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Preparation of self-assembled nanoparticles of chitosan oligosaccharide-graft-polycaprolactone as a carrier of bovine serum albumin drug

Fenghong Li*, Xinrui Zhang, Hongyi Li, Liying Xiang and Yanming Chen
School of Petrochemical Engineering, Shenyang University of Technology, Liaoyang 111003, P.R. China

Abstract. Chitosan oligosaccharides graft polycaprolactone copolymer (PHCSO-g-PCL) has been synthesized via initiating the polymerization of ε-caprolactone (CL) monomer through an amino group protection route using phthaloyl chitosan oligosaccharide (PHCSO) as intermediate. The grafting reaction was carried out in Pyridine at 120°C with the hydroxyl group of the chitosan oligosaccharide (CSO) as initiator and the tin 2-ethylhexanoate ($Sn(Oct)_2$) as catalyst. The PHCSO-g-PCL nanoparticles with and without bovine serum albumin (BSA) drug were prepared through the self-assembled approach in Dimethylformamide (DMF) organic solvents. PHCSO-PCL copolymer was investigated by Fourier transform infrared spectroscopy (FTIR), ^1H NMR spectrum and scanning electron microscopy (SEM). The physicochemical properties of the hydrophobized PHCSO-g-PCL nanoparticles were characterized by fluorescence spectroscopy and dynamic light scattering (DLS). The results of DLS showed that the hydrodynamic diameters and particle size distribution with various concentrations of PHCSO-g-PCL nanoparticles were from 69.82 nm to 195.9 nm with a narrow polydispersity factor of 0.212 to 0.172. The results of DLS also showed that the hydrodynamic diameters and particle size distribution of PHCSO-g-PCL (5 mg/ml) nanoparticles without and with BSA drug (0.4 mg/ml) were from 168.44 nm to 200.7 nm. The polydispersity factor was from 0.119 to 0.159.

Keywords: Chitosan oligosaccharide, preparation, self-assembled nanoparticles, bovine serum albumin, polycaprolactone

1. Introduction

Chitosan (CS) is a natural biodegradable polymer obtained from the chitin after its alkaline deacetylation. It has prospective applications in biomedical fields such as biomedicine, tissue engineering, controlled drug delivery system. However, its use suffers severe limitations because it is insoluble owing to strong hydrogen bonding between the molecules of polysaccharides. Many CS-based amphiphilic copolymers composed of hydrophilic and hydrophobic segments have been reported through the reactions of amide group or hydroxyl group to improve solubility and expand its pharmaceutical appli-

*Corresponding author: Fenghong Li, School of Petrochemical Engineering, Shenyang University of Technology, No. 30, Guanghua Street, Hongwei District, Liaoyang 111003, P.R. China. Tel.: +86 4195319252; Fax: +86 4195311989; E-mail: lfhby@aliyun.com.

cations [1–5]. They have a tendency to form micelles through the self-assembled method above the critical micelle concentration (CMC) at the boundary between two phases, and can be used as target drug delivery carriers [6–10]. Chitosan oligosaccharide (CSO) is easily produced from chitosan either by acid or by enzymatic degradation, which has the advantages of biological activities, low viscosity, and good solubility over chitosan. Recently, using amphiphilic modified CSO with various long chained organic acids to fabricate self-assembled polymeric nanoparticles has shown great promise in the applications of drug delivery system [11–16]. CSO with different molecular weight and cholesterol modified CSO/DNA ionic complex nanoparticles shows superior DNA neutralization in contrast to the effect of the unmodified CSO and can be considered as an efficient gene carrier [17].

The objective of this work was to synthesize low molecular weight CSO graft copolymers (PHCSO-g-PCL) and prepare nanoparticles as a carrier of protein drug with the flexible chain of polycaprolactone (PCL) as hydrophobic group. PCL has attracted much attention owing to its excellent biocompatibility and biodegradability. It has been used as a repair material during surgery and in drug delivery systems [18]. The strong rigidity of CSO backbone was decreased and the solubility of CSO backbone in the organic solvent was improved after modification. The structure of chitosan oligosaccharide graft copolymers (PHCSO-g-PCL) and the preparation of self-assembled nanoparticles with BSA drug were investigated.

2. Experimental methods and materials

2.1. Materials

CSO (Mw<1000) was purchased from Dalian Glycobio Co., Ltd. (Dalian, China). ε-Caprolactone, Pyrene and BSA (Sigma-Aldrich) were used without further purification. Phthalic anhydride and Stannous octoate (Sn $(Oct)_2$) were purchased from the First Reagent Factory of Shanghai (China). Dimethylformamide (DMF) and Pyridine were stored over molecular sieves (4 Å). CSO was dried in a vacuum oven at 50°C for 24h prior to use. All other reagents and solvents used in this study were of analytical grade. A dialysis bag (cutoff molecular weight: 3500 Da) was purchased from Beijing Solarbio Science & Technology (Beijing, China).

2.2. Preparation of graft copolymers

Phthaloylation of chitosan oligosaccharide (PHCSO): CSO (1 g) and phthalic anhydride (1.5 g) were added in 20 mL of anhydrous DMF and the mixture was stirred at 110°C for 6 h. The obtained PHCSO was precipitated with 500 mL water-free cold ethanol.

Preparation of PHCSO-g-PCL copolymer: Vacuum dried PHCSO (1 g), ε-caprolactone monomer (8 mL) and 10 mL pyridine were added into a dried glass reactor, and then tin 2-ethylhexanoate (stannous octoate) was added to the reactor dropwise. The graft copolymerization was stirred continually at 120°C for 20 h under the protection of nitrogen. The obtained product PHCSO-g-PCL copolymer was precipitated with methanol and extracted with acetone in a Soxhlet apparatus for 24 h to remove the homopolymers (PCL). The final product was dried in a vacuum for 24 h. The synthetic procedures were presented in Figure 1.

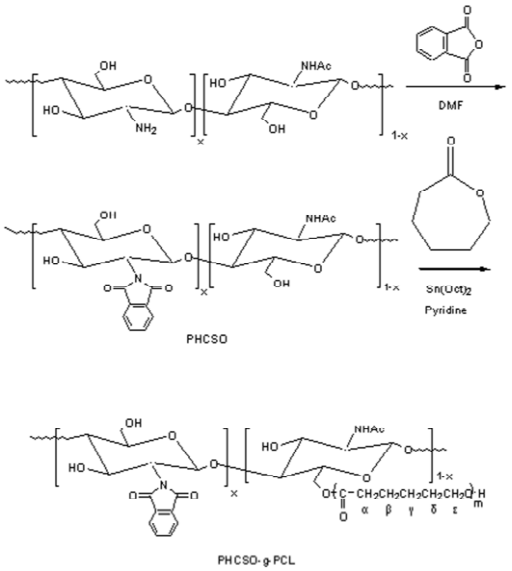

Fig. 1. Synthetic procedure of PHCSO-g-PCL.

2.3. Preparation of PHCSO-g-PCL self-assembled nanoparticles

The PHCSO-g-PCL nanoparticles with and without BSA drug were prepared by diafiltration method. The detailed process for PHCSO-g-PCL nanoparticles preparation was as follows: 10 mg of the PHCSO-g-PCL copolymer was weighed and dissolved in 2 mL DMF (C=5 mg/mL), then it was sonicated for 24h and the solution was dripped into 3mL distilled water while stirring. All of the resulting solutions were transferred to a dialysis bag (3500 Da molecular weight cutoff) and dialyzed in 4 L distilled water for two days to remove the DMF. During the dialysis process, distilled water was replaced every 24 h. Finally, the solution was filtrated through a 0.45 μm microfilter to remove the aggregates from the micelle solution.

For BSA drug loading, the PHCSO-g-PCL copolymer (10 mg) was weighed and dissolved in 2 mL DMF (C=5 mg/mL), then the PHCSO-g-PCL solution was sonicated for 24h at 4°C. The solution above was dripped into 3mL distilled water while vigorously stirring for 2h, then BSA (2 mg) was put into the solution. Then the solution was poured into a dialysis bag and dialyzed in 4 L distilled water for two days in order to remove the DMF. The distilled water was replaced every 24 h during the dialysis process and the PHCSO-g-PCL molecules self-assembled into particles slowly. With the presence of BSA, a portion of BSA was encapsulated into the hydrophobic cores of PHCS-g-PCL particles. Finally, the solution was filtrated through a 0.45 μm microfilter to remove the aggregates from the micelle solution.

2.4. Structure analysis of PHCSO-g-PCL

Infrared spectra analysis of the products was characterized by a Nicolet 470F7 FTIR Spectrometer (Nicolet Instruments, Madison, WI). ^{1}H NMR spectrum of the synthesized PHCSO-g-PCL was measured at 298 K using Bruker ARX-300MHz spectrometer and $CDCl_3$ deuteride as solvent. The content of PHCSO-g-PCL solution was 20 mg/mL.

2.5. Morphology studies of PHCSO-g-PCL

The surface morphology analysis of the sample was operated at Superscan SSX-550 microscope. The powder specimen was coated with gold to improve scanning electron microscopy (SEM) imaging.

2.6. Fluorescence measurements of self-assembly behavior

The self-assembly behavior of the PHCSO-g-PCL copolymer was studied by fluorescence measurement and pyrene was used as a probe. Pyrene (3 mg) was firstly dissolved in 500 ml acetone to prepare pyrene solution. The concentration of pyrene was 6×10^{-5} M. The PHCSO-g-PCL samples were dissolved in DMF to prepare PHCSO-g-PCL solutions with different concentrations from 0.05 to 1.0 mg/mL. Then PHCSO-g-PCL solutions were dialyzed to prepare PHCSO-g-PCL nanoparticles using the above method described in Section 2.3. The pyrene solution (0.1 mL) was poured into 10 mL of PHCSO-g-PCL nanoparticle solution, and then the solution was treated by water-bath ultrasonication for 30 min. The acetone in PHCSO-g-PCL nanoparticle solution was evaporated under 50ºC finally. The concentration of pyrene in PHCSO-g-PCL nanoparticle solution was 6×10^{-7} M. Fluorometer (LS-45/55, Perkin Elmer Co., USA) was used to measure the changes of fluorescence spectra intensity of the PHCSO-g-PCL nanoparticle solutions involved pyrene. The pyrene emission was monitored at a wavelength range of 340-620 nm. The excitation wavelength was 320 nm and the slit openings were set at 2.5 nm (emission) and 2.5 nm (excitation). Based on the change of intensity of pyrene excitation spectra with the increasing concentrations of PHCSO-g-PCL, the critical aggregation behavior of the PHCSO-g-PCL was measured.

2.7. Dynamic light scattering measurements of PHCSO-g-PCL nanoparticles

The average diameter and particle size distribution index (pdI) of the PHCSO-g-PCL nanoparticles were obtained through dynamic light scattering (DLS) measurements, which were determined by ZetasizerNano ZS90 (Malvern Co., UK) at 25°C. The disperse phase was water and the scattering angle was 90°.

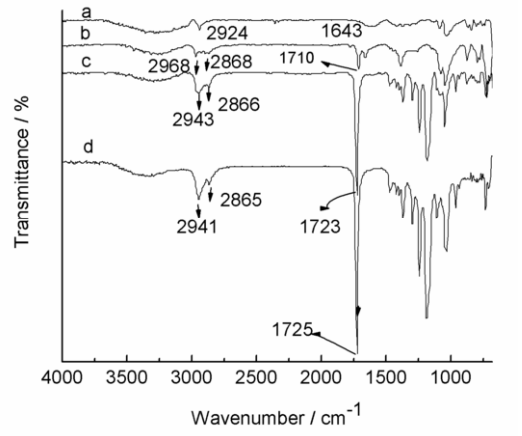

Fig. 2. FT-IR spectra of PHCSO-g-PCL.

3. Results and discussion

3.1. Chemical structure analysis of the PHCSO-g-PCL copolymers

Structure of PHCSO-g-PCL copolymers was characterized by FT-IR spectra (see Figure 2) and ^{1}H NMR spectra. The signals at 7.263 ppm belonged to the deuterated $CHCL_3$ solvent, the broad peaks at 3.651 ppm were assigned to the hydrogen atom of the structure unit of the polysaccharide ring [19]. The strong signals originated from PCL side chain were detected at 2.309 ppm (α-, –CO–CH_2–), 1.65 ppm (β- and δ-, –CH_2–), 1.383 ppm (γ-, –CH_2–) and 4.062 ppm (ε-, –CH_2O). The results evidenced that the modified CSO contained PCL side chain.

As shown in Figure 2(a), the absorption bands at 2924 cm^{-1} and 1643 cm^{-1} were described for C-H stretching and the amide group (C=O vibration mode) of CSO, respectively. As shown in Figure 2(b), the characteristic peaks of phthalimido referring to the carbonyl anhydride occurred at 1710 cm^{-1} and 1775 cm^{-1}, which clarified that PHCSO was synthesized and the amino group of CSO was protected by the phthalic anhydride. As shown in Figure 2(d), the characteristic peak of the stretching vibrations of crystalline carbonyl groups of pure PCL occurred at 1725 cm^{-1}, while the absorbance at 2800–3000 cm^{-1} (C–H of CH_2 group vibration mode) become stronger as shown in Figure 2(c). The new broad characteristic peak at 1723 cm^{-1} belonging to the carbonyl stretching band of PCL side chain also occurred. All these evidences implied that PCL has grafted onto the backbone of PHCSO successfully.

3.2. SEM studies

The SEM pictures of CSO and PHCSO-g-PCL were shown in Figure 3. The morphology of CSO particle was a sphere or sphere with small pits (Figure 3(a)). However, the independent particle of CSO disappeared after phthaloylation and graft copolymerization (Figure 3(b)), and the morphology of PHCSO-g-PCL showed a tightly packed structure. This indicated that the crystalline structure of CSO has changed due to PCL side chains. The surface morphology studies confirmed the homogeneous grafting of PCL into PHCSO.

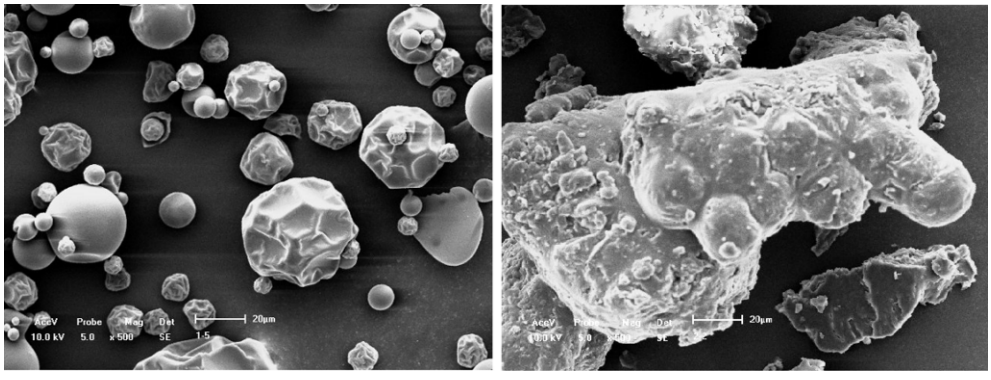

Fig. 3. SEM analysis of (a) CSO and (b) PHCSO-g-PCL (×500).

3.3. Determining the self-assembly behavior of the PHCSO-g-PCL copolymers

Pyrene was used as a hydrophobic fluorescence probe to investigate the self-aggregation behavior of PHCSO-g-PCL copolymers in an aqueous milieu. When exposed to a polymeric micelle aqueous solution, the pyrene molecules preferably participated into the hydrophobic domains of the micelle rather than the aqueous phase. Combined with strong fluorescence illumination of pyrene in a non-polar environment, the participated quantities of pyrene into hydrophobic domain depended on the concentration of micelle forming materials and would show different photophysical characteristics. The self-aggregation behaviors of the PHCSO-g-PCL copolymers in an aqueous phase were determined by the intensity of fluorescence excitation spectra of the PHCSO-g-PCL solutions with various concentrations, and the results were illustrated in Figure 4. The total fluorescence intensity increased with the increase of the concentration, which indicated that the pyrene was transferred from polar aqueous media to the less polar of the micelle such as the interior of hydrophobic domains of self-aggregate.

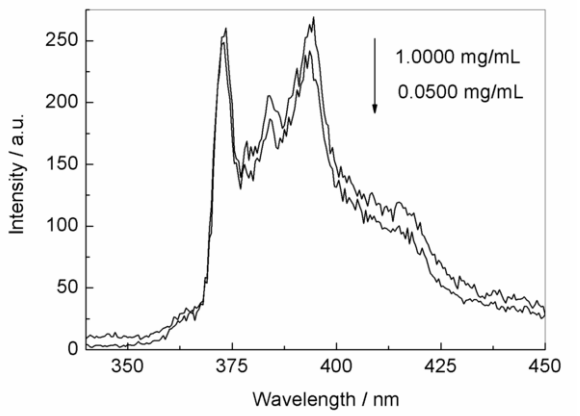

Fig. 4. Fluorescence emission spectra of pyrene as a function of PHCSO-g-PCL concentration.

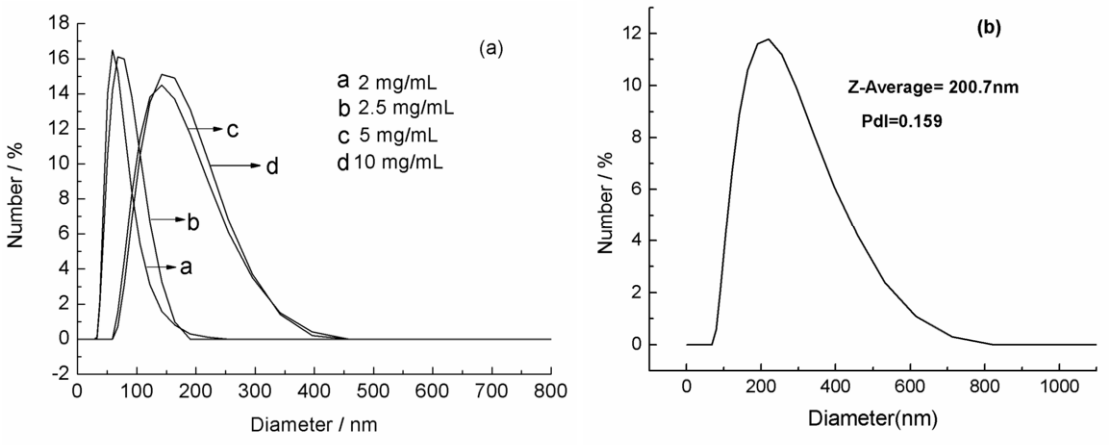

Fig. 5. Size and size distribution of PHCSO-g-PCL particles with (a) various concentrations and (b) BSA drug.

3.4. Characteristics of self-aggregated nanoparticles without and with BSA drug

The hydrodynamic diameters and particle size distribution with various concentrations of PHCSO-g-PCL in DMF solvent were determined using laser light scattering technique, which were shown in Figure 5. The hydrodynamic diameters were from 69.82 nm, 88.49 nm, and 168.44 nm to 195.9 nm with the increasing concentrations of PHCSO-g-PCL. The difference of hydrodynamic diameters between different concentrations of PHCSO-g-PCL indicated that DMF solvent can disturb the intra- and/or inter-molecular aggregate between the molecular chain and water phase. The concentration of PHCSO-g-PCL played a great role in determining the hydrodynamic diameters of the PHCSO-g-PCL nanoparticles.

Without the presence of BSA, PHCSO-g-PCL conjugates (5 mg/mL) forms particles with an average diameter of 168.44 nm and a narrow size distribution in an aqueous solution (see Figure 5(a)). The presence of BSA (0.4 mg/mL) leads to an increase of particle size to 200.7 nm (see Figure 5(b)) while the distribution of particle size remains narrow. The increase of particle size by BSA suggests that PHCSO-g-PCL entraps BSA molecules partly in its hydrophobic inner cores.

4. Conclusion

In this research, a kind of amphiphilic chitosan oligosaccharide graft copolymer (PHCSO-g-PCL) was successfully synthesized by introducing hydrophobic side chain PCL through the graft copolymerization. The hydroxyl group of PHCSO intermediate was used as the initiator and the ε-caprolactone was used as the monomer. It could form nano-sized polymeric micelles with narrower size distribution by self-aggregation in an aqueous medium owing to their amphiphilic characteristics. It has a great potential to be used as drug delivery carrier compared with chitosan. Further work are in progress, including the drug loading capacity, vitro drug release kinetic and the morphology of PHCSO-g-PCL nanoparticles with different structures controlled by the grafting ratio of PHCSO-g-PCL.

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