Colloid Polym Sci (2008) 286:819–825
DOI 10.1007/s00396-008-1843-9

SHORT COMMUNICATION

Uniform chitosan hollow microspheres prepared with the sulfonated polystyrene particles templates

Haiming Li · Mozhen Wang · Linyong Song · Xuewu Ge

Received: 2 November 2007 / Revised: 14 January 2008 / Accepted: 16 January 2008 / Published online: 21 February 2008

Abstract Biodegradable chitosan hollow microspheres have been fabricated by employing uniform sulfonated polystyrene (PS) particles as templates. The chitosan was adsorbed onto the surface of the sulfonated polystyrene templates through the electrostatic interaction between the sulfonic acid groups on the templates and the amino groups on the chitosan. Subsequently, the adsorbed chitosan was crosslinked by adding glutaraldehyde. After the removal of the sulfonated polystyrene core, chitosan hollow microspheres were obtained. The longer the sulfonation time used, the smaller the size of the hollow particles and the thicker the chitosan wall obtained. Fourier transform infrared spectrometry was used to characterize the component of the microspheres. The morphologies of the PS templates and the chitosan microspheres were observed by transmission electron microscopy and scanning electron microscopy. The controlled release behavior of the chitosan hollow microspheres was also primarily investigated.

Keywords Chitosan hollow microspheres · Sulfonated polystyrene template · Biodegradable · Controlled release

Introduction

Hollow spheres and capsules have spurred great interest because they can encapsulate large quantities of guest molecules—especially those spheres with functionalities within the empty core—so they have some potential applications in controlled release, artificial cells, light weight fillers, catalysis, and confined reaction vessels [1–6]. Some common approaches are used to obtain nano- or submicron-sized hollow spheres and capsules, such as self-assembly of block copolymers in the selective solvent [7–9], emulsion polymerization [10–12], templates polymerization [13–15], and so on.

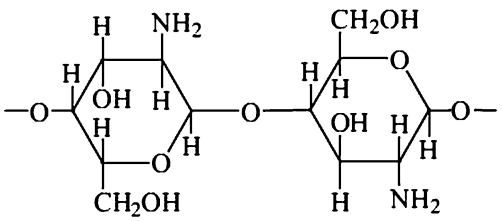
Templates method is an effective way to fabricate hollow microspheres, which can control the core size by selecting appropriate templates. It is very important to modify the surface of the template to make the template active, which can later interact with shell substance like monomer or polymer chain. Several research groups have now developed some ways to improve the surface character of templates, such as copolymerization with some other monomers to introduce active groups on the template surface [16], adding another special substance to adsorb polymer or monomers on the surface of templates [17–19], and directly modified the templates through some chemical reaction like sulfonation [20]. Utilizing the interaction between the modified surface of the templates and base substance, core–shell-structured particles could be finally produced. Wu et al. [16] used polystyrene (PS) particles terminated by COOH and NH_2 groups as core templates to prepare magnetic hollow silica spheres by sol–gel method. Armes et al. used poly(*N*-vinylpyrrolidone) (PVP)-stabilized PS latex particles as core templates. The oxidant and monomer were introduced on the surface of the templates via an aqueous dispersion. A thin overlayer of conductive polymer (such as polyaniline [17] or polypyrrole [18, 19]) formed on PS latex. Chu et al. [13] used sulfonated PS microspheres as templates. The sulfonic acid groups on the surface can interact with aniline and pyrrole through

H. Li · M. Wang (✉) · L. Song · X. Ge (✉)
Department of Polymer Science and Engineering,
Joint Laboratory of Polymer, Thin Films and Solution,
University of Science and Technology of China,
Hefei, Anhui 230026, China
e-mail: pstwmz@ustc.edu.cn
e-mail: xwge@ustc.edu.cn

electrostatic force. Polymerization of the monomer can form uniformed-sized core–shell-structured particles. The PS cores could be removed in the latter stage to form hollow spheres. The experiment results showed that the strategy based on modified PS spheres is a very effective and simple way in preparing narrow size distribution core–shell-structured particles or hollow spheres because it is easy to select templates with appropriate size and narrow size distribution.

Until now, most of the hollow spheres and capsules are not biodegradable and biocompatible, which limits their application in the fields, such as the drug controlled release. Chitosan (CS) is a kind of excellent natural hydrophilic polysaccharide among many degradable polymers. It is nontoxic and biocompatible. The chemical structure of chitosan is shown in Scheme 1. Some approaches have been developed to prepare the chitosan beads. Wang et al. [20] prepared uniform-sized chitosan microspheres containing insulin by membrane emulsion. The size of the microspheres was about 10 μm in diameter, and the size of microspheres was determined by the pore size of the glass membrane. Hu et al. [21] prepared chitosan–poly(acrylic acid) hollow microspheres with core-free strategy in acidic water solution. They started with chitosan–acrylic acid complex formed through the reaction between amino groups and carboxylic groups. Hollow polymeric nanospheres could be obtained by polymerizing the acrylic acid in the mixture initiated by potassium persulfate at 80 °C.

In this study, a sacrificial templates method was demonstrated to fabricate uniform-sized, biodegradable, and biocompatible chitosan hollow spheres which can be potentially used in biological fields such as drug controlled release and delivery. The polarity of the PS spheres was improved by sulfonation in concentrated sulfuric acid. Using the sulfonated PS microspheres as core templates, chitosan shell can be formed by electrostatic force between the amino groups and the sulfonic acid groups. The chitosan hollow spheres can be readily produced by removal of the PS core with tetrahydrofuran (THF). Furthermore, the release behavior of hollow chitosan microspheres in a medium of various pH values at 37 °C was also investigated.



Scheme 1 Chemical structure of chitosan

Experimental

Materials and methods

Chitosan was obtained from Yuhuan Ocean Biochemical Co. LTD, China. Its degree of deacetylation and viscosity molecular weight were 95% and 7.57×10^5. Styrene was purified by reduced pressure distillation before use. Poly (vinylpolypyrrolidone) and THF were obtained from Shanghai Chemical Reagents, China. The sulfuric acid (98%) and the other reagents were used as received.

Synthesis of uniform PS particles

The uniform PS particles were obtained by dispersion polymerization. PVP (3.0 g) was dissolved in the mixture of ethanol (60.0 g) and distilled water (20.0 g) in a glass container with a magnetic stirrer. Styrene (20.0 g) was added to the mixture, and nitrogen was bubbled to get rid of oxygen. Then, the polymerization was initiated by ^{60}Co γ-ray radiation (the ^{60}Co source is located in University of Science and Technology of China, China) at a dose rate of 80 Gy/min for a total absorbed dose of 40 kGy. The PS spheres were collected by centrifugation and washed with ethanol and water three times then dried in vacuum oven at 40 °C for 48 h.

Preparation of sulfonated PS particles

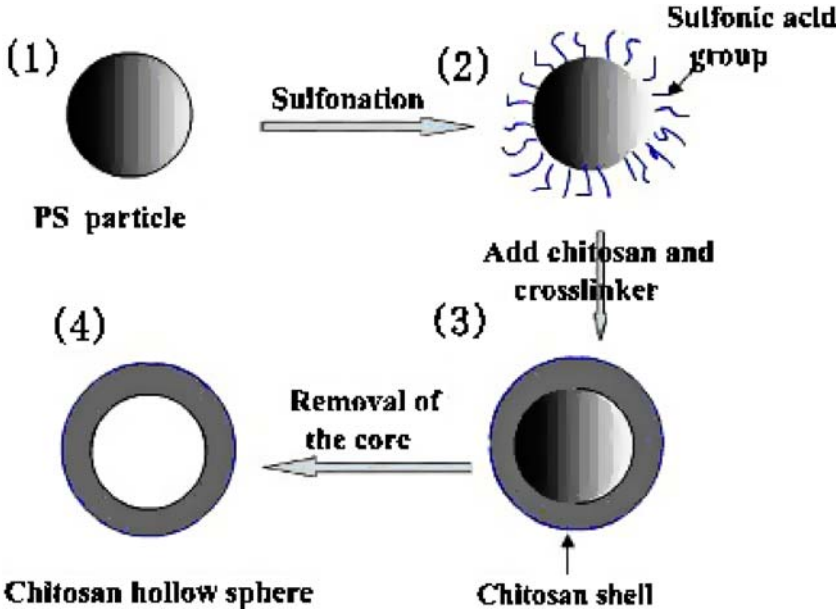
Uniform PS particles (4.0 g) synthesized as discussed above were dispersed in sulfuric acid (120 ml, 98%) with the aid of ultrasonic. The sulfonation took place at 40 °C with magnetic stirring. The degree of sulfonation was controlled by the sulfonation time. The samples A1, A2, and A3 were sulfonated for 10, 20, and 30 h, respectively. After having been diluted, the sulfonated PS spheres were separated by centrifugation and washed with ethanol and water three times. After drying in vacuum oven at 40 °C, yellowish fine powder is obtained.

Preparation of CS hollow microspheres

Step 1: CS (0.3 g) was dissolved in acetic acid aqueous solution (2%, *v*/*v*; 30.0 ml).

Step 2: Sulfonated PS particles (0.2 g) were fully dispersed in water (20.0 ml) under ultrasonic agitation. Then the suspension was added into the CS solution prepared in step 1 under vigorous stirring. This process would take 2 h. Finally, the CS–PS particles were separated by centrifugation to remove the excess CS. The samples were washed with distilled water three times.

The as-prepared CS–PS particles were dispersed in distilled water (30.0 ml) with magnetic stirring. Glutaraldehyde (2 ml, 2.5%) was added to crosslink the CS shell at



Scheme 2 Schematic illustration of the fabrication procedures of chitosan hollow microspheres

40 °C. After 2 h, the crosslinked particles were separated by centrifugation. Lastly, the gained product was alternately washed with THF and water three times, and the hollow spheres were dried in vacuum oven at 40 °C for 12 h before use. All the above procedures are shown in Scheme 2.

Controlled release experiment

The prepared CS hollow microspheres (used A3 as templates) were used as carriers to study their controlled release behavior. The sodium salicylate (SS) was chosen as a model drug. The encapsulation process was as follows: 0.1 g of CS hollow spheres and 0.05 g of SS were added into 10 ml distilled H_2O. The mixture is agitated for 3 days. Then the mixture was centrifuged.

The release process was as follows: the above product was re-dispersed in 10 ml distilled water. The mixture is placed into a dialysis membrane bag with a molecular cutoff of 10 kDa, then the bag was tied and placed in 300 ml water to make sure that it was immerged in the water. The system was kept at 37 °C with continuous magnetic stirring. After a certain period, 5 ml of the medium is removed, and the amount of the SS was analyzed by ultraviolet–visible spectrophotometer at 295.0 nm. To maintain the original

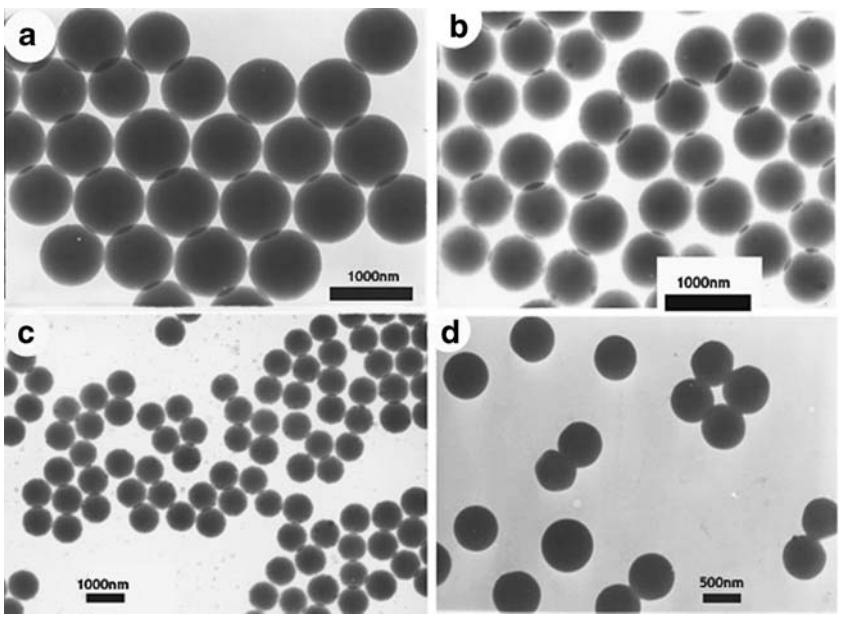


Fig. 1 TEM images of **a** PS, **b** A1, **c** A2, and **d** A3

Table 1 The average diameter and zeta potential of templates

Samples	Sulfonation time (h)	The average diameter (nm)	Zeta potential (mV)
PS	0	842±41	−20.1±0.8
A1	10	750±45	−49.5±0.9
A2	20	633±47	−72.2±1.8
A3	30	564±43	−76.5±0.7

volume, 5 ml of fresh water is put into the medium after each sampling. The sampling influence on the concentration of the SS is corrected with a mathematical method following Eq. 1:

$$C_n = C_n' + \sum C_{n-1} V_s / 300 \ (n > 1) \quad (1)$$

where C_n is the corrected concentration, C_n' is the measured concentration at the nth time, n is the order of sampling, and V_s is the sample volume [22].

Characterization

The morphology of the CS hollow microspheres and PS particles is observed by field-emission scanning electron microscopy (FESEM, JEOL JSM-6700) and transmission electronic microscopy (TEM, Hitachi H-800). For FESEM analysis, a drop of dispersed solution of CS hollow spheres is placed onto a copper tape. After evaporation of ethanol, the dried sample was coated with gold to reduce charging effect. The acceleration voltage of FESEM is 5 kV. For TEM analysis, the as-prepared samples are diluted in ethanol and dropped onto copper grid. The acceleration voltage is 200 kV. From the TEM analyses, different particle diameter moments (number average diameter, D_n) were calculated using the following equation:

$$D_n = \sum n_i D_i \Big/ \sum n_i \quad (2)$$

where n_i is the number of polymer particles with diameter D_i.

The particle sizes are examined by TEM, in which at least 100 particles were counted for every sample. The average particle sizes (D_n) are calculated from Eq. 2 according to the results in the TEM.

The infrared spectra are obtained from samples in KBr pellets using a Bruker EQUINOX 55 Fourier transform infrared (FT-IR) spectrophotometer.

Zeta potential, which is used as the characterization of the degree of sulfonation, is calculated from the measured electrophoretic mobilities using a Nano-ZS90 instrument (Malvern Instruments). Measurements (averaged more than 20 runs for each test, three times for each sample) are made after the sulfonated PS is neutralized with sodium hydroxide. UV–vis spectra were recorded at UV2401 PC (Sahimadzu corporation, Japan).

Results and discussions

Formation mechanism of the CS hollow spheres

The strategy of fabrication of the hollow microspheres includes four steps as illustrated in Scheme 2.

In the template method, the preparation of uniform-sized templates is the key step to produce narrow-size-distributed

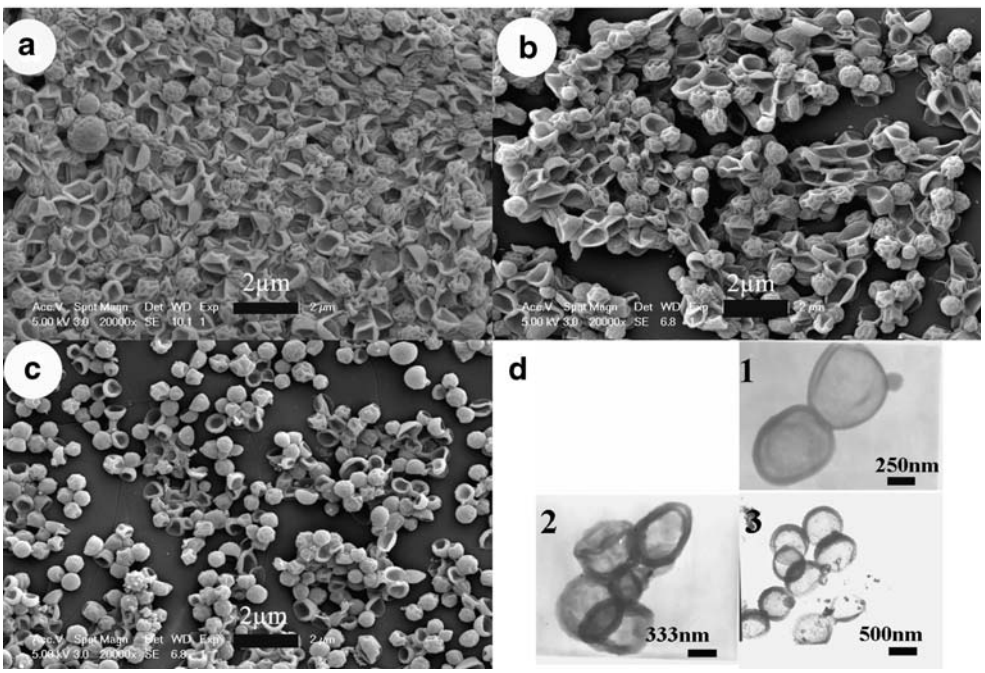


Fig. 2 SEM images of chitosan hollow microspheres used **a** A1, **b** A2 and **c** A3 as templates, and **d** TEM images of chitosan hollow microspheres used A1, A2, and A3 as templates, respectively

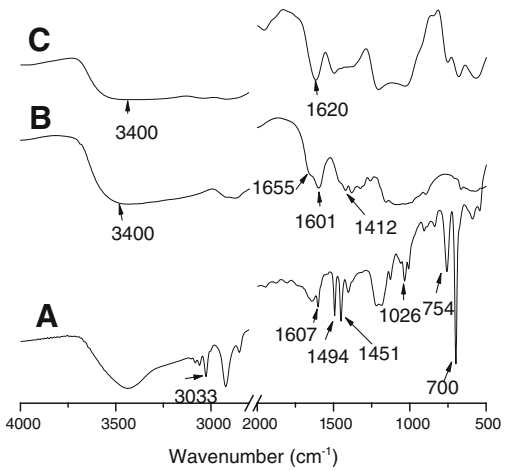


Fig. 3 FT-IR spectrum of sulfonated PS (*A*), pure CS (*B*), and chitosan hollow microspheres (*C*)

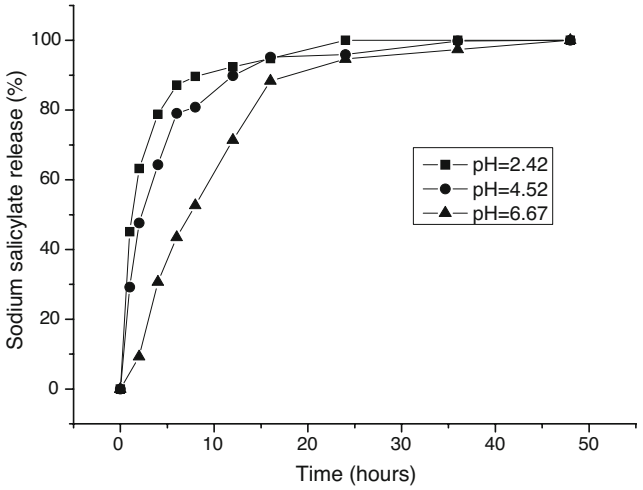
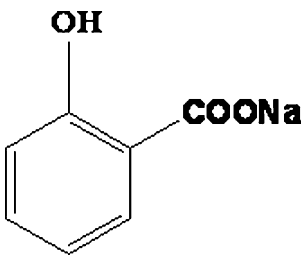


Fig. 4 Release profiles of sodium salicylate from CS hollow microspheres at various pH values at 37 °C

hollow particles. In this work, uniform-sized PS particles as templates were firstly synthesized by dispersion polymerization initiated with ^{60}Co γ-ray radiation. Radiation initiation is one of the most convenient initiation methods for polymerization owing to its large yield of radicals produced by γ-ray radiation and temperature-independent initiation process. It can easily result in a shorter nucleation stage during the dispersion polymerization. It is favorable to prepare the uniform polymer particles without the pollution of chemical initiators. The particle size could be controlled by the amount of the PVP and the ratio of the ethanol and water [23]. Figure 1a exhibited the morphology of the PS particles synthesized by the radiation dispersion polymerization. The particles seem to be uniform in size. Under present conditions, the average diameter of the particles is about 842±41 nm.

Secondly, the surface of the uniform PS templates was modified by introducing the sulfonic acid groups. This modification is important because the CS could not be adsorbed onto the surface of pure PS templates. The morphology of the modified PS templates is shown in Fig. 1b–d. Obviously, the particle size is still uniform after the sulfonation modification. But the particle size of all sulfonated PS samples is smaller than that of the corresponding PS particle. This could be related with the change of the hydrophilicity of PS chain before and after sulfonation.

Scheme 3 Chemical structure of sodium salicylate



The degree of sulfonation can be controlled by sulfonation time and reflected by the change of the surface zeta potential. The more sulfonic acid groups were introduced into the templates, the more charge on the templates, thus the zeta potential would be higher. From Table 1, it can be seen that the zeta potentials of PS, A1, A2, and A3 are −20.1±0.8, −49.5±0.9, −72.2±1.8, and −76.5±0.7 mV, respectively. It is shown clearly in Table 1 that, at the initial stage of the sulfonation, the zeta potential of the sulfonated PS increased quickly, and then it changed a little after 20 h. It means that the saturation of the sulfonic acid groups on the PS surface is leveling off after sulfonation for 20 h. With the prolongation of sulfonation time, the sulfuric acid can enter inwardly from the PS particles surface; meanwhile, the sulfonated site becomes hydrophilic [24, 25], and the hydrophilic sulfonated PS chain on the surface can be swollen and taken away from the particle surface during the sulfonation or washing in the later stage. Therefore, the sulfonated PS templates become smaller than the initial PS particles. Evidently, the longer the sulfonation time spent, the greater extent of the PS particle sulfonated; and, finally, the smaller size of sulfonated PS templates was obtained. It is consistent with the TEM images of the templates shown in Fig. 1. The sulfonated PS templates are about 750±45, 633±47, and 564±43 nm in diameter, respectively.

Thirdly, when the sulfonated PS templates and CS were mixed in acidic water, the protonized amino groups of CS can interact with sulfonic acid groups on the surface of the templates through the electrostatic force so that the CS can be adsorbed onto the surface of sulfonated PS spheres, forming a CS shell around the template particles. After being crosslinked by glutaraldehyde, the CS shell can firmly attach on the surface of the sulfonated PS core.

Finally, the CS hollow spheres can be produced after the removal of the cores by dissolving the abovementioned prepared core–shell-structured particles in THF solvent.

Figure 2 displays the TEM and SEM images of the CS hollow microspheres. What can be seen in these images are the uniform-sized hollow particles or collapsed spheres. The TEM images showed clearly that the hollow-structured microspheres were formed after the removal of the PS cores. But when the hollow CS microspheres were dried in vacuum, the CS shell was not rigid enough to keep the original shape and collapsed. From Fig. 2a to c, the burliness of the CS hollow spheres are getting better. It is also indicated in TEM images in Fig. 2d that the shell is becoming thicker when using templates sulfonated for a longer time. The shell thickness of samples using A1–A3 as templates is about 45±5, 64±6, and 81±11, respectively. It indicated that templates sulfonated for a longer time can adsorb more CS.

FT-IR was used to characterize the component of the CS hollow microspheres. The spectra of the sulfonated PS, the pure CS, and the CS hollow spheres are conducted as shown in Fig. 3. The peaks shown in Fig. 3(A) at 1,607 and 1,494 cm^{-1} are corresponding to the vibration of the C–C in the phenyl group; the strong absorbance peaks at 3,080, 3,075, 3,033, 750, and 700 cm^{-1} correspond to the vibration of hydrogen in the phenyl group of PS (Ar–H). The peaks at 1,451 and 1,025 cm^{-1} are attributed to the sulfonic acid groups of the sulfonated PS. The characteristic peaks in Fig. 3(B) at 1,655 and 1,601 cm^{-1} are assigned to the amide I and II band of CS, respectively, and a broad band concentrated on 3,400 cm^{-1} assigned to the OH bonded to with the contribution of NH absorption; the peaks at 1,412 cm^{-1} correspond to the vibration of the C–H of the methylene groups. The differences are obvious between the crosslinked core–shell particles and the particles which were treated with THF to remove the sacrificial cores. As shown in Fig. 3(C), all the characteristic absorbance peaks of the sulfonated PS disappeared, which indicated that the templates are removed basically, and a new peak at about 1,620 cm^{-1} appeared, indicating the formation of the Schiff's base structure [26].

Drug release behavior of the CS hollow spheres

One of the most important applications of hollow spheres is as drug carrier. CS hollow microspheres have peerless advantage in being used as drug carrier because it is biodegradable and biocompatible. Herein, sodium salicylate (SS) is chosen as model drug for it is easily detected by ultraviolet–visible spectrophotometer. Its structure is shown in Scheme 3. Figure 4 shows the release profiles of SS from CS hollow microspheres for various time intervals in media of various pH values at 37 °C. The SS release profiles are found to occur in a biphasic manner: an initial fast release phase and then leveling off. It is obvious that the release of the SS depends on the pH values of the release medium. The release rate decreases with the increase in pH value of the release medium. The release profile has the fastest release rate at pH=2.4. This can be explained by the fact that the release of the SS depends greatly on the swelling of the hollow microspheres. The hollow microspheres can be swollen to a great extent at acidic condition because the amino groups of CS are protonized, leading to the breaking of the strong hydrogen bonds between the CS chains and resulting in a fairly faster release of SS. However, at high pH value, the microspheres limitedly swelled. The SS is entrapped into the hollow microspheres and cannot be released easily.

Conclusions

In summary, the CS hollow microspheres which are biologically compatible and biodegradable have been successfully fabricated using sulfonated PS particles as templates. The size of the hollow spheres was determined by the size of the uniform-sized sulfonated PS templates and the thickness of shell was determined by the sulfonation time. The preliminary release experiment result of model drugs (sodium salicylate) indicated that the drug release rate is dependent on the pH value of the system.

Acknowledgement This work was supported by the National Natural Science Foundation of China (No. 50573070, 50773073) and the program for Changjiang Scholars and Innovative Research Team in University of Science and Technology of China.

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