molecular pharmaceutics

Article

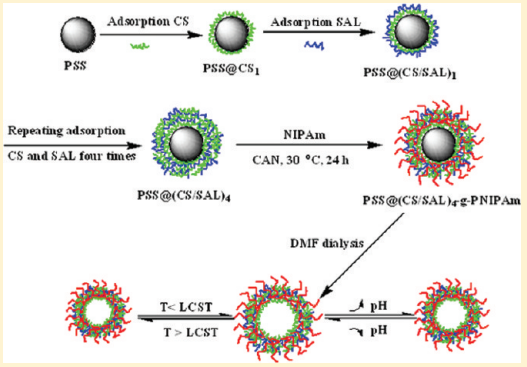
pubs.acs.org/molecularpharmaceutics

Fabrication of Flocculation-Resistant pH/Ionic Strength/Temperature Multiresponsive Hollow Microspheres and Their Controlled Release

Bin Mu, Peng Liu,* Xiaorui Li, Pengcheng Du, Yun Dong, and Yunjiao Wang

State Key Laboratory of Applied Organic Chemistry and Institute of Polymer Science and Engineering, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China

ABSTRACT: pH/ionic strength/temperature multiresponsive hollow microspheres were successfully prepared by the Ce(IV) initiated grafting polymerization of *N*-isopropylacrylamide (NIPAm) onto the multilayered polyelectrolyte shells encapsulating the polystyrene sulfonate (PSS) microsphere templates fabricated by the layer-by-layer assembly of chitosan (CS) and alginate (SAL), after etching the templates by dialysis. The hollow structure of the obtained multiresponsive hollow microspheres was characterized by transmission electron microscopy (TEM), which indicated that the inner diameter of the hollow microspheres was about 200 nm. The environmental responsive properties of the multiresponsive hollow microspheres were characterized with dynamic light scattering (DLS) in an aqueous system. The introduction of poly(*N*-isopropylacrylamide) (PNIPAm) brushes onto the pH/ionic strength dual-responsive hollow microspheres achieved temperature-responsive characteristics. It also could prevent flocculation among the obtained multiresponsive hollow microspheres in a solution with higher salt concentration. Their controlled release of drug molecules (a model hydrophobic drug, dipyridamole (DIP)) was also investigated.



KEYWORDS: *hollow microspheres, flocculation-resistant, multiresponsive, layer-by-layer assembly, grafting polymerization, controlled release*

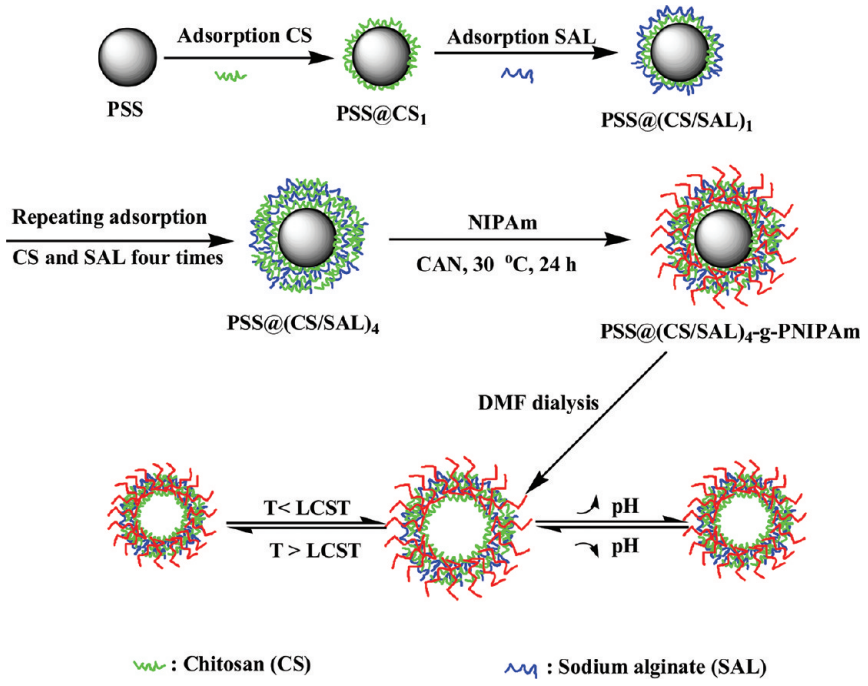
INTRODUCTION

The concept of stepwise deposition of opposite charged polyelectrolytes (PEs) onto a matrix surface was introduced by Decher and defined as a layer-by-layer (LBL) technique.[1] This approach was developed on a colloidal spherical core surface which could be decomposed after coating polyelectrolytes by electrostatic attraction between the oppositely charged polyelectrolytes to obtain hollow microspheres or microcapsules.[2−5] The layer-by-layer adsorption of polyelectrolytes (PE) is acknowledged as a convenient and versatile method to obtain polymeric microcapsules or hollow microspheres based on dissolvable colloidal templates. Compared with other relevant techniques, the layer-by-layer assembly has two prominent advantages. First, the loading capacity of the obtained hollow microspheres and the release kinetics of the loaded guest molecules could be conveniently and precisely controlled by tailoring the assembly parameters such as layers of adsorption, ionic strength, and pH of dipping solution.[6,7] Second, the obtained hollow microspheres or microcapsules have been prepared with controlling over the size ranging from 0.1 to 10 μm as well as controlling over the wall thickness from 10 to 100 nm.[8,9] Therefore, the encapsulation of the guest molecules (pharmaceuticals, protein, and so on) could be achieved by the assembly of the polyelectrolytes deposited on the surface of the colloidal particles (silica,[10] $CaCO_3$,[11] polystyrene latex,[12] and melamine formaldehyde particles[13]) or droplets,[14] and then the templates were removed to obtain the hollow shells, which could be refilled with the desired molecules.

In recent years, "smart" hollow microspheres and microcapsules have received increasing attention due to their potential applications in drug delivery and biomedical fields. The stimuli-responsive hollow microspheres permit the adjustable permeability of the guest molecules with the controllable release based on a designed mechanism under a given environmental stimulus. The release rate of the guest molecules was usually controlled by the diffusion rate of the guest molecules across the walls of the hollow microspheres. Therefore, the fast response of the wall structure of the hollow microspheres to external factors was indispensable. At present, stimuli-responsive hollow microspheres have been reported in response to specific stimuli, such as pH,[15,16] temperature,[17,18] glucose,[19] ionic strength,[20] and light.[21] Among them, pH and ionic strength dual-responsive hollow microspheres prepared via the LBL assembly technique have been reported;[21,22] and the temperature-responsive microcapsules were fabricated by layer-by-layer deposition onto colloid templates of the

Received: May 24, 2011
Revised: July 19, 2011
Accepted: November 20, 2011
Published: November 21, 2011

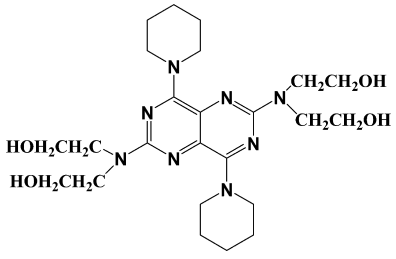
Scheme 1. Schematic Illustration of the Preparation of pH/Ionic Strength/Temperature Multiresponsive Hollow Microspheres



oppositely charged diblock copolymers each containing a poly(*N*-isopropylacrylamide) (PNIPAm) block via electrostatic self-assembly,[23] or tannic acid and temperature-responsive polymer through hydrogen-bonded self-assembly.[24] However, pH/ionic strength/temperature multiresponsive hollow microspheres prepared by LBL were scarcely reported.[25]

It is well-known that natural polymers such as chitosan,[26] sodium alginate,[27] carrageenan,[28] and konjac,[29] could complete the grafting polymerization of the vinyl monomers with cerium ammonium nitrate (CAN) as a redox initiator. On this basis, pH/ionic strength/temperature multiresponsive hollow microspheres with a controllable thermoresponsive PNIPAm layer were designed and prepared for the first time via the combination of the layer-by-layer (LBL) assembly of chitosan and alginate and Ce(IV) initiated grafting polymerization techniques after etching the templates (polystyrene sulfonate (PSS) microspheres) by dialysis (Scheme 1). The controlled release behavior of the obtained pH/ionic strength/temperature multiresponsive hollow microspheres was also investigated by adjusting the pH and temperature of the aqueous dispersions of the drug-loaded multiresponsive hollow microspheres, with dipyridamole (DIP) as the model hydrophobic drug; the molecular structure of the dipyridamole is shown in Scheme 2.

Scheme 2. Molecular Structure of the Dipyridamole (DIP)



EXPERIMENTAL SECTION

Materials. Chitosan (viscosity-average molecular weight was 6.0×10^5, and its N-deacetylation degree was 90%) was purchased from Yuhuan Ocean Biochemical Co. Ltd., Zhejiang, China. Sodium alginate (viscosity 30−80 cP for a 1 mg/mL solution and the viscosity-average molecular weight was about 4.5×10^5) was obtained from Xvdong Chemical Plant, Beijing, China. Styrene (St, analytical reagent, Tianjin Chemicals Co. Ltd., China) was purified by removing inhibitor by being filtered through an aluminum oxide column and then stirred with CaH_2 overnight and distilled under reduced pressure before use. *N*-Isopropylacrylamide (NIPAm, 99%, J & K Chemical Ltd.) was purified by recrystallization from a mixture of toluene and *n*-hexane (1/2, v/v). Dipyridamole (DIP, $C_{21}H_{40}N_8O_4$, maximum wavelength: 284 nm) was analytical reagent grade from J & K Chemical Ltd. Methacrylic acid (MAA), cerium ammonium nitrate (CAN) and other reagents were all of analytical reagent grade from Tianjin Chemical Co., China, and were used without further purification. Deionized water was used throughout.

Preparation of Uniform PS Particles. The polystyrene (PS) latex was prepared by the emulsifier-free emulsion polymerization of styrene (St) with methacrylic acid (MAA) as the surfmer according to the procedure reported previously.[30] Styrene (St, 10 mL) and 2 mL of methacrylic acid (MAA) were added into 95 mL of distilled water in a three-necked round-bottom flask fitted with a condenser and a magnetic stirrer (500 rps) and purged with nitrogen. A solution of ammonium persulfate (APS, 0.054 g) predissolved in water (5 mL) was added to the reaction vessel with vigorous stirring, bubbling with nitrogen. The polymerization was continued for 24 h at 72 °C. After being cooled to room temperature, the product was isolated by being centrifuged and washed with ethanol. A white fine powder (PS, with M_n of 1.18×10^4 by GPC analysis) was finally obtained after being dried in a vacuum oven at 50 °C.

dx.doi.org/10.1021/mp200269r | *Mol. Pharmaceutics* 2012, 9, 91−101

Preparation of Polystyrene Sulfonate (PSS) Microspheres. Uniform PS particles (2.0 g) synthesized as presented above were dispersed in sulfuric acid (60 mL, 98%) with the aid of ultrasonic irridiation. The sulfonation was allowed to take place at 45 °C under magnetic stirring for 8 h. After being cooled to the room temperature, the product was separated by being centrifuged and washed with a large excess of water after being diluted with distilled water. The transformation of the sulfonated PS into sodium polystyrene sulfonate was performed by adding an excess of sodium bicarbonate after being resuspended in water, and then separated by being centrifuged and thoroughly rinsed with water until the neutral pH. The obtained microspheres were finally dispersed and stored in 50 mL of distilled water.

Preparation of the Chitosan/Sodium Alginate Encapsulated Polystyrene Sulfonate Microspheres (PSS@(CS/SAL)$_4$). The layer-by-layer assembly technique was applied for the preparation of polyelectrolyte encapsulated polystyrene sulfonate microspheres by electrostatic interaction between the amino groups of chitosan (CS) and the carboxyl groups of sodium alginate (SAL), starting with CS. The adsorption of CS was conducted in a solution of 500 mL of deionized water containing 0.5 g of polystyrene sulfonate (PSS) and 0.5 g of chitosan at pH around 4 for 8 h followed by being centrifuged and washed three times with water to obtain the chitosan adsorbed polystyrene sulfonate (PSS-CS$_1$). The aqueous solution of 200 mL containing 0.5 g of sodium alginate (pH = 6) was added to 300 mL PSS-CS$_1$ dispersion under magnetic stirring for 8 h, and then the mixture was centrifuged and washed three times with water combined with ultrasonic irradiation to obtain the PSS@(CS/SAL)$_1$. Then chitosan and sodium alginate were alternately deposited a further three times onto the PSS microspheres to obtain the CS/SAL multilayer encapsulated PSS microspheres (PSS@(CS/SAL)$_4$).

Preparation of PNIPAm Grafted onto Polyelectrolyte Shell To Coat PSS Microspheres (PSS@(CS/SAL)$_4$-*g*-PNIPAm). Poly(*N*-isopropylacrylamide) brushes were grafted onto the polyelectrolyte shells by the grafting polymerization of *N*-isopropylacrylamide (NIPAm) onto the chitosan and sodium alginate backbones with cerium ammonium nitrate (CAN) as a redox initiator via the solution radical polymerization technique under nitrogen atmosphere.[27] While bubbling with nitrogen, NIPAm monomer and cerium ammonium nitrate were added into a 50 mL aqueous solution containing 0.3 g of PSS@(CS/SAL)$_4$, and the mixture was stirred at 30 °C for 24 h. The concentration of NIPAm monomer was varied to modify the polyelectrolyte shells with different content of the NIPAm moiety at fixed concentration of initiator CAN as shown in Table 1. After the grafting polymerization, the products were washed with excess water and separated by centrifugation until the homopolymer of NIPAm was completely removed. The resulting product was then dried under vacuum at 40 °C until a constant weight was attained.

Table 1. The Graft Polymerizing Conditions

samples	NIPAm (mmol)	CAN (mmol)
PSS@(CS/SAL)$_4$-*g*-PNIPAm-1	4.0	0.5
PSS@(CS/SAL)$_4$-*g*-PNIPAm-2	6.0	0.5

Preparation of pH/Ionic Strength/Temperature Multiresponsive Hollow Microspheres. To obtain the pH/ionic strength/temperature multiresponsive hollow microspheres, the sacrificial templates (PSS) were removed by dialysis. The process to etch the templates was illustrated as follows: the PSS@(CS/SAL)$_4$-*g*-PNIPAm aqueous dispersion was dialyzed against *N*,*N*- dimethylformamide (DMF) and deionized water using a dialysis membrane (MWCO = 14000) for 5 days with several changes of DMF and deionized water, respectively. The absence of the templates was confirmed by mixing the final dialysate with three times volume of water ensuring the absence of any precipitate. The resultant solution was centrifuged, washed with ethanol, and dried under vacuum at room temperature for 48 h to obtain the ultimate products ((CS/SAL)$_4$-*g*-PNIPAm-1 and (CS/SAL)$_4$-*g*-PNIPAm-2 hollow microspheres). Furthermore, PSS@(CS/SAL)$_4$ microspheres were dialyzed to remove the templates to obtain the pH/ionic strength dual-responsive hollow microspheres ((CS/SAL)$_4$) in order to compare with the pH/ionic strength/temperature multiresponsive hollow microspheres.

Drug Loading. The obtained pH/ionic strength dual-responsive and pH/ionic strength/temperature multiresponsive hollow microspheres (50 mg) were added into the DIP solution (5 mL, 1.0 mg/mL, pH = 4), respectively. After 24 h, the DIP-loaded hollow microspheres were centrifuged to remove the free excess DIP molecules. Then the drug concentration in the supernatant solution was analyzed using a UV spectrophotometer at a wavelength of maximum absorbance (284 nm) after being diluted. The drug loading capacity of the hollow microspheres was calculated from the drug concentrations in solutions before and after adsorption.

The Controlled Release of DIP-Loaded Multiresponsive Hollow Microspheres. A 10 mL aqueous solution containing the DIP-loaded pH/ionic strength/temperature multiresponsive hollow microspheres was transferred into dialysis tubes with a molecular weight cutoff of 14000 and immersed into 90 mL of buffer solution at the four different conditions: pH 1.8 at 25 °C, pH 1.8 at 37 °C, pH 7.4 at 25 °C, and pH 7.4 at 37 °C, respectively. The pH/ionic strength dual-responsive hollow microspheres (CS/SAL)$_4$ were separately placed into pH 1.8 and pH 7.4 buffer media at 25 and 37 °C to compare with the pH/ionic strength/temperature multiresponsive hollow microspheres. Aliquots (5.0 mL) of the solutions were taken at time intervals. The solution was further diluted, and then the drug molecule concentration in the dialysate was analyzed for monitoring the 284 nm absorption peak of DIP using UV−vis spectrometry in order to detect the rate of drug release. Furthermore, 5.0 mL of fresh solution with the same pH value was added after each sampling to keep the total volume of the solution constant. The cumulative release is expressed as the total percentage of drug molecule released through the dialysis membrane over time.

Characterizations. A Bruker IFS 66 v/s infrared spectrometer (Bruker, Karlsruhe, Germany) was used for the Fourier transform infrared (FT-IR) spectroscopy analysis in the range of 400−4000 cm^{-1} with a resolution of 4 cm^{-1}. The KBr pellet technique was adopted to prepare the sample for recording the IR spectra.

The morphologies of the polystyrene sulfonate (PSS) microspheres, the chitosan/sodium alginate multilayer encapsulated polystyrene sulfonate (PSS@(CS/SAL)$_4$), the PNIPAm grafted chitosan/sodium alginate multilayer encapsulated PSS microspheres (PSS@(CS/SAL)$_4$-*g*-PNIPAm), the pH/ionic strength dual-responsive hollow microspheres (CS/SAL)$_4$, the pH/ionic strength/temperature multiresponsive microspheres ((CS/SAL)$_4$-*g*-PNIPAm), and the drug-loaded stimuli-respon-

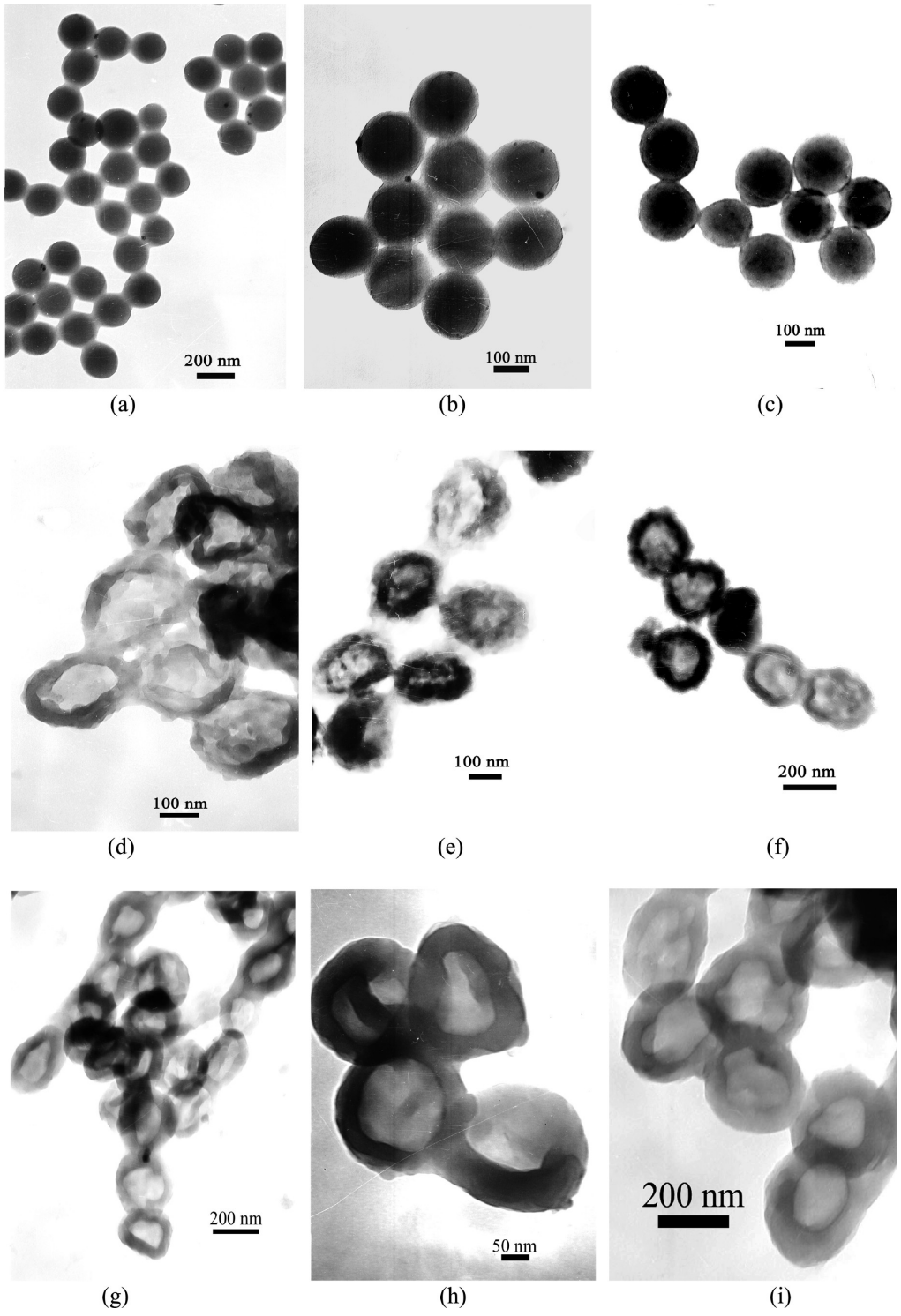


Figure 1. The TEM of (a) polystyrene sulfonate microspheres (PSS), (b) the polyelectrolyte multilayer encapsulated PSS microspheres (PSS@(CS/SAL)$_4$), (c) the PNIPAm grafted polyelectrolyte multilayer encapsulated PSS microspheres (PSS@(CS/SAL)$_4$-*g*-PNIPAm), (d) the pH/ionic strength dual-responsive hollow microspheres ((CS/SAL)$_4$), the pH/ionic strength/temperature multiresponsive hollow microspheres ((CS/SAL)$_4$-*g*-PNIPAm-1 (e) and (CS/SAL)$_4$-*g*-PNIPAm-2 (f)), (g) drug-loaded (CS/SAL)$_4$ hollow microspheres, (h) drug-loaded (CS/SAL)$_4$-*g*-PNIPAm-1 hollow microspheres, and (i) drug-loaded (CS/SAL)$_4$-*g*-PNIPAm-2 hollow microspheres.

sive hollow microspheres were characterized with a JEM-1200 EX/S transmission electron microscope (TEM) (JEOL, Tokyo, Japan). They were dispersed in water and stirred for 30 min, and then deposited on a copper grid covered with a perforated carbon film.

The zeta potentials of the polyelectrolyte encapsulated PSS microspheres and the PNIPAm grafted chitosan/sodium alginate multilayer encapsulated PSS microspheres in deionized water were determined with Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.).

The mean particle size and size distributions of the obtained hollow microspheres were determined by the dynamical mode (dynamic light scattering (DLS)) on the "Light Scattering System BI-200SM, Brookhaven Instruments" device equipped with the BI-200SM goniometer, the BI-9000AT correlator, temperature controller and the Coherent INOVA 70C argon ion laser at 20 °C. DLS measurements are performed using 135 mW intense laser excitation at 514.5 nm and at a detection angle of 90° using the emulsion directly at 25 °C. Particle size distribution is calculated using the Brookhaven Instruments Particle sizing software. The details of the laser light scattering (LLS) instrumentation and theory can be found in the literature.[31]

The behavior of drug loading and controlled release of the pH/ionic strength dual-responsive hollow microspheres (CS/SAL)$_4$ and the pH/ionic strength/temperature multiresponsive hollow microspheres ((CS/SAL)$_4$-*g*-PNIPAm) were detected by a Perkin-Elmer Lambda 35 UV/vis spectrometer (Perkin-Elmer Instruments, USA) at room temperature.

RESULTS AND DISCUSSION

The PS latex was prepared by emulsifier-free emulsion polymerization, and then the sulfonic acid groups were introduced onto the surface of the PS spheres to adsorb the polycation chitosan (CS). A typical TEM image of the polystyrene sulfonate (PSS) microspheres obtained is given in Figure 1a. The microspheres are spherical in shape and monodisperse in size, with a diameter of about 200 nm.

The layer-by-layer assembly technique was used to prepare the polyelectrolyte multilayer encapsulated PSS microspheres with chitosan (CS) and sodium alginate (SAL) alternately adsorbed onto the PSS templates by the electrostatic interaction between the amino groups of chitosan and the carboxyl groups of alginate. Initially, the polycation chitosan was adsorbed onto the PSS templates by the electrostatic interaction between the amino groups of CS and the carboxyl groups and sulfonic acid groups of the PSS templates. Next the polyanion sodium alginate was added to the aqueous solution containing PSS-CS$_1$ to achieve the layer-by-layer assembly between CS and SAL. Repeating this cycle four times, the chitosan/sodium alginate encapsulated polystyrene sulfonate microspheres (PSS@(CS/SAL)$_4$) were then obtained. The zeta potentials of the polyelectrolyte multilayer coated PSS microspheres were conducted to track the polyelectrolyte multilayer growth as shown in Figure 2. The odd layer numbers correspond to the CS deposition except for the ninth layer and the even layer numbers to SAL adsorption. The zeta potential was about −32.9 mV when the first layer of chitosan was deposited on the PSS cores, indicating that the deposition of chitosan could not completely cover the PSS microsphere surface. Then the zeta potential changed to a higher negative value (−80.3 mV) after the adsorption of the polyanion SAL over the chitosan coated PSS microspheres. The alternating zeta potential values indicated that the polyelectrolyte multilayer films were successfully deposited on the PSS templates. Furthermore, it also revealed that adsorption of the polycation (CS) did not lead to a reversal of the zeta potential signs as reported previously.[32] It could be ascribed to the fact that the adsorbed chitosan could not form a layer dense enough to hide the influence of the charges from the surface beneath.[33]

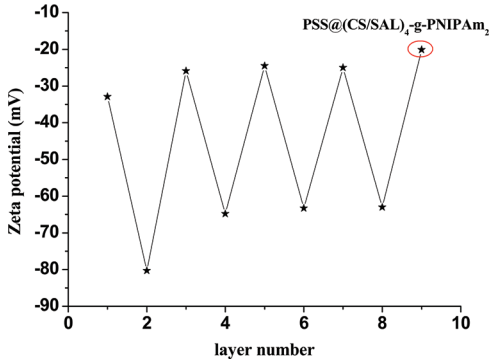


Figure 2. Zeta potentials of the CS/SAL multilayer coated PSS microspheres and the PSS@(CS/SAL)$_4$-*g*-PNIPAm as a function of the layer number.

The IR spectrum of the PSS@(CS/SAL)$_4$, as shown in Figure 3, revealed that the well-defined characteristic absorbance bands of benzene ring at 3080, 3060, 3020 cm^{-1} (C−H, stretching vibration), 1601, 1492, 1452 cm^{-1} (C═C, stretching vibration), 700 cm^{-1} (out-of-plane blending vibration, δ_{ring}), and the two characteristic peaks at 1699 and 1117 cm^{-1} are attributed to the carbonyl stretching of the carboxyl groups of MAA and sulfonic acid groups, respectively. The TEM image of PSS@(CS/SAL)$_4$ is shown in Figure 1b; the diameter of the PSS@(CS/SAL)$_4$ was around 210 nm with a core−shell morphology.

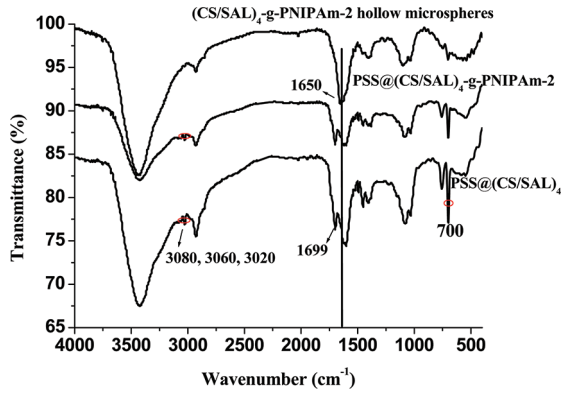
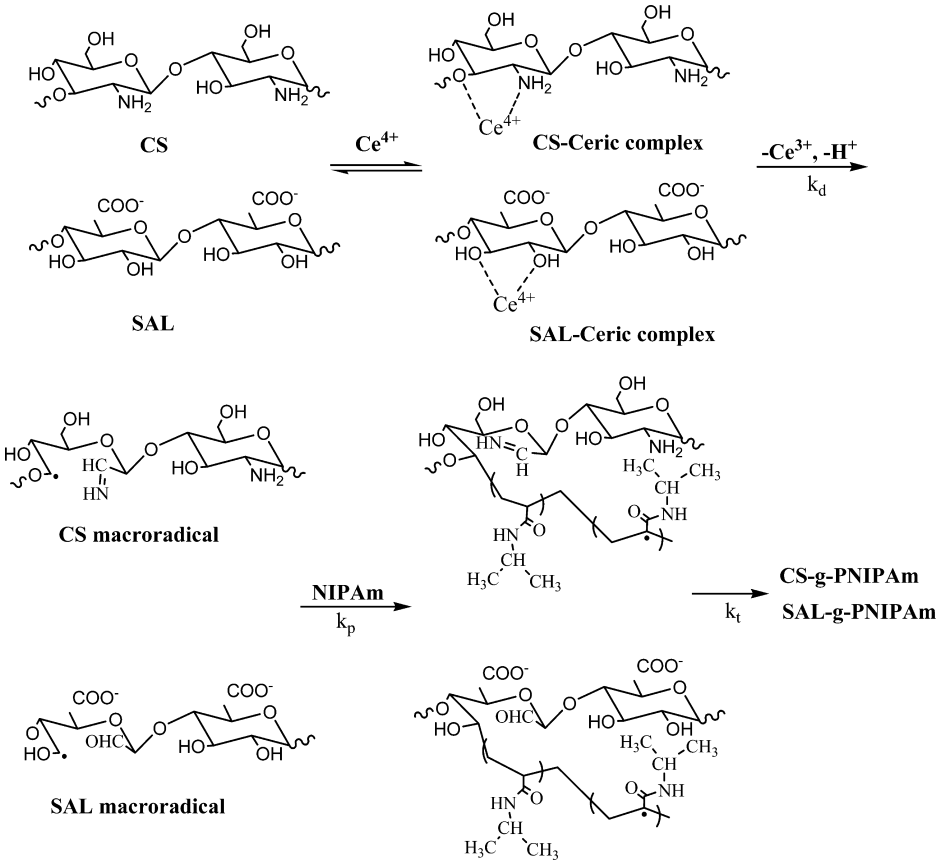


Figure 3. The FTIR spectra of the PSS@(CS/SAL)$_4$, PSS@(CS/SAL)$_4$-*g*-PNIPAm-2, and (CS/SAL)$_4$-*g*-PNIPAm-2 hollow microspheres.

To obtain the pH/ionic strength/temperature multiresponsive hollow microspheres, the temperature-sensitive PNIPAm brushes were introduced into the polyelectrolyte multilayers by the grafting polymerization of *N*-isopropylacrylamide onto the CS/SAL shell with cerium ammonium nitrate (CAN) as a redox initiator in an aqueous medium. Several papers reported on the grafting polymerization mechanisms of the vinyl monomer onto the natural polyelectrolyte macromolecules with cerium ammonium nitrate as a redox initiator.[34,35] The proposed mechanism used in this study was based on the same principle as reported by Ceresa.[36] The first step of the mechanism was that the cerium ion attacked the polyelectrolyte macromolecules and formed a polyelectrolyte−ceric complex. The Ce^{4+} ion in the complex was then reduced to Ce^{3+} ion, and a hydrogen atom was oxidized. Consequently, a free radical was formed onto polyelectrolytes while the C−C bond was broken. The polyelectrolyte free radicals (CS macroradical and SAL macroradical) formed might initiate the grafting polymerization

dx.doi.org/10.1021/mp200269r | *Mol. Pharmaceutics* 2012, 9, 91−101

Scheme 3. The Grafting Polymerization Mechanisms



of the monomer to form the natural polyelectrolytes grafted with PNIPAm (CS-*g*-PNIPAm and SAL-*g*-PNIPAm), as illustrated in Scheme 3.

The zeta potential of the PSS@$(CS/SAL)_4$-*g*-PNIPAm-2 increased to −20.1 mV, compared with the value of the PSS@$(CS/SAL)_4$ (−63.0 mV), as shown in Figure 2, which also indicated that PNIPAm was successfully grafted onto the polyelectrolyte shells. The diameter of the PSS@$(CS/SAL)_4$-*g*-PNIPAm-2 was around 216 nm (Figure 1c), and the microspheres were monodisperse in size with a typical core−shell morphology.

The templates were removed by dialysis in DMF to obtain the ultimate pH/ionic strength dual-responsive hollow microspheres ($(CS/SAL)_4$) and the pH/ionic strength/temperature multiresponsive hollow microspheres ($(CS/SAL)_4$-*g*-PNIPAm). The characteristic absorbance bands of the templates disappeared after the templates were etched by dialysis as shown in Figure 3, which indicated that PSS templates were completely removed, and the stretching vibration of amido bond was also observed at 1650 cm^{-1} from the FTIR spectrum of the $(CS/SAL)_4$-*g*-PNIPAm hollow microspheres, after the dissociative PNIPAm homopolymer was completely removed by washing with water. The hollow structure of the pH/ionic strength dual-responsive hollow microspheres and the pH/ionic strength/temperature multiresponsive hollow microspheres could be observed in the TEM analysis (Figure 1d−f). The inner diameter of the $(CS/SAL)_4$ hollow microspheres was about 250 nm with irregular spheres, which was larger than that of the templates (PSS, 200 nm). It might be ascribed to the swelling due to the lower ionic cross-linking density. However, the inner diameter of the pH/ionic strength/temperature multiresponsive hollow microspheres ($(CS/SAL)_4$-*g*-PNIPAm) was near 200 nm, which was consistent with that of the primitive PSS microspheres. Furthermore, it is worth mentioning that the shape of the obtained hollow microspheres changed from irregular spheres to regular ones with the increase in the grafting degree of PNIPAm. It was in agreement with the fact that the walls of hollow microspheres became rigid after grafting PNIPAm because chitosan and sodium alginate of every polyelectrolyte layer could initiate the grafting polymerization of PNIPAm to form denser shells.[27]

The influence of the ionic strength on the mean diameter of the pH/ionic strength dual-responsive hollow microspheres and the pH/ionic strength/temperature multiresponsive hollow microspheres was researched by dynamic light scattering (DLS). The results are given in Figure 4. It was found that introducing small molecule electrolytes (e.g., NaCl) had a significant influence on the size of the obtained hollow microspheres; the diameter of the pH/ionic strength dual-responsive hollow microspheres increased from 395 to 536 nm with increase of the ionic strength from the range of 0−0.20 mol/L NaCl while that of the pH/ionic strength/temperature multiresponsive hollow microspheres increased from 627 to 906 nm.

It was well-known that the ionic strength of the solution of the polyelectrolyte depended on the concentration of the polyelectrolyte and the small electrolyte molecules. The small molecule electrolytes could weaken the electrostatic repulsion and the salt bond between sodium alginate and chitosan among the shell of the hollow microspheres, hence the chain of the polyelectrolyte should be stretched and the size of obtained hollow microspheres would increase because salt usually had a

dx.doi.org/10.1021/mp200269r | *Mol. Pharmaceutics* 2012, 9, 91−101

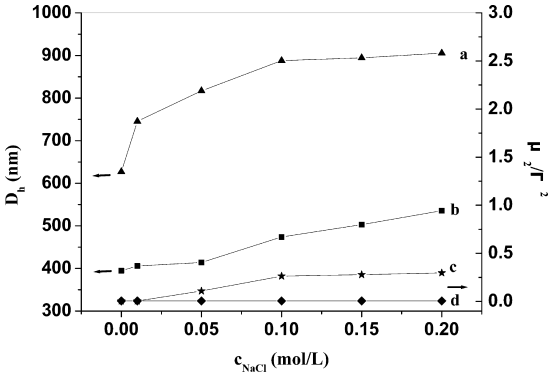


Figure 4. Ionic strength dependence of the average hydrodynamic diameter (D_h) and polydispersity index (μ_2/Γ^2) of the pH/ionic strength/temperature multiresponsive hollow microspheres ((CS/SAL)$_4$-*g*-PNIPAm-2) (a and d), and the pH/ionic strength dual-responsive hollow microspheres (CS/SAL)$_4$) (b and c).

shielding effect on the electrostatic force.[37] Furthermore, it also was observed that the polydispersity index (μ_2/Γ^2) of the pH/ionic strength dual-responsive hollow microspheres increased gradually with the increase of ionic strength (from 0.005 to 0.297), which indicated that the hollow microspheres aggregated during the process of measurement as reported previously.[22] However, the μ_2/Γ^2 of the pH/ionic strength/temperature multiresponsive hollow microspheres was a constant (0.005) with the increase of ionic strength. This further demonstrated that the grafted PNIPAm layer could prevent flocculation among the obtained multiresponsive hollow microspheres in the solution with higher salt concentration.

The influence of pH on the mean diameter of the pH/ionic strength/temperature multiresponsive hollow microspheres was investigated by dynamic light scattering (DLS). It could be seen from Figure 5 that the diameter of the (CS/SAL)$_4$-*g*-PNIPAm-

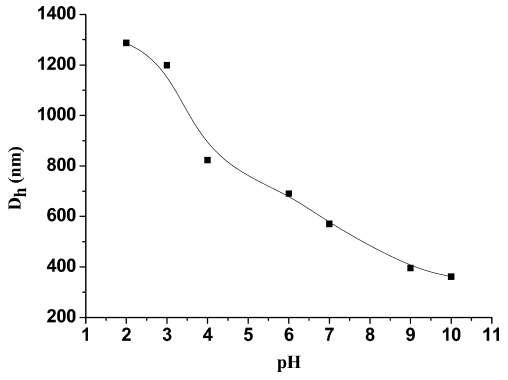


Figure 5. pH dependence of the average hydrodynamic diameter (D_h) of the pH/ionic strength/temperature multiresponsive hollow microspheres ((CS/SAL)$_4$-*g*-PNIPAm-2) at 25 °C.

2 hollow microspheres decreased from 1287 to 362 nm with increase of pH values in the range of 2−10. It is well-known that the pK_a values of the guluronic acid and the mannuronic acid of sodium alginate as a weak acid are 3.65 and 3.38, respectively.[38] However, the chitosan is a weak polybase and the pK_a value of CS is about 6.5,[39,40] and the charge densities of sodium alginate and chitosan are mainly controlled by the pH of solution. At low pH (<4), the ionization of the carboxyl groups was normally depressed. The size of the obtained pH/ionic strength/temperature multiresponsive hollow microspheres decreased more significantly because the decrease of pH weakened the salt bonds between sodium alginate and chitosan, and resulted in no ionic cross-linking among the shell of the hollow microspheres. Between pH 4 and 7, the extent of the decease in size of the hollow microspheres was smaller compared to pH < 4 and pH > 7 because of the significant electrostatic attraction between sodium alginate and chitosan among the shells of the hollow microspheres. The ionization of the amine groups of chitosan decreased greatly when the solution pH increased above 6.0 (around the pK_a of chitosan 6.5) and at pH higher than 7.5 usually less than 10% of the amine groups were ionized.[41] The salt bonds also weakened and led to the decrease in size of the obtained multiresponsive hollow spheres and the increase of pH over 7 due to the insolubility and shrinkage of the chain of CS under basic medium. The above phenomenon also indicated that the polyelectrolyte shells of the obtained multisensitive hollow microspheres were pH-sensitive.

The PNIPAm homopolymer would experience a coil-to-globule phase transition in dilute aqueous solution at its lower critical solution temperature (LCST) about 32 °C.[42] The PNIPAm chains carried out a random-coil conformation at temperatures below 32 °C, on the contrary, the intramolecular hydrogen-bonding interactions between the C═O and N−H groups helped the polymer chains to collapse and compact at above its LCST due to the reducing of their water solubility. The temperature responsive property of the multiresponsive hollow microspheres was also investigated by the change of size of the hollow microspheres in aqueous solution at different temperatures, characterized with dynamic light scattering (DLS). Figure 6 showed the influence of temperature on the

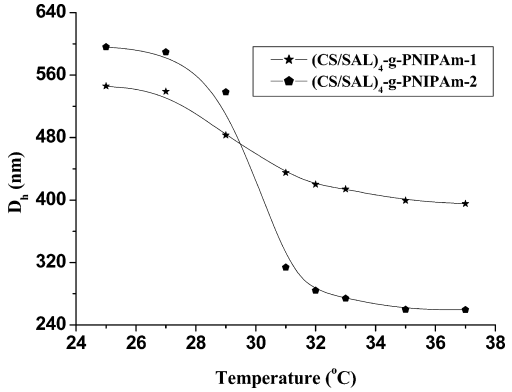


Figure 6. Temeperature dependence of the average hydrodynamic diameter (D_h) of the pH/ionic strength/temperature multiresponsive hollow microspheres ((CS/SAL)$_4$-*g*-PNIPAm).

mean diameter of the pH/ionic strength/temperature multiresponsive hollow microspheres. It obviously expressed the thermal phase transitions of the (CS/SAL)$_4$-*g*-PNIPAm hollow microspheres spanning a broad temperature range. As can be seen from Figure 6, the coil-to-globule phase transition in dilute aqueous solution took place at around 32 °C. Upon heating, the average hydrodynamic diameter (D_h) of the (CS/SAL)$_4$-*g*-PNIPAm-1 decreased monotonically from 546 to 395 nm in the temperature range of 25−37 °C. However, the D_h of the (CS/SAL)$_4$-*g*-PNIPAm-2 decreased from 596 nm at 25 °C to 259 nm at 37 °C. At the same time, it also could be calculated that the hydrodynamic volume of the (CS/SAL)$_4$-*g*-PNIPAm-1

dx.doi.org/10.1021/mp200269r | *Mol. Pharmaceutics* 2012, 9, 91−101

hollow microspheres shrank about 2.6 times upon heating from 25 to 37 °C compared with 12.2 times of the $(CS/SAL)_4$-*g*-PNIPAm-2 hollow microspheres in the same temperature range. It also reflected that the proportion of PNIPAm onto the polymeric shells of the $(CS/SAL)_4$-*g*-PNIPAm-2 hollow microspheres was higher than the $CS/SAL)_4$-*g*-PNIPAm-1 hollow microspheres, which could be attributed to the higher grafting degree of PNIPAm while the content of monomer (NIPAm) was increased in the grafting polymerization. Furthermore, the scattered light intensity also exhibited appreciable changes at around 32 °C due to the decrease in the diameter of the hollow microspheres (Figure 7A,B), and the

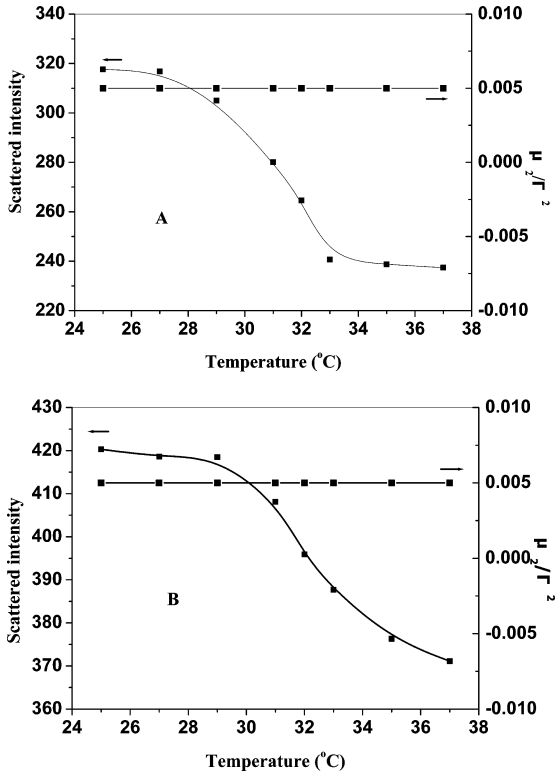


Figure 7. Temperature dependence of the scattered intensity and μ_2/Γ^2 of the pH/ionic strength/temperature multiresponsive hollow microspheres (A, $(CS/SAL)_4$-*g*-PNIPAm-1, and B, $(CS/SAL)_4$-*g*-PNIPAm-2).

polydispersity index (μ_2/Γ^2) was constant (0.005) in the whole temperature range, which further demonstrated that the hollow microspheres did not aggregate during the process of measurement. Therefore, it could be safely concluded that the obtained hollow microspheres were rapidly responsive to the external pH and temperature stimuli.

Although various types of hollow microspheres or microcapsules have been reported, very few studies concerning the drug release behavior of the pH/ionic strength/temperature multiresponsive hollow microspheres prepared by layer-by-layer assembly have been reported. The obtained multiresponsive hollow microspheres $(CS/SAL)_4$-*g*-PNIPAm with pH/ionic strength/temperature multiresponsive shells are anticipated to be drug carriers. The structure of the obtained multisensitive drug carriers was relatively stable compared with the pH/ionic strength dual-responsive hollow microspheres $(CS/SAL)_4$ due to the introduction of the PNIPAm brushes, which not only provided the temperature response but also stabilized the polyelectrolyte shell free from aggregation. In other words, the multiresponsive structure was similar to a cross-linking shell so that the variations of pH, temperature, and ionic strengths, etc., will not disintegrate the hollow structure. Therefore, we then investigated the drug release of the obtained pH/ionic strength dual-responsive and the pH/ionic strength/temperature multiresponsive hollow microspheres at different pH values and temperatures after being loaded with a model hydrophobic drug (DIP). Dipyidamole could rapidly dissolve at the low pH stage (pH = 2), but the amount of dissolved drug drastically decreased at about pH 5. At pH 8, dipyridamole was practically insoluble. The solubility of dipyridamole was 36.5 mg/mL and 0.02 mg/mL in pH 1.0 and 7.0 at 37 °C, respectively.[43,44]

The pH/ionic strength dual-responsive and the pH/ionic strength/temperature multiresponsive hollow microspheres were mixed with DIP in aqueous solution at pH = 4 and 25 °C. According to the UV−vis result, the drug loading of the $(CS/SAL)_4$, $(CS/SAL)_4$-*g*-PNIPAm-1 and $(CS/SAL)_4$-*g*-PNIPAm-2 hollow microspheres was calculated to be about 25.4%, 30.0% and 26.5%, respectively. The TEM images of the above drug-loading stimulus-responsive hollow microspheres are shown in Figure 1g−i. It could be seen that the morphologies of the drug-loading stimulus-responsive hollow microspheres did not change obviously after loading the DIP molecules.

The pH values of gastric juice, small intestine and colon are 1.2−2.0, 6.8−7.0, and 8.0, respectively.[45] Hence we choose pH values of 1.8 and 7.4 to investigate the pH-responsive controlled release of the drug-loaded hollow microspheres. Figures 8 and 9 show the time dependence of the cumulative

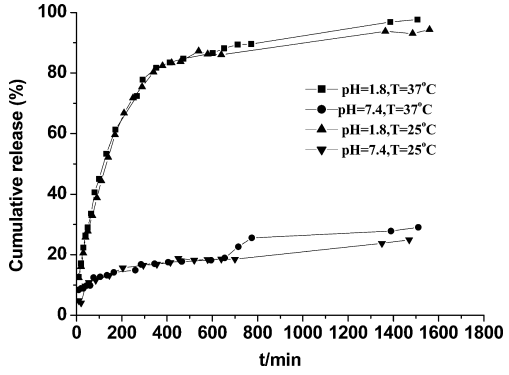


Figure 8. Cumulative dipyridamole (DIP) release from pH/ionic strength dual-responsive hollow microspheres $(CS/SAL)_4$ in pH 1.8 and pH 7.4 buffer media at 25 and 37 °C, repectively.

dipyridamole (DIP) release from the pH/ionic strength dual-responsive hollow microspheres and the pH/ionic strength/temperature multiresponsive hollow microspheres in aqueous solution pH 1.8 and 7.4 at 25 °C, and 37 °C, respectively. When the drug-loaded hollow microspheres ($(CS/SAL)_4$ and $(CS/SAL)_4$-*g*-PNIPAm) were placed in pH 7.4 at 25 and 37 °C, respectively, few drug molecules (<28%) were released from the drug carrier even after 30 h, and the amount of cumulative release was higher at 37 °C than 25 °C for the pH/ionic strength/temperature multiresponsive hollow microspheres. The same results were also observed at pH 1.8 and 37 °C compared with the ones at 25 °C. Okano[46] and Jiang[47] also observed that the release of ADR and DIP could be accelerated at elevated temperature when the drug molecules were loaded into the hydrophobic core covered with PNIPAm coronas, which mainly was attributed to the structural deformation of

dx.doi.org/10.1021/mp200269r | *Mol. Pharmaceutics* 2012, 9, 91−101

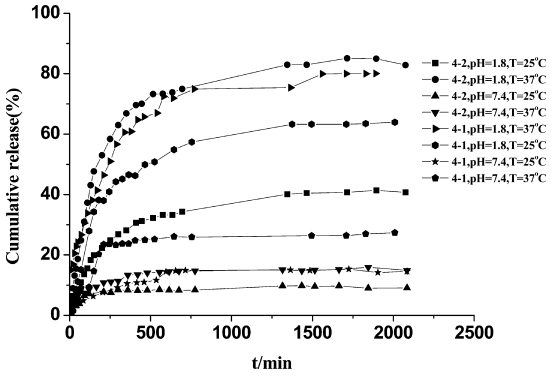


Figure 9. Cumulative dipyridamole (DIP) release from pH/ionic strength/temperature multiresponsive hollow microspheres (CS/SAL)$_4$-*g*-PNIPAm-1 and (CS/SAL)$_4$-*g*-PNIPAm-2 in pH 1.8 and pH 7.4 buffer media at 25 and 37 °C, repectively.

the core−shell micelles. The obtained pH/ionic strength/temperature multiresponsive hollow microspheres possessed a similar structure as the core−shell micelles with PNIPAm coronas reported. The mean diameter decreased with the increase of temperature (Figure 6) due to the collapse of the thermoresponsive PNIPAm chain. Moreover, the collapse of the PNIPAm layer might also lead to the formation of pores or channels throughout the thermoresponsive layer.[47] This factor would contribute to the enhanced permeability of the PNIPAm layer for the guest molecules, resulting in a faster release of DIP at 37 °C compared with 25 °C. Furthermore, the diffusion rate and the solubility of DIP are higher at 37 °C than 25 °C, which would further lead to the faster release of DIP at 37 °C.

Furthermore, it was also found that the releasing ratio and the cumulative release of dipyridamole from the drug carriers at pH 1.8 were quicker and higher than pH 7.4 (Figures 8 and 9) at the same temperature. The release of drug molecules through the hollow microspheres involved two processes as follows: the bulk solution diffused into the shell to dissolve the drug molecules, and the dissolved drugs diffused out of the polymeric shell. The results of the dipyridamole cumulative release at different pH media revealed that the release at low pH could partly be attributed to the fact that the polyelectrolyte shell was highly permeable at low pH but not at high pH, because the decrease of pH weakened the ionic cross-linking salt bonds between CS and SAL due to the decrease in the charge density of SAL and the cross-linking density, as a consequence, resulted in no ionic cross-linking among the shells of the obtained hollow microspheres as shown in Figure 6. Furthermore, the polyelectrolyte multilayer shell was positively charged at low pH, the shell and DIP molecules likely charged the same sign because of the decrease in charge density of alginate and the protonation of CS, which might make such a configuration unstable so that the DIP molecules were impelled across the polyelectrolyte shells.[48] However, this interpretation could not well explain the little release in alkaline medium. It also could be speculated that the little release also might be ascribed to the solubility of DIP molecules in different pH media. The DIP molecules became insoluble in water at high pH because DIP was an alkaline molecule which could dissolve in the acidic media. In other words, the difference in release rates also should partly be attributed to the solubility of the drug molecules in different pH media. The high concentration of dipyridamole inside or among the polyelectrolyte shells could lead to a large concentration gradient across the walls, and further cause a high osmotic pressure within the shells to accelerate the diffusion of dipyridamole through the polyelectrolyte shells.[49] On the basis of the above discussion, we could conclude that the drug release should be governed simultaneously by the saturation solubility of the drug and the permeability of the polyelectrolyte shells.

■ CONCLUSIONS

Hollow microspheres with pH/ionic strength/temperature multiresponsive shells were successfully prepared via the combination of layer-by-layer assembly techniques and grafting polymerization of NIPAm with cerium ammonium nitrate as a redox initiator. The introduction of the PNIPAm brushes in the pH/ionic strength dual-responsive polyelectrolyte shells not only achieved the controlled release of drug molecules that could be dually controlled by solution pH and temperature, but also prevented flocculation among the obtained multiresponsive hollow microspheres in the solution with higher salt concentration. The multiresponsive hollow microspheres could act as excellent and smart drug carriers with stable structure and unique dual control (pH and temperature) in real-world clinical application compared with the common polyelectrolyte hollow microspheres or microcapsules prepared by layer-by-layer assembly.

■ AUTHOR INFORMATION

Corresponding Author

*Lanzhou University, College of Chemistry and Chemical Engineering, Tianshui South Road 222#, Lanzhou 730000, China. E-mail: pliu@lzu.edu.cn. Tel: 86-931-8912516. Fax: 86-931-8912582.

■ ACKNOWLEDGMENTS

This project was granted financial support from the National Nature Science Foundation of China (Grant No. 20904017), the Program for New Century Excellent Talents in University (Grant No. NCET-09-0441), and the Fundamental Research Funds for the Central Universities (Grant No. lzujbky-2012-k12).

■ REFERENCES

(1) Decher, G. Fuzzy nanoassemblies: toward layered polymeric multicomposites. *Science* **1997**, *277*, 1232−1236.

(2) Hu, S. H.; Tsai, C. H.; Liao, C. F.; Liu, D. M.; Chen, S. Y. Controlled rupture of magnetic polyelectrolyte microcapsules for drug delivery. *Langmuir* **2008**, *24*, 11811−11818.

(3) Angelatos, A. S.; Johnston, A. P. P. R; Wang, Y.; Caruso, F. Probing the permeability of polyelectrolyte multilayer capsules via a molecular beacon approach. *Langmuir* **2007**, *23*, 4554−4562.

(4) Khapli, S.; Kim, J. R.; Monclare, J. K.; Levicky, R.; Porfiri, M.; Sofou, S. Frozen cyclohexane-in-water emulsion as a sacrificial template for the synthesis of multilayered polyelectrolyte microcapsules. *Langmuir* **2009**, *25*, 9728−9733.

(5) Becker, A. L.; Zelikin, A. N.; Johnston, A. P. R; Caruso, F. Tuning the formation and degradation of layer-by-layer assembled polymer hydrogel microcapsules. *Langmuir* **2009**, *25*, 14079−14085.

(6) Berg, M. C.; Zhai, L.; Cohen, R. E.; Rubner, M. F. Controlled drug release from porous polyelectrolyte multilayers. *Biomacromolecules* **2006**, *7*, 357−364.

(7) Tang, Z. Y.; Wang, Y.; Podsiadlo, P.; Kotov, N. A. Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering. *Adv. Mater.* **2006**, *18*, 3203−3224.

(8) Shenoy, D. B.; Antipov, A. A.; Sukhorukov, G. B.; Möhwald, H. Layer-by-layer engineering of biocompatible, decomposable core-shell structures. *Biomacromolecules* **2003**, *4*, 265−272.
(9) Mauser, T.; Déjugnat, C.; Möhwald, H.; Sukhorukov, G. B. Microcapsules made of weak polyelectrolytes: templating and stimuli-responsive properties. *Langmuir* **2006**, *22*, 5888−5893.
(10) Imoto, T.; Kida, T.; Matsusaki, M.; Akashi, M. Preparation and unique pH-responsive properties of novel biodegradable nanocapsules composed of poly(γ-glutamic acid) and chitosan as weak polyelectrolytes. *Macromol. Biosci.* **2010**, *10*, 271−277.
(11) Borodina, T.; Markvicheva, E.; Kunizhev, S.; Möhwald, H.; Sukhorukov, G, B.; Kreft, O. Controlled release of DNA from self-degrading microcapsules. *Macromol. Rapid Commun.* **2007**, *28*, 1894−1899.
(12) Mu, B.; Liu, P.; Dong, Y.; Lu, C. Y.; Wu, X. L. Superparamagnetic multilayer hybrid hollow microspheres with pH-sensitive shell for targeted controlled release. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 3135−3144.
(13) Radtchenko, I. L.; Sukhorukov, G. B.; Möhwald, H. Incorporation of macromolecules into polyelectrolyte micro and nanocapsules via surface controlled precipitation on colloidal particles. *Colloids Surf., A* **2002**, *202*, 127−133.
(14) Paiphansiri, U.; Tangboriboonrat, P.; Landfester, K. Antiseptic nanocapsule formation via controlling polymer deposition onto water-in-oil miniemulsion droplets. *Macromol. Symp.* **2007**, *251*, 54−62.
(15) An, Z. H.; Möhwald, H.; Li, J. B. pH controlled permeability of lipid/protein biomimetic microcapsules. *Biomacromolecules* **2006**, *7*, 580−585.
(16) Tong, W. J.; Gao, C. Y.; Möhwald, H. Stable weak polyelectrolyte microcapsules with pH-responsive permeability. *Macromolecules* **2006**, *39*, 335−340.
(17) Köhler, K.; Möhwald, H.; Sukhorukov, G. B. Thermal behavior of polyelectrolyte multilayer microcapsules: 2. insight into molecular mechanisms for the PDADMAC/PSS system. *J. Phys. Chem. B* **2006**, *110*, 24002−24010.
(18) Ichikawa, H.; Fukumori, Y. A novel positively thermosensitive controlled-release microcapsule with membrane of nano-sized poly(*N*isopropylacrylamide) gel dispersed in ethylcellulose matrix. *J. Controlled Release* **2000**, *63*, 107−119.
(19) De Geest, B. G.; Jonas, A. M.; Demeester, J.; De Smedt, S. C. Glucose-responsive polyelectrolyte capsules. *Langmuir* **2006**, *22*, 5070−5074.
(20) Mu, B.; Lu, C. Y.; Liu, P. Disintegration-controllable stimuli-responsive polyelectrolyte multilayer microcapsules via covalent layer-by-layer assembly. *Colloids Surf., B* **2011**, *82*, 385−390.
(21) Angelatos, A. S.; Radt, B.; Caruso, F. Light-responsive polyelectrolyte/gold nanoparticle microcapsules. *J. Phys. Chem. B* **2005**, *109*, 3071−3076.
(22) Lu, C. Y.; Mu, B.; Liu, P. Stimuli-responsive multilayer chitosan hollow microspheres via layer-by-layer assembly. *Colloids Surf., B* **2011**, *83*, 254−259.
(23) Glinel, K.; Sukhorukov, G. B.; Möhwald, H.; Khrenov, V.; Tauer, K. Thermosensitive hollow capsules based on thermoresponsive polyelectrolytes. *Macromol. Chem. Phys.* **2003**, *204*, 1784−1790.
(24) Kozlovskaya, V.; Kharlampieva, E.; Drachuk, I.; Cheng, D.; Tsukruk, V. V. Responsive microcapsule reactors based on hydrogen-bonded tannic acid layer-by-layer assemblies. *Soft Matter* **2010**, *6*, 3596−3608.
(25) Sukhishvili, S. A. Responsive polymer films and capsules via layer-by-layer assembly. *Curr. Opin. Colloid Interface Sci.* **2005**, *10*, 37−44.
(26) Kim, S. Y.; Cho, S. M.; Lee, Y. M.; Kim, S. J. Thermo- and pH-responsive behaviors of graft copolymer and blend based on chitosan and N-isopropylacrylamide. *J. Appl. Polym. Sci.* **2000**, *78*, 1381−1391.
(27) Shah, S. B.; Pate1, C. P.; Trivedi, H. C. Ceric-induced grafting of acrylate monomers onto sodium alginate. *Carbohydr. Polym.* **1995**, *26*, 61−67.
(28) Ceric-initiated free radical graft copolymerization of acrylonitrile onto kappa carrageenan. *J. Appl. Polym. Sci.* **2009**, *114*, 404-412.
(29) Duan, J. C.; Lu, Q.; Chen, R. W.; Duan, Y. Q.; Wang, L. F.; Gao, L.; Pan, S. Y. Synthesis of a novel flocculant on the basis of crosslinked Konjac glucomannan-graft-polyacrylamide-co-sodium xanthate and its applicationin removal of Cu^{2+} ion. *Carbohydr. Polym.* **2010**, *80*, 436−441.
(30) Davies, M. C.; Lynn, R. A. P.; Hearn, J.; Paul, A. J.; Vickerman, J. C.; Watts, J. F. Surface chemical characterization using XPS and ToF-SIMS of latex particles prepared by the emulsion copolymerization of methacrylic acid and styrene. *Langmuir* **1996**, *12*, 3866−3875.
(31) Jin, Y.; Zhang, L. N.; Zhang, M.; Chen, L.; Cheung, P. C. K.; Oi, V. E. C.; Lin, Y. Antitumor activities of heteropolysaccharides of poria cocos mycelia from different strains and culture media. *Carbohydr. Res.* **2003**, *338*, 1517−1521.
(32) Khopade, A. J.; Arulsudar, N.; Khopade, S. A.; Hartmann, J. Ultrathin antibiotic walled microcapsules. *Biomacromolecules* **2005**, *6*, 229−234.
(33) Zhou, J.; Romerob, G.; Rojas, E.; Ma, L.; Moya, S.; Gao, C. Y. Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles for antifouling protection and Folic acid binding to achieve selective cell targeting. *J. Colloid Interface Sci.* **2010**, *345*, 241−247.
(34) Dennerberg, R. J.; Bothast, R. J.; Abbort, T. P. A new biodegradable plastic made from starch graft poly(methyl acrylate) copolymer. *J. Appl. Polym. Sci.* **1978**, *22*, 459−465.
(35) Gao, J. P.; Tian, R. U.; Jiu, Y.; Duan, M. L.; Chuan, R. Graft copolymers of methy methacrylate onto canna starch using manganic pyrophosphate as an initiator. *J. Appl. Polym. Sci.* **1994**, *53*, 1091−1102.
(36) Ceresa, R. J. *Block and Graft Copolymerization*; John Wiley: New York, 1973; Chapter 1.
(37) Shu, X. Z.; Zhu, K. J.; Song, W. H. Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. *Int. J. Pharm.* **2001**, *212*, 19−28.
(38) Orive, G.; Ponce, S.; Hernandez, R. M.; Gascon, A. R.; Igartua, M.; Pedraz, J. L. Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. *Biomaterials* **2002**, *23*, 3825−3831.
(39) Drury, J. L.; Monney, D. J. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* **2002**, *24*, 4337−-4351.
(40) Lai, H. L.; Abu'Khalil, A.; Craig, D. Q. M. Preparation and characterisation of drug-loaded alginate and chitosan sponges. *Int. J. Pharm.* **2003**, *251*, 175−181.
(41) Yalpani, M.; Hall, L. D. Some chemical and analytical aspects of polysaccharide modifications. III. Formation of branched-chain, soluble chitosan derivatives. *Macromolecules* **1984**, *17*, 272−281.
(42) Schild, H G. Preparation of novel acrylamide-based thermoresponsive polymer analogues and their application as thermoresponsive chromatographic matrices. *Prog. Polym. Sci.* **1992**, *17*, 163−249.
(43) Hasegawa, A.; Kawamura, R.; Nakagawa, H.; Sugimoto, I. Application of solid dispersions with enteric coating agents to overcome some pharmaceutical problem. *Chem. Pharm. Bull.* **1986**, *34*, 2183−2190.
(44) Ren, H. Y.; Liu, Q.; Cai, W. W.; Wang, W. G. Studies on the solid dispersion of dipyridamole and its sustained release pellets. *Sci. Technol. Eng.* **2008**, *8*, 2340−2343.
(45) Shargel, L.; Yu, A. *Applied Biopharmaceutics and Pharmacokinetics*, 4th ed.; McGraw-Hill: New York, 1999; pp 110−111.
(46) Chung, J. E.; Yokoyama, M.; Yamato, M.; Aoyagi, T.; Sakurai, Y.; Okano, T. Thermo-responsive drug delivery from polymerc micelles constructed using block copolymers of poly(N-isopropylacrilamide) and poly(butyl methacrylate). *J. Controlled Release* **1999**, *62*, 115−127.
(47) Jiang, X. Z.; Ge, Z. S.; Xu, J.; Liu, H.; Liu, S. Y. Fabrication of multiresponsive shell cross-linked micelles possessing pH-controllable

core swellability and thermo-tunable corona permeability. *Biomacromolecules* **2007**, *8*, 3184−3192.
(48) Kim, B. S.; Vinogradova, O. I. pH-Controlled Swelling of polyelectrolyte multilayer microcapsules. *J. Phys. Chem. B* **2004**, *108*, 8161−8165.
(49) Qiu, X. P.; Leporatti, S.; Donath, E.; Möhwald, H. Studies on the drug release properties of polysaccharide multilayers encapsulated ibuprofen microparticles. *Langmuir* **2001**, *17*, 5375−5380.