Carbohydrate Polymers 75 (2009) 401–407

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Synthesis and self-assembly of chitosan-based copolymer with a pair of hydrophobic/hydrophilic grafts of polycaprolactone and poly(ethylene glycol)

Li Liu [a], Xian Xu [a], Shengrong Guo [a,*], Wei Han [b]

[a] School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China
[b] College of Life Science Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, PR China

ARTICLE INFO

*Article history:*
Received 29 May 2008
Received in revised form 28 July 2008
Accepted 31 July 2008
Available online 14 August 2008

*Keywords:*
Chitosan
Graft copolymer
Amphiphilic
Self-aggregation

ABSTRACT

Chitosan-based copolymers with binary grafts of hydrophobic polycaprolactone and hydrophilic poly(ethylene glycol) (CS-g-PCL&PEG) were prepared by a homogeneous coupling reaction of phthaloyl-protected chitosan with functional PCL-COOH and PEG-COOH, following deprotection to regenerate free amino groups back to chitosan backbone. They were characterized by $^1$H NMR, Fourier transform infrared and X-ray diffraction analysis. These CS-g-PCL&PEG copolymers could form nano-size self-aggregates in acidic aqueous solution without a specific processing technique, which were investigated using dynamic light scattering and transmission electron microscopy. The formed self-aggregates become smaller with weakened stability upon pH increasing. Moreover, the aggregates of copolymer with higher content of PEG and PCL grafts could remain stable for over 30 days in both acid and neutral condition. A possible mechanism was proposed for the formation of self-aggregates from CS-g-PCL&PEG and their structural changes as pH. It is warranted to find promising application of these self-aggregates based on chitosan as drug carriers.

## 1. Introduction

Research and development on a variety of amphiphilic copolymers containing hydrophobic and hydrophilic segments, have been very active owing to concerns related to their spontaneous self-assembly behaviors in aqueous media (Forster & Plantenberg, 2002). These smart transitions often leads to diverse functional compartment structures like micelles, vesicles and gels, which represent promising applications in the filed of biotechnology and pharmaceutics. So far, most of polymeric self-assembly systems are formed from amphiphilic block copolymers or hydrophobically modified water-soluble polymers. In particular, there has been a growing interest in the self-assemblies of natural polysacchrides and their derivatives, such as cellulose, starch and chitosan. For example, Jiang and their co-works (Dou, Jiang, Peng, Chen, & Hong, 2003; Wan, Jiang, & Zhang, 2007) have intensively studied poly(acrylic acid) grafted hydroxyethylcellulose, which displayed reversible pH-dependent micellization and the resultant micelles exhibited a reversible micelle-hollow sphere transition after partial crosslinking. Besheer, Hause, Kressler, and Mader (2007) reported synthesis of hydrophobically modified hydroxyethyl starch with fatty acid, which formed monodisperse self-aggregates by intra- and/or intermolecular association in a dilute aqueous solution.

Chitosan (CS), next to cellulose, is the second most plentiful biomass, with a repeated structure of β-(1,4)-linked 2-amino-2-deoxy-D-glucose. Significantly, much attention has been paid to utilize chitosan as drug or gene carriers because of its relatively good biocompatibility, biodegradability, low immunogenicity and biological activities. The extended applications of chitosan, however, are frequently limited by its poor solubility due to its highly crystallized structure. In order to overcome the problem above, chemical modification of chitosan has been carried out. Recently, Kwon's group (Kim et al., 2005; Lee, Kwon, Jo, & Jeong, 2005; Park et al., 2006; Yoo, Lee, Chung, Kwon, & Jeong, 2005) prepared hydrophobically modified chitosan derivates with deoxycholic acid and investigated physicochemical characteristics and applications of their self-aggregates, which possessed a promising potential in drug delivery.

In addition, graft modification of aminopolysaccharide with synthetic polymers has been used as an interesting alternative method to develop novel hybrid materials (Jenkins & Hudson, 2001). In recent years, we have made much effort to synthesize chitosan-based graft copolymers with well-defined structures, like chitosan-g-polycaprolactone, chitosan-g-poly(ethylene glycol) and so on, though a protection-grafting-deprotection route. (Liu, Chen, & Fang, 2006a; Liu, Li, Fang, & Guo, 2006b; Liu, Li, Liu, & Fang, 2004,) Polycaprolactone (PCL) is one of presented biodegradable polyesters with excellent mechanical strength, biocompatibility and non-toxicity. PCL grafted chitosan could also be used to prepare

---

* Corresponding author. Tel./fax: +86 021 3420 4793.
*E-mail address:* srguo@sjtu.edu.cn (S. Guo).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.07.038

nanoparticles of core/shell structure due to the hydrophobicity of PCL (Yu, Wang, Chen, Deng, & Jing, 2006). Nevertheless, there may exist a problem on the stability of self-aggregates from CS-g-PCL in biological solution (pH = 7.4), since the strong hydrophobicity of PCL and insoluble nature of chitosan. Poly(ethylene glycol) (PEG) is a highly water-soluble polymer and is one of the most useful hydrophilic blocks in amphiphilic block polymers. Ouchi, Nishizawa, & Ohya, 1998 ever reported the aggregation phenomenon of poly (ethylene glycol) (PEG) grafted chitosan in aqueous solution. The aggregates formed spontaneously by strong intermolecular hydrogen bonds between chitosan moieties in water, while the water-soluble PEG chains served as a hydrophilic shell stabilizing the nanoparticles.

In this paper, we develop a new binary graft copolymer, chitosan-*graft*- PCL&PEG (CS-g-PCL&PEG), which is composed of amino polysaccharide (chitosan) as the backbone and a pair of complementary amphiphilic polymers, hydrophobic PCL and hydrophilic PEG, as two grafts. It is worthy noting that few reports about such dual system of chitosan-based graft copolymers are present as far as we know. This graft copolymer is expected to forms self-aggregates in aqueous solution. Herein, we investigated in detail their self-aggregation behavior in aqueous media and the stability of self-aggregates.

## 2. Materials and methods

### 2.1. Materials and reagents

Chitosan (degree of deacetylation = 99.6%, determined by $^1$H NMR spectra and Element analyses; average molecular weight = $6.0 \times 10^5$, determined by the intrinsic viscosity assay) was purchased from JinKe Biochemical Co. Ltd. (Zhejiang, China). Monomethoxy-poly(ethylene glocol) (MPEG, $M_n$ 5000) was purchased from Fluka. Well-defined PCL terminated with hydroxyl group ($M_{n\ NMR} = 3000$) was synthesized from ring-opening polymerization of ε-caprolactone (from Aldrich) using benzyl alcohol as an initiator and stannous octoate as a catalyst in bulk at 130 °C in the lab according to our previous paper (Lu et al., 2006). 4-Dimethylaminopyridine (DMAP), 1-hydroxybenzotrizole (HOBt), 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC·HCL) were from the Shanghai Yanchang biotech developmental Co. Ltd. (China). Phthalic anhydride, succinic anhydride, maleic anhydride and hydrazine monohydrate were all from the Reagent Factory of Shanghai (China) and were used as received.

### 2.2. Preparation of carboxyl-capped MPEG precursors (MPEG-COOH) and carboxyl-capped PCL precursors (PCL-COOH)

Monohydroxy-terminated PEG and PCL macromonomers were transferred to carboxyl-capped precursors by reacting with anhydride, respectively.

For example, monomethoxy-poly(ethylene glocol) (MPEG, 1 mmol), succinic anhydride (1.2 mmol), DMAP (1 mmol) and triethylamine (1 mmol) were dissolved in dry dioxane (15 ml). The mixture was stirred at room temperature for 48 h under a dry nitrogen atmosphere. The dioxane was evaporated in vacuum, and the residue taken up in $CCl_4$, filtered, and precipitated by ether to produce MPEG-COOH.

PCL-COOH was obtained by the reaction of monohydroxy-terminated PCL (1 mmol) with maleic anhydride (2 mmol) in dry toluene (30 ml) at room temperature for 48 h, in the presence of DMAP (2 mmol) as a catalyst. Crude polymer obtained by precipitation into hexane was redissolved in DCM. Then the solution was washed with aqueous hydrochloric acid (10% in v/v) for three times and then four times with a saturated NaCl solution. Then the organic phase was isolated, and filtered. PCL-COOH recovered by precipitation into hexane was vacuum-dried to a constant weight at 40 °C.

### 2.3. Preparation of graft copolymers

Firstly, Chitosan was heated with excess phthalic anhydride in dried DMF to give phthaloylchitosan (PHCS) according to the previously reported procedure (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002; Nishimura, Kohgo, & Kurita, 1991). It was obtained as a brown–yellow powdery material and the degree of substitution (DS) of phthaloyl groups was determined to be about 1.05 by elemental analysis. Calculated for PHCS (DS = 1.05) (%): C, 53.68; H, 4.43; and N, 4.70. Found (%): C, 53.44; H, 4.80; and N, 4.33.

Then vacuum-dried PHCS and monocarboxy-capped macromonomers (MPEG-COOH and PCL-COOH) with different ratios were dissolved in DMF (PHCS 1 g with DMF 10 ml) containing HOBt, followed by EDC·HCL added (HOBt and EDC·HCL, each three moles equivalent to the macromonomers, according to the reference (Yoksan, Matsusaki, Akashi, & Chirachanchai, 2004). The reaction was continued at room temperature for 24 h under stirring. The mixture was poured into water, and the precipitate was separated by centrifuging and was washed by DCM to eliminate the free macromonomers. Then the product was vacuum-dried to a constant weight at 40 °C.

The obtained phthaloyl-protected graft copolymer (1 g) was stirred in 10 ml of DMF and heated to 100 °C under nitrogen. Hydrazine monohydrate was added and the reaction was continued for 2 h to deprotect the phthaloyl group. The mixture was dialyzed in water and alcohol for purification and freezing-dried to obtain powdery graft copolymers.

### 2.4. Chatacterization

All infrared spectra were obtained from samples in KBr pellets using a Perkin Elmer Paragon 1000 FT-IR spectrophotometer. $^1$H NMR spectra were taken by a Varian MERCURY plus 400 at 25 °C. X-ray powder diffraction diagrams were recorded with Bruker AXS X-ray diffractometer. Elemental analyses were performed using a Perkin Elmer PE 2400 II el elemental analyzer.

### 2.5. Determination of the primary amines in graft copolymers by ninhydrin assay

The suspension of CS-g-PCL/PEG with a concentration of 1.0 mg/ml was obtained by dispersing 10 mg graft copolymers to 10 ml deionized water. For the assay, 0.5 ml of the ninhydrin reagent (which was freshly prepared according to the previous references (Leane, Nankervis, Smith, & Illum, 2004; Prochazkova, Varum, & Ostgaard, 1999) was added to 0.5 ml of the suspension in a glass vial. The vial was immediately capped, briefly shaken by hand and heated in boiling water for 30 min to accelerate the reaction between ninhydrin and amino groups. After cooling, the content in each vial was diluted by adding 15 ml of a 50:50 mixture of ethanol and water. The absorbance of each mixture was measured on a Spectrumlab 54 UV–vis spectrophotometer (Lengguang Tech) at 570 nm, zero-set against a similarly treated blank of water. The concentration of amino group in the sample was obtained from a standard calibration curve, using glycine as standard.

### 2.6. Fabrication of aggregates from the graft copolymers

Generally, 10 mg of the graft copolymer (CS-g-PCL&PEG) was dispersed in 10.0 ml of 1% acetic acid (pH 2.8) aqueous solution under gentle shaking for 24 h to obtain the aggregates with a concentration of 1.0 mg/ml. All samples were prepared at room temperature and filtered using disposable 0.8 μm filters before size measurements. For stability test, the aggregates solution at pH 2.8 was dialyzed against Milli-Q water for 24 h to produce a stock suspension at pH 7.0.

### 2.7. Dynamic light scattering (DLS) measurement

The effective diameters of chitosan self-aggregates were measured by DLS using a Malvern Instrument Zetasizer Nano S laser scattering system with a 632 nm laser source. The intensity of the scattered light was detected at 173° to minimize the effect of multiple scattering. Mean diameter ($d$) was evaluated by the following Stokes-Einstein relationship.

$$d = \frac{k_B T}{3\pi\eta_0 D}$$

Where $D$ is the diffusion constant; $k_B$ is the Boltzmann constant; $T$ is the absolute temperature; $\eta_0$ is the solvent viscosity. The copolymer solutions were put in 12 mm diameter polystyrene cuvettes and the minimum sample volume required for the experiment was 1 mL. All analyses were run at 25 °C in six times, and the results are reported as the average values.

### 2.8. Transmission electron microscopy (TEM)

TEM observations were performed on a JEM-2010 Analytical Transmission Electron Microscope (JEOL, JAPAN) at an accelerating voltage of 200 kV. A small drop from the aggregates solutions was placed onto a carbon-coated copper grid. About 2 min after deposition, the grid was tapped with filter paper to remove surface water, followed by air drying. Staining was performed using a droplet of a 0.1 wt.% phosphotungstic acid aqueous solution.

## 3. Results and discussion

### 3.1. Synthesis and characterization of the chitosan-graft-PCL&PEG copolymers

Generally, in graft modification program of chitosan, it is important to maintain the primary amino groups in the polysaccharide backbone for biomedical use. These amino groups can produce positively charges in a biological fluid, thus make chitosan derivates possess the ability to encapsulate various macromolecular drugs containing negatively charged ones (e.g., peptides, proteins and genes). In the previous researches, a successful protection-graft-deprotection route has been reported to prepare graft copolymers of chitosan with PCL (Liu et al., 2004) or with PEG (Liu et al., 2006b) using phthaloylchitosan as an intermediate. This strategy was also explored in this work to conjugate two kinds of graft, hydrophobic PCL and hydrophilic PEG, onto chitosan. Since PCL and PEG are polymers having conflicting affinity with water, it is necessary to find an appropriate solvent for both chitosan and the reactants to carry out a homogeneous reaction. Fortunately, phthaloylchitosan is known as a derivative with improved solubility in some polar organic solvents like DMF and DMSO, which are also good solvents for PCL and PEG. Accordingly, phthaloylchitosan was introduced not only to protect the amino group but also enable the graft reaction proceed in a homogeneous system.

The synthesis route of CS-g-PCL&PEG was presented in Fig. 1. PCL and PEG macromonomers were functionalized to the corresponding carboxyl-capped prepolymers, followed by the esterification with hydroxyl group of PHCS in the presence of EDC·HCl in DMSO at room temperature. Then, phthaloyl was deprotected by incubation with hydrazine to regenerate active amino groups in the backbone of final graft copolymer. Fig. 2 presented a typical $^1$H NMR spectrum of CS-g-PCL&PEG copolymer. Peak assignment for the PCL and PEG segments was straightforward, where the sharp multiply peaks at 1.0, 1.2, 2.0 and 3.3 ppm respectively belonged to the γ-, (δ + β)-, α-, and ε-methylene protons next to the carbonyl group of PCL branch, the strong signal at 3.4 ppm was related to the methylene protons of PEG, and the weak signals of terminated methyl protons of MPEG was also detected at 3.1 ppm. Meanwhile, the proton signals of chitosan backbone were assigned at 4.6 ppm for $H_1$, at 2.8 ppm for $H_2$ and at 3.2–3.8 ppm for $H_3$-$H_6$ overlapped with ε-methylene protons of PCL. The characteristic signals of both PCL and PEG indicated the coexistence of two grafts with chitosan in the graft copolymer.

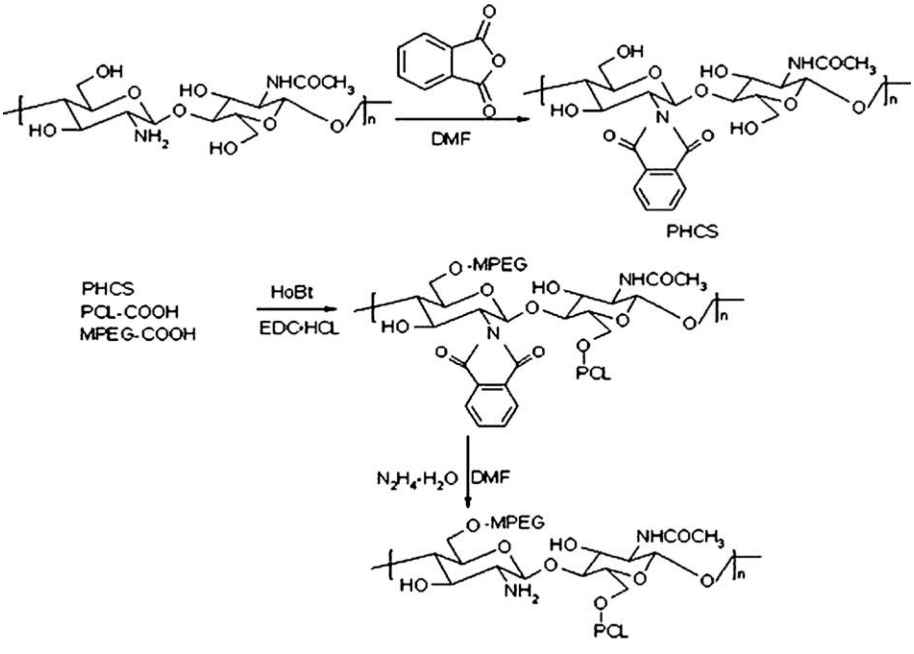

**Fig. 1.** Synthetic route of the CS-g-PCL&PEG copolymers.

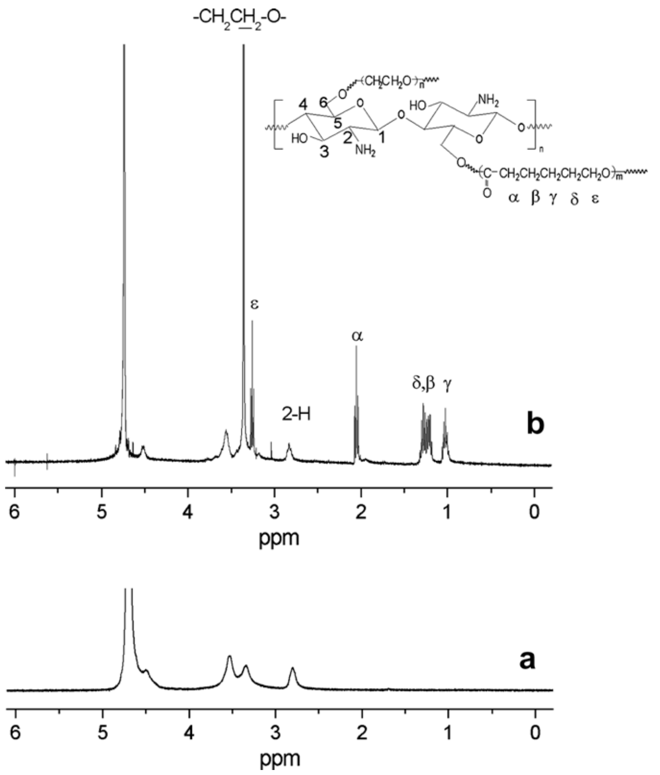

**Fig. 2.** $^1$H NMR spectra of chitosan (a) and graft copolymer of CS-g-PCL$_{4.0}$&PEG$_{3.0}$ (b) in $D_2O/CF_3COOD$ 95:5 v/v.

$^1$H NMR was also used to calculate degree of grafting (DG) of the PCL branches and the PEG branches, where DG was assumed as the number of grafting branches per 100 chitosan units. Thus,

$$\mathrm{DG_{PEG}} = \frac{I_{3.0\mathrm{ppm}}}{3I_{2.8\mathrm{ppm}}} \times 100$$

$$\mathrm{DG_{PCL}} = \frac{I_{1.2-1.5\mathrm{ppm}}}{6I_{2.8\mathrm{ppm}}} \times \frac{114}{3000} \times 100$$

Where 114 is the molecular weight of CL unit and 3000 is the average molecular weight of PCL prepolymer.

The hydrophilicity or hydrophobicity of graft copolymers could be controlled well by adjusting the feed ratio of PCL/PEG prepolymers to PHCS. Results in Table 1 showed two kinds of graft copolymers, CS-g-PCL$_{4.0}$&PEG$_{3.0}$ with the comparative content of PEG and PCL, while CS-g-PCL$_{2.1}$&PEG$_{0.3}$ with a relative lower content of PEG, which would be speculated to own the decreased hydrophilicity.

In addition, the graft conjugation was further confirmed by Fourier transform infrared (FT-IR) spectra. Fig. 3 presented the FT-IR spectra of chitosan, two CS-g-PCL&PEG copolymers, PEG and PCL homopolymers. Compared with original chitosan, the obtained CS-g-PCL$_{4.0}$&PEG$_{3.0}$ showed a characteristic sharp peak at around 1724 cm$^{-1}$ belonging to ester carbonyl stretching band (C=O), stronger absorbance at about 1150 cm$^{-1}$ for C—O and at 2800–3000 cm$^{-1}$ for C—H (of $CH_2$). This indicated the successful conjugation of both PCL and PEG branches with chitosan. Nevertheless, in the FT-IR spectra of CS-g-PCL$_{2.1}$&PEG$_{0.3}$, besides the sharp peak of ester carbonyl, the peaks at 2800–3000 cm$^{-1}$ and 1150 cm$^{-1}$ presented equal absorbance with chitosan, which may imply the low content of PEG segments.

**Table 1**
Compositions of the CS-g-PCL&PEG copolymers and average particle sizes of CS-g-PCLPEG copolymers in different aqueous solutions

| Copolymers | CS (wt.%) | Grafting content of PEG[a] | | Grafting content of PCL[a] | | Diameters by intensity (nm)[b] | |
|---|---|---|---|---|---|---|---|
| | | wt.% | DG | wt.% | DG | pH = 2.8 | pH = 7.0 |
| Chitosan | 100% | / | / | / | / | / | / |
| CS-g-PCL$_{4.0}$&PEG$_{3.0}$ | 37.6 | 35.1 | 3.0 | 27.3 | 4.0 | 269 | 248 |
| CS-g-PCL$_{2.1}$&PEG$_{0.3}$ | 67.2 | 6.3 | 0.3 | 26.5 | 2.1 | 354 | 346 |

[a] Determined by NMR. Graft content of PEG (wt.%) = weight of PEG segment/weight of graft copolymer. Graft content of PCL (wt.%) = weight of PCL segment/weight of graft copolymer.

[b] Determined by DLS.

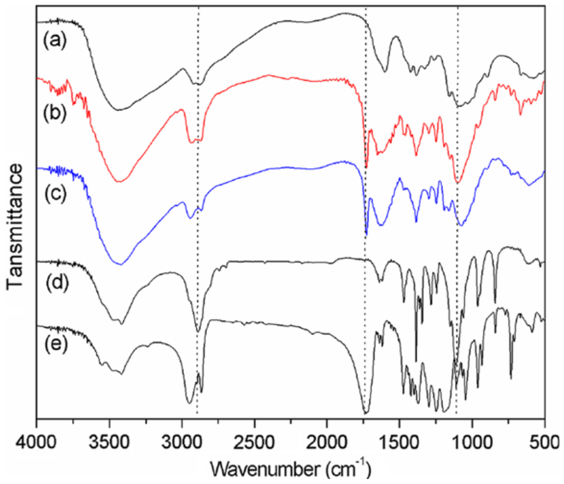

**Fig. 3.** FT-IR spectra of chitosan (a), CS-g-PCL$_{4.0}$&PEG$_{3.0}$ (b), CS-g-PCL$_{2.1}$&PEG$_{0.3}$ (c), PEG (d) and PCL (e).

It should be noted that free amino group would be regenerated to chitosan backbone in the final graft copolymers. By ninhydrin assay, the amount of $-NH_2$ was evaluated to be $1.91 \times 10^{-3}$ mol/g in CS-g-PCL$_{4.0}$&PEG$_{3.0}$., which is equivalent to the calculated value ($2.29 \times 10^{-3}$ mol/g) resulted from the assumption that all $-NH_2$ were regenerated. This case confirms the presence of free amino groups in the graft copolymers.

X-ray powder diffraction analysis (XRD) is another useful method to demonstrate the structure of graft copolymers in solid state. The XRD patterns of chitosan, CS-g-PCL&PEG copolymers, PCL and PEG were detected as illustrated in Fig. 4. It was known that homopolymers of PCL and PEG were all easy to crystallize, and chitosan

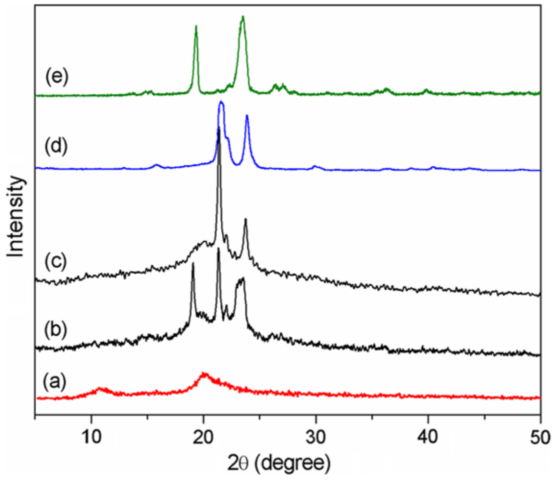

**Fig. 4.** X-ray diffraction of chitosan (a), CS-g-PCL$_{4.0}$&PEG$_{3.0}$ (b), CS-g-PCL$_{2.1}$&PEG$_{0.3}$ (c), PCL (d) and PEG (e).

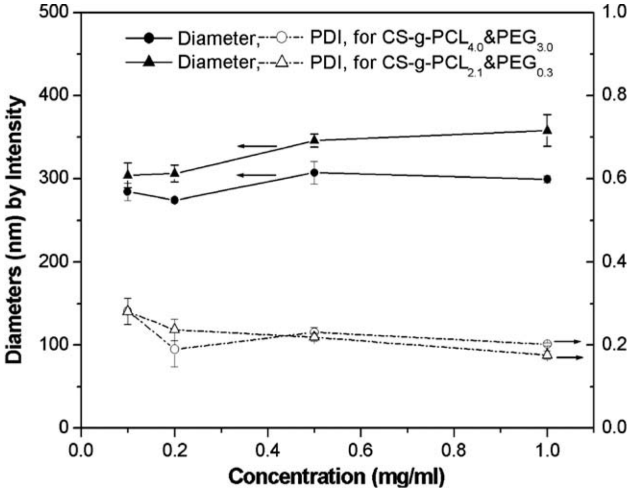

**Fig. 5.** Sizes of self-aggregates in water at various concentrations.

showed two broad diffraction peaks at $2\theta = 12°$ and 21°. In XRD curve of CS-g-PCL&PEG copolymers, there existed three sharp signals at $2\theta = 19.0°$, 21.4°and 23.7° besides the weak and broad peak in the $2\theta = 15–25°$ region. The disappearance of signal at $2\theta = 12°$ belonging to polysaccharide sequences of chitosan indicated that the introduction of PEG or PCL branches suppressed the crystallization of chitosan. Meanwhile, the new sharp signals coincided with the diffraction peaks of PCL crystals and PEG crystals, respectively, which demonstrated crystalline phases of PCL and PEG in the graft copolymers. Thus the CS-g-PCL&PEG copolymer was formed as micophase-separated crystalline materials.

### 3.2. Self-aggregation properties

Graft copolymers CS-g-PCL&PEG comprising natural polysaccharide as the backbone and a pair of amphiphilic branches, PCL and PEG, as the grafts, are speculated to aggregate spontaneously in water. It was observed that when graft copolymers were dispersed in the acid water (good solvent for CS and MPEG block, but poor solvent for PCL block), a colloidal solution was obtained without any specific processing technique. This course was monitored by DLS. As shown in Table 1, the distribution of aggregates diameters were unimodal, and the hydrodynamic diameters of aggregates were in the range of 200–400 nm. It is speculated that

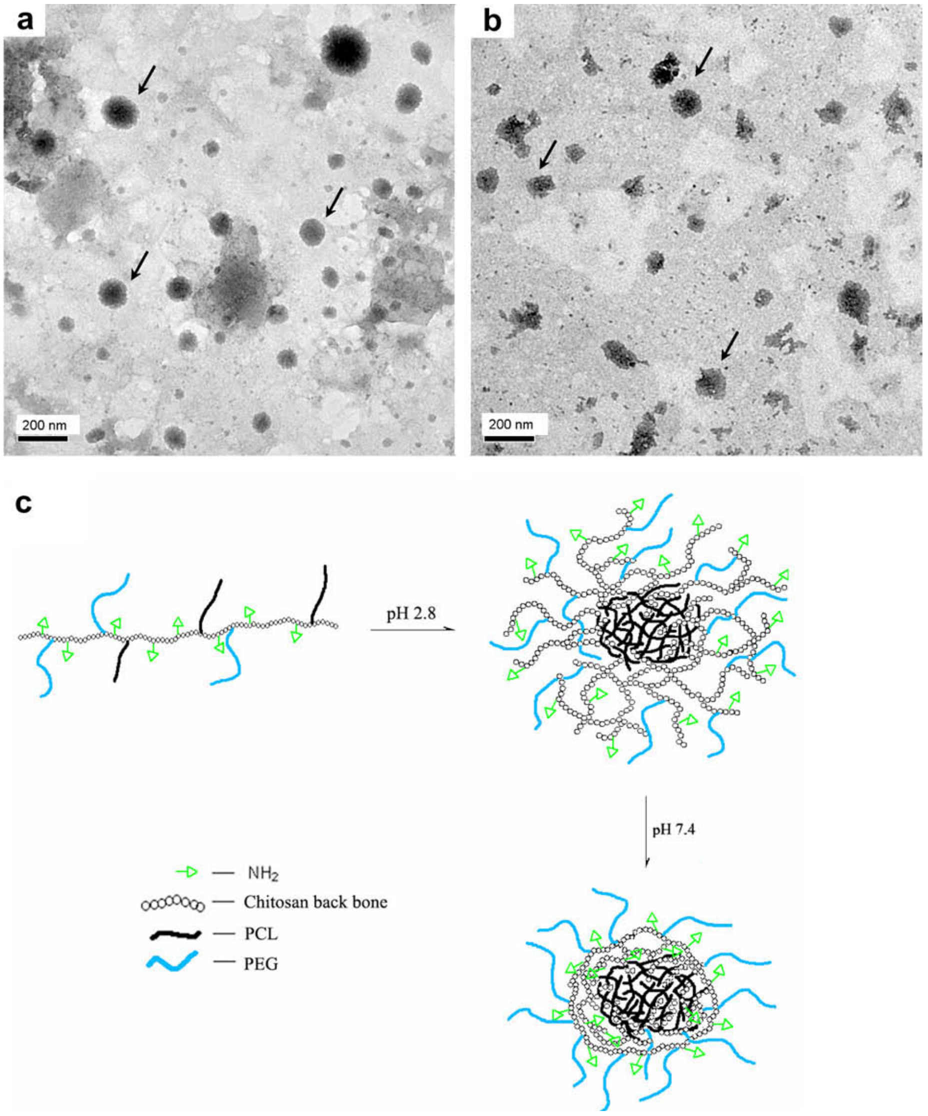

**Fig. 6.** Typical TEM images of the self-aggregates from CS-g-PCL&PEG copolymers: (a) from CS-g-$PCL_{4.0}$&$PEG_{3.0}$; (b) from CS-g-$PCL_{2.1}$&$PEG_{0.3}$. (c) The proposed scheme of self-aggregation of CS-g-PCL&PEG copolymers.

the self-aggregation of CS-g-PCL&PEG is induced by the hydrophobicity of PCL branches and by the interaction of chitosan backbone and hydrophilic PEG branches with water molecules. Specifically, the size of aggregates decreased as the grafting content increased, indicating formation of more dense hydrophobic cores. This phenomenon resembles the self-aggregation of hydrophobically modified glycol chitosan-deoxycholic acid conjugates, reported by Kim et al. (2005). On the other hand, the resulted aggregates are almost at the same sizes in graft copolymer solutions at various concentrations, as shown in Fig. 5. It implies that the aggregates are indeed self-assembly of CS-g-PCL&PEG graft copolymer.

The morphology of these aggregates was examined by TEM using negative staining. As shown in Fig. 6a, CS-g-PCL$_{4.0}$&PEG$_{3.0}$ gave the normally spherical aggregates with sizes around 150–300 nm in an acidic medium, a little smaller than the particle size measured by DLS analysis, which indicates that the particles collapse during drying. Note that the aggregates of CS-g-PCL$_{2.1}$&PEG$_{0.3}$ showed mainly irregular shapes and a few of spheres (Fig. 6b). This may be related to the lack of PEG branches compared with CS-g-PCL$_{4.0}$&PEG$_{3.0}$, where the interactions of both hydrophilic PEG and chitosan backbone with water molecules may offer a hydrated corona around the core, resulting in the formation of good spherical aggregates. This case could demonstrate the aggregates with core-corona structure where PEG and chitosan referred to the corona.

Then, the self-aggregates obtained in acid solution were dialyzed against ultra-pure water, to perform an increase in the pH value to 7. DLS analysis showed that the aggregates sizes shrank slightly after dialysis, which may be attributed to the conformational change of chitosan backbone from an extended state to a hypercoil depending on the pH increasing. The proposed process of self-aggregation of graft copolymer and their pH-induced transitions could be illustrated by the scheme shown in Fig. 6c. It is inferred that nano aggregates are formed with core-corona structure in acidic medium, in which the aggregation of hydrophobic PCL branches built up core, while the PEG branches and protonated chitosan backbones extend as corona. Nevertheless, the self-aggregates in neutral condition are expected to have a three-layer structure: the hydrophobic PCL chains form the core, and the insoluble complexes chitosan/PCL chains form the inner shell and hydrated PEG chains form the outer corona.

### 3.3. Stability of self-aggregates

The stability of self-aggregates is very important in their clinical application as drug delivery carriers. And it is more desirable to get stable self-aggregates from chitosan derivates, since the insoluble nature of chitosan in aqueous solution often led to aggregation and precipitation of self-aggregates within a few days. The stability of CS-g-PCL&PEG self-aggregates was investigated by measuring the mean diameter and the corresponding polydispersing index (PDI) of self-aggregates, both in acidic solution (pH = 2.8) and in neutral condition (pH = 7.0) (Fig. 7). In acidic condition, the diameter and PDI showed no significant changes for both CS-g-PCL$_{4.0}$&PEG$_{3.0}$ and CS-g-PCL$_{2.1}$&PEG$_{0.3}$, indicating their stability maintained over 30 days. Nevertheless, in neutral condition, the diameter and PDI of CS-g-PCL$_{4.0}$&PEG$_{3.0}$ self-aggregates increased with respect to the incubation time during the first 10 days, then showed no fluctuation in the following days. The initial data suggest some aggregation happened with the nanopaticles, resulting in a broad size distribution. Fortunately, their diameters could maintain with bigger sizes later, which may be attributed to the stabilizing of hydrated PEG corona. Moreover, the enhanced hydrophobicity of inner core may also counteract the further aggregation of self-aggregates. But for CS-g-PCL$_{2.1}$&PEG$_{0.3}$ with a rather low content of PEG, its self-aggregates would not remain stable in the neutral condition, which resulted in precipitation in three days. We can conclude that PEG plays an important role in the stability of self-aggregate.

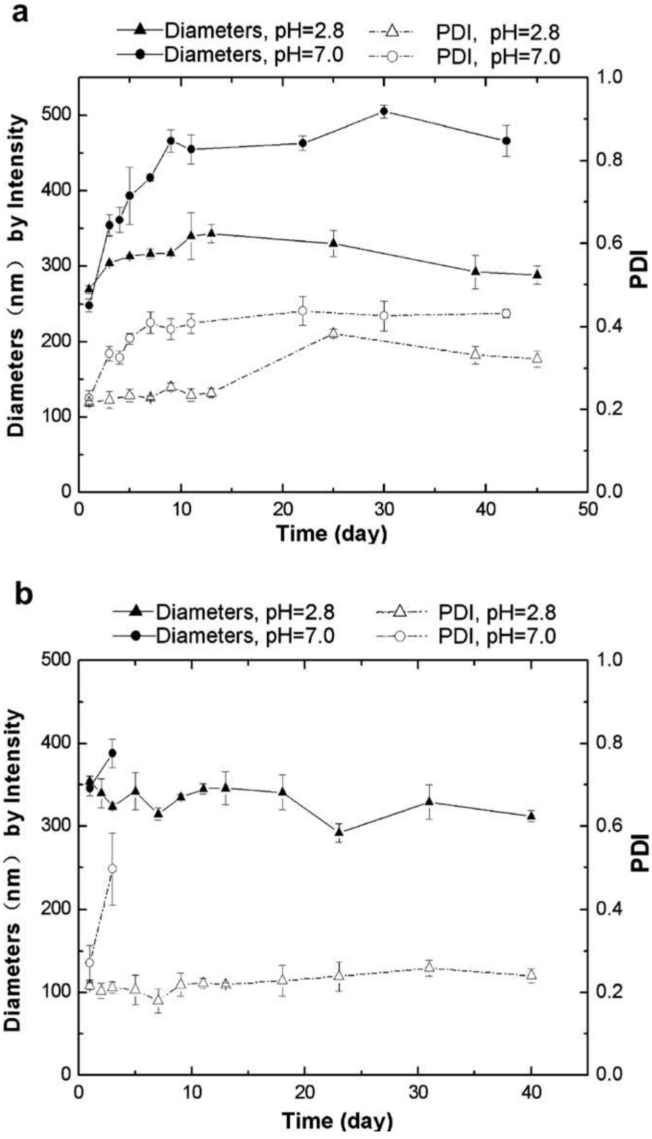

**Fig. 7.** Time course of changes in the diameter and PDI for self-aggregates from CS-g-PCL&PEG copolymers (1.0 mg/mL): (a) from CS-g-PCL$_{4.0}$&PEG$_{3.0}$; (b) from CS-g-PCL2.1&PEG$_{0.3}$.

## 4. Conclusion

Graft copolymers of CS-g-PCL&PEG, with chitosan as backbone and a pair of hydrophobic polycaprolactone and hydrophilic poly(ethylene glycol) as two grafts, were successful synthesized via a protection-graft-deprotection procedure. After deprotection, the amino group was regenerated in the final copolymer. The amphiphilic hybrid copolymers generated self-aggregates of about 300 nm in aqueous solution without a specific processing technique. The size, morphology and stability of self-aggregate could be controlled by adjusting the contents of hydrophobic PCL or/and hydrophilic PEG. And PEG played a crucial role in the stability of self-aggregates. It is worthy noting that the materials used here including chitosan, PCL and PEG are environmental-friendly polymers, thus, the resultant aggregates have great potential in applications as drug delivery carriers.

## Acknowledgements

Thanks for National 973 Basic Research Program of China (2004CB518802) and Research Start-up Grant for Young Scholars of Shanghai Jiao Tong University (05DBX027). Also thank the support from Innovative PRP Program (PRP-C07061) and Science Foundation for Youth Scholars (2006) in Shanghai Jiao Tong University. We thank Instrumental Analysis Center of Shanghai Jiao Tong University for TEM measurement.

## References

Besheer, A., Hause, G., Kressler, J., & Mader, K. (2007). Hydrophobically modified hydroxyethyl starch: Synthesis, characterization, and aqueous self-assembly into nano-sized polymeric micelles and vesicles. *Biomacromolecules, 8*, 359–367.

Dou, H. J., Jiang, M., Peng, H. S., Chen, D. Y., & Hong, Y. (2003). PH-dependent self-assembly: Micellization and micelle-hollow-sphere transition of cellulose-based copolymers. *Angewandte Chemie-International Edition, 42*, 1516–1519.

Forster, S., & Plantenberg, T. (2002). From self-organizing polymers to nanohybrid and biomaterials. *Angewandte Chemie-International Edition, 41*, 689–714.

Jenkins, D. W., & Hudson, S. M. (2001). Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers. *Chemical Reviews, 101*, 3245–3273.

Kim, K., Kwon, S., Park, J. H., Chung, H., Jeong, S. Y., & Kwon, I. C. (2005). Physicochemical characterizations of self-assembled nanoparticles of glycol chitosan-deoxycholic acid conjugates. *Biomacromolecules, 6*, 1154–1158.

Kurita, K., Ikeda, H., Yoshida, Y., Shimojoh, M., & Harata, M. (2002). Chemoselective protection of the amino groups of chitosan by controlled phthaloylation: Facile preparation of a precursor useful for chemical modifications. *Biomacromolecules, 3*, 1–4.

Leane, M. M., Nankervis, R., Smith, A., & Illum, L. (2004). Use of the ninhydrin assay to measure the release of chitosan from oral solid dosage forms. *International Journal of Pharmaceutics, 271*, 241–249.

Lee, K. Y., Kwon, I. C., Jo, W. H., & Jeong, S. Y. (2005). Complex formation between plasmid DNA and self-aggregates of deoxycholic acid-modified chitosan. *Polymer, 46*, 8107–8112.

Liu, L., Chen, L. X., & Fang, Y. E. (2006a). Self-catalysis of phthaloylchitosan for graft copolymerization of epsilon-caprolactone with chitosan. *Macromolecular Rapid Communications, 27*, 1988–1994.

Liu, L., Li, F. Z., Fang, Y. E., & Guo, S. R. (2006b). Regioselective grafting of poly(ethylene glycol) onto chitosan and the properties of the resulting copolymers. *Macromolecular Bioscience, 6*, 855–861.

Liu, L., Li, Y., Liu, H., & Fang, Y. (2004). Synthesis and characterization of chitosan-graft-polycaprolactone copolymers. *European Polymer Journal, 40*, 2739–2744.

Lu, C. F., Guo, S. R., Liu, L., Zhang, Y. Q., Li, Z. H., & Gu, J. R. (2006). Aggregation behavior of MPEG-PCL diblock copolymers in aqueous solutions and morphologies of the aggregates. *Journal of Polymer Science Part B-Polymer Physics, 44*, 3406–3417.

Nishimura, S., Kohgo, O., & Kurita, K. (1991). Chemospecific manipulation of a rigid polysaccharide: Syntheses of novel chitosan derivatives with excellent solubility in common organic solvents by regioselective chemical modifications. *Macromolecules, 24*, 4745–4748.

Ouchi, T., Nishizawa, H., & Ohya, Y. (1998). Aggregation phenomenon of PEG-grafted chitosan in aqueous solution. *Polymer, 39*, 5171–5175.

Park, J. H., Kwon, S., Lee, M., Chung, H., Kim, J. H., Kim, Y. S., et al. (2006). Self-assembled nanoparticles based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin: In vivo biodistribution and anti-tumor activity. *Biomaterials, 27*, 119–126.

Prochazkova, S., Varum, K. M., & Ostgaard, K. (1999). Quantitative determination of chitosans by ninhydrin. *Carbohydrate Polymers, 38*, 115–122.

Wan, S., Jiang, M., & Zhang, G. Z. (2007). Dual temperature- and pH-dependent self-assembly of cellulose-based copolymer with a pair of complementary grafts. *Macromolecules, 40*, 5552–5558.

Yoksan, R., Matsusaki, M., Akashi, M., & Chirachanchai, S. (2004). Controlled hydrophobic/hydrophilic chitosan: Colloidal phenomena and nanosphere formation. *Colloid and Polymer Science, 282*, 337–342.

Yoo, H. S., Lee, J. E., Chung, H., Kwon, I. C., & Jeong, S. Y. (2005). Self-assembled nanoparticles containing hydrophobically modified glycol chitosan for gene delivery. *Journal of Controlled Release, 103*, 235–243.

Yu, H. J., Wang, W. S., Chen, X. S., Deng, C., & Jing, X. B. (2006). Synthesis and characterization of the biodegradable polycaprolactone-graft-chitosan amphiphilic copolymers. *Biopolymers, 83*, 233–242.