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Crystalline and micellar properties of amphiphilic biodegradable chitooligosaccharide-*graft*-poly(ε-caprolactone) copolymers

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Abstract

Amphiphilic chitooligosaccharide-*graft*-poly(ε-caprolactone) (COS-*g*-PCL) copolymers were controllably synthesized using protection/deprotection technique of COS via trimethylsilyl groups and ring-opening polymerization of ε-caprolactone. Their crystalline properties were investigated with differential scanning calorimetry. With the increase of PCL content in graft copolymer, the values of ΔH and X_c became enhanced, and T_m shifted to higher temperatures. Micellar properties of amphiphilic graft copolymers were studied. Spherical micelles from COS_{11}-*g*-PCL_{132} were prepared. However, due to the higher PCL content, the spherical micelles from COS_{11}-*g*-PCL_{520} easily confused together so that the complicated network morphology was formed. Interestingly, hierarchical structure was observed in the formed network morphology, as being driven by the crystallization of PCL segments in micelles. Using pyrene as a model compound, the micelles from COS_{11}-*g*-PCL_{132} copolymers can be loaded by hydrophobic therapeutic agent, potentially offering an appropriate vehicle for drug delivery.
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Keywords: Amphiphilic copolymers; Biodegradable; Crystallization; Hierarchical structure; Micelles

1. Introduction

Recently, increasing interest is given to the study of amphiphilic biocompatible and biodegradable copolymers because of their controlled biodegradation rates and their potential applications in drug delivery and separations technology (Nie, Zhao, Xie, & Wu, 2003; Ouchi, Kontani, & Ohya, 2003; Rosler, Vandermeulen, & Klok, 2001). Up to now, amphiphilic linear copolymers have been extensively studied (Caillol et al., 2003; Chang, Bender, Phelps, & Allcock, 2002; Luo et al., 2004; Nie et al., 2003; Xiong, Tam, & Gan, 2003). In contrast, relatively few works were conducted on amphiphilic nonlinear copolymer such as graft copolymers (Jeong, Kang, Yang, & Kim, 2002; Li, Zhu, Sunintaboon, & Harris, 2002), although, they have many advantages in providing integrations of considerable functionality onto the polymer backbone (Breitenkamp & Emrick, 2003; Sato et al., 2005).

Chitosan (CTS), a hydrophilic natural polysaccharide containing an amount of amino and hydroxyl groups, is known to be nontoxic, biocompatible and biodegradable in animal tissues. Much attention have been paid to its biomedical, ecological, and industrial application such as wound healing and dressings, drug delivery agents, anti-cholesterolemic agents, blood anti-coagulants, anti-tumor agents as well as immunoadjuvants (Hua et al., 2002; Jae Hyung et al., 2004; Kim, Park, Nah, Choi, & Cho, 2004; Kockisch, Rees, Young, Tsibouklis, & Smart, 2003; Vongchan, Sajomsang, Subyen, & Kongtawelert, 2002). Especially, recent several studies on chitin and chitosan have been converted to chitooligosaccharides (COS) in view of its solubility in neutral aqueous solution, distinctive chemical and biological properties.

COS, the oligomer of chitosan, exhibits various interesting biological activities including anti-tumor activities, immuno-enhancing effects, increasing protective effect against pathogen infection, anti-microbial activities and DNA complexation capability. Due to lack of antigenicity and less accumulated tendency in the host animal, it is used more safely than polysaccharides. All these features make it quite attractive as special biomaterials (Ohara et al., 2004; Park, Je, & Kim, 2003; Roehrig et al., 1995).

At the same time, poly(ε-caprolactone)s (PCL), a kind of hydrophobic polymers, have been frequently applied as

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implantable carriers for drug delivery system as well as biomedical materials, based on high mechanical strength, biodegradable properties, biocompatibility and permeability to drugs (Nie et al., 2003; Ydens et al., 2000).

Thus, we are particularly interested in amphiphilic biodegradable graft copolymers consisting of hydrophilic COS backbones and hydrophobic PCL side chains. The integrations of these two kinds of biocompatible and biodegradable polymers may give a new functional copolymer combining the favorable properties of both COS and PCL.

So far, studies on the synthesis of biodegradable polyesters grafted onto chitin and chitosan backbones were limited in oligo(ε-caprolactone) or oligo(lactic acid) side chains grafted onto chitin and chitosan backbones, and the obtained copolymers are comb-shaped (Detchprohm, Aoi, & Okada, 2001; Yao et al., 2003). Recently, Liu et al. reported the synthesis of polylactide grafted onto chitosan backbone with brush-shaped structure (Liu, Tian, & Hu, 2004). However, as the polymerization was carried out in a heterogeneous system, the graft copolymers with controlled structure were difficultly obtained.

Recently, protection and deprotection of partial hydroxyl groups via trimethylsilyl (TMS) groups was successfully used in the preparation of polysaccharide-based polyester graft copolymers with controlled structures in a homogeneous system. For example, Ohya and Nouvel synthesized pollulan-*g*-poly(lactic acid) and dextran-*g*-poly(lactic acid) via ring-opening graft polymerization of lactide onto the corresponding trimethylsilyl (TMS) protected polysaccharides followed by the removal of TMS protection groups (Nouvel, Dubois, Dellacherie, & Six, 2004; Nouvel, Frochot et al., 2004; Ohya, Maruhashi, & Ouchi, 1998; Ouchi, Kontani, & Ohya, 2003; Ydens et al., 2000). In this respect, we have also synthesized hydroxylpropyl cellulose-*g*-poly(ε-caprolactone) (Wang, Dong, & Tan, 2003).

Hydroxyl groups on COS backbone can serve as the initiating points for the ring-opening polymerization (ROP) of CL. Partially protecting hydroxyl groups on COS backbone can control the number of the initiating points for polymerization and make the resulting graft copolymer with controlled structure possible. But also, the protection groups can render COS good solubility in common organic solvents and thereby, homogeneous chemical reaction is performable. Based on these considerations, we also use the protection/deprotection technique of part hydroxyl group via trimethylsilyl (TMS) groups and the homogenous ring-opening copolymerization of CL using $Sn(oct)_2$ as catalyst. So far, there are few reports on the synthesis of long PCL side chains grafted onto COS backbone. Their crystalline properties were investigated with differential scanning calorimetry. Micellar properties of amphiphilic graft copolymers. Interestingly, it is found that a complicated network with hierarchical structure were formed from COS_{11}-*g*-PCL_{520} with longer PCL chains, probably as being driven by the crystallization of PCL segments in micelles. Furthermore, the formed micelles show an efficient loading capability for hydrophobic agent, potentially offering an appropriate vehicle for drug delivery.

2. Experimental

2.1. Materials

Chitooligosaccharide ($M_n = 1800$, the degrees of deacetylation is 96%), purchased from Shenzhen Bright Way Novel Bio-Material Tech. Co., Ltd (PR China), was dried at 60 °C under vacuum before using. ε-Caprolactone (Acros Organics, 99%) was purified by vacuum distillation over CaH_2 and the fraction collected at 96–98 °C (5 mmHg) was used in polymerization. Stannous octoate [$Sn(oct)_2$] (Sigma, 95%) was used as received. Xylene, CH_3Cl and DMSO were purified by usual distillation method. Hexamethyldisilazan (HMDS), chlorotrimethylsilane, and other regents were used as received without further purification. Pyrene (99%) was purchased from Fluka Co. and was recrystallized three times from ethanol.

2.2. Preparation of COS-g-PCL copolymers

COS-*g*-PCL copolymers were synthesized using a three-step procedure. First, the hydroxyl and amino groups of COS were partially protected with hexamethyldisilazan and chlorotrimethylsilane, in which the number of the protected-OH groups was controlled by the adjustment of the molar ratio of COS to HMDS. The trimethylsilyl substitution of COS (D_{TMS}) was determined to be 2.3 based on the basis of the ratio of the integrated area of the signal for the methyl protons of the TMS groups at around $\delta = 0.1$ ppm to that for the methylene protons (H2) of monosaccharide residue at around $\delta = 2.7$ ppm. Secondly, the purified and dried TMSCOS was dissolved in freshly purified mixture of CH_3Cl and xylene, and a desired amount of ε-caprolactone monomer and a drop of $Sn(oct)_2$ were added under N_2. The reaction mixture in a capped vial under N_2 was placed in a preheated oil bath at 120 °C and stirred for 24 h. The resulting polymer was dissolved in $CHCl_3$ and precipitated twice times with CH_3OH to afford the purified TMSCOS-*g*-PCL. Finally, the obtained TMS-protected graft copolymer was dissolved in an isopropyl alcohol/H_2O/HCl mixture and stirred at room temperature. The solid was isolated, washed, and dried at 50 °C in vacuo. The average polymerization degree of ε-caprolactone grafted on every glucose unit of COS backbone (D_p) was calculated from the ratio of the integral areas of the methylene signal of PCL at 2.3 ppm to the methine proton signal (H2) of COS at 3.0 ppm (Table 1). Considering that the number of the remained hydroxyl groups in every glucose unit is about 0.7 and not every glucose unit was grafted onto caprolactone segments, the average length of PCL grafts (D'_p) in the copolymer is lower that the value of D_p.

2.3. Micellar preparation of COS-g-PCL

Micelles of COS-*g*-PCL in water were prepared as follows. COS-*g*-PCL copolymers were initially dissolved in THF. Deionized water was dropwise added to the COS-*g*-PCL solutions under vigorous stirring until pre-determined water contents were reached. After that, a large amount of water was

Table 1
Preparation and characterization of the COS-*g*-PCL

Sample	[CL]/[glucose unit of COS] (molar ratio)[a]	$M_n \times 10^4$ (M_w/M_n)[b]	T_m (°C)		ΔH (J/g)		X_c (%)[c]	
			T_{m1}	T_{m2}	ΔH_1	ΔH_2	X_{c1}	X_{c2}
g_1	0.5	–	–	–	–	–	–	–
g_2	6.0	0.9 (1.4)	54.6	45.0	44.7	28.0	32.9	20.5
g_3	12.0	1.4 (1.4)	56.1	47.9	47.0	30.8	34.6	22.6
g_4	23.0	4.1 (1.3)	59.0	55.8	83.6	59.9	61.5	44.0
g_5	47.3	8.5 (1.6)	60.3	55.4	93.4	69.9	68.7	51.4
PCL	–	6.2 (1.6)	64.5	56.6	86.8	56.1	63.8	41.3

[a] Calculated by ^{1}H NMR.
[b] Calculated by GPC.
[c] Calculated by DSC.

added to the solutions to quench the resulting micelles. The solution was then dialyzed against water to remove the organic solvent.

2.4. Measurements

^{1}H NMR analyses were carried out by means of a JOEL JNM-ECA300 spectrometer in DMSO (solvents without TMS). Molecular weight (M_n) and molecular weight distribution (M_w/M_n) were measured with a Viscotek TDA 302 GPC instrument equipped with tetrahydrofuran (THF) as the mobile phase and polystyrene was used as calibration standard. TEM was performed on a JEOL JEM-1200 electron microscope at an acceleration voltage of 120 kV. A copper grid with a carbon film was used. The copper grid was immersed in a drop of the aqueous polymer solution for 10 min and then removed and dried. A drop of phosorous tungstenic acid in water (2 wt%) was placed on the copper grid for 2 min. The copper grid was then dried at room temperature prior to measurement. Fluorescence spectra were recorded with a Perkin–Elmer LS55 luminescence spectrometer. The excitation wavelength was 335 nm and the emission spectra were recorded from 350 to 500 nm.

2.5. Encapsulation of pyrene in micelles

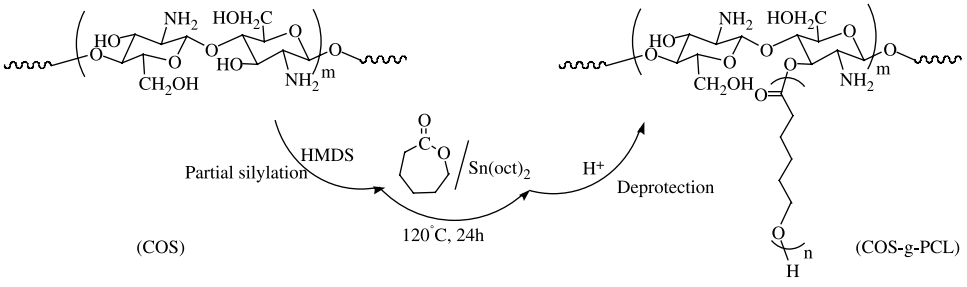
Pyrene, as a model compound of hydrophobic drug, was encapsulated into the micelle as follows. A saturated aqueous solution of pyrene was prepared. Then, the micelle aqueous solution of graft was dialyzed against water saturated with pyrene for scheduled time. Afterward, the solution was filtered through a 0.45 μm PTFE filter, which is known from separate experiments to remove microcrystals of pyrene. This solution was used for fluorescence measurement.

3. Results and discussion

3.1. Characterization and architecture of COS-g-PCL

The synthesis of COS-*g*-PCL was carried out by using a three-step procedure shown in Scheme 1. The trimethylsilylation of COS took place first onto the hydroxyl and amino groups, in which the number of protected-OH groups was controlled by the adjustment of the molar ratio of COS to HMDS. In our case, the trimethylsilyl substitution of COS was approximately 2.3. For this highly silylated COS, the glucose units were either trisilylated or disilylated with a majority of hydroxyl groups in the third position (OH^3), due to the relative weak reactivity of the OH^3 group in each glucose unit toward silylation (Nouvel, Dubois et al., 2004). This result means that there are remained about seven OH^3 groups per 10 glucose units and these remaining free hydroxyl groups can be served as the initiating points for the subsequent ROP reaction of ε-caprolactone. The obtained TMSCOS possesses a good solubility or swellability in common organic solvents such as pyridine, chloroform, THF, and xylene. Therefore, a homogeneous ROP reaction of ε-caprolactone was successfully performed on TMSCOS backbones in a mixed medium of chloroform and xylene. Finally, the TMS protection groups of TMSCOS-*g*-PCL were removed by the incubation of polymer samples into an isopropyl alcohol/H_2O/HCl mixture.

^{1}H NMR spectrum of TMS-deprotected COS-*g*-PCL was in good agreement with the expected structure as shown in Fig. 1. The deprotection of TMS groups was confirmed by the



Scheme 1. Synthesis of chitooligosaccharide-based graft copolymers (COS-*g*-PCL).

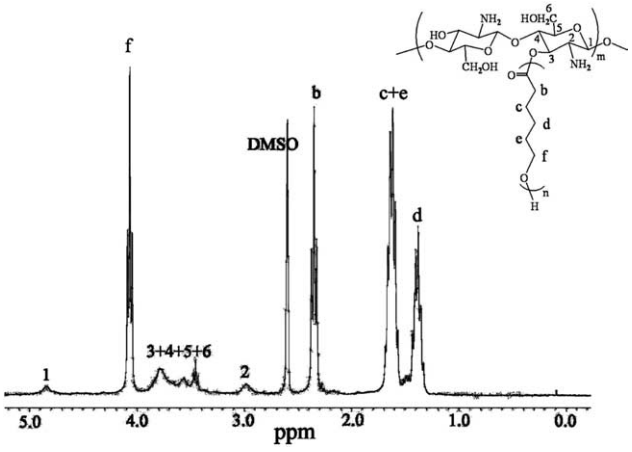


Fig. 1. ^{1}H NMR spectrum of TMS-deprotected graft copolymer COS-*g*-PCL (in DMSO-d_6), the molar ratio of CL to glucose unit of COS is about 6.

disappearance of methyl proton signals from TMS at 0.10 ppm. The methylene proton signals of PCL can be observed at 4.1 ppm (three peaks), 2.3 ppm (three peaks), 1.7 ppm (multipeaks), and 1.4 ppm (multipeaks). The methine and methylene proton signals of COS are at 3.0–5.0 ppm.

The incorporation of TMS groups ensures the control of the PCL grafts' number and the position on the COS backbone. The average degree of ε-caprolactone grafted on every glucose unit of COS backbone (D_p) was calculated from the ratio of the integrated area of the methylene signal of PCL at 2.3 ppm to the methine proton signal (H2) of COS at 3.0 ppm (Table 1). Considering that the number of the remaining hydroxyl groups in every glucose unit is about 0.7 and not every glucose unit was grafted by caprolactone segments, the average length of PCL grafts (D_p') in the resulting copolymer is lower than the value of D_p.

In this work, COS, which has a molecular weight of 1800, was used, meaning that the amount of glucose units per COS chain is about 11. When short oligo(ε-caprolactone) was grafted onto COS backbone, the structure of the obtained graft copolymer is comb-shaped. With the increasing of the length of ε-caprolactone segments, the structure of the obtained graft copolymer turns into brush-shaped and finally, becomes an asymmetric and unconventional graft copolymer structure consisting of a short main chain as backbone and long side chains as grafting segments. The schematic representation of their architecture is shown in Fig. 2.

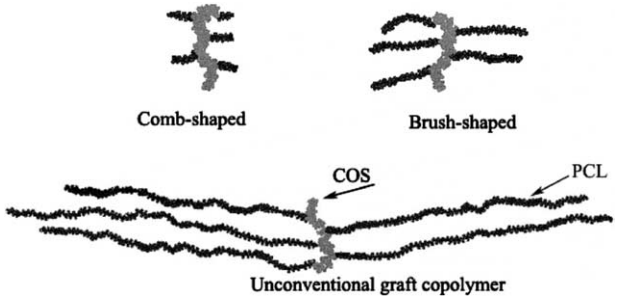


Fig. 2. Schematic representations of the graft copolymers with different PCL branch lengths.

The molecular weight and distribution of the synthesized graft copolymers measured by GPC instruments are listed in Table 1. In our experiments, due to the poor solubility of the graft copolymer containing short side length in THF, its GPC curve was not obtained. The contamination of the PCL homopolymer resulting from *trans*-esterification was not detected in GPC curves.

3.2. DSC analysis of COS-g-PCL

It is reported that the crystalline property of amphiphilic copolymer influences its biodegradation and hierarchical structure of its formed nanoparticles (Lin & Gast, 1996; Ohya et al., 1998; Portinha, Bouteiller, Pensec, & Richez, 2004; Richter, Schneiders, Monkenbusch, & Willner, 1997). So, thermal behavior of the synthesized new graft copolymers with different D_p was investigated by using DSC, where the PCL and COS homopolymers were selected as reference samples. It is well known that PCL homopolymer is a semicrystalline polymer (X_c, 41.3%). Since COS did not show any melting transition, the detected thermal phenomenon in DSC can only be attributed to PCL segments in COS-*g*-PCL. Fig. 3 shows the second melting curves of graft copolymers. The values of the melting temperature (T_m), melting enthalpy (ΔH) and the degree of crystallinity (X_c) of all samples are listed in Table 1. The values of X_c of PCL components were estimated from the assuming the ΔH value of completely crystalline PCL (136 J/g) (Ma et al. 2003). In comparison with COS homopolymer, we found that the grafting of PCL onto COS backbone gave rise to an increase in crystallinity as shown in Table 1. When a few PCL segments were grafted onto COS backbone, no detectable melting enthalpy were seen like that of COS homopolymer. With increasing PCL content in graft copolymer, the values of ΔH and X_c enhanced, and T_m shifted to higher temperatures. And, the value of T_m of the graft copolymer containing longer PCL branches is almost equal to that of PCL homopolymer. However, their values of ΔH and X_c are larger than those of PCL homopolymer. As a explanation it is believed that the existence of a little COS in COS-*g*-PCL

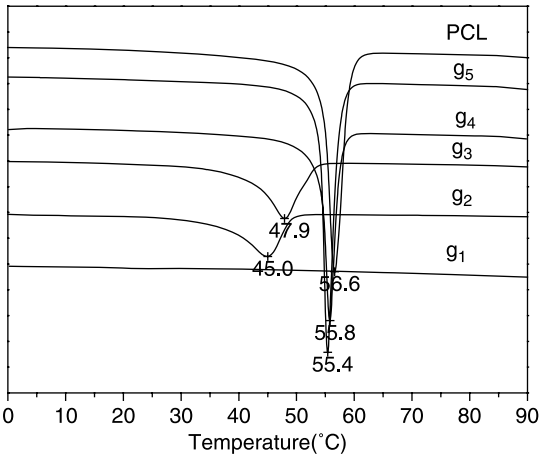


Fig. 3. DSC curves of the graft copolymers and PCL homopolymer.

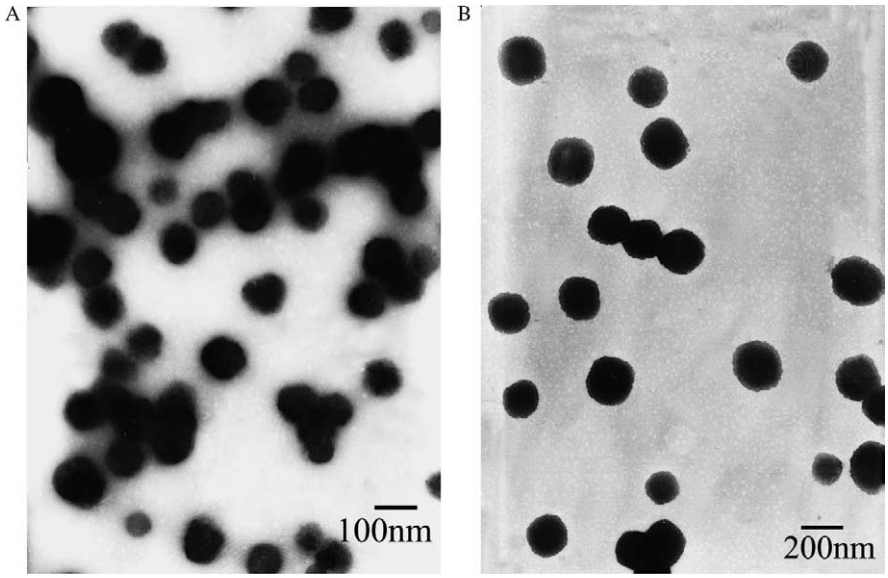


Fig. 4. TEM images of COS_{11}-*g*-PCL_{132} nanoparticles (dialysis against water) (A) 0.5% copolymer; (B) 0.8% copolymer.

promotes the PCL segments to form more regular crystalline domains.

3.3. Formation of micelles from COS-g-PCL copolymers

It is well known that chitosan with high degree of deacetylation is insoluble in neutral or alkaline aqueous solution or other organic solvents, but soluble in acid aqueous solution with rigid structure. When chitosan was degraded into COS, COS is soluble not only in acid aqueous solution but also in neutral aqueous solution. This means that micelles of our synthesized copolymers can be carried out in neutral aqueous solution.

As the synthesized COS-*g*-PCL consisting of short hydrophilic COS backbone and relative long branches was insoluble in water, micelles were prepared by using indirect method in our work. THF was used as the initial good solvent for copolymer samples, and neutral aqueous solution were used as the selective solvent. In the present work, two graft copolymers containing different PCL lengths were studied. One is COS_{11}-*g*-PCL_{132} and another is COS_{11}-*g*-PCL_{520}.

After THF was removed using dialysis against water, the resulting TEM images of these two graft copolymers (COS_{11}-*g*-PCL_{132} and COS_{11}-*g*-PCL_{520}) are shown in Figs. 4 and 5B. It appeared that nano-spherical micelles were formed in the case of COS_{11}-*g*-PCL_{132} with different initial copolymer concentration, respectively (Fig. 4). However, in the case of COS_{11}-*g*-PCL_{520}, a complicated network morphology, which clearly consists of the spherical micelles through fusion, was observed (Fig. 5B). For a comparison, the aggregation behavior before dialysis was further studied. It appeared that spherical micelles were formed and they well dispersed in the used solution due to the existence of THF (Fig. 5A). This result indicates that the high content of hydrophobic PCL part in COS_{11}-*g*-PCL_{520} led to instability of the formed micelles from copolymer in aqueous solution and the flocculation occurred.

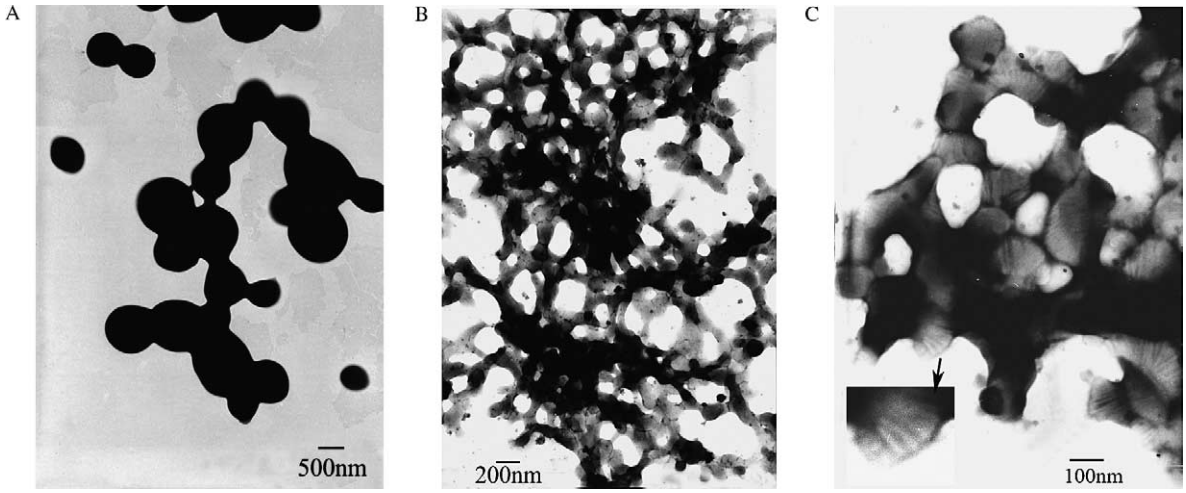


Fig. 5. Transmission electron micrographs of the aggregates from COS_{11}-*g*-PCL_{520} (A) before dialysis; (B) and (C) after dialysis (0.5% copolymer).

Additionally, it was observed that the micelles swelled with THF are larger than those after the removal of THF.

Interestingly, hierarchical structure was formed in the network morphologies from COS_{11}-*g*-PCL_{520} copolymer. At high magnification, it can be clearly seen that the lamellar sheets of PCL segments are vertical to the surface of micelles (Fig. 5C). Compared to other COS-*g*-PCL copolymers with short PCL chains, however, this phenomenon was not observed. Recently, similar hierarchical structure was also observed during the studies on self-assembly behavior of amphiphilic block copolymer in a select solvent. It is found that, if flexible blocks can crystallize, organized structure can occur within the core of the formed micelles (Lin & Gast, 1996; Portinha et al., 2004; Richter et al., 1997). For example, poly(ethylene oxide)–polystyrene diblock copolymers in hydrocarbon solvents form thin platelet structures consisting of chain-folded crystalline domains of the insoluble block polystyrene.

PCL is a kind of semicrystalline polymer whose crystalline temperature is about 60 °C. In our works, only in the case of the copolymer with longer PCL chains COS_{11}-*g*-PCL_{520} the hierarchical structure was observed. These facts drive us to believe that the strong tendency of PCL to crystallization may be the main reason for the formation of the observed hierarchical structure. Detailed studies on this observation are still undergoing.

3.4. Encapsulation of pyrene molecules in the formed micelles

Among mesospheric drug delivery systems, the micellar nanoparticles formed from the biodegradable and biocompatible COS-*g*-PCL copolymers in aqueous solution should be suitable as an appropriate vehicle for drug delivery. Hydrophobic therapeutic agent should be effectively encapsulated into the micelles. As a model molecule of hydrophobic drug, pyrene was chosen and successfully encapsulated in the formed micelles in this study.

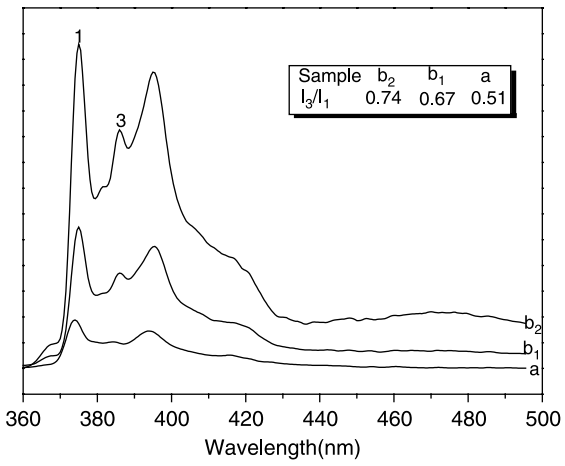


Fig. 6. Fluorescence spectra of (a) saturated pyrene in water and (b) pyrene-loaded nanoparticles solution of the graft copolymer (COS_{11}-*g*-PCL_{132}) (b_1, dialysis against water saturated with pyrene for 1 day; b_2, dialysis against water saturated with pyrene for 3 days).

Pyrene has been extensively used as a fluorescence probe to investigate the hydrophobic micro domains formed by amphiphilies. In particular, the ratio I_3/I_1 of the intensities of the third and first vibronic peak in pyrene spectrum is a sensitive indicator of the polarity of the pyrene microenvironment (Ma, Cao, & Webber, 1998; Matsui, Mitsuishi, & Miyashita, 2002). Thus, the change of I_3/I_1 can be used to characterize if pyrene molecule has been loaded into micelles. Fig. 6 shows the fluorescence spectra of pyrene in saturated aqueous solution and pyrene-loaded micellar solution of amphiphilic graft copolymer, respectively. It can be seen that not only the total emission intensity of pyrene-loaded micellar solution increases, but also the ratio I_3/I_1 of the intensities of the third and first vibronic peak increased from 0.51 to 0.74, after micelle solution was dialyzed against water saturated with pyrene for 3 days. This result clearly indicated that the pyrene molecule has been loaded into the hydrophobic PCL domains. Additionally, since more pyrene molecules were loaded into micelles, a broad structureless emission at longer wavelength (480 nm) attributed to the excited-state dimer of pyrene units was also observed in Fig. 6b. Loading content and release studies of these micelles are ongoing.

4. Conclusions

Amphiphilic graft copolymers with controlled structure, COS-*g*-PCL, were synthesized by using protection/deprotection of partial hydroxyl groups of COS via TMS groups and homogenous ring-opening polymerization of ε-caprolactone with stannous octoate as catalyst. A new kind of graft copolymers with controlled structure was obtained by adjusting the molar ratio of the CL to COS glucose unit. Studies on their crystalline property indicated that with the increase of PCL content in graft copolymer, the values of ΔH and X_c enhanced, and T_m shifted to higher temperatures. Spherical micelles from COS_{11}-*g*-PCL_{132} were formed. However, due to the higher PCL content, the spherical micelles from COS_{11}-*g*-PCL_{520} easily confused together so that the complicated network morphology was formed. Interestingly, hierarchical structure was observed in the formed network morphology, as being driven by the crystallization of PCL segments in micelles. Using pyrene as a model compound, the micelles from COS_{11}-*g*-PCL_{132} copolymers can be loaded by hydrophobic therapeutic agent, potentially offering an appropriate vehicle for drug delivery. Since the synthesized graft copolymers not only contain amino and hydroxyl groups on COS backbones but also are biodegradable and biocompatible, their micelles may be useful to design attractive functional nano-hybrid materials from the perspective of potential applications in biotechnology.

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