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ORIGINAL CONTRIBUTION

Manipulating the properties of coacervated polyelectrolyte microcapsules by chemical crosslinking

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Abstract The properties of coacervated sodium poly(styrene sulfonate) (PSS)/poly(allylamine hydrochloride) (PAH) microcapsules were manipulated by glutaraldehyde crosslinking at mild conditions. Although the crosslinking took place only between the PAH component, only 10% of PSS was lost from the 2-h crosslinked microcapsules. Significant variation in terms of capsule morphology, diameter, and wall thickness was not found by scanning electron microscopy and scanning force microscopy. Although all the microcapsules were not affected by annealing at 70 °C and incubation in 0.1 M HCl for 2 h, the crosslinked microcapsules indeed showed strong ability to resist osmotic-induced capsule invagination. Also, the 20-min and 2-h crosslinked capsule walls have elasticity modulii of 166 and 200 MPa, respectively, which are both larger than that of the original one (140 MPa). The crosslinked microcapsules also showed good stability in 0.01 M NaOH solution and poorer permeability for a large fluorescent probe.

Keywords Microcapsules · Polyelectrolytes · Coacervation · Crosslinking · Properties

Introduction

The capsules with a dimension ranging from nanometer to micrometer attract increasing interests because these hollow colloidal structures may find extensive applications in pharmaceutical, cosmetic, food, textile, adhesive, and agricultural industries [1–4]. Apart from the traditional methods such as nozzle reactor processes, emulsion/phase separation, and sol–gel processing [5–8], new techniques such as lipid vesicles, suspension and emulsion polymerization around latex particles, dendrimers or hyperbranched polymers for nanoencapsulation, or coacervation on interfaces have also been developed so far [6–11]. Many of these techniques involve the use of sacrificial colloid cores, i.e., deposition, adsorption, or polymerization on the hard templates followed by removal of the cores to yield hollow structures.

Layer-by-layer (LbL) assembly is a typical example of these approaches [12] in which oppositely charged polyelectrolytes are consecutively assembled onto sacrificial templates. The as-prepared microcapsules have accurately controlled size, wall composition, and thickness [13–15]. Yet, the fabricating process is rather time consuming. More recently, we have developed a polyelectrolyte coacervation method for fabrication of the hollow microcapsules [16] in which only one layer of polyelectrolyte (for example, poly(allylamine hydrochloride), PAH) is adsorbed onto sodium poly(styrene sulfonate) (PSS)-doped $CaCO_3$ particles. A subsequent core removal releases the entrapped PSS which encounters and combines with the adsorbed PAH immediately to form the microcapsule walls in situ. Therefore, the method is rather time and materials saving and can be easily scaled up.

It is known that the LbL-assembled microcapsules are susceptible to environmental stimuli such as pH [17–19] and temperature [20–25]. At extremely high pH, the PAH/PSS microcapsules are readily dissolvable. It is also observed that the microcapsules either shrink or swell upon annealing [20–25]. Since the microcapsule walls are very thin and are semipermeable to molecules, the capsules may deform their shapes in response to bulk osmotic pressure

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[26–28]. Recently, crosslinking of the LbL-assembled microcapsule walls by methods of thermal treatment [29], ultraviolet (UV) irradiation [30–32], and chemical reaction [33, 34] has been employed, yielding microcapsules with enhanced stability and mechanical strength. Recent works show that it is also possible to improve the multilayer/microcapsule stability by crosslinking just one component of the multilayers since there is a large overlap between the segments of the adjacent layers [35–37].

The structure of the microcapsules obtained by the polyelectrolyte coacervation is completely irregular compared with the "layered" structure of the LbL microcapsules. In this case, the microcapsule stability is expected to be poorer, at least not better than that of the LbL ones. Therefore, in this work, glutaraldehyde crosslinking of the polyelectrolyte-coacervated microcapsules shall be performed to improve their stability. Effects of the crosslinking on thermal, chemical, and mechanical stabilities shall be assessed in terms of microcapsule size, composition, morphology, and elasticity. This simple but effective manipulation, together with the ease of fabrication, endows the one-step adsorption technique as more competitive than others with respect to comprehensive performance and applications.

Experimental

Materials

Sodium poly(styrene sulfonate) (PSS, Mw 70 kDa), poly (allylamine hydrochloride) (PAH, Mw 70 kDa), rhodamine 6G (Rd6G), fluorescein isothiocyanate-labeled dextran (FITC-dextran, Mw 464 kDa), tetramethylrhodamine isothiocyanate-labeled dextran (TRITC-dextran, Mw 166 kDa), fluorescein isothiocyanate-labeled albumin (FITC-albumin), disodium ethylene diamine tetraacetate dihydrate (EDTA), and glutaraldehyde (GA, 25 wt.% solution in water) were all obtained from Sigma-Aldrich. All chemicals were used as received. PSS-doped $CaCO_3$ microparticles were fabricated according to [16]. The water used in all the experiments was triple distilled.

Methods

Hollow microcapsule preparation and GA crosslinking

The PSS-doped $CaCO_3$ microparticles (×0.5% *w*/*v* in suspension) were incubated in 2 mg/ml PAH/0.5 M NaCl solution for 30 min under shaking. The particles were separated from the solution by centrifugation at 500 ×*g* for 5 min followed by three washings with water. Then, the PAH-coated microparticles were treated with 0.02 M EDTA solution for 30 min under gentle shaking. The resultant capsules were separated by centrifugation at 1,500 ×*g* for 5 min followed by three washings in fresh EDTA solution. Finally, the capsules were washed with water three times. To obtain the crosslinked capsules, treatment in 2% GA solution at room temperature was performed followed by centrifugation at 1,500 ×*g* for 5 min and three washings. Variation of the absorbance during the reaction process was monitored by UV–vis spectroscopy (Shimadzu UV2550).

Stability of the capsules

To explore the thermal stability, the microcapsule suspension was incubated at 70 °C for 2 h. To test the chemical stability, the capsules were incubated in 0.1 M HCl or 0.01 M NaOH solution for 2 h.

Elasticity modulus measured by osmotic-induced microcapsule invagination

Elasticity modulus of the complex PAH/PSS walls was investigated by a method of osmotic-induced invagination [26–28]. The microcapsules were firstly covered by a layer of FITC-albumin which enables the observation of the microcapsules in PSS solution by confocal laser scanning microscopy (CLSM). The microcapsules were rapidly mixed with PSS solution of different concentration. The number of deformed and undeformed capsules was counted under CLSM. At least 200 microcapsules were counted for each PSS concentration. The deformation ratio was defined as the number of deformed capsules divided by the total number of capsules. The critical PSS concentration was defined as the concentration at which the invagination of 50% of the intact capsules occurred. The critical pressure was found by referring to a calibration curve [26–28].

Instrumental characterizations

The microcapsules were characterized by scanning electron microscope (SEM) (SIRION-100, FEI) and scanning force microscopy (SFM) (SPI3800N Probe Station, SPA400 SPM Unit, Seiko). Fluorescent images were taken with a Bio-Rad Radiance 2100 confocal laser scanning microscope. Rd6G was used to stain the microcapsules. The ζ-potentials were measured by Zetasizer Nanoinstrument Nano Z from Malvern. The UV–vis absorption spectra were recorded by a Shimadzu UV2550 UV–vis spectrophotometer. The absorbance of PSS at 225 nm was used as the inner standard to normalize the curves. Elemental analyses were carried out on a Flash EA 1112 instrument.

Results and discussion

It is known that aldehyde can react with amine rapidly to form Schiff base (−C=N−) at very mild conditions (room temperature and neutral pH) [38–40]. Figure 1a shows the UV–vis spectra of the microcapsule suspension crosslinked with GA at different times. To eliminate the influence of microcapsule amount and light scattering, here all the spectra are normalized to the absorption of PSS at 225 nm. It shows that the absorbance at 250–350 nm increased along with the treatment time due to formation of the Schiff base structure. Figure 1b presents the maximum absorbance at 270 nm as a function of reaction time. It shows a faster increase during the initial 20 min, then a slower increase till 2 h. This would mean that the crosslinking degree of the microcapsules can be roughly modulated by the reaction time. Therefore, the microcapsules crosslinked for 20 min and 2 h shall be used for further studies.

Elemental analysis recorded a N:C:S weight ratio of 3.89:45.57:11.60 for the microcapsules crosslinked for 20 min, manifesting that the amount of the incorporated GA in the microcapsules was 6%. After crosslinking for 2 h, a N:C:S weight ratio of 4.32:46.58:11.58 was found. This confirms that the amount of GA does not further increase. Since GA reacts only with the amine group of PAH, it is reasonable to assume that the amount of PAH during the crosslinking keeps constant. Thus, in comparison with that of the original PSS/PAH complex microcapsules (the weight ratio of N:C is 4.05:46.55), the PSS amount did not change for the microcapsules crosslinked for 20 min, whilst it was reduced to 90% for the microcapsules crosslinked for 2 h. It has to be noted that the loss of PSS may cause more or less inaccuracy of the UV–vis analysis, yet it is not obvious since the value is within a small range.

The ζ-potentials of the microcapsules were measured as −33±8.3 mV, −26.2±3.2 mV, and −18.3±4.4 mV for the original ones and those crosslinked for 20 min and 2 h, respectively. This implies that the crosslinking weakens the negative charge on the microcapsule surfaces. Since the PSS amount does not change after 20-min crosslinking, the reason why the negative charge decreased is not clear. One possible reason could be that the reaction between the GA and PAH may cause the reorganization of the capsule wall, leading to the exposure of the PAH component. Further reaction causes the partial release of PSS, most possibly from the very surface layer, resulting in the reduction of the surface negative charge. The release of PSS should be caused by the diminishment of electrostatic interaction, since GA crosslinking reduces the number of amine groups in the capsule wall.

Figure 1c presents the shape persistence ability of the microcapsules in response to osmotic pressure of the bulk solution. While both of sigmoidal curves of the crosslinked microcapsules shifted to higher PSS concentration than that of the original one [16], the longer crosslinked microcapsules have a stronger ability against bulk osmotic pressure. By fitting the curves in a sigmoidal manner and analyzing them with a tri-tangent method [26–28], the critical PSS concentrations, at which the shape transition occurs, were found to be 0.33 and 0.41 wt.% for the microcapsules crosslinked for 20 min and 2 h, respectively. The critical pressures, P_c, are thus 2.6×10^4 and 3.2×10^4 N/m^2 respectively by referring to a standard osmotic pressure curve of PSS [26–28].

Figure 2 presents the SEM and SFM images of the original microcapsules (Fig. 2a and b) and 2-h crosslinked microcapsules (Fig. 2c and d). No difference in the capsule morphology can be observed. Further quantification found no difference in the capsule diameter and wall thickness either, regardless of the crosslinking time. Therefore, we can use the same capsule diameter d (7.5±0.8 μm) and wall thickness h (18 nm) to calculate the wall elastic modulus (μ)

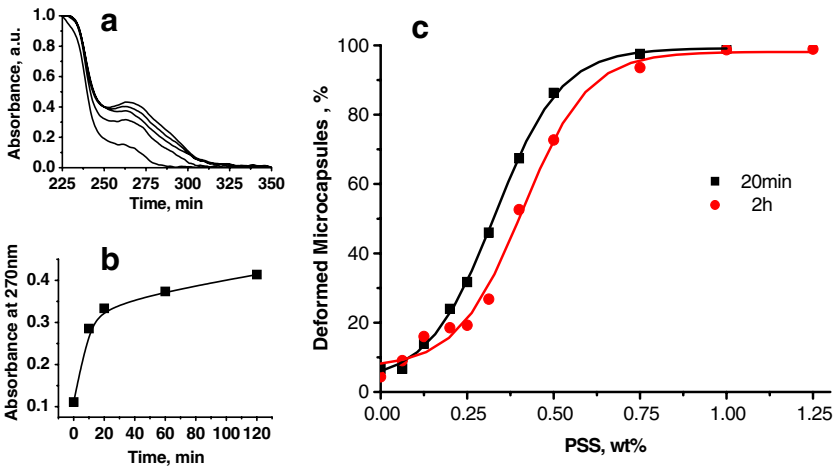

Fig. 1 **a** UV–vis spectra of PSS/PAH microcapsules treated with 2% GA at different times (0, 10, 20, 60, and 120 min from bottom to top, respectively). **b** The absorbance maxima at 270 nm as a function of GA treatment time. **c** Percentage of deformed microcapsules as a function of PSS concentration. *Filled squares* and *circles* represent the crosslinking time of 20 min and 2 h, respectively

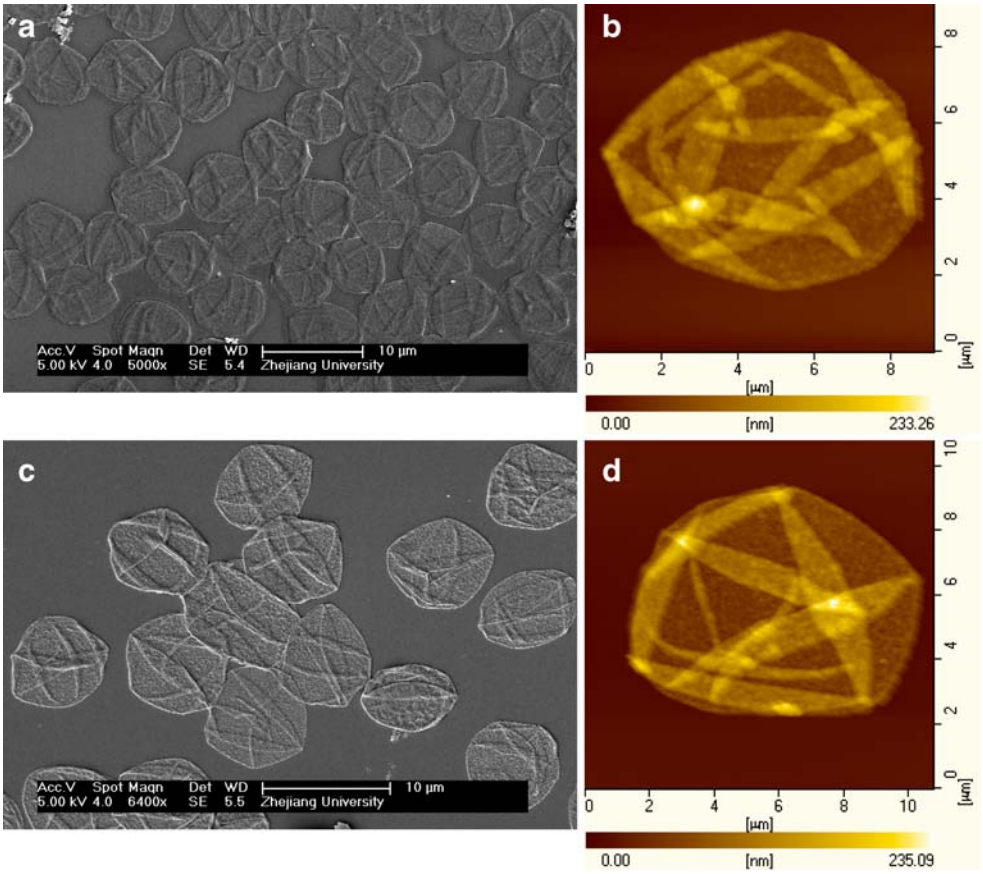

Fig. 2 SEM (**a**, **c**) and SFM (**b**, **d**) images of (**a**, **b**) uncrosslinked and (**c**, **d**) crosslinked microcapsules. Crosslinking conditions: 2% GA for 2 h at room temperature

according to $\mu=(1/4)P_c(d/2h)^2$ [26–28]. It is known that the wall thickness in a wet state should be 1.4 times the value in a dry state owing to the shell swelling nature [37]. Therefore, the elasticity modulii of the microcapsule walls are found to be 166 and 200 MPa after 20-min and 2-h crosslinking, respectively. They are both considerably larger than that of the original one (140 MPa) [16], demonstrating the enhancement of the wall strength. However, the improvement degree (200/140=1.4 for 2 h crosslinking) is much lower than that of its orderly assembled and GA-crosslinked PSS/PAH counterpart (680/290=2.3) [37]. Moreover, we observed that the double-wall thickness of the GA-crosslinked $(PSS/PAH)_5$ microcapsules is about 50% higher than that of the original ones in a dry state [37]. All these results imply that the complex structure is softer than that of the orderly assembled multilayers regardless of the crosslinking. The relatively less crosslinkable amount of PAH (26 wt.% in the coacervated microcapsules and 40 wt.% in the multilayer microcapsules, respectively [41, 42]) might be an important reason. Moreover, the elasticity modulus of the 2-h crosslinked coacervated microcapsules is not only lower than that of the crosslinked LbL microcapsules but also lower than that of the uncrosslinked LbL ones. This fact can explain why no difference in capsule morphology and wall thickness was observed between the uncrosslinked and the crosslinked coacervated microcapsules.

Both the original and the crosslinked microcapsules were subjected to annealing at 70 °C for 2 h (Fig. 3a and b). No variation of the capsule size and composition (by elemental analysis) was found regardless of the crosslinking and crosslinking time. It has been reported that the PSS/PAH multilayer capsules undergo significant heat-induced shrinkage and wall thickness increases under the same treatment conditions [20]. After crosslinking with GA, the capsule shrinkage is completely inhibited [37]. This discrepancy is exclusively caused by their capsule structures. Köhler et al. found that the shrinkage of PSS/poly (diallyldimethylammonium chloride) microcapsules is governed by the even or odd number, i.e., shrinking and swelling, respectively [23, 24]. Intrinsic reason for the geometric change is related to the charge repulsion and building manner. Unlike the layered structure, charge balance within the complex walls is well achieved. Furthermore, according to the fabrication procedure, most of the PAH molecules should be embedded spatially in between the outer and inner PSS layers though no distinct "layer" can be definitely figured out as that of the multilayer microcapsules [16, 37]. We believe that this

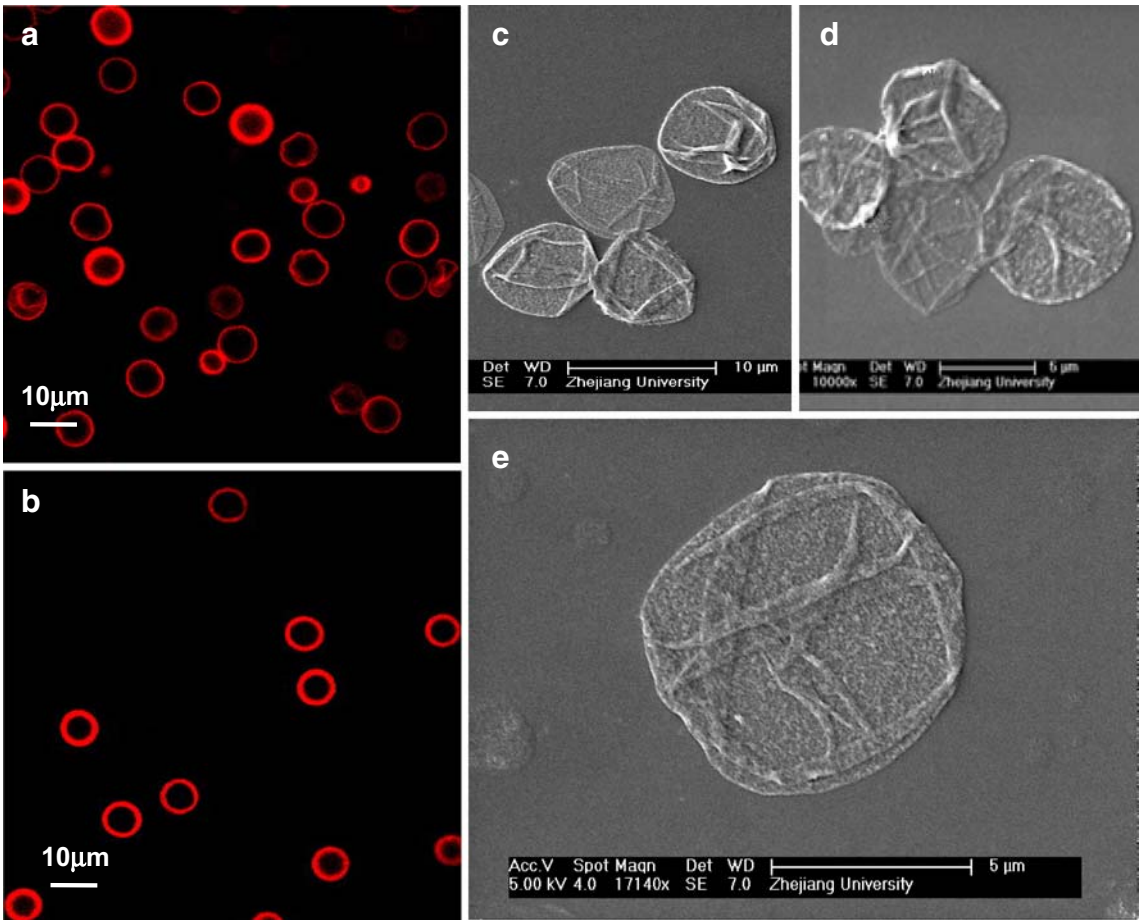

Fig. 3 CLSM images of uncrosslinked (**a**) and crosslinked (**b**) microcapsules after treated at 70 °C for 2 h. SEM images of uncrosslinked microcapsules (**c**) and crosslinked microcapsules (**d**) after incubation in 0.1 M HCl for 2 h. **e** SEM images of crosslinked microcapsules after incubation in 0.01 M NaOH for 2 h

"symmetric" structure is less sensitive to the thermal stimuli, which facilitates the release of stress such as charge repulsion and entropy-favored order to tangle transition.

Although the stability of the microcapsules at low pH has no difference, at high pH, however, the stability is significantly different before and after crosslinking. Both the crosslinked and the original microcapsules can survive through 0.1 M HCl for at least 2 h (Fig. 3c and d). After this treatment, both kinds of the microcapsules can disperse in water very well and keep their intact structures.

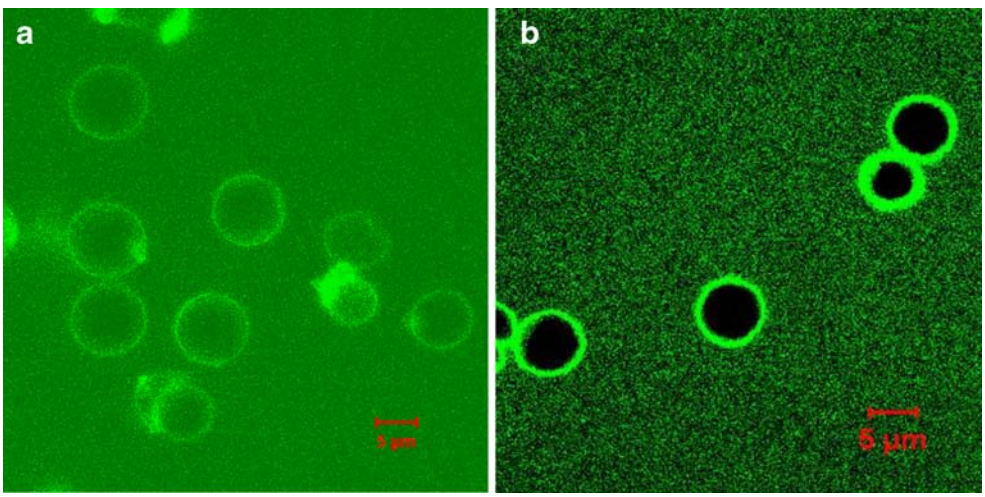

Fig. 4 CLSM images of uncrosslinked (**a**) and 2% GA 2-h crosslinked capsules (**b**) after incubation in FITC-dextran (Mw 464 kDa) for 20 min

Elemental analysis revealed that only a very little amount of PSS (3%) was released from the original microcapsules, whereas no PSS loss occurred for the 2-h crosslinked ones. Upon being treated with 0.01-M NaOH solution, however, the uncrosslinked microcapsules were quickly dissolved within 30 s. The reason is the deprotonation of PAH, leading to the disappearance of the charge interaction between the PSS and PAH components. The microcapsules crosslinked for 20 min could survive longer, whereas those crosslinked for 2 h could survive for at least 2 h (Fig. 3e). However, elemental analysis still detected that a large amount of PSS (33%) was released. GA crosslinking produces covalent bonds between the PAH chains which form a 3-D meshwork with a dense and stable structure even at high pH. Since PSS and PAH are intertwined with each other below a molecular level (a segment level, for example), the physical entanglement in this 3-D meshwork becomes strong enough to slow down or even retard the release of the PSS molecules, at least some of them. The relatively fast released PSS should be the part which is less entangled with the PAH molecules.

To detect variation of the meshwork of the capsule walls brought by crosslinking, permeability of the microcapsules was qualitatively studied by CLSM. As shown in Fig. 4a, the capsule interiors became as bright as the bulk solution, revealing that the original capsule walls are permeable to FITC-dextran with a molecular weight of 464 kDa. After crosslinking for 2 h, however, the capsule interiors remain dark, implying that the probe molecules are not obviously permeable at least within 20 min (Fig. 4b). While both the original and the 2-h crosslinked capsules are permeable to TRITC-dextran with a molecular weight of 166 kDa (images not shown), it can be clearly seen that the crosslinked capsules are less permeable to larger molecules. The initial permeability for the large dextran molecules is attributed to the coacervated wall structure, which is less ordered and possesses larger defects. Crosslinking may partially rearrange the polymer chains and reduce or even seal the large defects. Hence, the permeability of larger molecules is retarded.

Conclusions

In conclusion, the properties of the PSS/PAH microcapsules fabricated by polyelectrolyte coacervation can be effectively improved by GA crosslinking. The Schiff base amount, or namely the crosslinking degree, increases along with the incubation time. Also, 6% of GA is incorporated into the capsule walls after crosslinking for ≥20 min. The weight loss of PSS reaches 10% after crosslinking for 2 h. The crosslinked microcapsules show stronger ability to resist invagination caused by osmotic pressure. Yet, these variations do not bring significant influence on capsule morphology and geometric parameters such as diameter and wall thickness. Quantitative analysis confirms that the elasticity modulii of the capsule walls increase from 140 MPa (the original one) to 166 MPa (the 20-min crosslinking one) and further to 200 MPa (the 2-h crosslinking one). The microcapsules have a good ability to resist thermal and acid treatments regardless of the crosslinking, yet the original microcapsules are readily dissolvable within 15–30 s in 0.01 M NaOH solution. By contrast, the 2-h crosslinked microcapsules can survive in the same solution for at least 2 h, although up to 33% loss of PSS is found. Another effect brought by crosslinking is the decrease of permeable molecular size, e.g., permeable and impermeable for FITC-dextran with a molecular weight of 464 kDa before and after crosslinking, respectively. Therefore, the GA crosslinking can easily manipulate the properties of the coacervated microcapsules which are important in improving the performance of these hollow structures. This simple but effective manipulation, together with the ease of fabrication, endows the one-step adsorption technique more competitive with other capsule fabrication approaches.

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