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Thermal treatment of galactose-branched polyelectrolyte microcapsules to improve drug delivery with reserved targetability

Fu Zhang, Qi Wu, Li-Jun Liu, Zhi-Chun Chen, Xian-Fu Lin *

Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China

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Abstract

A novel multilayered drug delivery system by LbL assembly of galactosylated polyelectrolyte, which is possible to have the potential in hepatic targeting by the presence of galactose residues at the microcapsule's surface, is designed. Thermal treatment was performed on the capsules and a dramatic thermal shrinkage up to 60% decrease of capsule diameter above 50 °C was observed. This thermal behavior was then used to manipulate drug loading capacity and release rate. Heating after drug loading could seal the capsule shell, enhancing the loading capacity and reducing the release rate significantly. Excellent affinity between galactose-binding lectin and heated galactose-containing microcapsules were observed, indicating a stable targeting potential even after high temperature elevating up to 90 °C.

Keywords: Drug encapsulation; Galactose; Layer-by-layer; Microcapsules; Thermal properties

1. Introduction

All the modern drug delivery systems at least include two requisite functions: controlled drug release and drug targeting, by which the desired curative effect is enhanced, whilst the pharmaceutical toxicity to other healthy organs or tissues can be reduced (Sutton et al., 2007). Polymer–drug complexes, hydrogels, lipid vesicles, and amphiphilic micelles have shown great usage in drug delivery areas (Moreno-Villoslada et al., 2005; Serpe et al., 2005; Greish et al., 2004; Lo et al., 2006).

Recently, many efforts have been devoted for investigating the possibility of the microcapsules fabricated on removable templates with layer-by-layer (LbL) technique for the usage of controlled release, owing to the facile preparation, variety of potential layer compositions, and possibility to tune the thickness in the nanometer (Sukhorukov and Möhwald, 2007; De Geest et al., 2007; Johnston et al., 2006; Kharlampieva and Sukhishvili, 2006). Berg et al. (2006) successfully loaded ketoprofen and cytochalasin D into nanoporous films and a zero-order release kinetics was observed over a period of many days. Zelikin et al. (2006) reported a facial method to deliver DNA with high concentrations by degradable LbL capsules. Ma et al. (2005) incorporated polymer micelles as building blocks for LbL assembly to encapsulate and to release water-insoluble dyes.

Consequently, there is a particularly important need for understanding the internal structure of microcapsules as well as the response of these films to external stimuli influencing the interaction between the oppositely charged polyelectrolytes. Many methods have been demonstrated to manipulate the properties of the capsules especially the permeability, by exposing them to different pH values (Déjugnat and Sukhorukov, 2004), ionic strengths (Antipov et al., 2003), different salts (Heuvingh et al., 2005), solvents of different polarities (Kim et al., 2005), crosslinking agents (Tong et al., 2005) and different temperatures (Glinel et al., 2003), to get better usage in modern drug delivery system. Several researches have described the thermal behaviors of microcapsules with no thermosensitive blocks (Gao et al., 2003; Köhler et al., 2004, 2006). Shells terminated with poly(styrenesulfonate) (PSS) or poly(allylamine) (PAH) shrink upon heating, whereas poly(diallyldimethylammonium chloride) (PDADMAC)-terminated ones swell. Recently, Ye et al. (2006) reported a novel two-temperature loading procedure to increase the insulin loading capacity and slow down its release rate in chitosan/alginate (CHI/ALG) microcapsules. Doping charged materials such as PSS (Liu et al., 2005; Wang et al., 2006) inside the preformed hollow capsules are also a convenient

* Corresponding author. Tel.: + 86 571 87953001; fax: +86 571 87952618.
E-mail address: llc123@zju.edu.cn (X.-F. Lin).

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way to enhance loading from bulk solution. Zhu et al. (2005) reported a spontaneous loading technique for encapsulating positively charged molecules in alginate-templated PAH/PSS microcapsules. Nayak and McShane (2007) carried out the polymerization of acrylic acid monomers within the capsule cavity to form an anionic interior and encapsulation of large quantities of cationic horseradish peroxidase by electrostatically driven attraction.

Multilayers made of PSS, PAH, PDADMAC, CHI and ALG have been the most widely used systems in the pharmaceutical area to encapsulate drugs (Qiu et al., 2001; Tiourina and Sukhorukov, 2002; Zhu et al., 2005; Liu et al., 2005; Wang et al., 2007). However, there is a need for biologically targeted surface modifications of the capsules to control specific and unspecific adhesions to biological organisms (Sukhorukov and Möhwald, 2007). Functional ligands were introduced to microcapsule systems to achieve desired specific properties. Magnetic particles (Kim et al., 2006), near-infrared (NIR) light responsive materials (Skirtach et al., 2006), copolymers (Greish et al., 2004), peptides (Toublan et al., 2006), proteins (Benkirane-Jessel et al., 2005) and antibodies (Cortez et al., 2006) can be embedded in multilayers to selectively release target drugs. Besides the above mentioned targeting approaches, D-galactose, a well-known targeting molecule directing to the hepatic cells through strongly binding with large numbers of asialoglycoprotein receptors which are exclusively expressed by liver parenchymal cells (Hashida et al., 1997; Schneider et al., 2006), has been effectively incorporated into the multilayers by LbL assembly of galactosylated polyelectrolyte by our group (Wu et al., 2006; Zhang et al., 2006, 2008). The strong potential of such multilayers in the application of modern drug delivery systems with controlled drug release and hepatic targeting is expected. However, their properties need to be further investigated, and especially the response of the mutilayers to external stimuli such as heating is attractive in order to adjust the internal structure suitable for the better drug encapsulation, sustained release, and effective targetability.

In this work, we studied the thermal behavior of galactose-branched polyelectrolyte multilayered microcapsules, and found that the thermal treatment on the capsules induce a dramatic shrinking up to 60% decrease of capsule diameter above 50 °C. Such thermal behavior was used to realize enhanced loading and effectively controlled release of a nonselective beta-blocker, propranolol hydrochloride (PRH) (Hayes et al., 1987; Garcia-Pagan et al., 2003). Stable affinity between capsules and lectin after heat-treating was also confirmed (Fig. 1).

2. Materials and methods

2.1. Materials

Poly(sodium 4-styrenesulfonate) (PSS, M_W 70 000) (Sigma–Aldrich), 2-(metharcyloyloxy) ethyltrimethylammonium chloride (DMC, 72% aqueous solution) (Alfa Aesar), propronolol hydrochloride (PRH, Xingyuan chemical plant, Jurong, Jiangsu, P.R. China), fluorescein labeled peanut agglutinin (FL-PNA, β-galactose-binding lectin) (Vector, USA). Other chemicals were of the finest grade available.

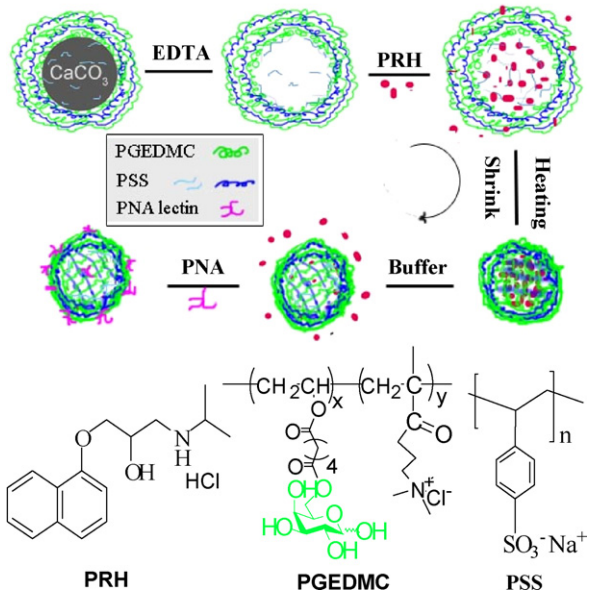

Fig. 1. Schematic diagram of enhanced drug delivery and reserved lectin-affinity by galactose-branched polyelectrolyte microcapsules via thermal treatment.

2.2. $CaCO_3$ preparation

Spherical microparticles of $CaCO_3$ were prepared by precipitating the Ca^{2+} and CO_3^{2-}. 100 mL of 0.025 M $Ca(NO_3)_2$ solution were mixed with equal volume of Na_2CO_3 solution (0.025 M, containing 0.2 g PSS) under intensive ultrasonication for 10 s. After standing, the liquid was carefully decanted. The precipitated particles were resuspended in ethanol by ultrasonication and collected by membrane filtration. Particles with different diameters were obtained by varying standing time from several minutes to several hours.

2.3. Polycation preparation

Vinyl hexanedioyl galactose ester monomer was regioselectively synthesized by the transesterification of galactose with divinyl hexanedioate catalyzed by Alkaline protease from *Bacillus subtilis* in pyridine at 50 °C (Wu et al., 2001). Poly(vinyl galactose ester-*co*-methacryloxyethyl trimethylammonium chloride) (PGEDMC, containing 19-mol% galactose residues, M_w 54 200, $M_w/M_n = 2.4$, GPC data) was prepared by copolymerization of vinyl hexanedioyl galactose ester with 2-(metharcyloyloxy) ethyltrimethylammonium chloride using redox initiator in H_2O (Zhang et al., 2006, 2008).

2.4. Hollow capsule fabrication

The hollow capsules used for drug encapsulation in this article were prepared according to the procedure of literature (Sukhorukov et al., 2004). Briefly, PGEDMC and PSS multilayers were coated on $CaCO_3$ particles by alternate assembly

with oppositely charged polyelectrolytes (2 mg/mL, containing 0.5 M NaCl). The $CaCO_3$ cores were then removed by 0.2 M EDTA (pH 7.0 by adding NaOH), and the hollow capsules were centrifuged at 5000 rpm for 5 min and rinsed with water three times.

2.5. Thermal treatment

For heating, 120 μL of hollow capsule suspensions were dispersed under short ultrasonication in 1 mL Eppendorf tube and incubated at desired temperatures in a thermostatic water bath for 30 min. The morphology of the capsules was measured by SEM.

2.6. Enhanced drug loading and sustained release

200 μL of hollow capsules suspensions ($\sim 2 \times 10^8$ capsules, counted by using a cell counting chamber under the microscope) was centrifuged and the supernatant was removed. 1 mL of PRH solution was added and the mixture was gently agitated for 1 h at 25 °C. Then the mixture was incubated at higher temperatures (50 or 70 °C, 25 °C for control) for another 1 h. After the above thermal treatment, the capsules were centrifuged and rinsed with water to remove the excess drug. All the supernatants were collected to measure the unloaded drug amount by UV–vis at 289.5 nm. The morphologies and double-wall thicknesses of microcapsules before and after PRH loading were recorded by TEM and AFM, respectively.

The release of encapsulated PRH was followed by UV–vis. PRH loaded capsules was dispersed in pH 7.4 PBS solution (0.02 M) with a final volume of 1 mL at 37 °C under gentle shaking (100 rpm). 0.5 mL of the supernatant was taken out each time from the release system by centrifugation and the concentration was determined at 289.5 nm, whilst same volume of fresh buffer solution was rapidly supplemented.

2.7. Reserved lectin recognition

Different multilayers were assembled on silica slides (1 cm × 2 cm) (methods see Supporting Information) and $CaCO_3$ microparticles using LbL technology. Keeping PGEDMC or PSS as the outmost layer, the slides and the capsules after core removal were incubated in 200 μg/mL fluorescence labeled PNA lectin solution for 1 h at 25 °C. Here the hollow capsules were preheated at 30, 50, 70 and 90 °C for 0.5 h before incubation with lectin. The substrates were rinsed with PBS several times to remove uncombined lectin. Fluorescence microscopy was employed to characterize lectin recognition results.

2.8. Characterization

A Shimadzu UV2550 UV–vis spectrophotometer was used to measure the release amount of encapsulated PRH from microcapsules at 289.5 nm.

The microcapsules were loaded on a carbon coated 200 mesh copper grid and observed under a transmission electron microscope (TEM, JEM 200CX) at 100 kV electron beam accelerating voltage. Typically, 15 μL of sample suspension was carefully dropped on the grid, the extra solution was then allowed to air dry for 12 h.

Samples were prepared by loading 20 μL of the capsule suspension onto a glass slide freshly cleaned by a mixture of H_2SO_4 and H_2O_2 (3:1, v/v). For samples with negatively charged surface, a precursor of PGEDMC was coated onto the glass before sample loading. After vacuum drying overnight, the samples were sputtered with a thin gold layer and measured by scanning electron microscopy (SEM) using a SIRION-100 instrument (FEI, USA) at the acceleration voltage of 5.0 kV.

The double-wall thickness of microcapsules was recorded in air at room temperature using a Nanoscope IV multimode AFM (Veeco, Santa Barbara, CA). The used silicon cantilever was 125 μm in length with a resonance frequency of 325 kHz and the images were acquired in a constant force mode at a scan rate of 1 Hz. The images were processed with Nanoscope software and the average height of the capsule was obtained by section analysis. Samples were prepared by dropping 30 μL of a capsule suspension onto a freshly cleaved mica surface and dried.

The fluorescence images of microcapsules were taken by a Zeiss LSM 510 scanning device (Zeiss, Germany) mounted on a Zeiss Axiovert 100 inverted microscope equipped with external argon laser (for excitation at 488 nm). Observations were taken by using a 40× water immersion objective with a numerical aperture of 1.75 for capsules under fluorescence plus transmission mode. Typically, 20 μL of lectin adsorbed capsule suspension was dropped on a freshly cleaned glass slide and a cover glass was coated before observation.

The lectin processed quartz slide samples were digitally scanned as 2D images using a Typhoon-9200 fluorescence image scanner (Amersham Phamacia Biotech, USA) and were then analyzed using ImageMaster software (Amersham Pharmacia Biotech) automatically. Notably, herein the fluorescence signal of FL-PNA was output in orange color, and the background of the slide was shown as green color.

3. Results and discussion

3.1. Preparation of galactosylated polyelectrolyte microcapsules

To provide a polyelectrolyte having high potential targetability to hapatoma cells, the polycation PGEDMC carrying galactose units was synthesized by copolymerization of vinyl hexanedioyl galactose ester with 2-(metharcyloyloxy) ethyltrimethylammonium chloride (^{1}H NMR of PGEDMC see Supporting Information). We have reported that PGEDMC was well alternated with PSS on planar substrates and polystyrene (PS) particles (Zhang et al., 2006). PS template has good dispersity and spherical shape but the organic solvents such as tetrahydrofuran have to be employed to remove it, and usually the core removal process takes long period. There is an ongoing search for new templates with high surface charge density, easily decomposing property and nice biocompatibility. And recent studies have shown that the $CaCO_3$ template fulfills many of

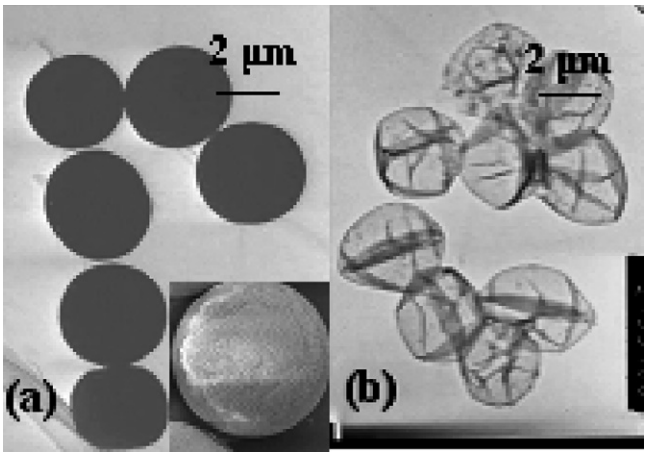

Fig. 2. TEM images of dried (a) $CaCO_3$ templates and (b) hollow capsules of $(PGEDMC/PSS)_{4.5}$ made on 2.8 μm $CaCO_3$. The inset in (a) shows the SEM image of a typical $CaCO_3$ particle.

the desirable properties (Sukhorukov et al., 2004). The uniform and homogeneously sized spherical $CaCO_3$ microparticles were obtained by direct mixing soluble salts of Ca^{2+} and CO_3^{2-}. The TEM image in Fig. 2a shows the $CaCO_3$ particles so prepared with good dispersity and an average diameter of 2.8 μm, which can result in a short and "clean" core removal by EDTA solution. SEM image in Fig. 2a inset shows that the surface is very rough, and it was reported that the internal structure was canal-like and the average pore size was about 35 nm, which facilitated the adsorption of deposited polyelectrolytes (Volodkin et al., 2004). However, these referenced pore structures could not be observed here by TEM measurement, since the cavity did not impenetrate the whole particle and the electronic beam was not able to penetrate a thickness of micrometer scale.

Hollow microcapsules were obtained after repeated EDTA treatments. $CaCO_3$ core was easily dissolved and the small solutes could penetrate the shell wall. TEM measurements were performed to investigate the inner structure of hollow capsules. Fig. 2b shows that the capsule integrity is kept, and the folds and drapes are formed due to the collapse of capsule shell in the drying process. Here, $(PGEDMC/PSS)_{4.5}$ represents that the capsule consisting of four bilayer of (PGEDMC/PSS) and one monolayer of PGEDMC, the outermost layer of which is PGEDMC. Similarly, $(PGEDMC/PSS)_4$ represents that the capsule consisting of four bilayer of (PGEDMC/PSS), the outermost layer of which is PSS. The "clean" and intact interior thus created a capacity for next drug loading.

3.2. Thermal treatment

The effect of heating treatment on the morphology of hollow shells, which was composed of $(PGEDMC/PSS)_4$ and $(PGEDMC/PSS)_{4.5}$ multilayers assembled on negatively charged $CaCO_3$ particles, was investigated. A larger core with average diameter of 15.6 μm was used in this part in order to facilitate the observations since the initial size of capsules did not influence the general thermal behavior of capsule shells (Köhler et al., 2006). Hollow capsule suspensions were incubated in sealed vessels at 30, 50, 70, and 90 °C for 0.5 h, respectively. The corresponding SEM micrographs of dried $(PGEDMC/PSS)_4$ capsules before and after heat treatment can be seen in Fig. 3a–e. The initial 15.6 μm-capsules collapse during interior water evaporation forming a flat structure with folds and aggregates, and have a remarkable surface roughness (Fig. 3a). The capsules keep their original shape after heating but shrink gradually. A reduction of diameter from 15.6 μm to approximately 5 μm can be observed when heating to 90 °C. However, capsules shrunk less at lower temperature, where the shrinkage percent is less than 10% below 50 °C (see Fig. 3f). Higher temperature treating results in a distinct shrinkage of around 70%, and capsules have reached the final size at above 70 °C. At the endpoint of shrinkage smooth and dense particles with spherical shape are observed compared to a somewhat rougher and flatter surface below 50 °C, indicating a more rigid shell that overcomes collapse during drying, which points to a healing of defects within multilayered shell structure.

Fig. 4a–e shows a similar correlation between temperature and shrinkage of capsules terminated with positively charged PGEDMC layer. The overall morphology of capsule changes little still showing many folds and rough surface when treating temperature is below 50 °C. However, when higher temperature (>50 °C) reaches, the diameter dramatically decreases almost 60% of untreated ones (see Fig. 4f). It can be seen obviously the samples exhibit bulky spherical shape and smoother surface after the end size is more or less reached.

Up to now, two model polyelectrolyte systems have been discussed in detail on their thermal behaviors mainly by Köhler and Sukhorukov (Köhler et al., 2004, 2006). Two polycations, PDADMAC and PAH, assembled with PSS, respectively, exhibited oppositely during heating. In PDADMAC/PSS capsules, when PSS formed the outer layer their walls shrunk at elevated temperatures, while PDADMAC-terminated shells swelled until rupture. First it was considered as the effect of odd and even layer number (Köhler et al., 2005) but further investigation by the same group showed that either a swelling or a shrinkage in water upon heating depended on the polyelectrolyte forming the outer layer but not depending on the total number of layers (Köhler et al., 2006). Surface overall charge either surface tension resulted in an expansion or shrinkage of the capsules. PSS terminated capsules were more electroneutral, thus the shrinking was mostly driven by minimization of the water–polymer interface. The polyelectrolyte chains softened with heating and the network rearranged to a structure (i.e., spheroid) with less surface energy. Same thermal behavior was observed in PAH-capped PSS/PAH pairs that a drastic shrinkage was achieved after incubation at 120 °C (Köhler et al., 2004).

In our system, PGEDMC/PSS deposition favored a linear growth regime after the full coating of substrates (the initial two bilayers), and the polycation was not fully charged due to an uncharged side chain (galactose-containing) which led to a nearly neutral overall charge (see Supporting Information). Hence the dominant driving force tended to be an unfavorable polymer–solvent interaction upon heating, resulting in shrinkage and rearrangement of capsules terminated with PGEDMC or PSS. However, there were too few points to really reveal the

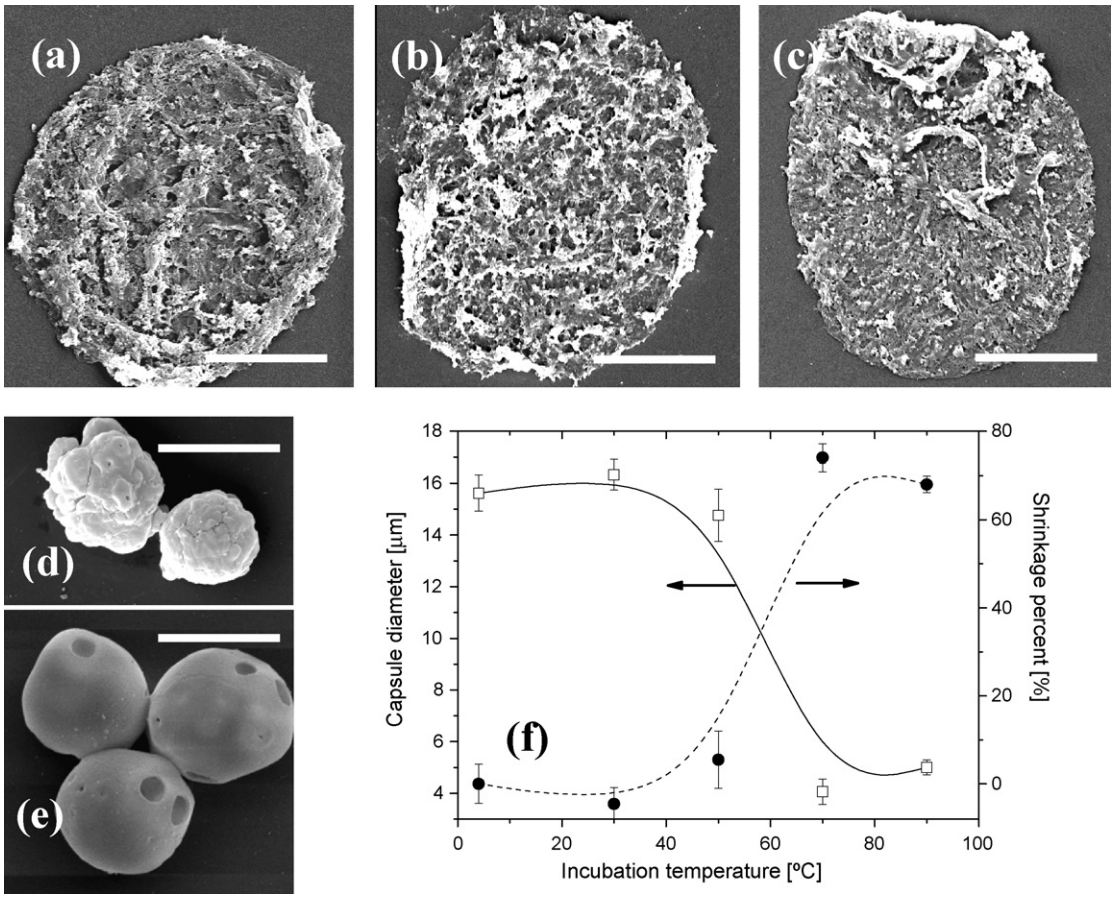

Fig. 3. SEM images of dried $(PGEDMC/PSS)_4$ polyelectrolyte capsules capped with PSS made on 15.6 μm $CaCO_3$ cores before (a) and after heating at (b) 30, (c) 50, (d) 70, and (e) 90 °C for 0.5 h. The scale bar is 5 μm. (f) is the average diameter of $(PGEDMC/PSS)_4$ capsules (left axis) and the corresponding capsule shrinkage percent (right axis) as a function of temperature after 0.5 h of incubation.

thermal response regimes of more polyelectrolyte capsule systems, and further research would be taken to explain the behavior after heating.

3.3. Enhanced drug loading and controlled release from capsules

Hydrophilic drugs were difficult to be used as templates because of the large loss during assembly cycles, and they could be loaded from feeding solution into pre-formed hollow capsules. The encapsulation and release of compounds from polyelectrolyte microcapsules are governed by a variety of conditions. By changing the permeability of capsule walls with different surrounding pH, temperatures, electrolytes and solution polarities, or using residues left inside the capsule, many molecules can be temporarily attracted inside the capsules and then sustained release into the media (Sukhishvili, 2005). Herein we simply used spontaneous deposition of water-soluble drugs from bulk drug solution to load a model drug of propranolol hydrochloride (PRH) by using PSS-doped $CaCO_3$ templates as reported elsewhere (Liu et al., 2005). The hollow microcapsules were stored in water for several days and rinsed before use to exclude any possibility of doped micro-PSS release together with PRH during the next processes. Owing to the electrostatic attraction between negatively charged PSS residual inside the capsule and the positively charged PRH molecule in bulk solution, the drug could be largely and temporarily concentrated into the capsule interior. The encapsulation was first observed under TEM after 2 h incubation in 1 mg/mL PRH solution at 25 °C. As shown in Fig. 5, the capsule walls are intact, and the typical nature of polyelectrolyte capsules with folds and creases is visualized. However, compared to hollow capsules before drug encapsulation in Fig. 2b, the capsule interior is not as "clean" as before but with shadows and lots of aggregates especially inside the capsule. This TEM image is a qualitative proof which indicates that the microcapsules were filled with drugs. AFM studies show that the hollow capsules of $(PGEDMC/PSS/)_{4.5}$ have a double-wall thickness of 30.5 ± 4.4 nm, and after drug loading, the thickness increases to 108.2 ± 15.5 nm (images shown in Supporting Information). PRH encapsulation resulted in a z-height raise of about 78 nm on the double-wall thickness.

The previous part of our study had shown that the microcapsule shells would shrink and become more compact after heating. Here, this temperature-responsive behavior was applied for drug loading and sustained release. The hollow capsules of $(PGEDMC/PSS)_{4.5}$ were first incubated in 1 mg/mL PRH bulk solution for 1 h at 25 °C which allowed a drug distribution balance between the capsule interior and exterior. Then they

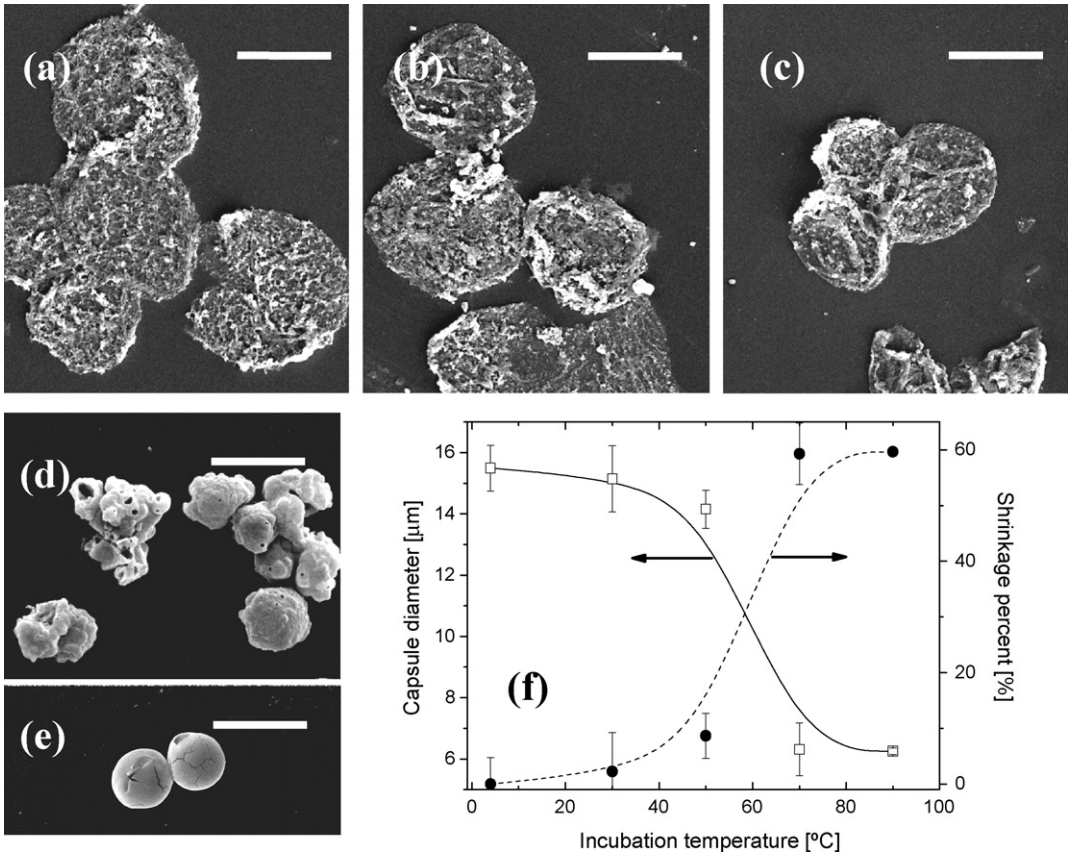

Fig. 4. SEM images of dried $(PGEDMC/PSS)_{4.5}$ polyelectrolyte capsules capped with PGEDMC made on 15.6 μm $CaCO_3$ cores before (a) and after heating at (b) 30, (c) 50, (d) 70, and (e) 90 °C for 0.5 h. The scale bar is 10 μm. (f) is the average diameter of $(PGEDMC/PSS)_{4.5}$ capsules (left axis) and the corresponding capsule shrinkage percent (right axis) as a function of temperature after 0.5 h of incubation.

were incubated at elevated temperature for another hour. Fig. 6a presents the concentration interior per capsule after 1 h heat treatment. The PRH loading capacity increases from 4.92×10^8 to 7.66×10^8 molecules per capsule when the incubating temperature is elevated from 25 to 70 °C. Considering the interior volume per capsule of 1×10^{-11} mL calculated from the average diameter of 2.8 μm and a total capsule number of 2×10^8 per sample, the PRH loading capacity was approximate to 24 mg/mL at 25 °C and 38 mg/mL at 70 °C, which were 30–48 folds of the bulk concentration. On the other hand, the average capsule diameter was much less than 2.8 μm after heating, thus the total volume of capsules in the same sample was decreased. If we calculated the concentration interior from the diameter value of shrunk capsule, the drug loading capacity would be revalued to 42 mg/mL at 50 °C and 557 mg/mL at 70 °C. Temperature elevating not only accelerated the molecular motion of PRH penetrating, but much more dominantly, it triggered the polyelectrolyte to shrink. During the rearrangement of capsule shell, more PRH nearby the microcapsule surface or intertwined within the shell layers were engulfed inside the reshaped compartment. Increased wall thickness of capsules along with smoothened and densified multilayer shell led to a reduced film permeability, and the loss of encapsulated drug during centrifugal separation and rinsing was largely suppressed. Therefore, the loading capacity was gradually enhanced by the next thermal treatment. Although this procedure provides a relatively simple way to increase the drug loading capacity of LbL system, one needs to understand what kind of thermo-response the microcapsule shows before heat manipulation. The system with PDADMAC as the outmost layer is thus not suitable to use this method, since the capsule

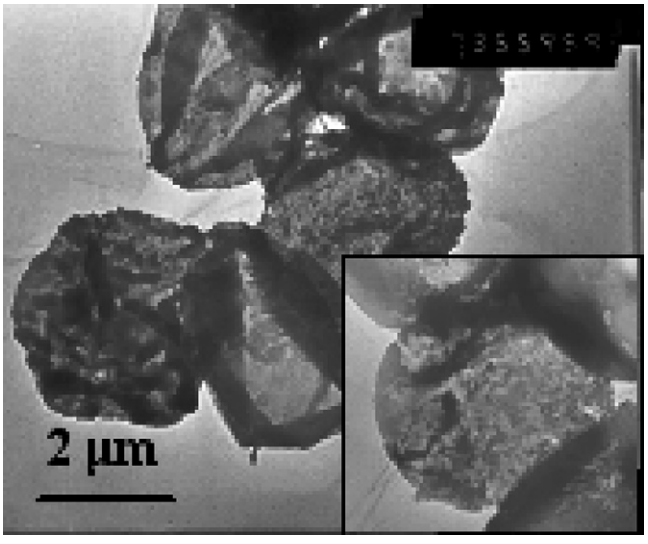

Fig. 5. TEM observations of propranolol deposited $(PGEDMC/PSS)_{4.5}$ capsules at 25 °C. The inset is the magnification image.

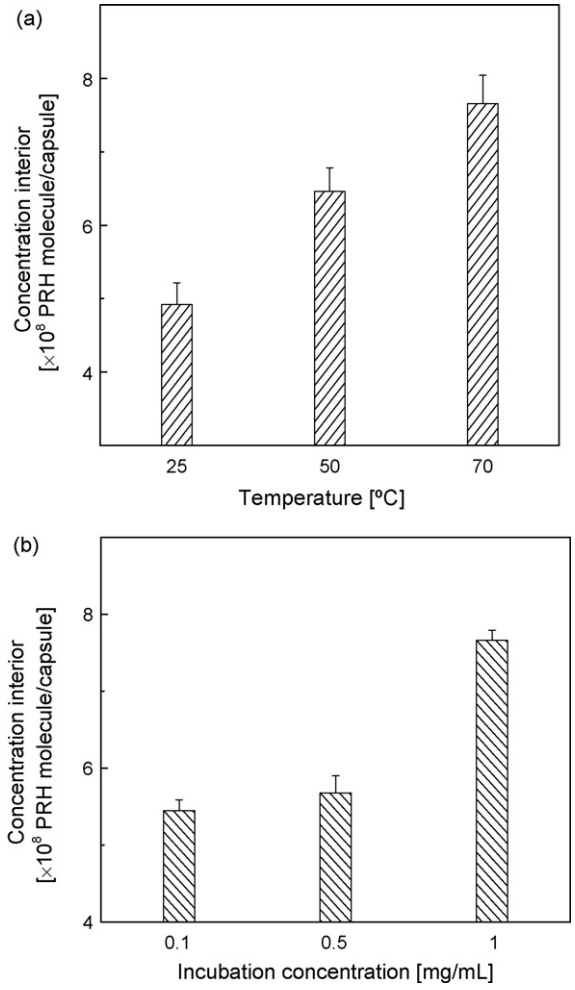

Fig. 6. Loading capacity of $(PGEDMC/PSS)_{4.5}$ microcapsules as a function of (a) heating temperature after 1 h incubation at 25 °C, and (b) PRH concentration in the bulk solution after incubating at 25 °C and then 70 °C for 1 h each.

would swell to burst at higher temperature and the loss of loaded drug would be greatly increased oppositely.

Bulk solution concentration had some affect on PRH loading capacity as illustrated in Fig. 6b. The drug concentration interior the microcapsule increased from 5.45×10^8 to 7.66×10^8 molecules per capsule, namely, 27–38 mg/mL (calculated from fixed diameter of 2.8 μm) when the bulk concentration was increased from 0.1 to 1 mg/mL after heating at 70 °C for 1 h. The results show that a remarkable enrichment of PRH in the capsules can still be achieved even at low incubation concentration of 0.1 mg/mL. Higher bulk concentration was not involved here since it was reported that the loading capacity would reach an equilibrated value and no increment was observed in bulk solutions higher than that critical concentration (Ye et al., 2006). The interior volume per capsule was no larger than 1×10^{-11} mL as calculated from the average diameter of 2.8 μm, therefore, drug molecules could not be enriched everlastingly in such a confined compartment.

The release profiles of encapsulated PRH at pH 7.4 treated by temperature elevating were depicted in Fig. 7. The release rate slows down significantly after the treatment at high temperature. The higher the temperature is, the slower and the less

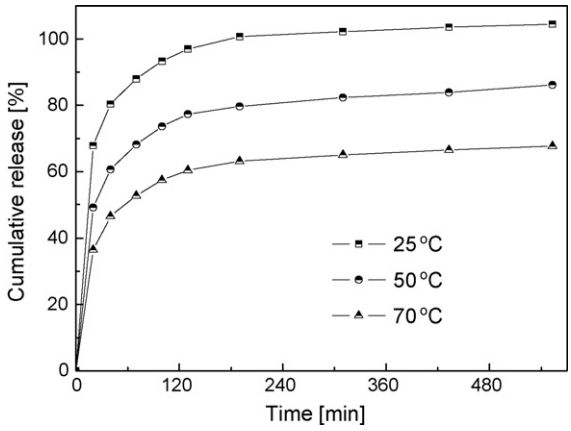

Fig. 7. Release profiles for propranolol from $(PGEDMC/PSS)_{4.5}$ capsules loaded by heat-treating.

it releases. At 70 °C treatment, only ~60% of the total loaded amount can be cumulatively released. As discussed above, thick and compact outer shell induced by heating treatment restrained PRH interior from fast burst into the release medium. One should note that drug release performed by such way is limited by the kinetic equilibrium involving the drug and the capsule shell. The reduced cavity size and thicken capsule wall within the shell did hinder the diffusion of encapsulated drug, and meanwhile partial of those hindered drug molecules interacted with thermal-entwisted polyelectrolyte chains for long-term by versatile affinities (Drug residuals found in the capsules after 10 h release by TEM). Hence, it was not strange if the cumulative release percentage was getting smaller upon heating. Thus, we believe that using proper heat treatment, the drug capacity of (PGEDMC/PSS) multilayered system could be largely increased, and the release rate could be controlled more precisely.

3.4. Reserved lectin recognition

Galactosylated polymers showed strong interactions with galactose-binding lectins such as RCA_{120} and PNA (Miura et

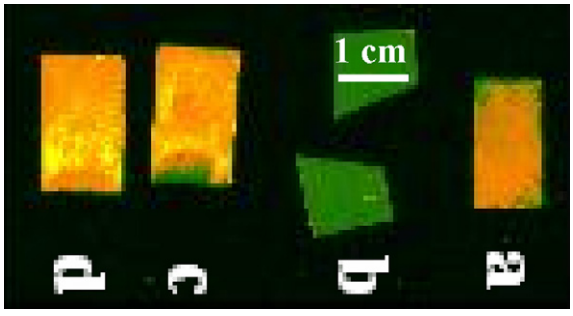

Fig. 8. Fluorescence images of (a) $(PGEDMC_{5\%}/PSS)_3PGEDMC_{5\%}$ multilayer-PNA; (b) $(PGEDMC/PSS)_3$ multilayer-PNA; (c) $(PGEDMC/PSS)_3PGEDMC$ multilayer-$PNA_{pH\,4.5}$; (d) $(PGEDMC/PSS)_3PGEDMC$ multilayer-PNA. The galactose content of unstated PGEDMC was 19 mol%, and unstated lectin pH value was 7.8. The multilayers on silica substrates (as backgrounds) were colored green and the fluorescent signals were colored orange by softwares. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

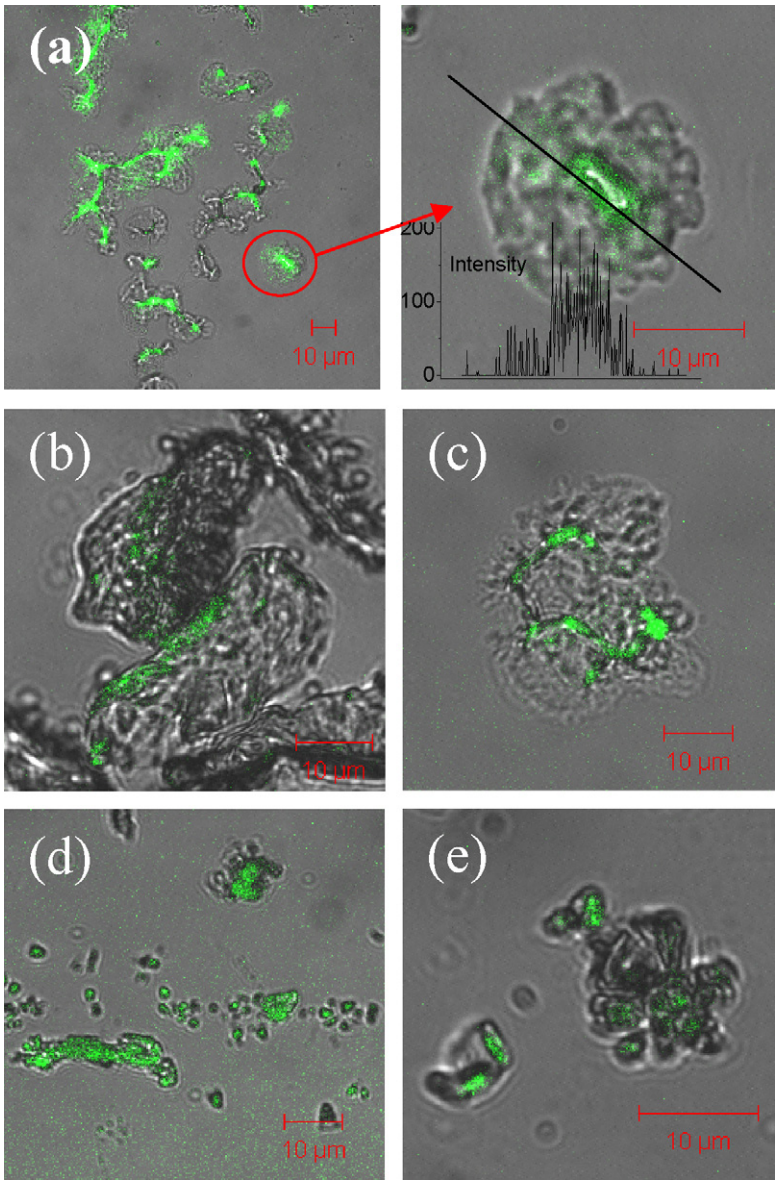

Fig. 9. Fluorescence images of dried PGEDMC-capped $(PGEDMC/PSS)_{4.5}$ polyelectrolyte capsules before (a) and after heating at (b) 30, (c) 50, (d) 70, and (e) 90 °C for 0.5 h and sequential incubation in FITC-PNA lectin solution for 1 h. The red arrow points to a local magnification image of (a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

al., 2004; Ambrosi et al., 2005). Here we used peanut agglutinin (PNA), a plant protein composed of four identical subunits each of which can specifically combine one galactose residue to investigate the hepatic target possibility in vitro. PGEDMC/PSS multilayered films were deposited on quartz slides and then they were incubated in PNA solutions to actuate the possible lectin adhesions. Four groups of PNA adhesion to different multilayers were recorded by fluorescence scanning observation (Fig. 8). In this test, the lectin fluorescence signal and the background signal were expressed as orange color and green color, respectively. It was shown that strong lectin signal was visualized when PGEDMC being the outmost layer (see orange color along the whole multilayer coated slides in Fig. 8a, c and d). Oppositely, the fluorescence signal of PNA lectin was quite weak on the slide when PSS became the outmost coating (Fig. 8b). Copolymers with different galactose contents both exhibited high lectin-adhesion. As seen in Fig. 8, multilayers consisting of large mole ratio of galactose group (19% in Fig. 8d), show stronger fluorescence intensity (bright orange color) than multilayers with low mole ratio of galactose group (5% in Fig. 8a). Thus it is shown that the galactose groups of PGEDMC remain their biological ability during LbL assembly with polyanions. Furthermore, we adjusted the pH of PNA lectin from basic (pH 7.8) to acidic (pH 4.5), and PSS/PGEDMC multilayers bound firmly the PNA lectin at both pH values (Fig. 8c and d). Since PNA may bring more negative charge at neutral or basic buffer and more positive charge at acidic buffer, the unspecific binding of PNA (mostly the electrostatic interaction) to galactose-branched polymer multilayer could be excluded to a certain extent. Actually, all the experiments were carried out preparing sample of PNA

in 10 mM phosphate buffer, containing 0.15 M NaCl. The addition of salt would largely screen contributory electrostatic effect and further decrease electrostatic protein surface–surface interactions (Ambrosi et al., 2005). Hence the protein–carbohydrate affinity was greatly enhanced. In addition, our previous study (Zhang et al., 2006) had proved that multilayers terminated with PGEDMC showed weak adhesion to non-galactose-binding lectin such as Con A. Recent data (Zhang et al., 2008) also indicate a minor adhesion between PNA and multilayers capped with galactose-free polycation.

However, it ought to be considered seriously whether the galactose ligands still play a role after different thermal treatments, although their lectin-affinity was proved as mentioned above. The designed drug delivery system was expected to possess two functions: sustained drug release and site-targetability. The former could be achieved via thermal treating; however, the target potential should not be screened during this procedure. According to this consideration, the heat-treated hollow capsules were mixed with PNA solution to examine their lectin-affinity. Fig. 9a–e depicts the fluorescence images of dried PNA adsorbed capsules capped with PGEDMC, which had previously incubated at 4, 30, 50, 70 and 90 °C, respectively. The images were captured under fluorescence plus transmission mode, and both of the morphology and fluorescence signal were observed. Before heat-treating, the capsule surfaces were combined with lectin, especially on the folds and creases, as seen in Fig. 9a. The green signals were excited from fluorescence labeled on PNA molecules and hence represented the adsorbed lectin. After heating, the capsules are still able to bind lectin molecules, shown as green fluorescence in Fig. 9b–e. Typically, lectin was firmly adhered to surfaces of shrinking microcapsules that were preheated at 50 °C (Fig. 9c) and 70 °C (Fig. 9d). That means, during the rearrangement of capsules upon thermal elevating, the branched galactose chains had not been engulfed inside the capsules or cleaved from the polymer chains which could possibly malfunction the ligand targetablity. Delightfully, enough galactose residues were exposed on the outermost layer of the capsule after full shrinkage. Regardless of how polymer chains actually behave upon heating, these microcapsules remained their bioactivity (namely, potential targetablity). We did not perform any ultrasonic assistance to disperse PNA bound capsules in order to avoid possible lectin desorption. What's more, the capsule suspensions were clipped between two glass slides and the liquid was rapidly volatilized so the capsules were actually recorded in dried forms. Both of the above mentioned factors caused some aggregations and confocal difficulties which resulted in an unclarity of single microcapsule observation. But strong fluorescence signals were all detected in these samples, which was the most important evidence to show the potential targetability of galactose-branched capsules.

All the above lectin-multilayer interaction researches showed excellent lectin-affinity to the galactose-containing polyelectrolyte multilayers, indicating that the bioactivity of galactose still worked after graft polymerization on a polymer chain and alternately assembly with a polyanion onto different substrates, and even the treatment with high temperature. We believed that the galactose-functioned multilayers had the potentials for further application in hepatocyte-targeting drug delivery.

4. Conclusions

Thermal responsive behavior of novel microcapsules, alternately deposited from oppositely charged polyanion of PSS and galactose-branched polycation of PGEDMC on spherical calcium carbonate particles, was studied in detail. After the core removal, the obtained hollow capsules were treated in elevated temperature and the corresponding surface morphology and diameter changes were recorded. Capsules capped with either PGEDMC or PSS layer both showed a thermo-shrinkage in water due to an unfavorable polymer–solvent interaction upon heating. This result was referenced in drug delivery. A model drug, PRH, was first spontaneously loaded in $(PGEDMC/PSS)_{4.5}$ microcapsules at room temperature. When the incubation temperature was elevated later, the loading capacity was largely increased and the release rate was greatly suppressed. What's more, the affinity between galactose-binding lectin and galactose-containing microcapsules did not lose even after high temperature incubation, which gave us much confidence to apply thermal treating to our designed drug delivery model with hepacyte-targeting potential.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2008.01.021.

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