Fabrication of Micro Reaction Cages with Tailored Properties

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Abstract: Hollow polyelectrolyte capsules in micro- and submicrometer size were prepared. Their interior was functionalized by a "ship in bottle" synthesis of copolymers. While the monomers permeated the capsule wall easily, the formed polymers remained in the capsule cage. The physicochemical properties of the capsule interior such as ion strength, pH, light absorption, and fluorescence could be controlled independently from the surrounding solvent by means of the chemical nature of the captured polymer. In case of polyelectrolytes the osmotic pressure of the counterions led to a swelling of the capsules which can be important for micromechanics. The functionalization with light-sensitive materials allowed selective photoreactions inside the capsules. Synthesis of polyelectrolytes at high concentration resulted in an intertwining of the capsule wall with the polymer. The modified walls behaved like ion exchange membranes and showed selectivity toward adsorption and permeation of organic ions. The modified capsules offer many possibilities for novel applications as containers for controlled precipitation, as nanoreactors for catalyzed reactions, or as sensors.

Introduction

A novel type of hollow polyelectrolyte capsules has recently been prepared[1] by covering templates ranging from 60 nm to 10 μm in size with alternating layers of polycations and polyanions.[2] In a second step the templates are removed by changing the pH.[3] These capsules offer broad perspectives in nanoscale encapsulation of drugs, minerals, dyes, and proteins. In addition, they are promising mesoscopic reaction cages. Such nanoreactors[4] attract considerable interest and some progress has been made recently in their preparation. The physicochemical properties of both the wall and the interior of the new polyelectrolyte capsules can be customized widely. Polyelectrolytes, nanoparticles, or multivalent ions as layer building materials offer a broad variability to tailor the wall design.[5,6] The properties of the capsule interior could be changed by filling them with low molecular weight compounds and subsequent precipitation,[7] micelle formation, or solvent exchange,[8] but these processes are reversible and depend on the exterior environment. For applications as nanoreactors a permanent adjustment of the physicochemical properties of the interior is desired. This could be achieved by inclusion of macromolecules because the capsule wall is impermeable for polymers whereas monomers penetrate the wall easily. Exploiting this property, we have developed a general procedure for loading the capsules by a "ship in bottle" synthesis (Figure 1). Different polymers and functionalized copolymers have been synthesized inside the capsules from water-soluble anionic, cationic, and neutral monomers. This will be demonstrated by polymerization of sodium styrene sulfonate

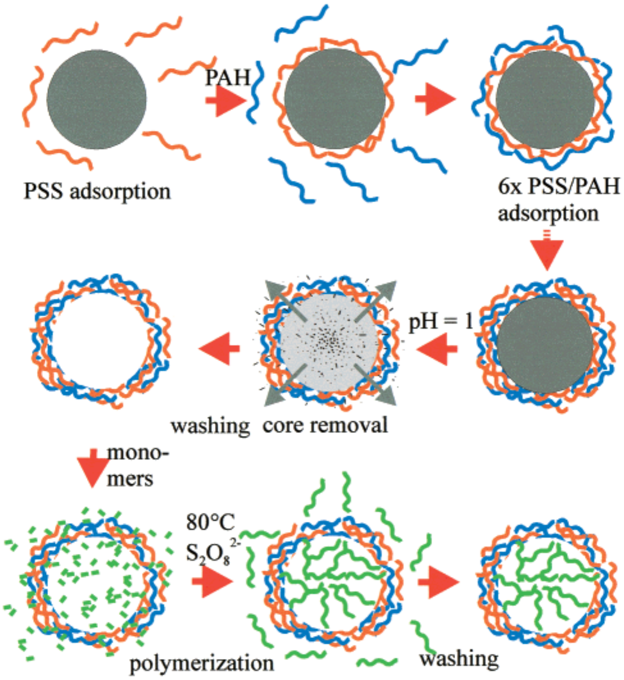

Figure 1. Scheme of capsule preparation and filling with polymers via a "ship in bottle" synthesis.

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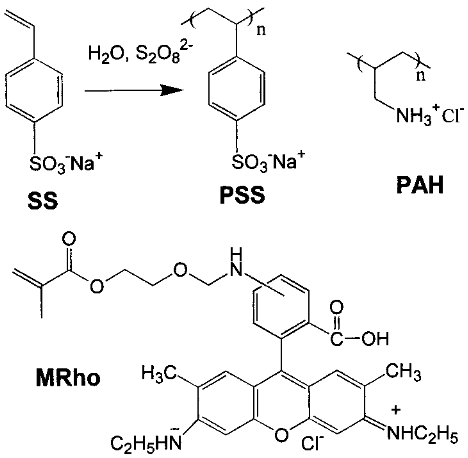
Scheme 1. Reaction Scheme and Formulas of the Chemical Materials Used[a]



[a] Polymerization of sodium styrene sulfonate (SS) to PSS initiated by thermal decomposition of potassium peroxodisulfate $K_2S_2O_8$; PAH = poly(allylamine), MRho = methacroyloxyethyl thiocarbamoyl rhodamine B.

(SS) to sodium poly(styrenesulfonate) (PSS, Scheme 1) as the model system.

Experimental Details

Capsule Preparation. Hollow capsules were prepared as described in the literature (Figure 1).[9] Monodisperse spheres ($d = 5.94$ μm) of weakly polymerized melamine-formaldehyde resin (Microparticle GmbH) were covered by 9 layers of PSS (MW 70 000 g mol^{-1}, Aldrich) and poly(allylamine hydrochloride) (PAH, MW 70 000 g mol^{-1}, Aldrich). The resin core was dissolved in 0.1 M hydrochloric acid and the remaining capsules were thoroughly washed with water until pH 6 was reached.

Polymer Synthesis in the Capsule Interior. Polymerization was carried out at various SS concentrations in the presence of capsules for 4 h at 80 °C under nitrogen atmosphere according to standard procedures in the literature.[10] As initiator 1% potassium peroxodisulfate, related to the monomer concentration, was used. Optically sensitive polymers were prepared by copolymerization of SS and 0.5 mol % methacroyloxyethylthiocarbamoyle rhodamine B (MRho, Scheme 1, Polyscience) in 20% methanol. After polymerization, the capsules were separated from the PSS in the bulk solution by centrifugation. Several washing cycles with water followed (Figure 1).

Confocal Laser Scanning Microscopy. The size, integrity, and degree of filling of individual capsules were determined by an inverse Confocal Laser Scanning Microscope (CLSM, Leica, Germany) with either a 100× or a 40× oil immersion objective with a numerical aperture of 1.4. The capsules were visualized by electrostatic adsorption of the fluorescence dyes rhodamine 6G or fluorescein to the polyelectrolytes and their complexes. From the image of the equatorial plane of the capsules (see Figure 2c), the amount of incorporated copolymer was determined by integration of the fluorescence intensity in the capsule interior using the LEICA software package.

Scanning Force Microscopy. Scanning force microscopy was applied to capsules air-dried on freshly cleaved mica. SFM images have been recorded at room temperature using a Dimension 3000 SFM (Digital Instruments, Santa Barbara). Both tapping mode and contact mode have been employed. Microlithographed tips on silicon nitride cantilevers with a force constant of 0.58 N/m were used. The contact force between the tip and the sample was kept as low as possible (<10 nN) and images were acquired in constant force mode.

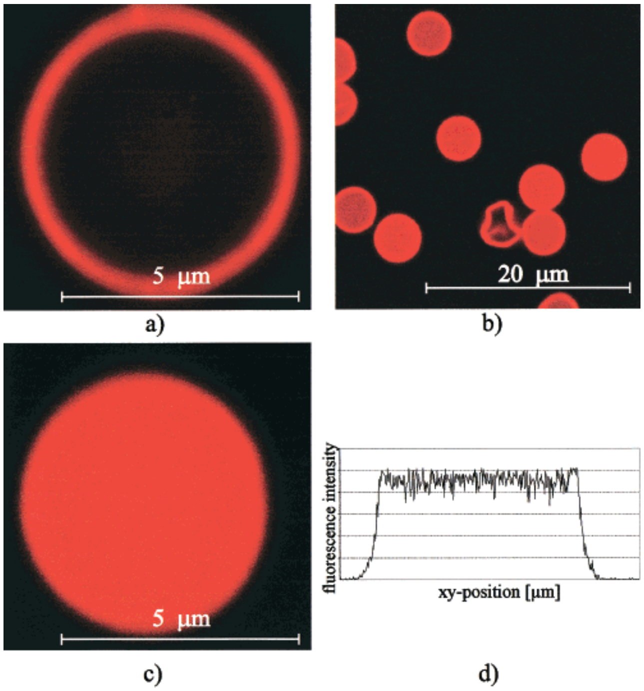


Figure 2. CLSM images taken at the equatorial plane of the capsules: (a) hollow capsules (electrostatic adsorption of rhodamine 6G was used for the visualization); (b) capsules filled by copolymerization of a 0.5 M SS solution and MRho (the unfilled capsule is broken); (c) homogeneously distributed MRho-PSS in one capsule; and (d) fluorescence intensity profile along a line through the capsule center.

Photochemistry. The photochemical bleaching of the capsule interior was performed by the ArKr ion laser of the CLSM. The laser beam of 488 and 568 nm was focused with full power onto individual capsules. Afterward images of the capsules were taken at reduced laser power. At usual scanning conditions the laser beam bleached the rhodamine dye less than 0.5% per scan.

Other Methods. The molecular weight distribution of the synthesized polymers was determined by gel permeation chromatography (Thermo Separation Products). A combination of the columns HEMA-BIO 40/100/300/1000 was used. The polymer concentration was determined by a refractive index detector Shodex RI-71 calibrated with PSS standard samples (Polyscience).

The number of capsules for the titration was determined using a noncommercial apparatus for Single Particle Light Scattering.[12] The PSS polymer was titrated with the positively charged dye acridine orange, which binds efficiently to the negative sulfonate groups.[13] After each titration step including 20 min incubation time, the supernatant was separated by centrifugation and its fluorescence was measured. The measurements were done on a plate reader HTS 7000 (Perkin-Elmer). If the fluorescence of the supernatant increased, the equivalence point was reached.

Results and Discussion

Synthesis. The copolymerization of 0.5 M SS with MRho in the presence of monodisperse hollow capsules (Figure 2a) yielded PSS with a molecular weight of $M_w = 120\,000$ g mol^{-1} labeled at every 650th position with MRho. The capsules were isolated from the free polymers in solution by centrifugation and by four subsequent washing cycles with water. The copolymer, which is too large for permeating the capsule wall, remained inside the capsules. Remains of monomers and

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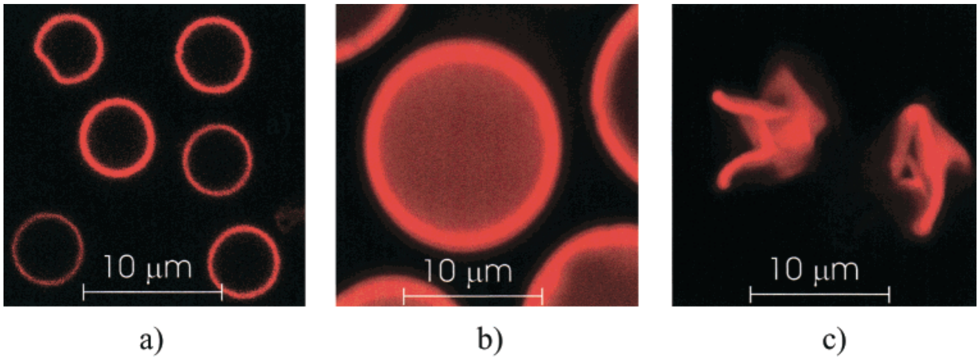

Figure 3. CLSM images of the same capsules (a) before and (b) after they were filled by polymerization of a 1 M SS solution and (c) shrinking of the capsules in b caused by the osmotic pressure of a 2 M polydimethyldiallylammonium chloride solution in the exterior.

oligomers were “dialyzed” from the capsules by incubation for 7 h in pure water. The CLSM image shows that the synthesized MRho-PSS copolymer is captured in the interior of the capsules whereas in the exterior solution the polyelectrolytes are completely removed (Figure 2b). Even after several months leakage of capsules was not observed. The synthesized polymer is homogeneously distributed in the capsules, as demonstrated by the confocal image in Figure 2c and the cross section of the capsule (Figure 2d). However, the concentration of synthesized PSS is usually higher in the wall than in the interior of the capsules (see Figure 3b).

The amount of PSS in the capsules was quantified by titration of approximately 10^6 filled capsules with the positively charged dye acridine orange. The titration of capsules which were filled by polymerization in a 0.2 M SS solution yielded an average concentration of 0.12 M PSS. The concentration of PSS in capsules filled in 1 M SS solution was only 0.05 M. However, the capsules were swollen to 14 μm in diameter (see next paragraph). Taking the initial capsule diameter of 5.7 μm into account, the concentration would have been 0.8 M which agrees well with the polymer concentration in the reaction solution. The slightly decreased amounts compared with the starting monomer concentration have been caused probably by a loss of small polymers (oligomers) from the interior during the washing process.

By means of the polyelectrolyte concentration the ion strength in the capsule interior can be controlled selectively. Incubation of the filled capsules in solutions of various salts led to an complete exchange of the PSS counterions in the capsule against the ions in solution. In the case of protons a high concentration could be achieved in the absence of other ions, which allows the pH to be adjusted in the capsule interior down to 2−3, in a neutral, salt-free bulk solution. The opposite effect of a pH difference between a PSS bulk solution and the interior of the hollow capsules was reported already in the literature.[14]

Osmotic Effects. As shown in panels a and c of Figure 2, the diameter of capsules filled with 0.5 M PSS is about 15% smaller than the diameter of the original hollow capsules. It is known that hollow PAH/PSS capsules shrink at higher temperatures.[11] In a reference experiment the temperature conditions of the synthesis were applied to capsules in water, resulting in a reduction of the capsule diameter by more than 40% of the original size. The less shrinking of the filled capsules indicates an additional process working in the opposite direction. Indeed, synthesis of PSS in concentrations above 0.6 M even increased the capsule diameter (Figure 3a,b) up to a factor of 4, before the capsules burst. The strong swelling is induced by the osmotic pressure of the sodium counterions in the capsule interior. This osmotic effect can be used for controlling the swelling and shrinking of capsules by means of an external counter-pressure. Applying a 2 M poly(dimethyldiallylammonium) chloride solution to the swollen capsules, the higher osmotic pressure in the bulk caused a complete shrinking of the capsules (Figure 3c). After the polymer was washed away, the capsule re-swelled to its initial size (Figure 3b). These “micro-mechanics” could be useful for the construction of osmotically driven nanopumps or switchable stoppers for capillarities.

Modification of the Capsule Wall. The 4-fold increase of the capsule diameter is equivalent to an enlargement of the capsule wall surface by a factor of 16. Assuming a constant amount and density of polyelectrolyte complex in the wall, this stretching would reduce the wall thickness from 15 nm[11] to less than 1 nm, which is difficult to imagine. To investigate the wall thickness in dependence on the surface enlargement scanning force microscopy was applied to dried capsules. During the drying process the water evaporates from the interior and the capsules collapse. The remaining two walls of the empty capsules had together a height of 30 nm (Figure 4a), which is in good agreement with previous measurements.[11] The same capsules filled with 0.1 M PSS showed an average height of 148 nm (Figure 4b). If 30 nm is attributed to part of the original capsule wall, the additional 108 nm corresponds to dried, synthesized PSS. From these numbers and the capsule diameter the amount of PSS can be estimated. For the individual capsule shown it was approximately 85% of that have expected from a concentration of 0.1 M PSS in the capsule.

The wall thickness of the swollen capsules filled by polymerization of a 1 M SS solution can be determined after the wall was ruptured by means of sonication and subsequent washings to remove the released PSS. The dried remains of the capsule wall had a thickness of 12 nm (Figure 4c), which is only slightly smaller than the original wall thickness despite of the 16-fold increase in surface area by the swelling. Therefore, a remarkable amount of the synthesized PSS was either adsorbed on the wall surface or intertwined in the polyelectrolyte layers as shown in the scheme in Figure 5. Due to the repulsion of the negative charges the PSS chains stick into the solution like a brush. The assumption of a high PSS content around the wall is confirmed by the CLSM image (Figure 3b) showing a larger amount of synthesized MRho-PSS copolymer in the capsule wall than in the interior. The explanation of the enrichment of PSS in and on the capsule wall is provided by considering the 2-fold negative charged initiator-ion $S_2O_8^{2-}$, which is adsorbed on the remaining positive charges at the wall surface and in the wall interior. Due to the two negative charges this initiator ion has a higher binding constant to the wall than the competing SS^-

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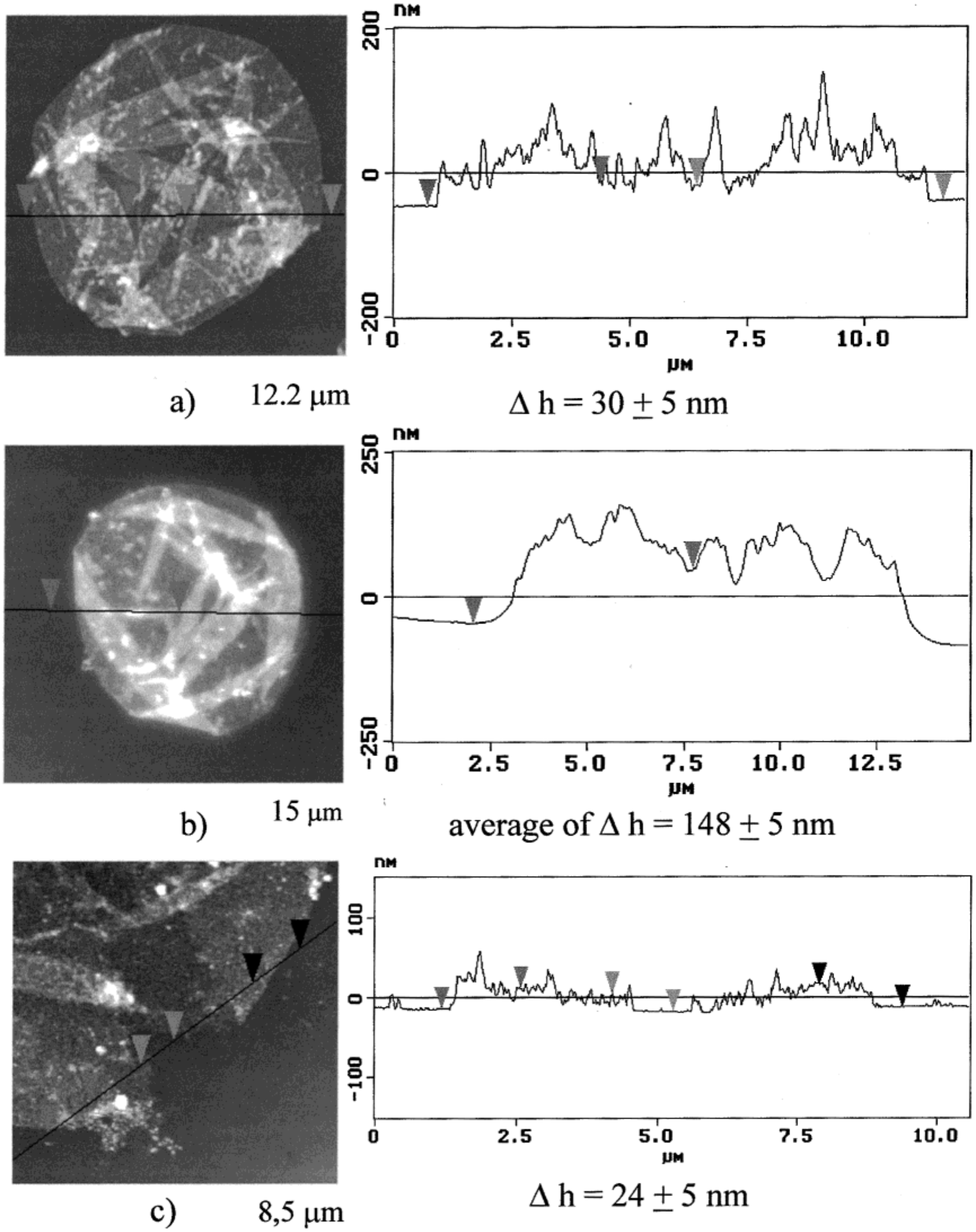

Figure 4. SFM images and corresponding height profiles of dried capsules on mica: section profiles of (a) the hollow capsule, (b) the capsule filled with 0.1 M PSS, and (c) the broken capsule that was originally filled with 1 M PSS. After the capsules were broken the polymer was washed away from the interior.

ions. The higher initiator concentration in the wall results in a larger polymerization rate than in the bulk solution.

An intertwining of the polyelectrolyte complex wall with PSS chains increases the bounded negative charges in the wall. The capsules become similar to an ion exchange membrane (Figure 5). As a result of this modification, the capsules become selective toward the charge of the ions. This was demonstrated for the positively charged rhodamine 6G and the negatively charged fluorescein. Both dyes were well adsorbed at the nonmodified initial capsules. An excess of the dye molecules permeates immediately into the capsules, yielding the same fluorescence intensity inside and outside the capsules. The treatment of the charged capsules with rhodamine 6 G, however, yielded at low dye concentration a strong adsorption in the capsule wall. The addition of a larger amount of dye resulted in an inhomogeneous distribution of the dye in the capsule wall. Islands were formed which consist probably of the hydrophobic dye−PSS complex. This was accompanied by an increase of porosity. Panels a and b in Figure 6 show the surface and the cross section of such capsule. After the formation of large pores the captured polymers were released into the bulk solution. In contrast, even a large excess of the negatively charged fluorescein did not result in adsorption. The fluorescein also did not readily permeate the negatively charged capsule wall (Figure 6c). Only after several hours were a small number of the fluorescein molecules found inside the capsules (Figure 6d).

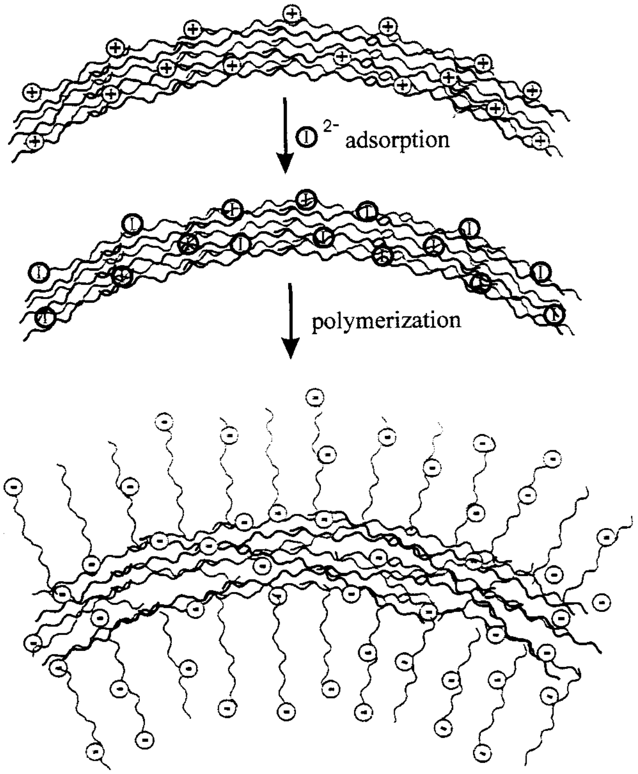

Figure 5. Model of increased polymerization rate in and on the wall due to surface adsorption of the initiator (I) counterions. The resulting walls are intertwined with PSS chains such as an ion exchange membrane with a brush of PSS chains sticking in solution due to the electrostatic repulsion.

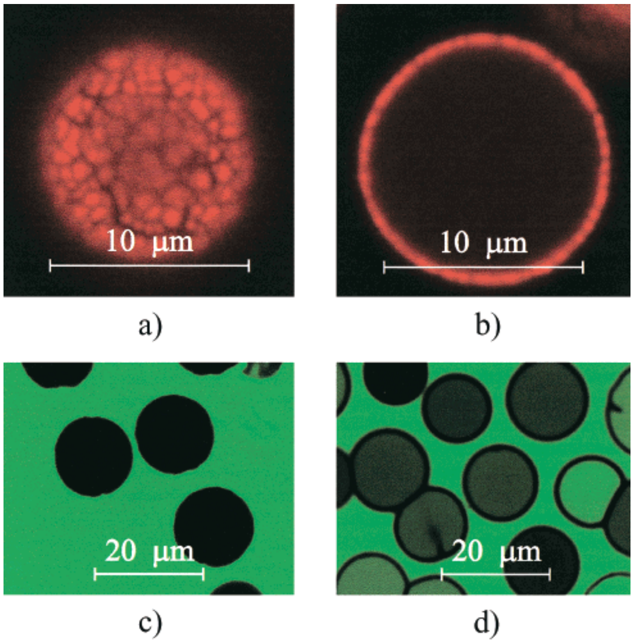

Figure 6. Treatment of the negatively charged walls with equivalent amounts of organic cations leads to the formation of hydrophobic islands and many pores, through which the interior material is immediately released: (a) surface and (b) cross section through the center of a capsule, filled with 1 M PSS and treated with an excess of Rho 6G and (c) treatment of the same capsules with the anionic dye fluorescein and (d) after 4 h incubation time. From panel d an overall wall thickness of 350 nm was estimated.

A further remarkable result was the wall thickness of the modified capsules in solution. The wall thickness of the capsule shown in Figure 6d was about 350 nm, which confirms the model of a highly charged wall with a PSS brush on top (Figure 5).

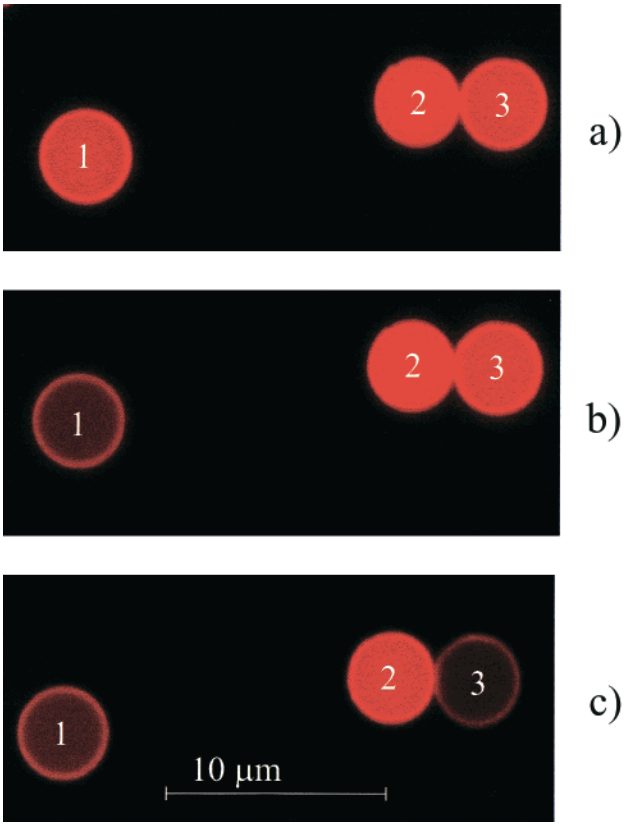

Figure 7. Photochemistry in capsules filled with 0.5 M MRho-PSS copolymer: (a) all capsules contained the same amount of rhodamine molecules; (b) capsule 1 was selectively irradiated for 2 min with the focused laser beam of the CLSM resulting in an decrease of the original fluorescence to 40%; and (c) capsule 3 was irradiated for 4 min, which decreased the rhodamine amount to 15% of the original value.

Photochemistry in Microreactors. The incorporation of copolymers containing photoactive groups such as fluorescent dyes, photoinitiators, or azocompounds allows every capsule to be selectively addressed by light and photoreactions to be induced in the small capsule volume independent of the bulk properties. The start and degree of the reaction could be controlled by the wavelength and the light intensity. This was demonstrated by photobleaching the MRho-PSS copolymer. Three capsules filled with the same amount of 0.5 M MRho-PSS (Figure 7a) were irradiated independently with the focused laser beam of the CLSM. First, capsule 1 was bleached for 2 min reducing the amount of the rhodamine to 40% (Figure 7b). In the second step, capsule 3 was irradiated for 4 min, yielding only 15% of the original fluorescence intensity. In this way the initially identical capsules could be selectively marked. The photobleaching is one of the simplest reactions, but photopolymerization[15] or catching bioactive compounds via insertion reactions of nitrenes by photodecomposition of azides[16] should work in a similar way.

Summary

The incorporation of functionalized polymers into polyelectrolyte microcapsules provides new opportunities for tuning the

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physicochemical properties of the capsule interior in a wide range. A general method for polymer incorporation is a “ship in bottle” synthesis from small monomers, which can easily permeate the capsule wall. The captured polymers modify the properties of the interior permanently independent of the bulk properties. The number of polymers in the interior correlates with the initial monomer concentration before polymerization. The incorporation of polyelectrolytes in the capsules enables the selective control of ion strength, pH, viscosity, etc. in the interior. These properties can be used for acid-catalyzed reactions or for selective nucleation and precipitation of organic acids in the capsule interior. A high concentration of polyelectrolytes increases the osmotic pressure inside the capsules to such an extent that swelling of the capsules up to 4 times in diameter occurred. If a counter-pressure from the bulk solution is applied, the capsules exhibit reversible collapsing, which might be useful in micromechanics. Due to the synthesis of polymers in and on the capsule wall, it can be strongly charged like an ion exchange membrane. The charging results in an increase in thickness. The walls become sensitive to the adsorption and permeation of organic ions. While oppositely charged ions destroy the capsule wall and release the interior material, ions of the same charge are excluded from the adsorption or penetration through the capsule wall. The labeling of the captured polymers by photoactive substances allows a selective light-guided addressing of each capsule. Various photoreactions can be induced. Due to the novel tailored properties and the almost unlimited variability of the modified capsules, these are very promising candidates for use as cages for controlled chemical reactions or for selective precipitation of solid materials in confined systems.

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