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Preparation, characterization and aggregation behavior of amphiphilic chitosan derivative having poly (L-lactic acid) side chains

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Abstract

The amphiphilic graft copolymer using chitosan (CS) as hydrophilic segment and poly (L-lactic acid) (PLLA) as hydrophobic segment was prepared through a protection-graft-deprotection route. PLLA macromonomer which molecular weight ($\overline{M}_n$) and polydispersity index (PDI) are 4267, 1.30, respectively, reacted with hydroxyl groups on phthaloylchitosan (PHCS) regionselectively. The graft reaction was carried out in homogeneous system using *N*,*N*-dimethylformamide (DMF) as solvent. The grafting content increases with the PLLA/PHCS ratio and reaction time. Furthermore, the grafting content soared from 80 to 90 °C and then leveled off. Under the present preparation method, it varied from 43.4% to 342.6%. The aggregation of the copolymers in aqueous solutions was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results indicated both hollow and solid spherical micelles were present in aqueous solutions. The ratio between the two kinds of spheres involved with the grafting content of PLLA. The number of hollow spheres reduced with the increase of the grafting content.

Keywords: Chitosan; Poly (L-lactic acid); Aggregation behavior

1. Introduction

As a well-known abundant natural polymer, chitosan has many biological (bioactive, biocompatible, biodegradable) and physicochemical (polycationic, reactive OH and NH_2 groups) properties, which makes chitosan an excellent material for the biomedical applications. Much attention has been paid to the use of chitosan in biomedical applications, for example, as a wound dressing, skin grafting template, hemostatic agent, hemodialysis membrane, drug delivery vehicle, etc. (Agnihotri & Aminabhavi, 2004; Kumar, 2000). Grafting with synthetic polymers has been explored as an interesting alternative method for developing novel hybrid materials of chitosan (Don, Hsu, & Chiu, 2001; Jenkins & Hudson, 2001; Silva, Menezes, & Garcia, 2003). Poly (lactic acid) (PLA) which was approved by the US FDA as far back as the 1970s has since been widely utilized in sutures, clips, plates and screws, ultrasound contrast agents, nerve guides and in drug delivery devices in clinical applications (Wang et al., 2003a, 2003b). Therefore, it is promising to combine chitosan with PLA to produce a new biosynthetic polymer hybrid applicable for a variety of purposes.

Several studies have been conducted on the PLA modification of chitosan and its derivates. Qu (Qu & Wirsen, 1999; Qu, Wirsen, & Albertsson, 2000) synthesized the copolymers consisting of chitosan with low molecular weight and D,L-lactic acid, and studied their properties. Yao, Chen, Liu, and Yao (2003) reported the synthesis and characterization of an amphoteric pH sensitive biodegradable chitosan-g-(L-lactic-co-citric) acid hydrogel. Liu, Tian, and Hu (2004) prepared a brush-like copolymer of poly (D,L-lactide) grafted onto chitosan by graft copolymerization with triethylaluminum as catalyst at 70 °C. All above mentioned investigations were based on the reaction

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on amino groups of chitosan. Considering the unique structure of chitosan, it is important to maintain the aminosaccharide unit for various specific functions, including biological activities and cationic polymer properties.

It is known that the aggregate morphologies of polymers are vital for the potential application in many fields, such as, drug delivery system. Since 1995, Eisenberg et al. (Zhang & Eisenberg, 1995) found the multiple morphologies of crew-cut micelle-like aggregates of polystyrene-*b*-poly (acrylic acid) diblock copolymers in solution, the preparation and assembly of amphiphilic copolymers of various architectures has become a research area of high interest and importance (Duan et al., 2001; Huang & Fang, 2006; Liu, Zhang, & Jiang, 1999).

In this article, the hydrophobic PLLA macromonomer was grafted onto the hydrophilic chitosan regionselectively at the hydroxyl group through a protection-graft-deprotection route. Since the resultant graft copolymers were amphiphilic, the aggregation behavior of the graft copolymers in aqueous solution was studied also.

2. Experimental

2.1. Materials and reagents

Chitosan (degree of deacetylation = 95%) was purchased from Yuhuan Ocean Biochemical Co. Ltd. (Zhejiang, China). L-lactic acid(LLA) supplied by Bengbu Fengyuan Biochemical Co. Ltd. (Anhui, China) was used as an 80 wt% aqueous solution of the monomer, 99% optically pure according to the manufacturer. 2,4-Tolylene diisocyanate (TDI) and phthalic anhydride were supplied by the First Reagent Factory of Shanghai (China). Chitosan and PLLA were dried in vacuum oven at 50 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried by magnesium sulfate and purified by distillation and stored over molecular sieves (4 Å). The other commercially available chemicals were used as received without further purification.

2.2. Synthesis of PLLA macromonomer

Hundred milliliters of 80 wt% aqueous solution of LLA was dehydrated at 150 °C under the atmospheric pressure for 4 h and then under a reduced pressure of 10 kPa for another 4 h. The above treated solution was heated up to 200 °C under a magnetoelectric stirring. The pressure was reduced subsequently stepwise to 300 Pa, at which the reaction continued for 12 h. At the end of the reaction, the product was dissolved in chloroform and subsequently precipitated in ethanol. The resultant solid yields were filtered and dried under vacuum.

2.3. Phthaloylation of chitosan

Chitosan was heated with excess phthalic anhydride in dried DMF to yield phthaylchitosan (PHCS) (Kurita, Ikeda, Yuga, Manabu, & Manabu, 2002). It was obtained as a yellow powdery material and the degree of substitution (DS) of phthaloyl groups was 98% determined by elemental analysis.

2.4. Preparation of graft copolymers

Step 1: The prepolymers were prepared by dropping TDI into PLLA solution at the NCO/OH ratio of 2:1 (mol/mol). The reactions in DMF were performed under N_2 at 60 °C for 30 min.

Step 2: Vacuum dried PHCS was dissolved in anhydrous DMF, and then added into the above prepolymers solution. The reaction continued with stirring at different temperature for different time under a nitrogen atmosphere. The obtained product was poured into ice water and separated by filtration. The unreacted PLLA–NCO was removed by Soxhlet's extraction with acetone for 24 h.

The graft content ($G\%$) was calculated as follows:

$$G\% = (W_g - W_0)/W_0 \times 100$$

where W_g and W_0 are the weight of graft copolymers and PHCS, respectively.

2.5. Deprotection of the graft copolymers

The obtained phthaloyl-protected graft copolymer (1 g) was added into 50 ml DMF under stirring, and heated to 100 °C under a nitrogen atmosphere. Hydrazine monohydrate (2 ml) was added into the system and the reaction continued for 2 h to deprotect the phthaloyl groups. The solution cooled down to room temperature and was poured into ethanol for precipitation. The precipitate of CS-g-PLLA was collected, and washed thoroughly with ethanol and dried.

2.6. Aggregation of CS-g-PLLA graft copolymers

CS-g-PLLA was dissolved in DMF again. The resultant solution was dialyzed against deionized water using a dialysis membrane (molecular weight cut-off 12 kDa; dialysis tubings, cellulose membrane, Sigma) for 3 days to remove most of the DMF. Deionized water was replaced every 12 h.

2.7. Characterization

All Fourier-transform infrared (FT-IR) transmission spectra were obtained from samples in KBr pellets using a Bruker EQUINOX55 FT-IR spectrophotometer (Germany). ^{1}H NMR (nuclear magnetic resonance) spectra were recorded on an AVANCE 300 NMR Spectrometer in d_6-DMSO. Elemental analyses were performed with an Elementar Vario EL-III elemental analyzer. X-ray powder diffraction diagrams were recorded with a Japan D/Max-γA X-ray diffractometer using graphite-monochromatized Cu–K radiation ($\lambda = 1.54$ Å). GPC analyses were performed with gel permeation chromatography apparatus (GPC, Waters 150C) operating with chloroform and calibrated with polystyrene standards. The morphology of the copolymers aggregates was studied by a JSM-6700F scanning electron micrograph (SEM) and a H-800 transmission electron micrograph (TEM). The sample for observations was prepared by the placement of a 10 μl micellar solution at a concentration of 1.0×10^{-3} g/ml on copper grids. The sizes of spherical micelles were determined by a dynamic light scattering measurement (Zetasizer Nano ZS-90, Malvern Co., Ltd.).

3. Results and discussion

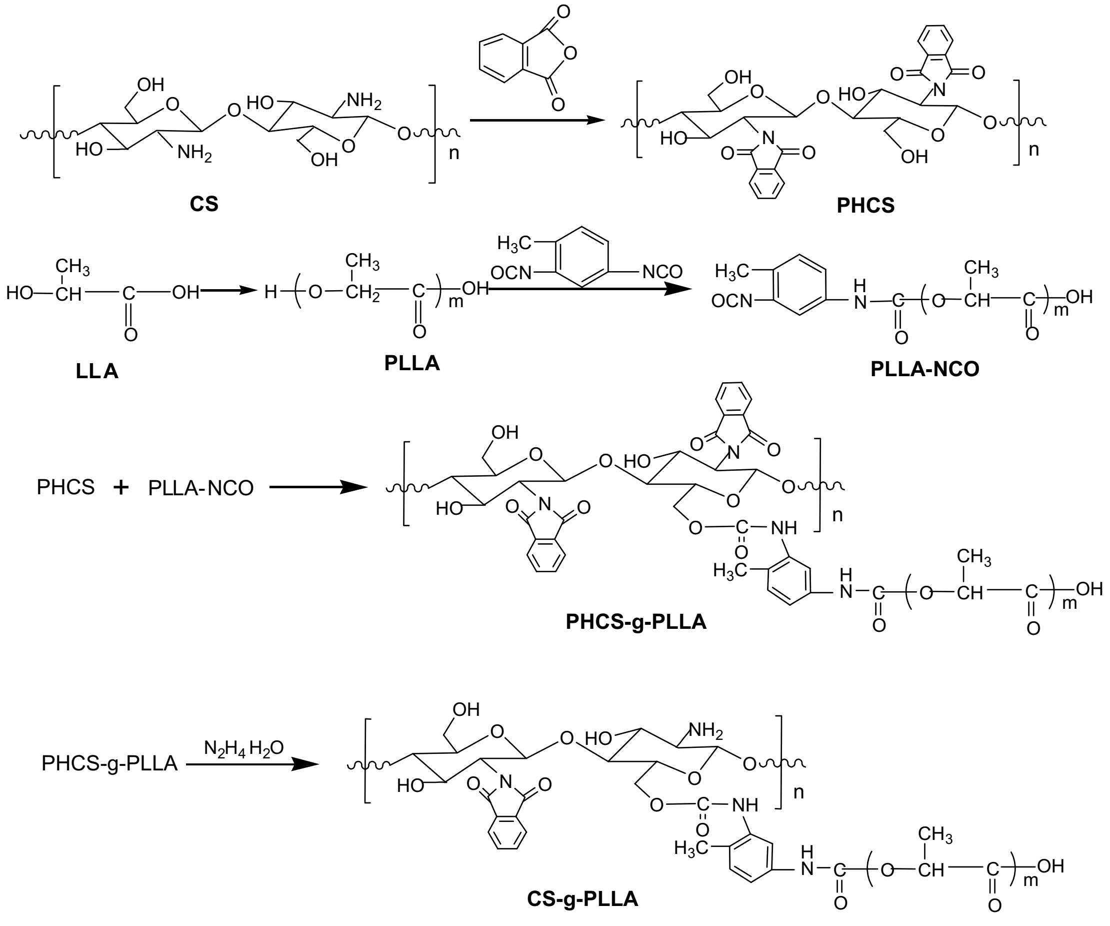
3.1. Synthesis of PLLA macromonomer

Poly (L-lactic acid) (PLLA) is generally produced by ring opening polymerization of L-lactic acid (Schwach, Coudane, Engel, & Vert, 1997; Zhang, Gan, Zhong, & Jing, 1998). This process needs a rigid condition even though it can produce a high molecular weight PLLA. In this work, PLLA macromonomer is prepared successfully through melt polycondensation of LLA (Moon, Lee, Miyamoto, & Kimura, 2000). The molecular weight $(\overline{M}_n)$ and polydispersity index (PDI) are 4267, 1.30, respectively, determined by gel permeation chromatography (GPC) measurement.

3.2. Preparation of graft copolymers

The whole grafting procedure is shown in Scheme 1. As a chitosan derivative, PHCS not only protect the abundant amino groups in the graft procedure but also enable the graft reaction to be carried out in a homogeneous system. The graft reaction starts from the reaction of PLLA macromonomer with TDI to produce PLLA–NCO prepolymer. In DMF homogeneous solution, the PLLA chains are grafted onto PHCS in virtue of the reaction between OH groups on PHCS and the terminated NCO groups on prepolymer. Thus, the PHCS-g-PLLA copolymers having hydrophobic PLLA side chains are obtained.

The deprotection of *N*-phthaloyl groups is carried out by incubation with hydrazine to bring active amino groups back to chitosan. However, the deprotection of phthalimido groups by hydrazine may compete with the reduction of carbamate bonds. Further more, the ester groups in PLLA could hydrolyze in alkaline condition, the PLLA side chains maybe degrade in deprotection of the copolymers. The appropriate condition is determined in DMF solution



Scheme 1. Synthetic procedure of the graft copolymers.

at 100 °C under nitrogen for 2 h to selectively deprotect the phthalimido groups (Liu, 2005). In this condition, the removal of phthalimide was prior to the reduction of carbamate bonds and the degradation of PLLA side chains.

3.3. Characterization of CS-g-PLLA copolymers

As indicated in FT-IR spectrum (Fig. 1), Spectrum (b) clarified that PHCS showed the phthalimido characteristic peaks at 1712 and 1777 cm^{-1}, referring to the carbonyl anhydride, and at 721 cm^{-1}, belonging to the aromatic ring. Compared to that of PHCS, new peaks at 1538 and 1758 cm^{-1}, come out in the spectrum of PHCS-g-PLLA, which indicates the formation of amide ester linkage (–OCONH–) and the ester groups of PLLA branches. After the deprotection of phthaloyl groups in PHCS-g-PLLA, the peaks at 1712, 1777, and 721 cm^{-1} almost disappeared, whereas the peaks at 1538 and 1758 cm^{-1} still exist in the final grafted product. It proved that the PLLA chains were grafted onto chitosan successfully.

The ^{1}H NMR spectrum of the CS-g-PLLA copolymer, shown in Fig. 2, also confirmed that PLLA chains had grafted onto CS. The peaks detected at 2.0–4.0 ppm belonged to the CS backbone hydrogens. The peak at 2.5 ppm belonged to solvent d_6-DMSO. The strong signals at 1.4 ppm were detected which was caused by the methyl protons of the PLLA side chain. The peaks at 5.2 ppm should be assigned to the –CH– hydrogen of lactyl moiety located at the PLLA chains. These testified PLLA macromonomer were grafted onto chitosan chains successfully. What is more, the ^{1}H NMR data suggested that the deprotection was incomplete since there were weak aromatic phthalimido peaks at 6.5–8.0 ppm associated with phenyl ring of TDI.

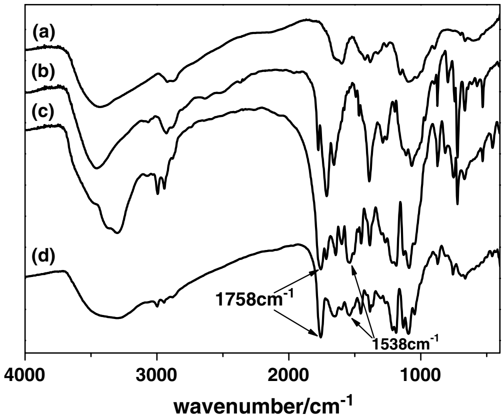


Fig. 1. FT-IR spectra of (a) chitosan, (b) PHCS, (c) PHCS-g-PLLA and (d) the final CS-g-PLLA.

The X-ray powder diffraction patterns of CS and CS-g-PLLA copolymers with different grafting content were illustrated in Fig. 3. Compared with chitosan (a), the X-ray diffraction spectrum of CS-g-PLLA(b and c) with low grafting contents showed a weaker and broader peak in the $2\theta = 10°–30°$ region, which implied that the conjugation of PLLA with CS suppressed the crystallization of both CS and PLLA to some extent. But a strong peak at about $2\theta = 17°$ could be detected in the CS-g-PLLA (d) sample with high grafting content. It is recognized as the (200, 110) reflection of the α-form crystals of PLLA (Huda, Yasui, Mohria, Fujimura, & Kimura, 2002; Xu et al., 2005).

3.4. Grafting content of PLLA in PHCS-g-PLLA copolymers

The graft reactions were conducted under various conditions. The effect of temperature, time and PLLA/PHCS feed ratios on the grafting content of PLLA was studied.

It is found that temperature has great effect on the graft reaction. Fig. 4 showed the grafting content of PLLA varied with the temperature under the same reaction time. Obviously, the grafting content soared from 80 to 90 °C, and then leveled off. The reason is that the terminated NCO groups on PLLA–NCO prepolymers would not be activated completely until 90 °C. Thus the copolymerization reactions of all the PHCS-g-PLLA samples were handled at 90 °C.

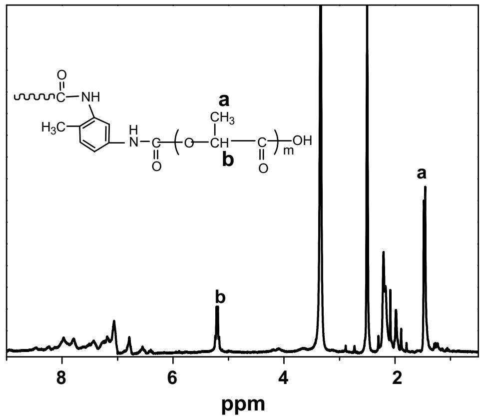


Fig. 2. ^{1}H NMR spectrum of the CS-g-PLLA copolymer.

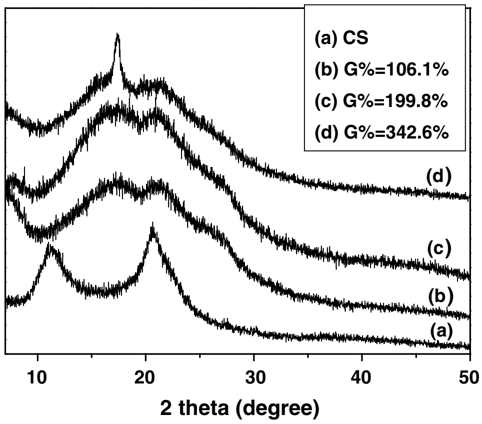


Fig. 3. X-ray diffraction of CS (a) and CS-g-PLLA copolymers.

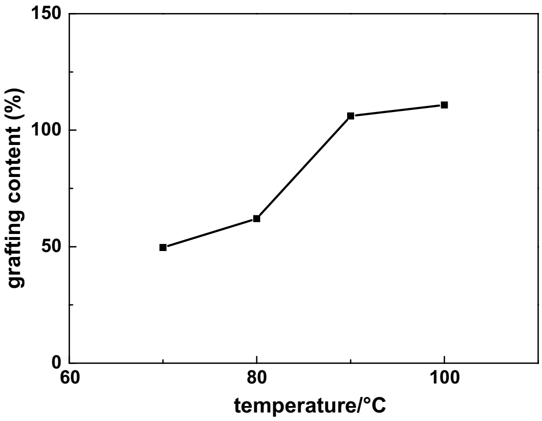


Fig. 4. Effect of temperature on the grafting content of PLLA (condition: time 3 h; PLLA/PHCS feed ratio 1:4 (w/w)).

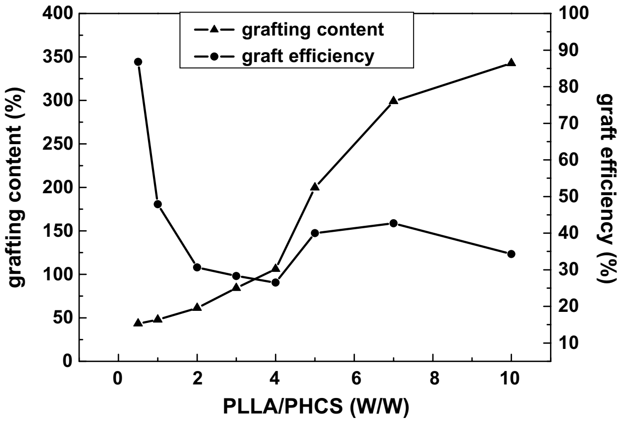


Fig. 6. Effect of PLLA/PHCS feed ratios (condition: temperature 90 °C; time 3 h).

The effect of the reaction time was also studied. As shown in Fig. 5, the grafting content of PLLA increased with the reaction time and tended to be unchanged after 9 h. In fact, the grafting content after 3 h had already been around 80% of the value after 9 h.

The grafting content and efficiency of PLLA changed with the feed amount of PLLA macromonomer, which was displayed in Fig. 6. With the increase of the feed of PLLA, the grafting content of PLLA enhanced, and the graft efficiency of PLLA fluctuated in 24–49% in the main. Furthermore, the grafting content could easily reach a high value. For example, the grafting content could beyond 100% when the PLLA/PHCS ratio was only 4:1 (w/w) without any additional catalyst. Under the present preparation method, the grafting content of PLLA varied from 43.4% to 342.6%.

3.5. Aggregation behavior of CS-g-PLLA copolymers

The different hydrophilicity between main chains and side chains results in phase separation of amphiphilic copolymers. The prepared graft copolymers consisted of hydrophobic linear PLLA side chains and hydrophilic chitosan backbones, which provided a possibility for the copolymers to disperse in the aqueous phase to form micelles. By dissolving graft copolymers in appropriate solvents, in which the main chains and the side chains have different solubility, various morphologies could be obtained. Scheme 2 provides a schematic representation of a possible mechanism for the formation of CS-g-PLLA nanospheres.

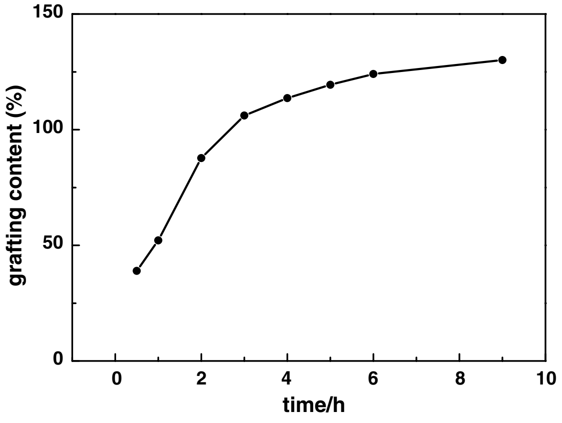
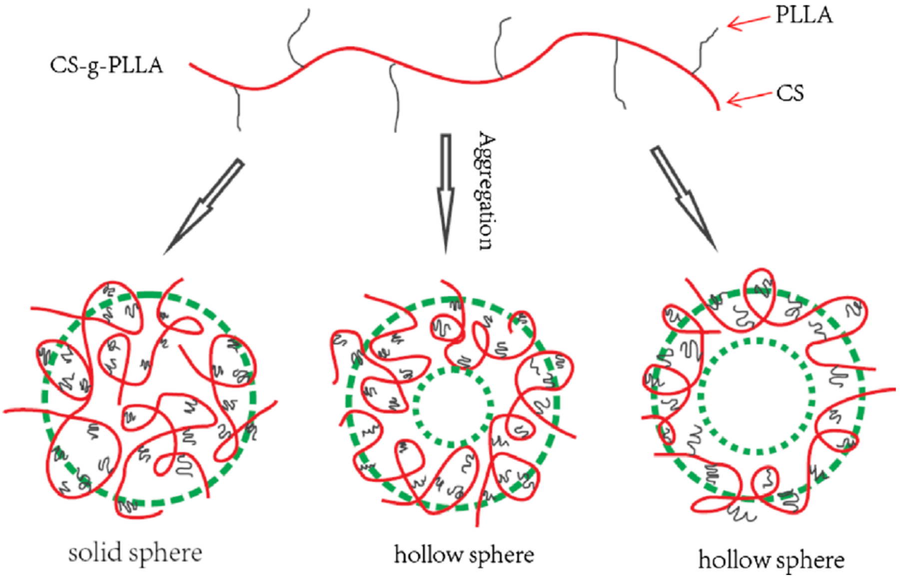


Fig. 5. Effect of time on the grafting content of PLLA (condition: temperature 90 °C; PLLA/PHCS feed ratio 1:4 (w/w)).

In a typical procedure, the CS-g-PLLA (20 mg) was dissolved in DMF (20 ml). The resultant solution was dialyzed against deionized water using a dialysis membrane to remove most of the DMF. The resulting micelle has an average diameter of 280 nm [PDI ($\mu_2/\Gamma^2) = 0.16$], determined by a dynamic light scattering measurement (Zetasizer Nano ZS-90). Typical electron microscopy images of micelles with different grafting content were shown in Fig. 7. The results indicated both hollow and solid spherical micelles were present in aqueous solutions. The ratio between the two kinds of spheres involved with the grafting content of PLLA. The number of hollow spheres reduced with the increase of the grafting content. The ratio of hollow spheres was less than 5% when the grafting content of PLLA was more than 300%. This could be comprehended that most of the PLLA chains trend to aggregate in the inner of the sphere because they are hydrophobic chains. The higher the grafting content of PLLA is, the more PLLA chains in the inner space of the spherical micelles. Therefore, the ratio of solid sphere to hollow sphere would increase with the grafting content.

4. Conclusion

Hydrophobic linear PLLA side chains were grafted onto chitosan successfully through a protection-graft-deprotection route. The graft reaction was carried out in homogeneous system and yielded copolymers with high grafting content due to solubilization. It is a potential method to



Scheme 2. Schematic showing the aggregation of CS-g-PLLA in aqueous solutions.

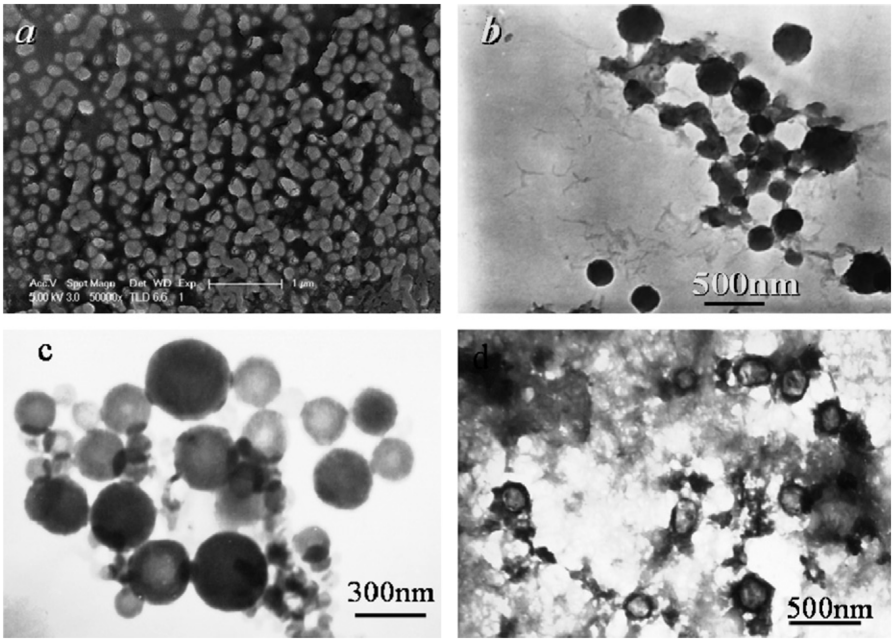


Fig. 7. Typical SEM (a) and TEM (b, c, d: the grafting content of PLLA are 342.6%, 199.8%, 106.1%, respectively) images of micelles formed from CS-g-PLLA copolymers in water.

combine chitosan with the hydrophobic synthetic polymers. In an aqueous solution, amphiphilic CS-g-PLLA aggregates into spherical micelles as observed by SEM and TEM. The results indicated both hollow and solid spherical micelles were present in aqueous solutions. The ratio between the two kinds of spheres involved with the grafting content of PLLA. Since both chitosan and PLLA are biodegradable, biocompatible and nontoxic, the graft copolymer is expected to be a nature/synthetic hybrid material of considerable importance in many fields, such as drug release, microreactor. Further work on drug delivery is in progress on these graft copolymers.

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