J Mater Sci (2011) 46:6758–6765
DOI 10.1007/s10853-011-5632-3

Sacrificial functional polystyrene template to prepare chitosan nanocapsules and in vitro drug release properties

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Received: 24 November 2010 / Accepted: 21 February 2011 / Published online: 20 May 2011

Abstract In this study, biocompatible and biodegradable chitosan (CS) nanocapsules are successfully prepared in abundant and easily using carboxyl-functionalized polystyrene (PS) as sacrificial template and cross-linked CS with glutaraldehyde as the shell. First, the monodisperse functionalized PS templates be about 200 nm are made by emulsifier-free emulsion polymerization. Second, nanocapsules are accomplished by fabricating on the basis of chemical cross-linking on the surface of the PS template and removing the core via tetrahydrofuran. The templates and nanocapsules were characterized by FT–IR, ^{1}H NMR, FESEM, and TEM. All the results confirmed that the nanocapsules are accomplished via this method. By dissolution of ibuprofen in the chloroform droplets when prepare the carboxyl-functionalized PS, drug-loaded nanocapsules are also fabricated. It is found that the loaded drug can be released again in a sustained manner for up to 80 h. The nanocapsules walls have a prominent effect in slowing down the drug release rate.

Introduction

Hollow nanocapsules have been widely used in fields of pharmaceutics, cosmetic, food, textile, adhesive, agricultural industry, the nanocapsules of artificial cells, and protection of proteins, enzymes, DNA, and catalysis [1–3]. All these are based on their isolating property, large inner volume, and tunable permeability [4–6]. To obtain the nanocapsules with versatile structures and properties, new efforts are continuously tried to explore various techniques for fabrication. The template method as a common method to prepare nanocapsules often needs a template such as micelles [7], calcium carbonate [8], polymer particles [9], and miniemulsion technique [10]. In the template method, the target material is precipitated or polymerized on the surface of the template. Then the template is removed to form a cavity, leading to a hollow sphere structure [11, 12]. For example, the hollow spheres are generally fabricated by inorganic material of silica or non-silica oxides directly deposited on the surface of the polystyrene (PS) templates [13]. Another common method to fabricate nanocapsules by the layer-by-layer (LBL) assembly [14, 15], multilayer nanocapsules with ultrathin wall thickness and tunable wall structures and properties has been fabricated. However, fabrications of biocompatible and biodegradable hollow microspheres from natural polymers and their derivatives have been scarcely published. It is important to develop a new methodology for the preparation of shell cross-linked hollow microsphere using natural materials for a sustainable development and human safety.

Chitosan, a biodegradable, nontoxic, and renewable linear polysaccharide, has been considered for various biomedical and pharmaceutical applications [16, 17]. To these ends, particles of CS in hundreds of micrometers have been prepared in different ways, including coacervation precipitation, spray-drying, emulsion cross-linking, emulsion droplet coalescence, reverse micellization, ionic gelation, and sieving method [18]. Complexation between CS and oppositely charged polysaccharides in solution is another

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way to synthesize CS particles; however, this process often leads to the formation of fibers [19]. Because of its advantages of fine control of the compositions and the thickness of the capsules, the LBL method received more and more attention in recent years [20]. Zhang et al. [21] fabricated a single component microcapsule of CS hydrogel from the CS/poly(acrylic acid) (PAA) LBL assembly by using SiO_2 as the template. The CS was cross-linked, and then PAA was removed while removing the SiO_2 core template with carbonate buffer solution. However, this strategy needed many times deposition cycles and the morphology of the CS nanocapsules was not approving. Peng et al. [22] prepared cross-linked N-methylated CS hollow microspheres using cyclohexane droplets as a template, but the sizes of these microspheres were inhomogeneous, the morphology and the burliness of the hollow microspheres were not well because of fluidity of the droplet core. Efforts should be still made to develop safe and facile approaches to fabrication of nanosized capsules of natural biopolymers.

For this purpose, we propose a new strategy to selectively cross-link the CS chains and remove the other polymer components that were built up in the capsule walls. Using this strategy, the monodisperse carboxyl-functionalized PS particles which made by emulsifier-free emulsion polymerization were used as the sacrificial template and single component CS nanocapsules were successfully synthesized by one step deposition cycle. More importantly, although many shell cross-linked capsules have been made from synthetic block copolymers through a strategy of self-assembly of block copolymers with selective cross-linking on the shell block [23–25], it is very challenging, if not impossible, to synthesize well defined block copolymers from natural polysaccharides. The novel strategy here can provide a general method to synthesize monodisperse shell cross-linked nanocapsules from natural polysaccharides that have many applications in the pharmaceutical and medical field due to their biocompatibility and degradability. So, by incorporation of ibuprofen into the nanocapsules interiors in the polymerization process, drug-loaded nanocapsules are obtained and the drug release properties are also assessed. With a mild and less toxic fabricating environment, these biodegradable and biocompatible nanocapsules might be expected to be favorable carriers for drug delivery systems.

Experimental

Materials

Styrene (St), methacrylic acid (MAA), potassium persulfate (KPS), anhydrous tetrahydrofuran (THF), glutaraldehyde (GA), and other reagents were all from Shanghai Chemical Reagents Co., Ltd. China. St was washed with NaOH (10 wt%) aqueous solution, then distilled water until pH = 7. After dried with anhydrous $MgSO_4$, St was distilled under vacuum prior to use. MAA was distilled under vacuum before use. KPS was re-crystallized before use. Chitosan was obtained from (San Huan Ocean Biochemical Co. Ltd. China). Its degree of deacetylation and the molecular weight were determined to be 95% and 5.0×10^5, respectively. All other agents were analytical grade and used as received. Distilled Water (DI) was obtained from a Milli-Q® Gradient System from Millipore equipped with a Quantum™ cartridge.

Preparation of monodisperse carboxyl-functionalized PS particles and ibuprofen-loaded functional PS

The monodisperse carboxyl-functionalized PS particles were prepared via traditional emulsifier-free polymerization of styrene and MAA [26]. In a typical experiment, St (10 g) and MAA (1.0 g) were added to 95 mL of Milli-Q® water in a 250 mL three-neck round-bottom flask equipped with a mechanical stirring, a nitrogen inlet and a condenser. After the mixture was deoxygenated by bubbling nitrogen gas at room temperature for 1 h, the flask was placed in a 70 °C oil bath and stirred mechanically at 300 rpm. An aqueous solution of KPS (0.100 g in 5 mL) was subsequently added to the reacting medium, all the reaction under a nitrogen atmosphere. The reaction was continued for 12 h to assure a maximum reaction. For the system of drug loading, 1 g ibuprofen was dissolved in chloroform and then added into the monomers of St and MAA. The same process as described above was adopted to obtain the ibuprofen-loaded functional PS particles.

Preparation of cross-linked CS nanocapsules and ibuprofen-loaded CS nanocapsules

0.5 g CS was dissolved in 50 mL of 2.0 wt% acetic acid under stirring and made into 1.0 wt% CS solution. 0.5 g carboxyl-functionalized PS particles were subjected to centrifugation at ~6000 rpm/min for 5 min and re-dispersed in Milli-Q® water by sonication and centrifuged. This dispersion centrifugation collection cycle was repeated four times to remove the water-soluble oligomer. The particles were then re-dispersed in 25 mL water and made into 2.0 wt% latexes. Then the PS latexes (2.0 wt%) were dropped into the CS solution with burette under stirring for 1 h. The mixture was subjected to centrifugation at ~6000 rpm/min for 5 min and re-dispersed in water by sonication and centrifuged. This dispersion centrifugation collection cycle was repeated four times to remove the excess CS which was not adsorbed on the surface of carboxyl-functionalized PS particles.

The resulting CS absorbed on PS surface particles was treated with 3.0 mL 2.5% GA for 2 h at 40 °C. After washing with water and anhydrous THF for four times orderly, they were added to the 0.2 M carbonate buffer of pH = 9. A decrease in pH value was observed upon the addition of particles. NaOH was added to maintain the pH at 9. After 2 h, they were washed with DI three times. The procedure was repeated to guarantee the complete removal of water-soluble oligomer [26].

The same method was used to prepare ibuprofen-loaded CS nanocapsules. The same process as described above just used the ibuprofen-loaded functional PS particles replace the unloaded functional PS particles. The drug loading efficiency of CS nanocapsules was measured as 75.5%.

In vitro ibuprofen release

The in vitro ibuprofen release behaviors were determined using a dialysis technique. The solutions of drug-loaded nanocapsules were ultrafiltered equipped with a cellulose membrane having a pore size of 50 nm and washed before lyophilization. Four hundred milligrams nanocapsules were dispersed in 40 mL PBS solution (pH 7.4). The resultant dispersions were introduced into a sealed filter membrane with a cut-off molecular weight of 10,000 Da. The sealed membrane was immersed into 400 mL PBS solution at 37 °C. At each time interval, 4 mL released medium was taken out for HPLC measurement taking the maximum absorbance at 265 nm. The total leaching solution was maintained at 400 mL by addition of 4 mL fresh PBS solution. Ibuprofen crystals were also put into the same filter membrane as a control. The mass of the released drug was quantified by HPLC measurement referring to a calibration curve recorded at the same conditions. The samples were then analyzed by HPLC. The dissolution specification of the pellets was that each capsule contained 40 μg ibuprofen. Chromatographic conditions: Diamonsil C18 column (5 μm, 250 × 4.6 mm^2), mobile phase methanol:water (25:75), flow rate 1 mL/min; UV detector wavelength 265 nm. Cumulative drug release percentage as a function of time was recorded.

Characterization

FTIR spectra were recorded on a Vector 22 FT–IR spectrometer. X-ray photoelectron spectroscopy (XPS) was performed on a VGESCALAB MKII X-ray photoelectron spectrometer with a nonmonochromatic Al K_α radiation (1486.6 eV) under high vacuum of 10^{-11} mbar. TEM measurements were carried out on a Japan Hitachi Model H800 microscopy with an accelerating voltage of 200 kV. Samples were prepared by dropping a suspension onto Formvar-coated copper grids. The morphology observation of the samples was carried out on a field-emission scanning electron microscopy (FESEM, JEOL JSM-6700) at an accelerating voltage of 10 kV. Nuclear magnetic resonance spectroscopy (^{1}H NMR) was performed on a Varian-500 MHz spectrometer.

Results and discussion

Characterization of monodisperse carboxyl PS particles

In order to confirm the copolymerization of MAA and St to prepare carboxyl-functional PS, the copolymers were extracted with Milli-Q® water for 5 days to remove the homo-polymer of MAA and dried under vacuum. FT–IR spectra of the samples functional PS are shown in Fig. 1a. Absorbance peaks at 3025, 1605, 1493, 1450, 750, and 699 cm^{-1} correspond to the phenyl group; the peaks at 2923 and 2850 cm^{-1} correspond to the methylene and

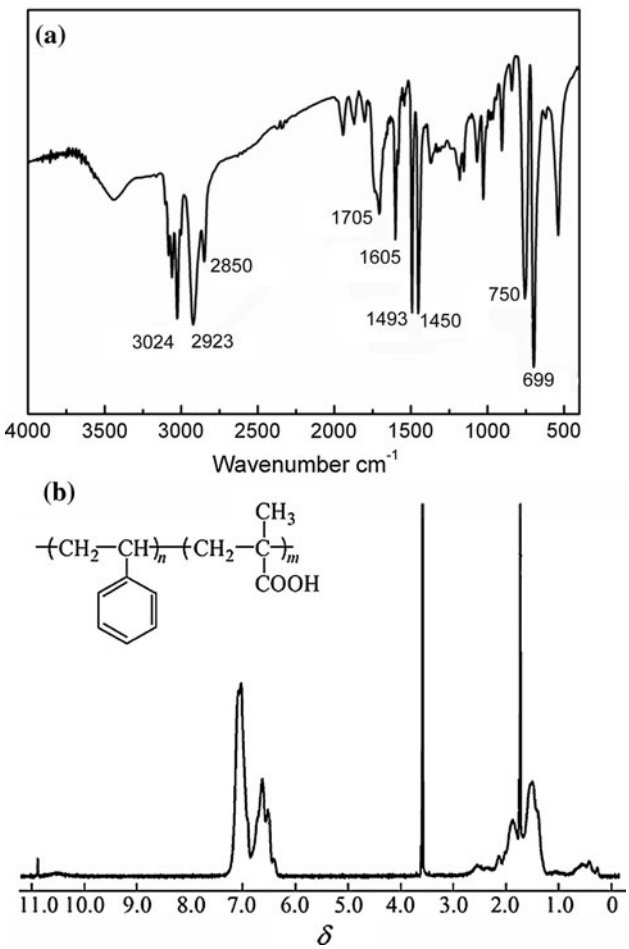


Fig. 1 **a** The FT–IR spectra of carboxyl-functionalize PS particles and **b** ^{1}H NMR spectrum of carboxyl-functionalize PS particles

methenyl groups. The characteristic peaks at 1705 cm^{-1} (C=O) are assigned to the carboxylic group of carboxyl-functionalized PS particles. The composition of the P(S-co-MAA) copolymers was determined using nuclear magnetic resonance spectroscopy (^{1}H NMR). The copolymer spectra were obtained using a Varian-500 MHz spectrometer. Deuterated tetrahydrofuran was selected as the reference solvent. The solid copolymer sample was dissolved in a 5-mm-diameter ^{1}H NMR test tube at a concentration of 0.1 g/mL. Figure 1b shows us the proton magnetic resonance spectroscopy of P(S-co-MAA) copolymers. From the figure, the chemical shift between $\delta = 0 \sim 1.0$ attribute to the proton ($-CH_3$) of MAA; $\delta = 1.0 \sim 3.0$ attribute to the proton ($-CH_2$) of styrene and MAA, respectively; and $\delta = 6.0 \sim 7.5$ attribute to the benzyl proton of styrene.

X-ray photoelectron spectroscopy is employed to determine the surface composition of carboxyl PS templates. The typical information depth of XPS is ~5 nm [27]. Figure 2a shows the XPS survey spectra of carboxyl PS templates. All spectra reveal the presence of only carbon (284.6 eV) and oxygen (532.3 eV). Figure 2b offers XPS spectrum of cross-linked CS particles after the extraction with water and reveals the existence of the element of N at the electron binding energy of 423 eV, indicating that CS was incorporated onto the surface of carboxyl-functionalized PS templates.

The monodisperse morphologies of carboxyl-functionalized PS particles are investigated by TEM and FESEM. The TEM and FESEM images of the samples are shown in Fig. 3. From TEM and FESEM images, we can see that all polymer particles are spherical with a very narrow size distribution.

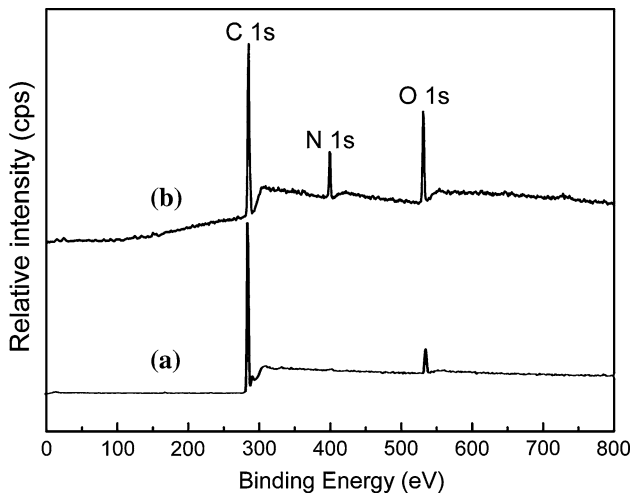


Fig. 2 XPS full-scan spectra of (*a*) the carboxyl PS templates and (*b*) PS cross-linked CS shell particles

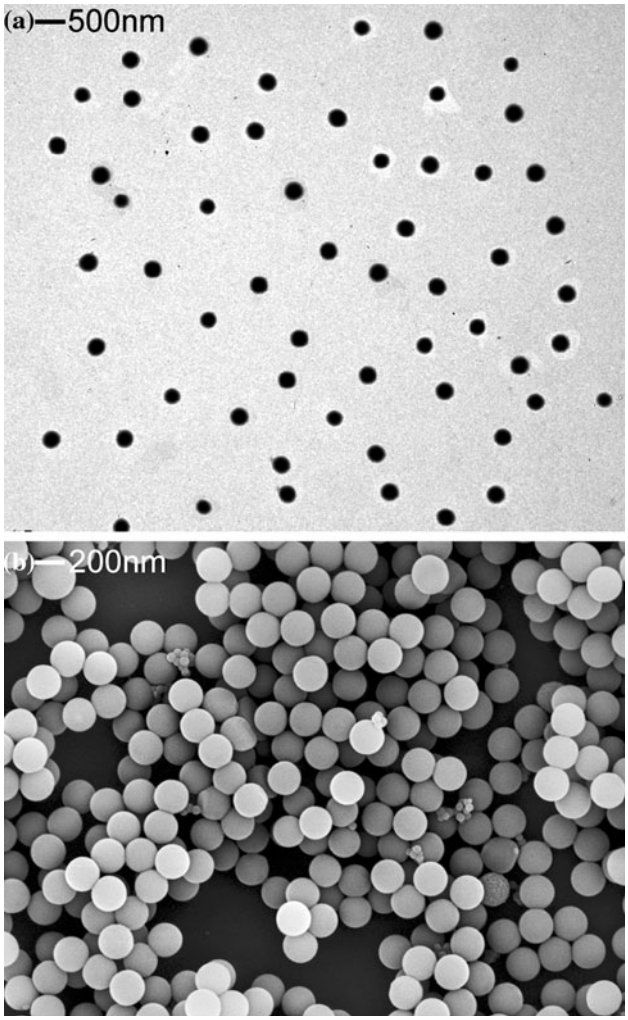


Fig. 3 TEM (**a**) and FESEM (**b**) images of carboxyl-functionalize PS particles

Characterization of cross-linked CS particles and cross-linked CS nanocapsules

We use the carboxyl-functional PS as the templates to absorb CS onto their surface to prepare CS nanocapsules. The absorbed CS particles are treated with 3.0 mL 2.5% GA for 2 h at 40 °C to get the cross-linked CS particles. Then the sacrificial cores are removed with solvent to get the cross-linked CS nanocapsules. The FT–IR spectra of CS, the cross-linked CS particles, and the cross-linked CS nanocapsules are shown in Fig. 4A. The characteristic peak at 1655 and 1600 cm^{-1} are assigned to the amide I and II band of CS, respectively. From Fig. 4b, the strong absorbance peaks at 1950, 1870, 1800, 1600, 1498, and 1450 cm^{-1} correspond to the phenyl group, the characteristic peaks at 1705 cm^{-1} (C=O) are assigned to the carboxylic group of carboxyl-functionalized PS particles. Significant changes are found after the cross-linked core–shell particles are treated with

anhydrous THF and carbonate buffer of pH = 9 to remove the sacrificial cores. As shown in Fig. 4c, all the absorbance peaks of phenyl group and carboxylic group of the carboxyl-functionalized PS particles disappear, and a new peak at about 1640 cm^{-1} appeared, indicating the formation of the Schiff's base structure [28]. The peaks at 1447 and 2925 cm^{-1} correspond to the methylene groups, the strong intensities are considered to be related with the excessive GA. From the comparison, we can see that the carboxyl-functionalized PS particles cores are removed basically. Figure 4B shows the 1H NMR spectra of CS in a mixed solvent of CD_3COOD and D_2O were recorded on a 500 NMR spectrometer (Varian, Inc., USA). The relevant chemical shifts were marked in the figure.

Figure 5a shows the typical FESEM graphs of the cross-linked CS particles (the core have not removed), contrast Fig. 5a and the FESEM pictures (Fig. 3b) uncross-linked carboxyl-functionalized PS particles, we can find the surface of cross-linked are rougher. Figure 5b shows the typical TEM graphs of the CS nanocapsules which are made from the functional PS as the sacrificial cores. Obviously, all the cross-linked CS nanocapsules exhibit the hollow inner structure. The size of the nanocapsules lies in the range from 190 to 250 nm. The average particle diameters of the

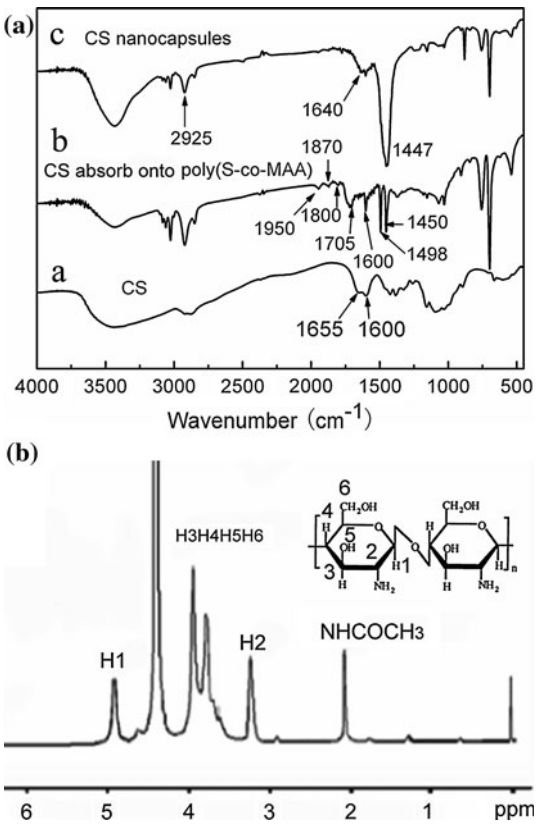


Fig. 4 **A** FT–IR spectra of (*a*) CS, (*b*) the carboxyl-functionalize PS cross-linked CS shell particles, (*c*) the hollow cross-linked CS nanocapsules. **B** ^{1}H NMR of the CS

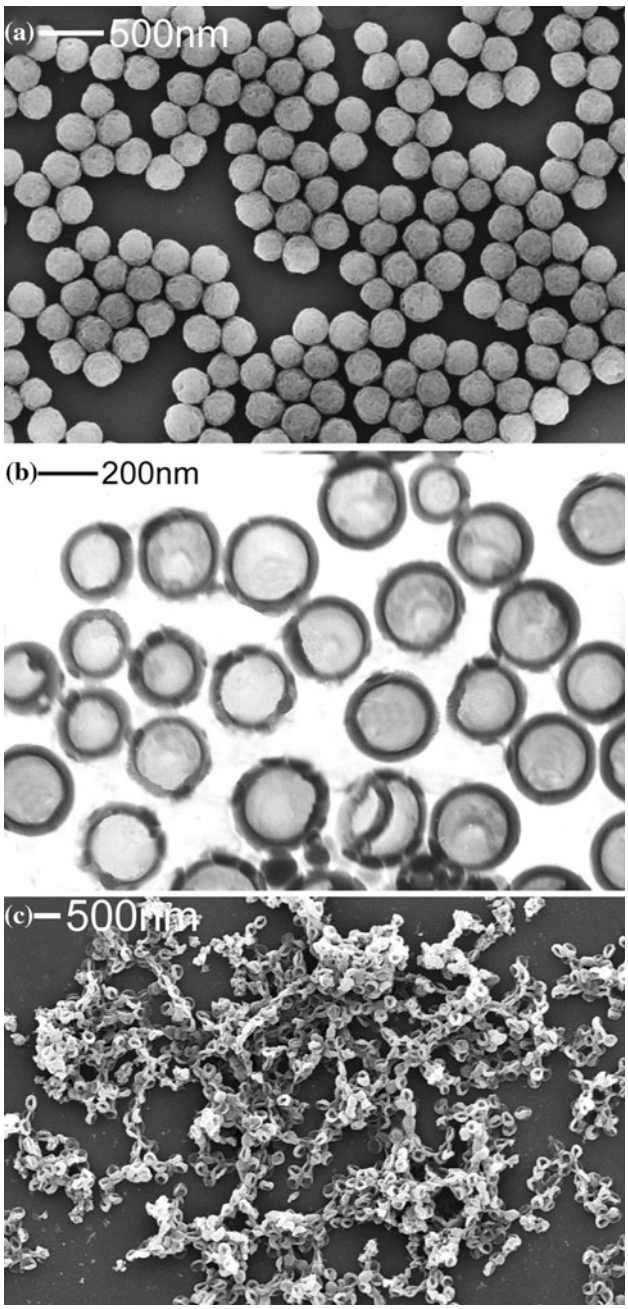
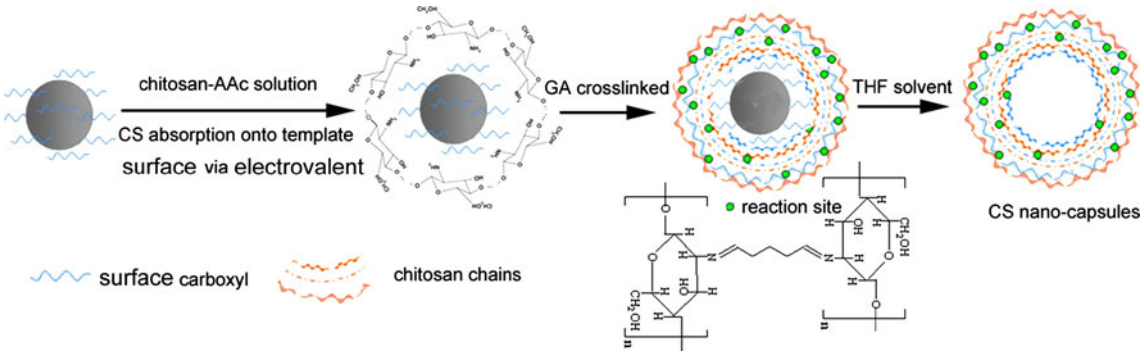


Fig. 5 **a** FESEM image of the cross-linked CS particles, TEM (**b**), and FESEM (**c**) images of the cross-linked CS nanocapsules

nanocapsules are about (230 ± 20 nm). From the data, we can see that the average diameters of the nanocapsules are smaller than the cross-linked CS particles and PS template (270 nm). This can be explained that the CS shell will shrink in some degree during the gradual drying process in air [21]. The shell thickness of nanocapsules is about ~35 nm. Figure 5c shows FESEM images of the morphology of CS nanocapsules. From the images, we can see the nanocapsules have been collapsed. At the same, the thickness and the burliness of the nanocapsules can indicate some



Scheme 1 Schematic for preparation cross-linked CS nanocapsules via sacrificial polymer template

microspheres keep the spherical shape perfectly. Moreover, the microspheres aggregate together in the images Fig. 5b. It because that aldehyde group of predominant GA is cross-linked with CS. And the other hydrophilic –CHO group remains untapped on the microspheres' surface as reported [29]. From the images, we can see that the nanocapsules seem to be somewhat more polydisperse than those of the template particles. In the author's opinion, the authors think this is because there have different contractibility rate and become deformed under TEM electron beam. At the same, the carboxyl PS templates can absorb different amount CS and GA cross-linked between CS.

Mechanistic description of cross-linked reaction to prepare CS nanocapsules

The new strategy is to selectively cross-link the CS chains and remove the polymer components that were built up in the capsule walls (see Scheme 1). Using this strategy, the monodisperse functional PS templates were first made by emulsifier-free emulsion polymerization. Because there have many carboxyl on the surface of copolymers, CS can absorb onto the surface of copolymers via electrovalent function. So there have many CS on the surface of polymers, GA was used as cross-linking agent to cross-link the amino of CS to form polymer shell. THF used as solvent to remove core. Thus, single component CS nanocapsules were successfully synthesized by a single step deposition cycle. The novel strategy here can provide a general method to synthesize monodisperse shell cross-linked microcapsules from natural polysaccharides that have many applications in the pharmaceutical and medical field due to their biocompatibility and degradability.

Ibuprofen-loaded CS nanocapsules and in vitro ibuprofen release

Ibuprofen, a widely used drug for aches and pains and for antipyretic purposes, was used as a model drug and incorporated into the cross-linked CS nanocapsules. TEM images (Fig. 6) show the structure of these drug-loaded capsules. The diameter of the nanocapsules is ~230 nm, which is same as that of the pure cross-linked CS nanocapsules as shown in Fig. 5b, c. The size distribution of the nanocapsules is also quite narrow. These results have indicated that incorporation of ibuprofen has little effect on the topology of the capsules. However, compared with the obvious hollow structure (Fig. 5b), these nanocapsules possess gray interiors and have some black clusters. This can be regarded as a sign of ibuprofen loading.

Release of ibuprofen from CS nanocapsules (Fig. 7b) and from pure ibuprofen crystals (Fig. 7a) was performed in PBS solutions at pH 7.4 and 37 °C. Figure 7a shows the pure ibuprofen crystals has a burst release behavior in PBS solutions. Figure 7b shows the release property of ibuprofen from CS nanocapsules. From Fig. 7b, we can see that a burst release behavior was recorded at the initial stage (within 3 h) in release profiles of the cross-linked CS nanocapsules. This is same as that of the control ibuprofen crystals without polymeric covering layer, except that the control drug was completely released within ~3 h with a much faster rate. After the burst release, the ibuprofen-loaded capsules

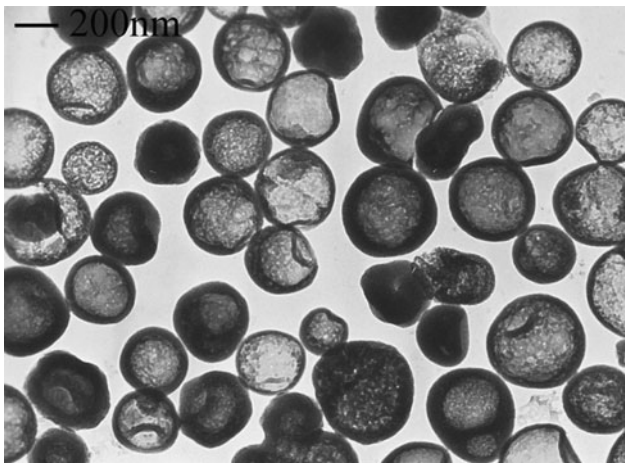


Fig. 6 TEM images of ibuprofen-loaded CS nanocapsules

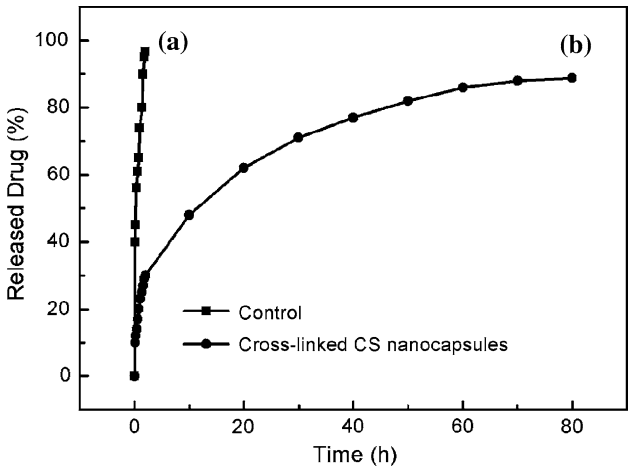


Fig. 7 Release profiles of ibuprofen from CS nanocapsules (*a*) and from pure ibuprofen crystals (*b*). The release was performed in PBS solutions at pH 7.4 and 37 °C

exhibited continuous slow release profiles lasting for at least 80 h. The results have roughly demonstrated that diffusion of the ibuprofen molecules from the capsules to the bulk solution is retarded by the polymeric shells. The burst release at the initial stage, a quite normal phenomenon in many drug release systems, should be ascribed to rapid dissolution of the ibuprofen molecules near the external surface of the capsules [30]. Compared to the release profile of the pure ibuprofen crystals, a slower release rate was observed in the profile of the CS nanocapsules. The cumulative drug percentage within the burst release (2 h) was 18% for the CS nanocapsules, which is smaller than that of the pure ibuprofen crystals (75%). Furthermore, the final released percentage (85%) at 80 h from the CS nanocapsules is lower than that from the pure ibuprofen crystals (98%) too, demonstrating that CS nanocapsules have more effective for sustained release.

Morphology of nanocapsules after ibuprofen release

After drug release for 80 h, the nanocapsules were subjected to TEM observations to check their structure change (Fig. 8). Compared with their filled counterparts (Fig. 6), the nanocapsules interiors became more transparent, which should of course be the result of drug release. For the cross-linked CS nanocapsules (Fig. 8), after drug release their structure is very similar with that of pure CS nanocapsules as shown in Fig. 5b. Their wall thickness was measured as ~30 nm, a value consistent with that of the pure cross-linked CS nanocapsules (Fig. 5b). Considering their similar particle size also, one can conclude that incorporation of ibuprofen has little influence on the wall structure and the geometric properties of the CS nanocapsules.

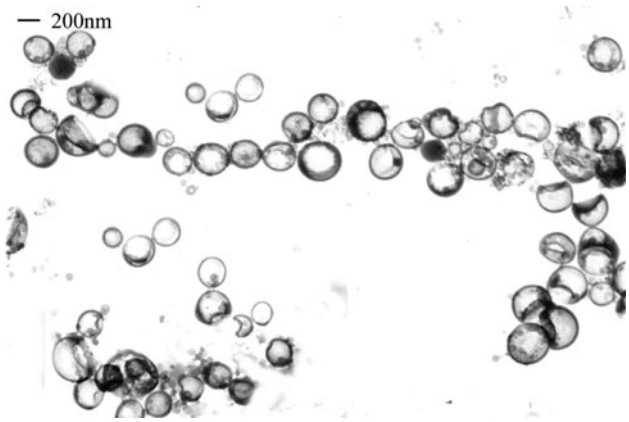


Fig. 8 TEM images to show the nanocapsules structure after release of ibuprofen

Conclusion

In conclusion, a strategy to prepare cross-linked CS nanocapsules with carboxyl PS template was proposed. It involves an initial cross-linking of CS followed by removal of the carboxyl templates. Results from SEM and TEM revealed the formation of nanocapsules. The potential of such materials in drug delivery or chromatography are currently under investigation. By dissolution in the oil phase, ibuprofen is incorporated in order to obtain drug-loaded nanocapsules. Characterizations by TEM demonstrate that nanocapsules with roughly the same diameter and wall thickness have been fabricated regardless of the introduction of the drugs. For nanocapsules made from cross-linked CS, the incorporated ibuprofen can be released. A burst release behavior is recorded at the initial stage (within 3 h), followed by sustained release for up to 80 h. Therefore, these biodegradable and biocompatible nanocapsules may find practical applications as drug delivery carriers.

Acknowledgements We greatly appreciate the help from Central Laboratory of Analysis and Structure Research in University of Science and Technology of China (USTC). This study is financially supported the National Science Foundation for Post-doctoral Scientists of China (no. 20100471000).

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