

## To prepare chitosan capsules via interfacial initiated chitosan macromonomer in situ polymerization

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Received: 21 May 2011 / Revised: 11 July 2011 / Accepted: 21 August 2011 /

Published online: 31 August 2011

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**Abstract** In this study, we present a method interfacial in situ polymerization of chitosan macromonomer (CM) to prepare biocompatible and biodegradable chitosan capsules, *N*, *N'*-methylene-bis-acrylamide (Bis) used as cross-linking agent. Methyl acrylic acid (MAA) is grafted onto the chitosan chain to obtain a water soluble double bond CM derivative. The molecular structure of chitosan derivative is confirmed by FT-IR and <sup>1</sup>H NMR. The oil soluble cumene hydroperoxide (CHPO) oxidizer and the water soluble reductant tetraethylenepentamine (TEPA) are used as redox initiation couple for producing radical at the oil/water emulsion interface when them encounter to initiate CM polymerization. The interfacial radical can in situ initiate CM to prepare capsules. The structure and morphology of the chitosan capsules are characterized by FESEM and TEM. All the results confirmed that the interfacial in situ polymerization can initiate double bond to prepare capsule at room temperature and under ambient pressure.

**Keywords** Capsules · Chitosan · Interfacial · In situ polymerization

### Introduction

Hollow capsules have been widely used in fields of pharmaceuticals, cosmetic, food, textile, adhesive, agricultural industry, the microcapsules of artificial cells, and protection of proteins, enzymes, DNA, and catalysis [1–3]. All these based on their isolating property, large inner volume, and tunable permeability [4–6]. To obtain the microcapsules with versatile structures and properties, many efforts are continuously tried to explore various techniques for fabrication. The template method as a

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common method to prepare microcapsules often needs a template such as polymer beads [7], radical suspension polymerization [8], electrocapillary emulsification [9], and emulsion droplets [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]. The hollow spheres are generally fabricated by inorganic material of silica or non-silica oxides directly deposited on the surface of the polystyrene templates [13]. Another common method to fabricate microcapsules by the layer-by-layer (LBL) assembly [14, 15], multilayer microcapsules with ultrathin wall thickness, and tunable wall structures and properties has been fabricated. They have shown potential applications in materials and life science as microreactors, microcontainers, drug-delivery vehicles, protective shells for cells or enzymes, transfection carriers for gene therapy, and biosensors, etc. 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 (CS), linear copolymer consisting of  $\beta$ -1,4-linked 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose units, a weak cationic polysaccharide produced by the deacetylation of the natural polymer chitin, has many useful biological properties, such as biocompatibility, biodegradability, and bioactivity [16, 17]. To these ends, microparticles 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 way to synthesize CS microparticles; 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], and so on. Most of them are involved in a multi-stage and time-consuming strategy. There are few reports to prepare capsules containing liquid cores via interfacial *in situ* polymerization at room temperature and ambient pressure.

In this study, we described the preparation of core shell CS capsules containing liquid cores through redox interfacial initiated *in situ* polymerization of CM in emulsion. Redox initiation pairs have been used extensively in the emulsion polymerization of vinyl monomers [21–23]. Radicals can be produced rapidly compared with thermally decomposed initiators such as persulfate. Among the redox initiation pairs, the couples composed of one water-soluble component and one oil-soluble component exhibit different characteristics, i.e., radicals are formed mostly at the water–oil interface. In previous articles, several groups have taken advantages of this method to prepare different polymer colloids particles and hybrid materials [24, 25]. In those studies, several pairs of such redox initiators are employed. They are cumene hydroperoxide (CHPO)/iron (II) sulfate (FS), CHPO/tetraethylpentamine, tert-butyl hydroperoxide with different water-soluble reductants [26, 27]. Our strategy here can provide a general method to synthesize

capsules from natural polysaccharides that have many applications in the pharmaceutical and medical field due to their biocompatibility and degradability.

## Experimental section

### Materials

CS was obtained from San Huan Ocean Biochemical Co. Ltd. China, its degree of deacetylation and the molecular weight were determined to be 95% and  $5.0 \times 10^5$ , respectively. Carbodiimide (EDAC), *N*, *N'*-methylene-bis-acrylamide (Bis), and Cumene hydroperoxide (CHPO) were purchased from Sigma-Aldrich. Tetraethyl-eneptamine (TEPA), octylphenyl poly (ethylene glycol) ether ( $n = 4$ ) (OP-4), and octylphenyl poly (ethylene glycol) ether ( $n = 10$ ) (OP-10) were purchased from Shanghai Chemical Reagent Company. All other agents were analytical grade and used without any further purification. Milli-Q water was obtained from a Milli-Q<sup>®</sup> Gradient System from Millipore equipped with a Quantum<sup>™</sup> cartridge.

### Preparation of water soluble CS macromonomer via EDAC as condensation reagent and determination of the graft density of MAA

The synthesis of CS macromonomer (CM) was prepared by grafting MAA onto CS chain via condensation between the amine groups and the carboxyl groups under the catalysis of carbodiimide. The preparation described as follows: 1 g CS was dissolved in 100 mL water containing 1 mL acetic acid, 1 mL MAA, 1 g EDAC was added. The reaction took place over 24 h at room temperature under agitation. In order to remove the free MAA and other byproducts, the mixture was dialyzed in a filter membrane with a molecular weight cut off of 7000 Da against a large amount of triple-distilled water for 5 days. Finally, MAA grafted CM was obtained by freeze-drying.

We can determine the graft density of MAA onto CS via element analysis. Quality percentage content (C and N %) in CS and CM can be determined via element analysis. Mole ration ( $r$ ) of C and N:  $r = \frac{n_C}{n_N} = \frac{m_C/M_C}{m_N/M_N} = \frac{m_C}{m_N} \cdot \frac{14}{12}$ . The contents of N has not changed in CS chains after MAA has been grafted into CS, there just add four carbon atoms in each cyclose. So, graft density of MAA(%) =  $\frac{r_{CM} - r_{CS}}{4} \times 100\% = 32\%$ .

### Preparation of CS capsules via interfacial in situ polymerization

The typical procedure for the preparation of CS capsules is as follows: CHPO (150 mg) was dissolved in hexane (3.5 mL) to obtain the dispersed liquid. The nonionic surfactants of OP-4 and OP-10 were selected as emulsifiers, and Milli-Q water was used as the continuous phase. In a typical experiment, OP-4 (1.5 g), OP-10 (2.5 g), the water soluble components of CM (0.5 g), and crosslinking reagent of Bis (20 mg) were dissolved in Milli-Q water (100 mL) to form the continuous

phase. The dispersed liquid was dropped into the continuous phase and the obtained mixture was sonified to obtain normal emulsion. Then, the emulsion was purged with nitrogen for 1 h. The water components of CM (0.1 g) and TEPA (100 mg) were dissolved in Milli-Q water (10 mL). The obtained solution was added dropwise into the normal emulsions continuously with a Sp1001 syringe pump in 1 h under N<sub>2</sub> atmosphere. The polymerization was continued for another 12 h to ascertain the maximum conversion of monomers. In order to remove the oil phase hexane or other smaller molecules, the mixture was dialyzed in a filter membrane with a molecular weight cut off of 10000 Da against a large amount of triple-distilled water for 5 days. Finally, CS capsules was obtained by freeze-drying.

### Characterization

Fourier transform infrared spectrometer (FT-IR) was obtained on a Vector 22 FT-IR spectrometer with KBr pellets. Nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR) spectrum of CM was recorded on an ANAVCE DMX500 with D<sub>2</sub>O as solvent working at 500 MHz. For laser light scattering, the obtained emulsions were thinned with Milli-Q water. Then, the thinned latex was passed through a Millipore filter (0.45 micron) to remove any dust. Dynamic radius ( $R_h$ ) and gyration radius ( $R_g$ ) of the capsules were determined on a modified commercial laser light scattering spectrometer (ALV/SP-125) equipped with an ALV-5000 multi- $\tau$  digital time correlator and a He–Ne laser (632.8 nm). In dynamic LLS, intensity–intensity time correlation function  $G(2)(t,q)$  was measured and its Laplace inversion resulted in a line-width distribution  $G(\Gamma)$ .  $G(\Gamma)$  can be directly converted to the translational diffusion coefficient distribution  $G(D)$  or the hydrodynamic radius distribution  $f(R_h)$ . Polydispersity index (PDI) of  $R_h$  was defined as Eq. 1.

$$PDI = \mu_2/\bar{\Gamma}^2 \text{ and } \mu_2 = \int (\Gamma - \bar{\Gamma})^2 G(\Gamma) d\Gamma \quad (1)$$

In static LLS,  $z$ -average root-mean square radius of gyration ( $\langle R_g^2 \rangle^{1/2}$  or written as  $\langle R_g \rangle$ ) in a dilute solution/dispersion from Rayleigh ratio  $R_{vv}(q)$  was coarsely determined at certain concentration according to Eq. 2.

$$\frac{KC}{R_{vv}(q)} \approx \frac{1}{M_w} \left( 1 + \frac{1}{3} \langle R_g^2 \rangle - q^2 \right) + 2A_2 C \quad (2)$$

where  $K = 4\pi n^2 (dn/dC)^2 / (NA \lambda_0^4)$  and  $q = (4\pi n / \lambda_0) \sin(\theta/2)$  with NA,  $dn/dC$ ,  $n$ , and  $\lambda_0$  being Avogadro constant, specific refractive index increment, solvent refractive index, and light wavelength in vacuum, respectively, and  $A_2$  is the second virial coefficient [28].

For observation the morphology of capsules via FESEM, silicon (Si) wafers were cleaned in a piranha solution (70/30 v/v of concentrated H<sub>2</sub>SO<sub>4</sub>/30% H<sub>2</sub>O<sub>2</sub>). During the process, we should be carefully because piranha solution reacts violently with organic compounds and should not be stored in closed containers, thoroughly rinsed with Milli-Q water, and then blown dry with nitrogen gas. Then, the purified products were thinned with ethanol to 1000 times and one drop of the thinned latex

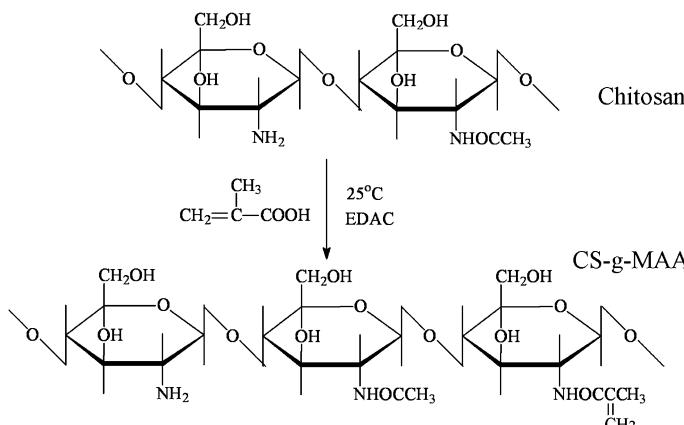
was attached to the cleaned silicon wafers. After the evaporation of solvent naturally, the silicon wafers was sputter coating Platinum for 70 s. The morphology of products was carried out on a field-emission scanning electron microscopy (FESEM, JEOL JSM-6700) at an accelerating voltage of 10 kV. The latex was thinned with ethanol to 1000 times and one drop of the thinned latex was attached to a copper grid. After the evaporation of solvent naturally, the morphology of products were observed under Hitachi Model H-800 Transmission Electron Microscopy with an accelerating voltage of 200 kV.

## Results and discussion

### Synthesis and characterization of CM via EDAC as condensation reagent

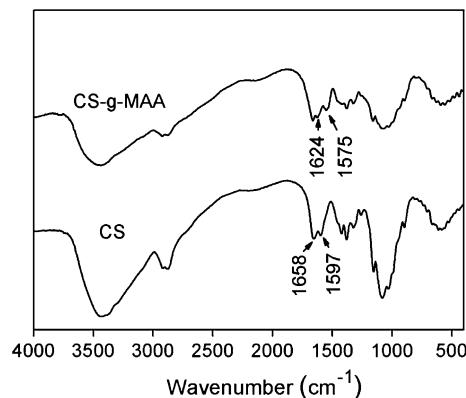
CS can be regarded as a copolymer of *N*-acetylglucosamine and *N*-glucosamine units randomly distributed throughout the molecular chain. It is dissolved only in acidic solution for its strong intermolecular hydrogen bonding. It contains abundant amino groups, through which both polymerizable (e.g., acrylate) and water-soluble groups can be conveniently introduced. In the present study, MAA is grafted onto the CS chains via the combination between the carboxyl groups and the amino groups to yield water soluble and polymerizable CS–MAA the catalysis of carbodiimide (Scheme 1). Since MAA is weak acids and CS can be directly dissolved in their solutions, reaction is easy to carry out without involvement of other acid. The byproducts of small molecular weight and unreacted monomers are then removed by dialysis [29, 30].

FT-IR and  $^1\text{H}$  NMR characterizations confirmed the structure of CS and its derivatives (Fig. 1). The IR spectrum of CS (Fig. 1a) illustrates peaks assigned to the saccharide structure at 1154, 1082, 1030, and 898  $\text{cm}^{-1}$  [31]. The peaks at around 1654 and 1598  $\text{cm}^{-1}$  are assigned to amide I band and  $\text{NH}_2$  group,



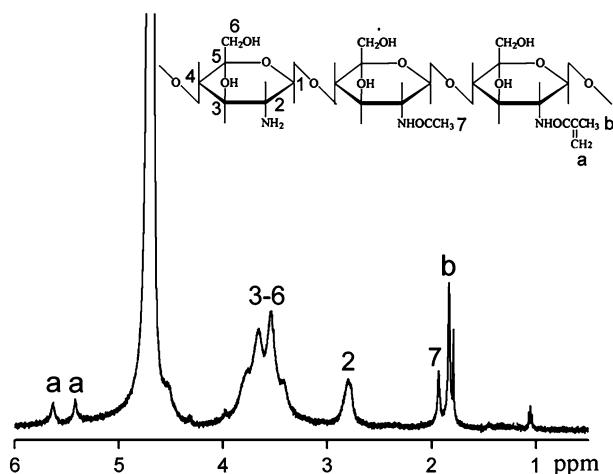
**Scheme 1** MAA grafted onto CS to synthesize macromonomer CM via EDAC as condensation reagent

**Fig. 1** FT-IR spectra of CS and CM



respectively. Accompanying with the weakening of absorbance at  $1598\text{ cm}^{-1}$ , new peaks at  $1624$  and  $1575\text{ cm}^{-1}$  emerge in the IR spectrum of CS-g-MAA (Fig. 1b) which should be assigned to the double bond and the amide II band, respectively. This result demonstrates that MAA has been successfully grafted.

Characterization of CS-g-MAA under  $^1\text{H}$  NMR confirms also its molecular structure. Chemical shifts belonging to the saccharide structure are assigned as follows:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta = 2.80$  ( $\text{H}_2$ ),  $\delta = 3.43\text{--}3.91$  ( $\text{H}_3\text{--H}_6$ ),  $\delta = 1.91$  ( $-\text{NCOCH}_3$ ) [32]. Chemical shifts at  $\delta = 5.64$  and  $\delta = 5.42$  are assigned to  $\text{H}_2\text{C}=\text{C}$ – (a) of MAA, respectively. Chemical shifts at  $\delta = 1.84$  and  $\delta = 1.20$  are assigned to methyl groups of MAA (b), respectively. The detailed peaks are marked in Fig. 2. All these results have confirmed that MAA has been grafted onto the CS chains.



**Fig. 2** The  $^1\text{H}$  NMR of CM macromonomer

## Characterization of the cross linked CS capsules

CS capsules were prepared through interfacial in situ polymerization of CS macromonomer in emulsion as summarized in Table 1. The core–shell hollow morphology of the capsules containing liquid cores is clearly observed under TEM and FESEM as shown in Fig. 3a, b, c. In those images, CS appears as the black loops. The capsules are hollow due to the removal of liquid and the cores appear white to gray. Figure 3a shows the typical FESEM graphs of the CS capsules via interfacial in situ polymerization of CS macromonomer (CS-g-MAA). From the FESEM, we can see that the capsules have been collapsed under the effects of electron beam of electron microscope. Figure 3b shows us a magnified picture of Fig. 3a. Figure 3c shows the typical TEM graphs of the CS capsules which are made via CHPO and TEPA as redox interfacial initiator to initiate CS-g-MAA macromonomer in situ polymerization. Obviously, all the CS capsules exhibit the hollow inner structure. The size of the capsules lies in the range from 200 to 250 nm. The average particle diameters of the capsules are about 220. From the date, we can see that the average diameters of the capsules are smaller than the data via DLS measure (Table 1). This can be explained that the CS shell will shrink in some degree during the gradual drying process in air and under the effect of electron beam of electron microscope. The shell thickness of capsules is about  $\sim 30$  nm. By counting 50 capsules, the averaged radius ( $r_0$ ) and shell thickness ( $t_{\text{shell}}$ ) were obtained and listed in Table 1. In this polymerization initiated by the redox couple, the oil-soluble oxidant component of CHPO stayed in the media of inverted emulsion while the reductive water-soluble component of TEPA existed in the water phase. Primary radicals would be produced only at the oil–water interface where both of them encountered. The primary radicals initiated the polymerization of monomers CM near the interface to form polymer propagating chains. Chitosan propagating chains might be anchored to the surfactant monolayer or escape to the media bulk. In our case, the anchoring could be caused by the interaction between the chains and the octyl group of OP. In the appropriate polymerization conditions, the anchoring of chitosan chains would form a chitosan-enriched layer at the oil–water interface and absorb the coming monomer.

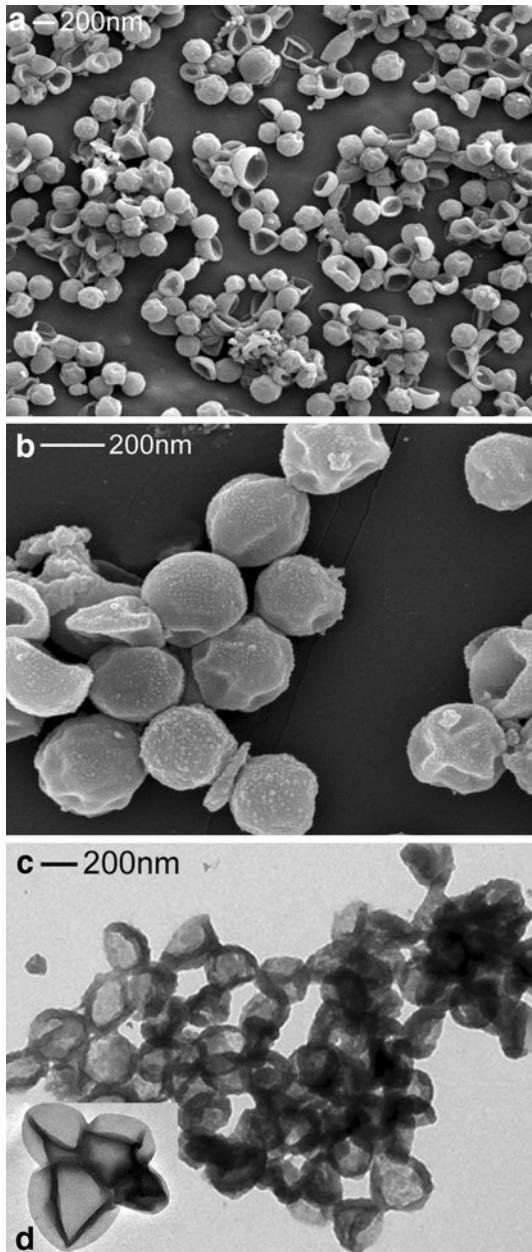
The data of hydrodynamic radius distribution ( $f(R_h)$ ) of CS capsules containing liquid cores are listed in Table 1, indicating a little broad distribution of those capsules with  $R_h$  156 nm. The values of their  $R_g$  are very close to those of their  $R_h$ , i.e., the ratios of  $R_h/R_g$  are about unity for capsule. These results might suggest that the hollow structure of CS capsules containing liquid cores. The diameter of capsules via DLS is about 300 nm, this is caused by the swelling of Milli-Q water.

**Table 1** Interfacial in situ polymerization of CS macromonomer in emulsion

Bis (mg)	CM (g)	Size by TEM (nm)		$R_g$ (nm)	$R_h/PDI$ (nm)
		$r_0$	Thickness		
Capsule	20	0.6	120	30	148
					156/0.18

The polymerizations were carried out with CHPO (150 mg) as the initiation oxidant in hexane (3.5 mL), OP-4 (1.5 g) and OP-10 (2.5 g)/Milli-Q water (110 mL), and TEPA (100 mg) as initiation reductant

**Fig. 3** **a** FESEM images of the cross linked core shell CS capsules; **b** a magnified FESEM of (a); **c, d** TEM images of the cross linked core shell CS capsules



## Conclusion

In conclusion, a novel strategy for the fabrication of cross linked CS capsules with liquid core was proposed. First, Water-soluble CM is successfully synthesized via grafting MAA under the catalysis of water-soluble carbodiimide. FT-IR and  $^1\text{H}$

NMR analysis confirm MAA has been grafted onto chitosan to form CM macromonomer. Second, CS capsules were successfully prepared via one stage interfacial in situ polymerization. This interfacial initiated polymerization provides an efficient, one step route to synthesize polymer capsules containing liquid cores. The formation feature of primary radicals and the anchoring effect of CS chains to the interface were suggested to be the reasons for the formation of capsules with the liquid cores. Results from FESEM and TEM revealed the formation of capsules.

**Acknowledgments** The authors greatly appreciate the help from Central Laboratory of Analysis & Structure Research in Zhejiang University. This study is financially supported by the Natural Science Foundation of Taizhou University and the National Science Foundation for Post-doctoral Scientists of China (no. 20100471000).

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