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Synthesis and characterization of a novel amphiphilic chitosan–polylactide graft copolymer

Yan Wu*, Yongli Zheng, Wuli Yang, Changchun Wang, Jianhua Hu, Shoukuan Fu*

Department of Macromolecular Science, Key Laboratory of Molecular Engineering of Polymers of Educational Ministry, Fudan University, Shanghai 200433, People's Republic of China

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Abstract

Water soluble chitosan (CS) derivatives containing polylactide unit were synthesized by reacting DL-lactide (DLLA) with chitosan in dimethyl sulfoxide solution in the presence of triethylamine. The chemical structure and physical properties of chitosan derivatives were characterized by FTIR, ^{1}HNMR, TGA and XRD. Formation and characteristics of polymeric micelles of graft copolymers were studied by fluorescence spectroscopy and dynamic light scattering (DLS) method. The critical micelles concentration (cmc) of polymeric amphiphiles was determined by measuring the fluorescence intensity of pyrene as a fluorescent probe. The cmc value of DLLA/CS (11:1) (DL-lactide/aminoglucoside units molar ratio = 11:1) in water was 6.49×10^{-2} mg/ml. The size of micelles and their size distribution in water were measured by DLS. Mean diameter of the polymeric amphiphiles micelles of DLLA/CS (11:1) in water was 154 nm with an unimodal size distribution (polydispersity = $\mu/\Gamma^2 = 0.03$). The spherical micelles were observed by transmission electron microscopy (TEM).

Keywords: Chitosan; Polylactide; Polymeric micelle; Characterization

1. Introduction

Amphiphilic copolymers consisting of hydrophilic and hydrophobic segments can form micelle structures with the hydrophobic inner core and the hydrophilic outer shell in aqueous media. The hydrophobic inner core is surrounded by a hydrophilic outer shell, and the hydrophilic outer shell provides a stabilizing interface between the micelle core and the aqueous environment (Janes, Calvo, & Alonso, 2001; Kwon & Kataoka, 1996). Polymeric micelles have received special attention due to their potential application and academic interest in many interdisciplinary field (Nishikawa, Akiyoshi, & Sunamoto, 1994; Zhang, Jackson, & Burt, 1996). Moreover, through adjusting the structure of the amphiphilic copolymers, the size and morphology of the polymeric micelles can be easily controlled. In addition, polymeric micelles were also considerably more stable than surfactant micelles (Batrakova, Han, Alakhov, Miller, & Kabanov, 1998; Kataoka, Kwon, Yokoyama, Okano, & Sakurai, 1993). Thus, these core-shell type micelles may be used as drug delivery vehicles for poorly water-soluble drugs, especially when the micelles are made with suitable biodegradable polymers.

Numerous studies reported that the core which generally consists of a biodegradable polymer such as poly(D,L-lactic acid) (PDLLA), poly(β-benzyl-L-aspartate) (PBLA) or poly(ε-caprolactone) (PCL) (Ryu et al., 2000; Zhu, Lin, & Yang, 1990), serves as a reservoir for a poorly water soluble drug, and thereby protects the drug from the aqueous environment. In contrast, the shell normally consists of water-soluble biocompatible polymer, such as PEG and PEO (Calvo, Remunan-Lopez, Vila-JATO, & Alonso, 1997). It has been reported that polymeric micelles could also be formed by modification of natural polymer (Lee, Kwon, Kim, Jo, & Jeong, 1998).

Polylactide is a kind of biodegradable materials with low toxicity, excellent biocompatibility and bioabsorbability in vivo. It has been widely used in biomedical applications, such as sustained drug delivery systems, implants for

* Corresponding authors. Tel.: +86 21 6564 2100; fax: +86 21 6564 2385.
E-mail addresses: wuyan166@eyou.com (Y. Wu), skfu@fudan.edu.cn (S.Z. Fu).

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orthopedic devices and absorbable fibers. However, the low hydrophilicity and high crystallimity of polylactide reduce its degradation rate, which results in poorer soft tissue compatibility (Suh, Hwang, Lee, Han, & Park, 2001).

Chitosan, next to cellulose, is the second most plentiful biomass and has a repeated structure of (1,4)-linked 2-amino-2-deoxy-β-D-glucan. Since chitosan is already known as a biocompatible, biodegradable and almost non-toxic material. It has been widely used in pharmaceutical research and industry as a carrier for drug delivery and as biomedical material (Muzzarelli et al., 1988). Chitosan is soluble in aqueous solutions of various acids, but chitosan molecules have no amphiphilic property and cannot form micelles in water. Apart from its biodegradable character in physiological conditions, chitosan has reactive amine and hydroxyl groups, which offer possibilities of modifications, graft reactions and ionic interactions. For instance, Qu (Qu, Wirsen, & Albertsson, 2000) has synthesized the copolymer of low molecular chitosan and D,L-lactic acid and studied its properties. Yao (Yao, Chen, Wang, Liu, & Yao, 2003) has reported the synthesis and characterization of an amphoteric pH sensitive biodegradable chitosan-g-(L-lactic-co-citric acid) hydrogel, and in vitro fibroblast stationery cultivation on its membrane showed that the cell growth rate was much faster than that on chitosan membrane.

Very little result has been reported about the synthesis of amphiphilic chitosan–polylactide graft copolymer. To combine the advantages of synthetic and natural polymers and at the same maintain the favorite properties of natural polymers such as biodegradation and bioactivity, in this paper a novel amphiphilic graft copolymer was synthesized by reacting DL-lactide (DLLA) and water soluble chitosan in dimethyl sulfoxide solution. The chemical structure and physical properties of graft copolymers were characterized and the micellar formation of the amphiphilic graft copolymers in water was investigated. All the results investigated may be used to evaluate the possibility of the amphiphilic graft copolymers as potential materials for biomedical applications.

2. Experimental

2.1. Materials

Chitosan (M_w = 340 kDa) from KaBo Biochemical Co (Shanghai, China) with deacetylation degrees of 90%. Chitosan oligomer (M_w 6500 Da) was prepared according to reference Tsuguhei and Tatsuaki (1997). Chitosan oligomer was prepared by oxidative degradation of chitosan with hydrogen peroxide. Chitosan powder (10 g) was dispersed in 300 ml water with magnetically stirred at room temperature for 1 h. The 30% hydrogen peroxide solution (10 ml) was added dropwise to the mixture and allowed to react at 60 °C for 6 h under the same stirring, and then the reaction mixture was adjusted with 1 M NaOH aqueous solution to pH 7. The insoluble solid was filtered off. The supernatant was evaporated with a rotary evaporator and precipitated with absolute ethanol. The obtained precipitate was repeatedly washed with absolute ethanol and then dried at vacuum to obtain the chitosan oligomer. The molecular weight and degree of deacetylation (DD) of the chitosan oligomer were measured by a gel permeation chromatography (GPC) and elemental analysis, respectively. DL-lactide (99.5%) from PURAC (Holland) was recrystallized twice from acetic ether. Dimethyl sulfoxide was distilled under reduced pressure from calcium hydride (CaH_2) and stored over molecular sieves (3 Å). All other reagents and solvents used in the study were analytical grade and obtained from commercial sources.

2.2. Synthesis of chitosan–polylactide graft copolymer

The polymerizations were carried out under magnetic stirring for 12 h in dimethyl sulfoxide at 80 °C. Water soluble chitosan was degassed for 1 h in vacuum below 1 mm Hg, and added to the reactor. A mixture of water soluble chitosan and DL-lactide (in the molar ratio of 6:1–20:1(DLLA/CS)) was suspended in 30 ml dimethyl sulfoxide solution with magnetic stirring at room temperature. The mixture was kept under a vacuum below 1 mm Hg for 1 h, then 0.5 mol triethylamine was added dropwise via a syringe through a rubber septum. The solution was reacted at 80 °C with magnetic stirring in nitrogen atmosphere. After a further 12 h continuous stirring, the mixture became a clear solution. The precipitate obtained by pouring the solution into ice-water was collected by filtration and thoroughly washed with distilled water, successively extracted with toluene in a Soxhlet's apparatus for 48 h. Dried at 40 °C for 48 h under vacuum, a pale yellow powder was obtained. The graft copolymers are soluble in DMSO, DMF, acetic acid and acetone.

2.3. Characterization of chitosan–polylactide graft copolymer

IR spectra were recorded on Fourier-transform infrared (FTIR) spectrometer (Nicolet, Magna-550). Chitosan and its graft copolymer were mixed with KBr and pressed to a plate for measurement.

^{1}H NMR was peformed on a Bruker, DMX-500 spectrometer, ^{1}H-NMR chitosan was dissolved in the mixed solvent D_2O and $(CD_3)_2SO$. The chitosan graft copolymer was dissolved in $(CD_3)_2SO$.

X-ray diffraction spectrometry was obtained using an XD-3A powder diffraction meter with Cu K_α radiation in the range 5–40° (2θ) at 40 kV and 30 mA.

Themogrammetry (TG) analysis was obtained with PE, Pyrolysis-1 equipment. The temperature range was 30–900 °C and the heating rate is 20 °C/min.

2.4. Preparation of chitosan–polylactide graft copolymer micelles

The copolymer micelles were prepared through phase separation-dialysis method. A given amount of copolymer was dissolved in DMF. Then a measured amount of copolymer solution was added dropwise into 10 ml distilled water under magnetic stirring to form the micelles. The micelles were then transferred into a membrane (cut off molecular weight, 12,000) and were dialyzed against 3 l of deionized water for 48 h to remove the DMF, and the micelle was obtained.

2.5. Transmission electron microscopy

Transmission electron microscopy (TEM) (Hitachi, H-600) was used to observe the morphology of the micelles. Samples were placed onto copper grill covered with nitrocellulose. They were dried at room temperature, and then were examined using a TEM by negative staining with an aqueous solution of sodium phosphotungstate.

2.6. Determination of micelle size

The size of polymeric micelles and their size distribution were measured by DLS with an argon ion laser system (Malvern Autosizer 4700). For size measurements, 5 ml micelles were diluted with 10 ml distilled water and measured for a minimum of 180 s. All DLS measurements were done with a wavelength of 532 nm at 25 °C with an angle detection of 90°. The measurement for each solution was repeated at least three times.

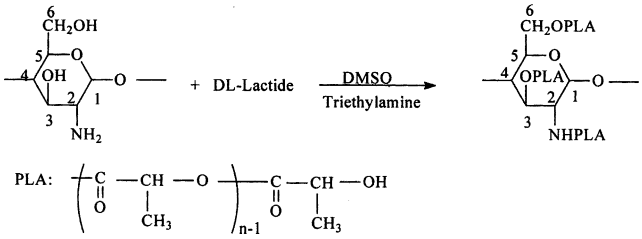
2.7. Fluorescence measurement

A solution of chitosan graft copolymers which containing 6×10^{-7} M of pyrene was placed in a square cell and the fluorescence spectrum was obtained with a fluorometer (FL-920 England). The concentrations of sample solution were varied from 3.02×10^{-5} to 1.0 mg/ml. For measurement of intensity ratio of the first and the third highest energy bands (I_1/I_3) in the pyrene emission spectra, the slit openings were set at 1 mm (excitation) and 0.5 mm (emission). The excitation wavelength (λ_{ex}) was 336 nm.

3. Results and discussion

3.1. Synthesis and characterization of chitosan–polylactide graft copolymers

The chitosan–polylactide grafting copolymers were prepared by reacting of DL-lactide (DLLA) on chitosan. The synthesis of copolymers were carried out as shown in Scheme 1. The polymerization in various molar ratio took



Scheme 1. Graft copolymerization of DL-lactide onto chitosan.

place easily under the designated conditions. The results are shown in Table 1.

Grafting percentage and the amount of lactide introduced to chitosan increase with the molar ratio of DL-lactide to structural unit of chitosan are shown in Table 1. When the molar ratio of lactide to chitosan increased from 6:1 to 20:1, the grafting percentage rose from 95 to 323%; meanwhile, the molar ratio of polylactide to chitosan in the copolymer also rose from 2.12 to 7.22. This indicates that the higher the concentration of the lactide, the higher the opportunity for the lactide to react with chitosan reactive centers. Grafting percentage and molar ratio of polylactide to chitosan in the copolymer could approach 198% and 4.43, respectively, when the molar ratio of lactide/aminoglucoside units is 11:1.

Structure changes of chitosan and its graft copolymer were confirmed by FTIR spectra (Fig. 1). Compared to the IR spectrum of chitosan, the copolymers have a new absorption peak appearing around 1746 cm^{-1}, corresponding to the carbonyl group of the branched polylactide. The methyl asymmetric deformation of polylactide appears at $\sim$1454 cm^{-1}. The $\sim$1197 and $\sim$1256 cm^{-1} doublets observed in the copolymer are assigned to the symmetric C–O–C stretching modes of the ester group. There are two other peaks at $\sim$1131 and $\sim$1046 cm^{-1} attributed to the methyl rocking and C–CH_3 stretching vibration, respectively. The increase of the amide I peak (1668 cm^{-1}) indicated increase of the amidation by reacting chitosan with lactide. That demonstrates the formation of amide group between chitosan and lactide. This evidence suggests that the lactide can indeed react with chitosan with triethylamine as catalyst. Increasing of the feed ratio of lactide to chitosan made the absorption at $\sim$1197 cm^{-1}

Table 1
Graft copymerization of lactide onto chitosan

Sample	DLLA/CS molar ratio	Total yield (%)	Grafting percentage (%)[a]	$F_{lactide}/F_{chitosan}$[b]
1	6:1	35.7	95	2.12
2	8:1	36.3	134	3.0
3	11:1	42.2	198	4.43
4	13:1	42.7	237	5.30
5	15:1	45.5	272	6.08
6	20:1	46.1	323	7.22

[a] Grafting percentage (%) = ((Mass of graft copolymer (g) − Mass of chitosan (g))/Mass of chitosan (g)) × 100%.
[b] Molar composition in graft copolymer = grafting percentage × 161/72.

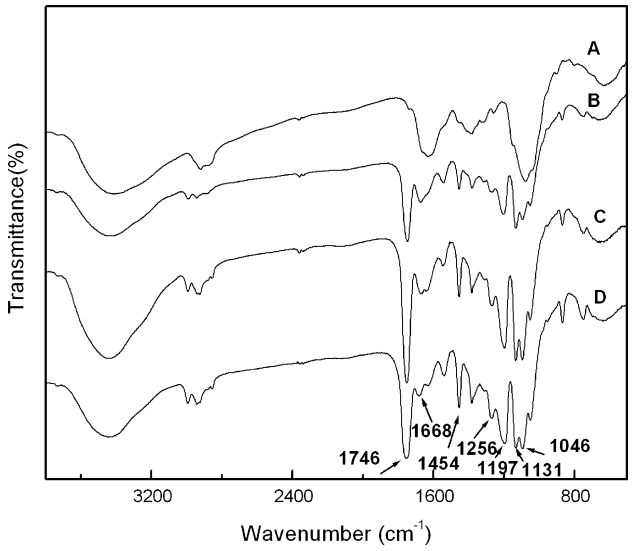


Fig. 1. IR spectra of chitosan and graft copolymers: (A) chitosan and (B) DLLA/CS = 8:1, (C) DLLA/CS = 11:1, (D) DLLA/CS = 15:1.

rise, which means that the more lactide had been grafted to chitosan.

The ^{1}H-NMR spectra of the chitosan and grafted copolymer of 8:1 are copmpared in Fig. 2. Chitosan shows a singlet at 3.13 (H-2) and mutiplets at 3.3–3.6 ppm (H-3, H-4, H-5, H-6) and a small singlet at 4.4 ppm (H-1) corresponding to the ring methenyl protons. The singlet at 1.9 ppm is due to the survival of the *N*-acetylglucosamine units of chitin (Hiral, Odani, & Nakajima, 1991). Compared with chitosan, the ^{1}H-NMR spectra of the graft copolymer showed that the signals at 4.2 and 5.1 ppm were assigned to the terminal methenyl protons of the branched polylactide and repeat units of it in the chain, respectively. The signals at 1.3 and 1.4 ppm were attributed to the methyl protons of the polylactide moiety located at the terminal groups and the backbones (Qu, Wirsen, & Albertsson, 1999; Yasugi, Nakamura, Nagasaki, Kato, & Kataoka, 1999). All these results evidenced that the chitosan derivatives contained polylactide side chains.

The integral intensity ratio between peak 5.1 and 4.2 ppm, that is the ratio of methenyl amount of polylactide in the chain and the terminal methenyl, is determined by the graft branch length. In the samples of 8:1, 11:1, 15:1, the integral intensity ratio between peaks 5.1 and 4.2 ppm is 3.2, 4.5 and 6.3, respectively. The results are correlated well with the results of gravimetric method (Table 1). The amount of branched polymer increases with an increase in lactide content in the feeding ratio.

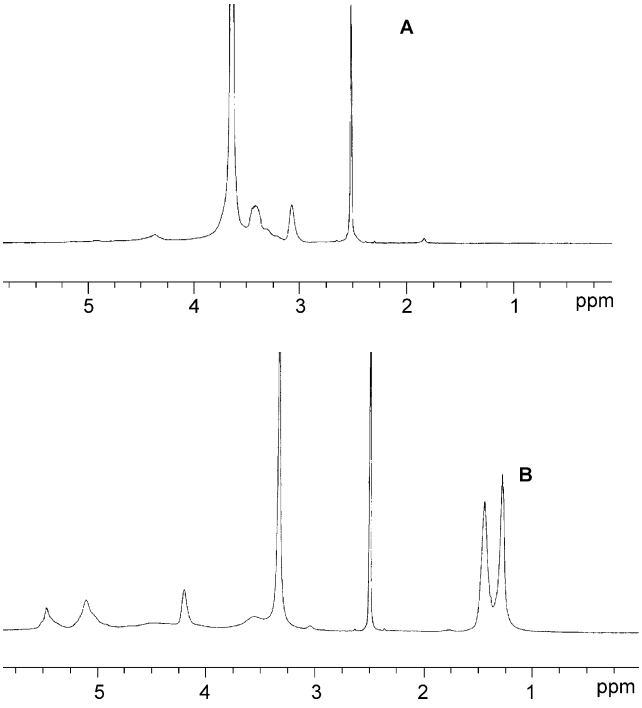


Fig. 2. The ^{1}H-NMR spectrum of (A) chitosan and (B) graft copolymer (DLLA/CS = 8:1).

3.2. Physical properties of chitosan derivatives

X-ray diffraction profiles of chitosan and its graft copolymer are shown in Fig. 3. Chitosan has two reflection fall at $2\theta = 10$ and 20°. The reflection fall at $2\theta = 10°$ was assigned to crystal forms I. The strongest reflection appears at $2\theta = 20°$, which correspond to crystal forms II (Dung, Rinaudo, & Desbriers, 1994). Compared with chitosan, the grafting decreases the intensity at both peaks. When the feed ratio reaches DLLA/CS = 11:1, the graft copolymer shows only one broad peak at around $2\theta = 12°$. It suggested that the ability of forming hydrogen bond of chitosan was decreased after grafting. When lactide was grafted onto chitosan, the original crystallinity of chitosan was destroyed.

TG curves for chitosan and grafted copolymer (11:1) are shown in Fig. 4. Compared to chitosan, graft copolymer has lower thermal degradation temperatures. A fast process of weight loss appears in the TG curves response for the graft

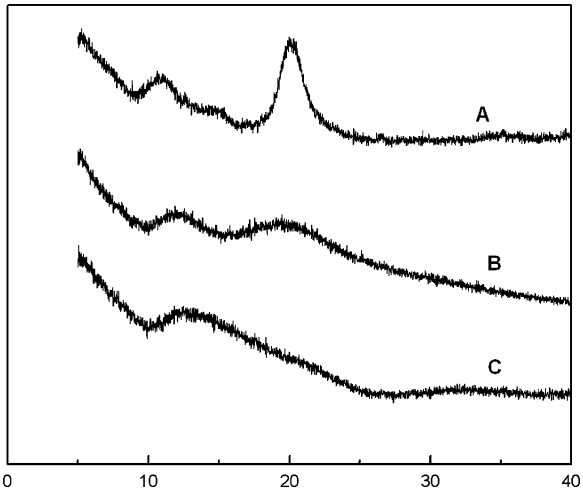


Fig. 3. X-ray diffraction profiles of chitosan and its graft copolymer: (A) chitosan, (B) DLLA/CS = 8:1, (C) DLLA/CS = 11:1.

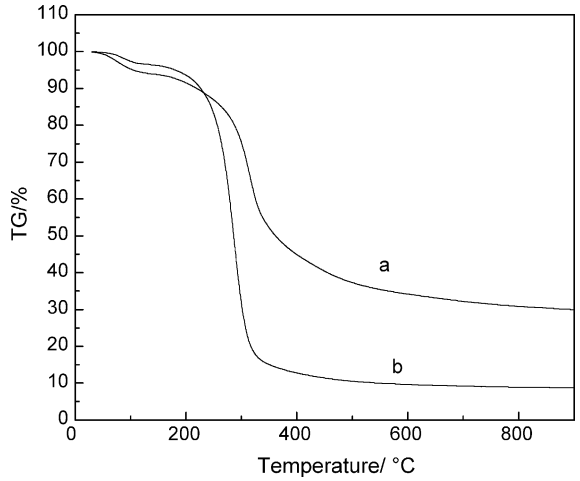


Fig. 4. TG thermograms of (a) chitosan and (b) chitosan–polylactide graft copolymer (DLLA/CS = 11:1).

copolymer in thermal degradation ranges. These results show some decrease of the thermal stability for chitosan–polylactide graft copolymer relative to the original chitosan. Introduction of substituents into polysaccharide structures should disrupt the crystalline structure of chitosan, especially by the loss of the hydrogen bonding.

3.3. Critical micelle concentration of chitosan–polylactide graft copolymer

It is well known that amphiphilic copolymers with a suitable hydrophilic/hydrophobic balance can form a micellar structure when exposed to a selective solvent. The amphiphilic nature of the chitosan–polylactide graft copolymers, consisting of hydrophilic chitosan and hydrophobic polylactide segments, provided an opportunity to form micelles in water. The micelle behavior of chitosan–polylactide graft copolymer in aqueous media was monitored by fluorometry in the presence of pyrene

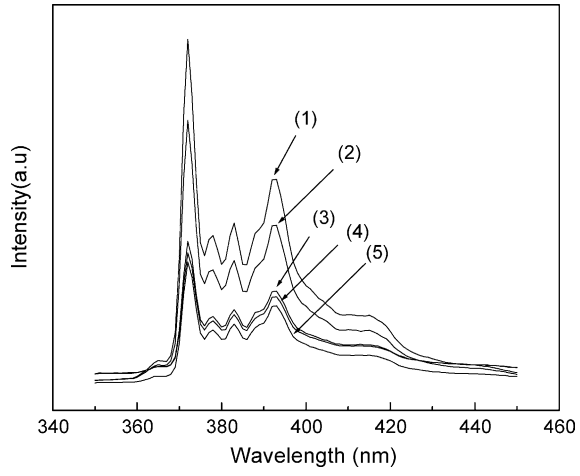


Fig. 5. Effect of polymer concentration on the fluorescence emission spectra of pyrene (6.0×10^{-7} M) in water in the presence of chitosan–polylactide graft copolymer (DLLA/CS = 11:1) at 25 °C: DLLA/CS (11:1) = (1) 0.5, (2) 0.25, (3) 0.1, (4) 0.01, (5) 0.001 mg/ml.

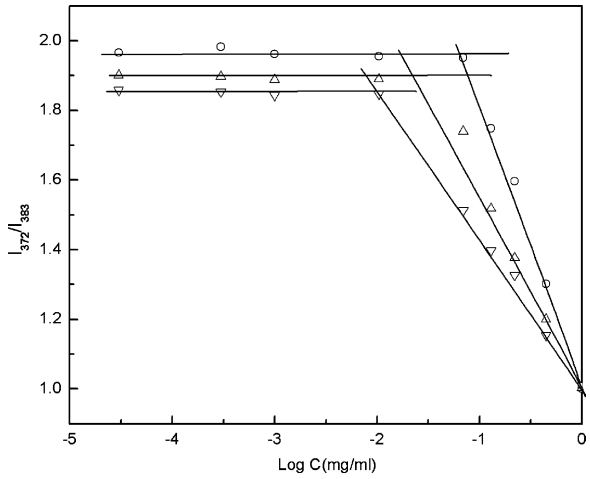


Fig. 6. Change of intensity ratio (I_{372}/I_{383}) versus the concentration of chitosan–polylactide graft copolymers: a (○) DLLA/CS = 11:1; (△) DLLA/CS = 15:1; (▽) DLLA/CS = 20:1.

as a fluorescence probe. Fig. 5 shows the fluorescence emission spectra of pyrene incorporated into chitosan–polylactide polymeric micelles (DLLA/CS = 11:1) in water at 25 °C. If micelles or other hydrophobic microdomains are formed in an aqueous solution, the pyrene preferably lies close to (or inside) these micro-enviroment and strongly emits, while it is quenched in polar media. When the pyrene coexists with polymeric micelles, the total emission intensity increases and, especially, the intensity of the third highest vibrational band at 383 nm (I_3) starts to drastically increase at a certain concentration of polymeric amphiphiles. In studying the formation of micelles from hydrophobically modified graft coplymer in aqueous solution, pyrene is generally used as a molecular probe, and the variation in the ratio of intensity of first (372 nm) to third (383 nm) vibronic peaks I_{372}/I_{383}, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where pyrene is located. Thus, the change of I_{372}/I_{383} can characterize the formation of micelle. The change of the intensity ratio (I_{372}/I_{383}) is shown in Fig. 6. For chitosan graft copolymer, at lower concentrations, I_{372}/I_{383} values remain nearly unchanged. Further increasing concentration, the intensity ratio start to decrease, implying the onset of micelle from grafted chitosans. The critical micelle concentration (cmc) is determined by the interception of two straight lines. The cmc values of graft copolymers are listed in Table 2. From the table, it can be seen that the cmc values of polymeric

Table 2
Effect of DLLA/CS molar ratio on the properties of polymeric micelles

Sample	DLLA/CS	Mean diameter (nm)	Polydispersity (μ/Γ^2)	cmc$\times10^2$ (mg/ml)
3	11:1	154	0.03	6.49
5	15:1	178	0.07	2.25
6	20:1	181	0.06	1.01

amphiphiles are lower than the critical micelle concentration (cmc) of low molecular weight surfactants (Lee et al., 1998), indicating the stability of micelles from graft copolymers at dilute conditions. The increasing hydrophobicity by introduction of a large amount of hydrophobic groups further reduces the cmc values (Table 2).

3.4. Size and its distribution of chitosan graft copolymer micelles in water

The size of polymeric micelles and their size distribution in aqueous media were measured by DLS. Since the unmodified chitosan does not form micelles, it cannot be used as a control. The polymeric micelles (DLLA/CS = 11:1) in water has a mean diameter of 154 nm. The size distribution of polymeric micelles is shown in Fig. 7. It can be seen from Fig. 7 that the micelles possess a narrow unimodal distribution (polydispersity $= \mu/\Gamma^2 = 0.03$).

The morphology of the polymeric micelles was investigated by the transmission electron microscopy technique. Fig. 8 shows the TEM image of polymeric micelles (DLLA/CS = 11:1). It could be confirmed that polymeric micelles are spherical in shape. A similar morphology was also observed in other polymeric micelles of DLLA/CS with different molar ratio (data not shown). The size of these micelles is smaller than that determined by DLS in water, presumably arising from the dry state of the TEM measurement.

The mean diameter of micelles and its distribution in water are listed in Table 2. The DLS data demonstrate that the micelle sizes get larger as the DLLA/CS molar ratio increase, suggesting the elongation of hydrophobic polylactide side chain facilitates the growth of the hydrophobic core of polymeric micelles.

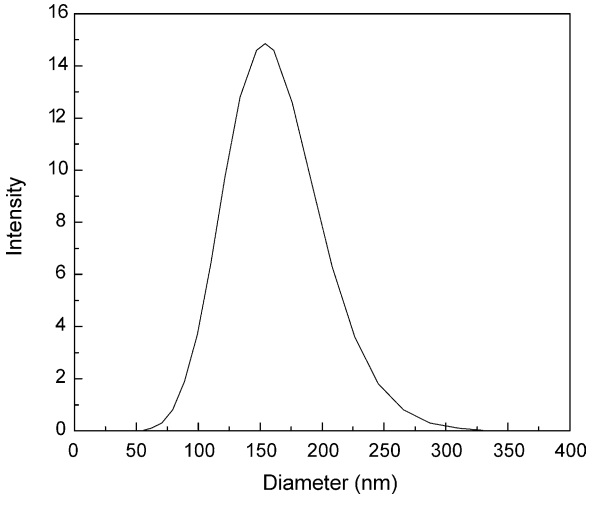


Fig. 7. The size distribution of polymeric micelles (DLLA/CS = 11:1) in water.

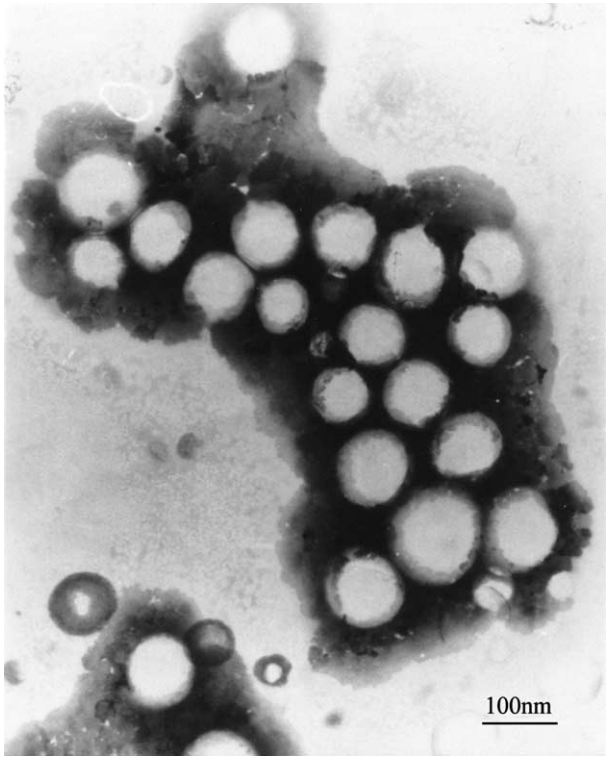


Fig. 8. Transmission electron microscopy photographs of chitosan–polylactide copolymer micelles (DLLA/CS = 11:1).

4. Conclusion

Through grafting DL-lactide onto water soluble chitosan, a novel amphiphilic chitosan–polylactide graft copolymers which can form the polymeric micelles were prepared, and the micelles with an unimodal size distribution. Since chitosan–polylactide polymeric micelles have a hydrophobic core, they can be used as a promising delivery carrier for the entrapment and controlled release of hydrophobic drugs.

Acknowledgements

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