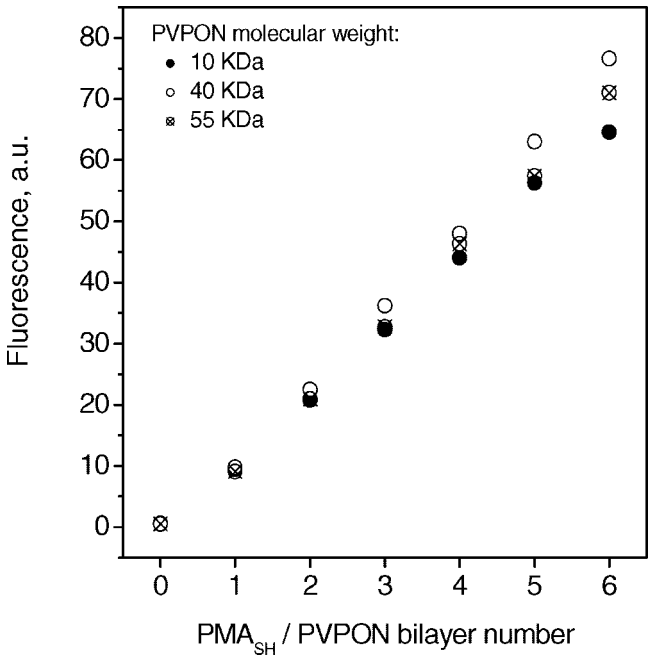

Figure 1. Fluorescence of 1 μm diameter SiO_2 particles coated with PMA_{SH}/PVPON multilayers as a function of bilayer number, as measured by flow cytometry. The effect of PVPON with different molecular weights (10, 40, and 55 kDa) on multilayer buildup is shown. PMA_{SH} was labeled with AF488; the multilayers were deposited from 1 g L^{-1} polymer solutions at pH 4 (10 mM sodium acetate) and measurements were taken after each PVPON layer was deposited.

irrespective of the PVPON M_w used. The multilayer buildup was regular for all three systems examined. In these experiments polymer deposition was accomplished at pH 4. At pH 5 (data not shown) the cumulative amount of PMA_{SH} adsorbed was less than 10% of that deposited at pH 4, probably as a result of increased ionization of PMA_{SH}. Thus, in all further experiments, multilayer assembly was performed at pH 4, and PVPON with a M_w of 10 kDa was selected for use.

Multilayer Cross-Linking. The formation of disulfide linkages via oxidation of two thiol groups is widely used in organic chemistry and for bioconjugation. This is largely due to the biodegradable nature of disulfide bonds;[14,31] that is, live cells have the ability to intracellularly degrade disulfides. Utilizing this process opens opportunities to design and construct drug delivery platforms.[32–34] However, the controlled oxidation of thiols into disulfides using inexpensive and common reagents can be challenging, and is not well-established. The first challenge is the need to avoid overoxidation of sulfur into oxygen-containing species, which do not have the properties of disulfide linkages, such as biodegradability. For the reagents that convert the thiol groups into disulfide linkages without overoxidation, the process can be rather slow, requiring extended reaction times. Also, the oxidation process can be pH-sensitive, accelerating at pH 7−9, and slowing down at lower pH values. Oxidation with hydrogen peroxide and other peroxides is pH-dependent

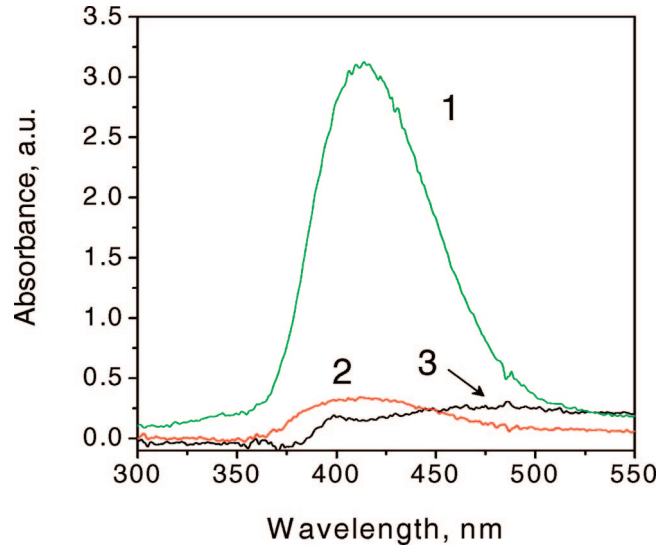

Figure 2. UV–vis spectra corresponding to the Ellman's test performed to quantify the thiol groups within the PMA_{SH}/PVPON multilayers. Fully reduced form (1) and after oxidation with 2 mole equivalents of Chloramine T (2) or 2 mM solution (excess) of Chloramine T (3) in 10 mM MES buffer, pH 6 for 1 min.

and often leads to overoxidation.[35,36] Dimethylsulfoxide (DMSO), a water miscible solvent, presents an alternate pathway,[37] yet efficient cross-linking requires 30−50 vol % of DMSO. These conditions cause the PMA/PVPON multilayers to become unstable. Chloramine T (CaT) has been shown to controllably oxidize thiols into disulfide linkages without overoxidation.[35,38] The reaction between CaT and thiols (for example, the natural thiol-containing molecule glutathione, GSH) is near quantitative and at pH 6, 2 mole equivalents of CaT oxidize more than 90% of glutathione thiol moieties within 1 min.

To study and quantify the oxidation of thiols within the multilayers, 1 μm silica particles coated with five bilayers of PMA_{SH}/PVPON were used. In reduced form, the multilayers are unstable at pH 7 and rapidly disassemble when placed into a 10 mM solution of Ellman's reagent in 1 M phosphate buffer, pH 7.5. The resulting solution has a characteristic absorption at 412 nm (Figure 2, spectrum 1), which is used to quantify the number of thiol groups by using a calibration curve obtained with standard solutions of glutathione. The addition of CaT to the particles with PMA_{SH}/PVPON multilayers at different pH (4–6) results in a decreased number of thiol groups, reflecting their oxidation into disulfide linkages. We found that the addition of 2 mole equivalents of CaT oxidized 90% of thiols within 1 min (Figure 2, spectrum 2), and a moderate excess of CaT (2 mM solution) converts the thiols into disulfides nearly quantitatively (Figure 2, spectrum 3).

To demonstrate the drastic difference in the ability of hydrogen peroxide[13] and CaT[15] to cross-link PMA_{SH} within the multilayers, the five bilayer multilayers were oxidized

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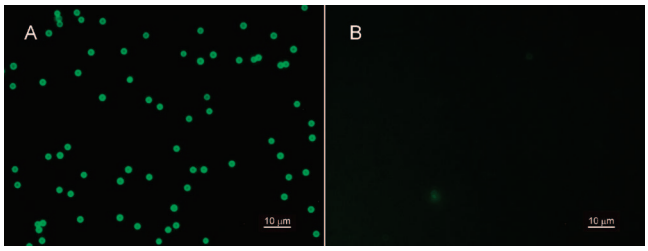

Figure 3. Fluorescence microscopy images of 3 μm SiO_2 particles with five PMA_{SH}/PVPON bilayers cross-linked with (a) 10 mM hydrogen peroxide at pH 4 for 15 min or (b) 2 mM chloramine T at pH 6 for 1 min and further incubated with a solution of AF488 maleimide (0.1 g L^{-1}, 10 mM phosphate buffer, pH 6.5, 2 h). Corresponding flow cytometry AF488 intensities are: bare silica <0.1; CaT cross-linked multilayers = 1; hydrogen peroxide cross-linked multilayers = 125.

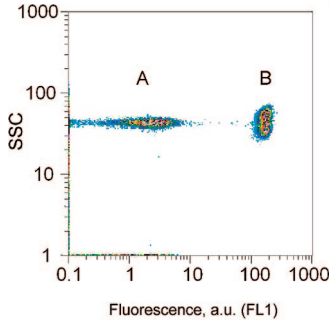

Figure 4. Flow cytometry dot plots (*Y* axis: light scattering parameter SSC; *X* axis: particle fluorescence) corresponding to the PMA_{SH}/PVPON multilayers exposed to pH 7.2 buffer (A) before and (B) after oxidation of thiol groups into disulfide linkages. The multilayers were assembled on nonfluorescent SiO_2 particles using a PMA_{SH} sample labeled with AF488 and PVPON. While (A) the reduced form of the multilayers disintegrates and is washed of the nonfluorescent SiO_2 particles, (B) the oxidized, cross-linked multilayers remain intact around silica core particles, which is registered by high fluorescence intensity.

using these reagents. The particles with oxidized multilayers were then exposed to a solution of AF488 maleimide, a thiol-reactive fluorophore, and analyzed by flow cytometry. In this experiment, the fluorescence of the particles reflects the reaction between the thiol groups within the multilayers with the fluorophore (Figure 3). While CaT-treated particles exhibit minor fluorescence, which is only marginally higher than that of nonfluorescent SiO_2 particles, the sample obtained via oxidation with hydrogen peroxide is 2 orders of magnitude more fluorescent, reflecting the presence of a large number of nonoxidized thiols within the multilayers after H_2O_2 treatment. Therefore, in all subsequent experiments, to achieve cross-linking of PMA_{SH} within the assembled multilayer films, we used a 2 mM solution of CaT at pH 6 for 1 min.

To examine the effect of cross-linking and verify the stability of the obtained multilayers at elevated pH, we prepared PMA_{SH}/PVPON multilayers on 1 μm silica particles using AF488-labeled PMA_{SH}. The sample oxidized with CaT and the nonoxidized counterpart were placed into a pH 7.2 buffer and analyzed by flow cytometry (Figure 4). As expected, the noncrosslinked multilayers desorb from the particle surface and the particles exhibit minor fluorescence (population A). In contrast, the particles with cross-linked multilayers (population B) show 2 orders of magnitude higher fluorescence and have similar fluorescence to the nonoxidized samples at pH 4 (data not shown), where the multilayers

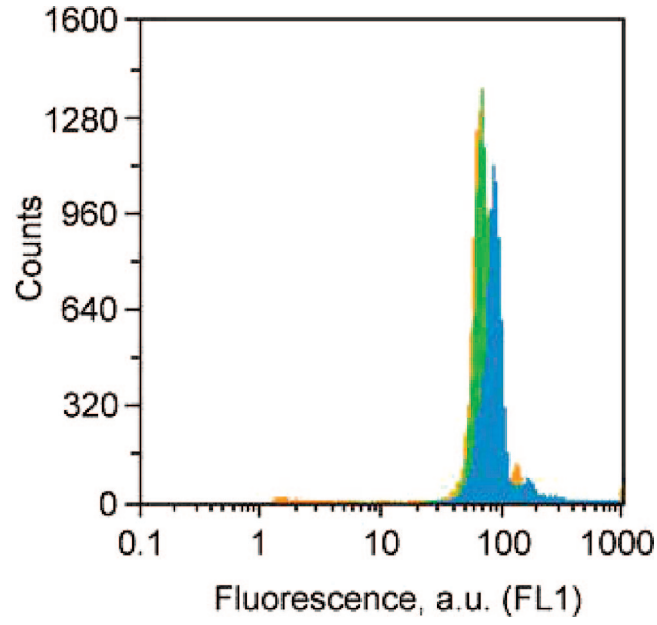

Figure 5. Flow cytometry histograms of fluorescence intensity for the 1-μm-diameter SiO_2 particles with PMA_{SH}/PVPON multilayers, pH 4 (green), and the corresponding capsules at pH 4 (orange) and 7.2 (blue). The multilayers were formed using AF488-labeled PMA_{SH}.

are stable without cross-linking. Thus, the PMA_{SH}/PVPON multilayers are similar to their PMA/PVPON counterparts, in that the reduced noncrosslinked form of the multilayer is "erasable",[24] i.e., the assembled films fall apart at pHs above the pK_a of PMA. However, oxidation of thiol groups imparts stability to the multilayers at pH > pK_a of PMA.

Formation of PMA Capsules. The silica core particles, coated by PMA_{SH}/PVPON multilayers and followed by oxidation with CaT to form disulfide bonds, were rapidly removed by buffered hydrofluoric acid to form capsules.[18] The core removal process did not lead to any significant loss of material from the assembled multilayers, as is evident from the histograms corresponding to the fluorescently labeled PMA_{SH} within the multilayers before and after removal of the particles (Figure 5). The fluorescent signal shows minimal change upon dissolution of the silica particles, indicating minimal loss of PMA_{SH} upon core removal. Removal of the core particles also does not lead to degradation of the disulfide linkages, as the fluorescence of the prepared capsules does not decrease when the capsules are transferred from pH 4 to pH 7.2 buffer solutions.

To independently ascertain the fate of PVPON, we synthesized a sample of PVPON with M_n = 12 kDa via reversible addition-fragmentation chain transfer polymerization (RAFT),[17] a technique that allows precise control over the molecular weight of the polymer and provides each of the polymer chains with a terminal group, which is easily converted into a thiol. The terminal thiol functionality allows for labeling of the polymer with a fluorescent marker. In this work, we labeled PVPON with AF488 and separately labeled PMA_{SH} with AF633 to allow spectral separation of the two dyes. We then used fluorescently labeled PVPON as the first layer in the assembled multilayer film. Upon dissolution of the template silica particles, this PVPON layer constitutes the inner layer of the capsules. When the prepared capsules were placed into a pH 7.2 buffer, the absence of

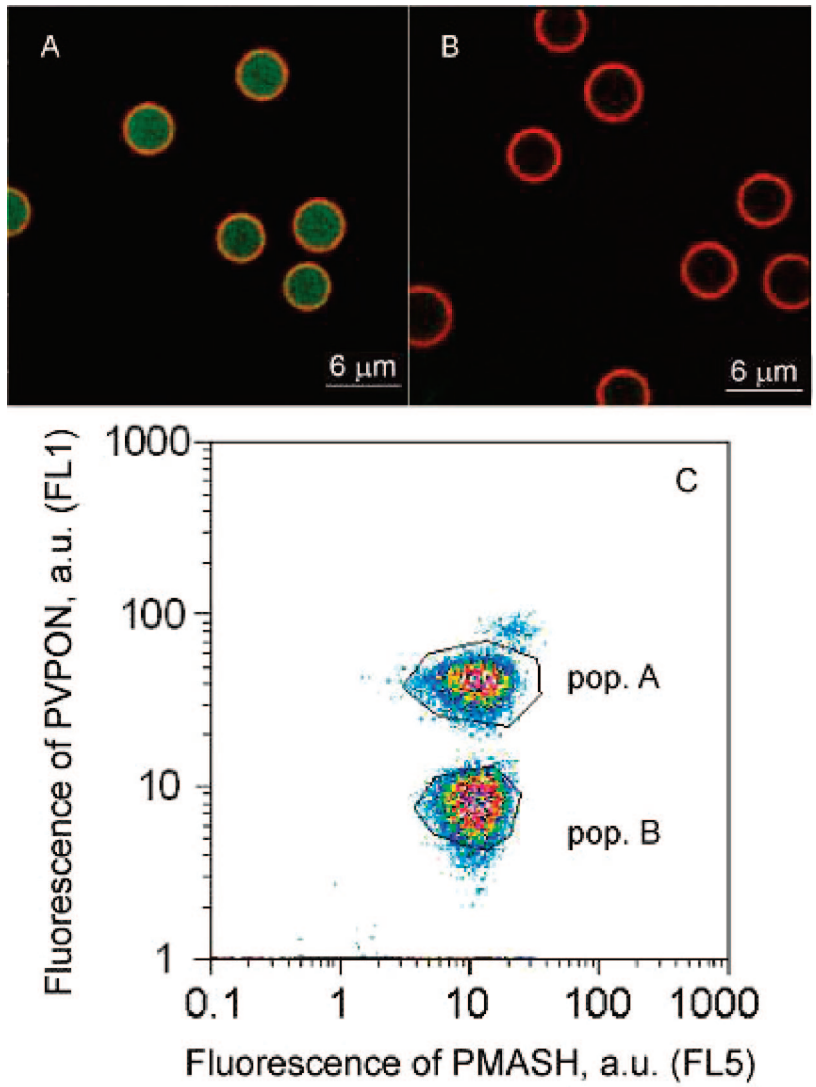

Figure 6. CLSM images corresponding to the PMA capsules (AF633 labeled, FL 5) with PVPON chains (AF488 labeled, FL 1) expelled from the inner surface of the multilayered film into the capsule interior (image A) and after release of PVPON (image B), and the corresponding flow cytometry dot plot (image C). The images were obtained by placing the PMA_{SH}/PVPON capsules with a AF488 labeled PVPON as the innermost layer into pH 7.2 buffer. The data validate the release of PVPON from the cross-linked PMA multilayers to result in the single-component capsules.

attractive forces between the polymers causes the PVPON chains to be expelled from the multilayer. At first, they fill the interior of the capsule, as shown in Figure 6A. Following this, the PVPON chains start diffusing through the PMA_{SH} "hydrogel" film and, over time, the interior of the capsules becomes free of the PVPON chains (Figure 6B). These CLSM observations are substantiated by the flow cytometry analysis of the capsules, which shows that the fluorescence of PMA_{SH} (FL5, *X* axis) remains at a constant level (Figure 6C). In contrast, the fluorescence of PVPON (FL1, *Y* axis) drastically diminishes (Figure 6C), indicating the release of PVPON chains. Thus, within the resolution of CLSM and flow cytometry, the PMA_{SH}/PVPON capsules lose PVPON upon exposure to pH 7.2 buffer and become single-component PMA capsules, stabilized by disulfide bonds. The importance of this lies in that the capsules are held together solely through disulfide linkages and are expected to disintegrate upon degradation of the disulfide linkages. As will be demonstrated below, this can be achieved using an intracellular concentration of the natural thiol-containing metabolite, GSH at pH 7.2. Yet, disassembly of the disulfide-stabilized PMA capsules is expected to occur at any pH upon the action of reducing agents. We have previously shown that PMA capsules effectively retain peptide, oligonucleotides, and polynucleotides (ss and ds DNA) within the capsule interior.[13,15,16] However, these capsules are permeable to uncharged macromolecules (PVPON) and small molecules (buffer salts), and therefore represent semipermeable single-component capsules.

As shown above, the PMA capsules are single-component, with the capsule wall consisting of PMA interlinked within the film via disulfide cross-links. The PMA capsules inherit the properties of PMA, such as swelling in response to solution pH. Swelling of the capsules proceeds in a narrow pH range with an inflection point around pH 6.5, close to the pK_a of PMA, resulting in more than a 1.3-fold increase in capsule radius upon changing the pH from 4 to 9. This swelling caused by ionization of PMA is reversible: when placed back into pH 4 buffer (Figure 7), the capsules attain a similar size as that before exposure to pH 7−9. Recently, we reported similar swelling characteristics for single component poly(acrylic acid) "click" capsules.[39] The pH-sensitive nature of the synthesized capsules can serve as

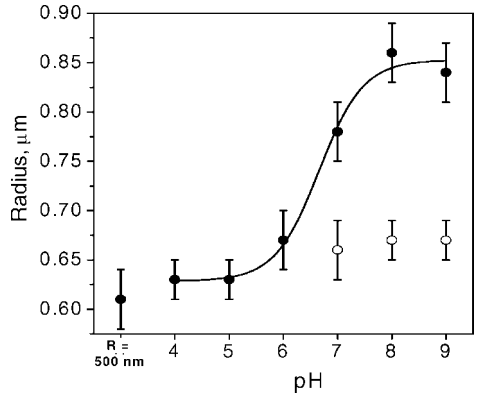

Figure 7. Swelling curve obtained using PMA capsules composed of five PMA_{SH} layers incubated in buffer solutions with varied pH (varied symbols). The first data point ($R = 500$ nm) corresponds to the SiO_2 particles with a 1 μm diameter. The open symbols correspond to the size of the capsules incubated at the specified pH and placed back into the pH 4 buffer.

the basis for pH-controlled permeability of the capsules and ionization-controlled adsorption–desorption phenomena.

Degradation of PMA Capsules. For biomedical applications, it is important to consider degradation of the PMA capsules at conditions close to those within live cells. Although the overall potential in the blood stream is oxidative,[31] the intracellular cytosolic space is reductive,[14] which provides a platform for the creation of drug delivery vehicles that remain stable in the blood stream but deconstruct once internalized by a living cell.[32,34]

Single-component PMA capsules comprising five layers of PMA_{SH} labeled with AF488 were placed in 5 mM GSH solution in phosphate buffered saline at pH 7.2. The fluorescence of the capsules was monitored over time with flow cytometry, and the size of the capsules, i.e., their swelling, was quantified using fluorescence microscopy (Figure 8). In the absence of GSH, the capsules exhibited a negligible change in size or fluorescence over time, indicating the high stability of the capsules and no release of PMA from the capsule wall. In the presence of GSH, during the first 3 h, the fluorescence of the capsules decreased by ca. 25%, accompanied with a pronounced swelling of the capsules (1.3-fold increase in radius). At longer times, the capsules were no longer spherical, making sizing of them impractical, and the fluorescence of the particles decreased to the background level within the next 1 h. The observed trends indicate a possible degradation mechanism of the PMA capsules. First, GSH action leads to the emergence of free thiol groups and a global reshuffling of disulfide linkages[40] within the multilayer, accompanied with swelling of the capsules. The degradation of the capsules occurs rapidly after the thiol-disulfide reshuffling yields PMA_{SH} chains not linked to the membrane.

Conclusions

We have demonstrated the preparation of monodispersed, single-component degradable capsules consisting of PMA chains cross-linked via disulfide linkages. Altering the PVPON M_w (10, 40, or 55 kDa) in the PMA_{SH}/PVPON multilayer buildup did not affect the amount of PMA_{SH} adsorbed, with regular film buildup observed for all three multilayer systems. A number of oxidation reagents were examined to cross-link the multilayers, with CaT found to be a highly efficient and suitable reagent to form stable, highly cross-linked PMA_{SH}/PVPON multilayers. Removal of PVPON from the capsules, resulting in single-component PMA capsules, was confirmed by fluorescence measurements. The PMA capsules exhibit reversible swelling in response to the solution pH, with a 1.3-fold increase in capsule radius observed when the solution pH was altered from 4 to 9. The PMA capsules remain stable at physiological pH. We also demonstrated that in the presence of a physiological concentration of GSH, the capsules swell within the first 3 h of exposure, followed by degradation. Together with our previous reports on strategies for the encapsulation of proteins,[13] oligonucleotides,[15] and double-stranded DNA,[16] the current work outlines new opportunities for the creation of responsive, single-component degradable capsules with potential for various biomedical applications.

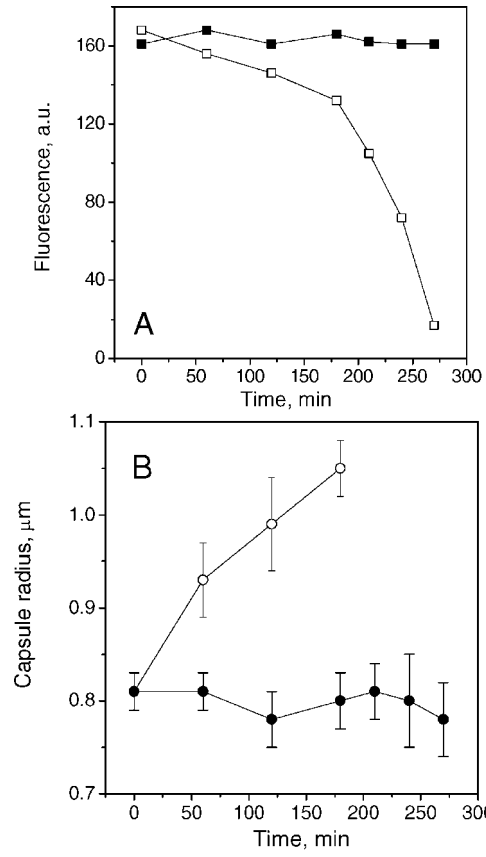

Figure 8. (A) Fluorescence and (B) radii of the PMA capsules in 5 mM GSH/PBS (open symbols) and PBS (closed symbols) at pH 7.2 as a function of incubation time.

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Note Added after ASAP Publication. There were minor text errors in the version published ASAP March 15, 2008; the corrected version was published ASAP March 21, 2008.

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