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A novel method of controlled grafting modification of chitosan via RAFT polymerization using chitosan-RAFT agent

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Abstract

The controlled grafting modification of chitosan has first been achieved by RAFT polymerization using chitosan-RAFT agent. The chitosan was first modified with *S*-1-dodecyl-*S*′-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DDACT) to serve as RAFT agent, and then the controlled grafting polymerization of acrylic acid were performed. The resultant copolymers were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared spectrometer (FT-IR), X-ray powder diffractometer (XRD), high performance particle sizer (HPPS), and transmission electron microscopy (TEM). The results indicate that the graft copolymers were successfully synthesized and that the grafting polymerization was a first-order reaction with respect to monomer concentration. The size distribution of chitosan-*g*-PAA in ethanol is very narrow, which may be associated with the grafting density and the "well-defined" PAAs onto chitosan from RAFT polymerization. TEM shows chitosan-*g*-PAA in dilute ethanol dispersions is roughly 80 nm in size with some aggregation. This work provides a new method to prepare chitosan grafting copolymers with controlled molecular weights and "well-defined" structures.

Keywords: Chitosan; Controlled modification; RAFT polymerization

1. Introduction

Chitosan is a well-known abundant natural polymer with relatively good biodegradability, biocompatibility and bioactivity. But the low solubility in water, the insolubility in common organic solvents and non-thermal plasticity of chitosan have limited its utilization and basic research. In order to overcome these problems, many efforts have been made to prepare functional derivatives by chemical modifications (Kumar, 2000; Kurita, 2001; Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002; Sashiwa et al., 2002). Among them, graft modification with synthetic polymers has been an important method to develop novel hybrid materials. Recently, there is a growing interest in grafting modification of chitosan for biomedical, industrial and agriculture applications(Jenkins & Hudson, 2001; Lim & Hudson, 2003; Lu, Liu, & Guo, 2007; Luckachan & Pillai, 2006; Park et al., 2001; Radhakumary, Nair, Mathew, & Nair, 2007). Polk et al. (Ding, Lian, Samuels, & Polk, 2003) performed the grafting of a non-linear optical mesogen onto chitosan with 4-(6-methacryloxyhexyloxy) -4'-nitrobiphenyl by radical polymerization under thermal condition. Hudson et al. (Jenkins & Hudson, 2002) reported the heterogeneous graft copolymerization of chitosan powder with methyl acrylate using trichloroacetyl-manganese carbonyl Co-initiation under heterogeneous conditions. Huang et al. (Huang & Fang, 2006a, 2006b; Huang, Liu, Zhang, Yuan, & Fang, 2006) prepared the graft copolymers, such as chitosan-*g*-polyethylene glycol, chitosan-*g*-poly(vinyl alcohol) and chitosan-*g*-poly(butylene glycol adipate) by the esterification reaction between 6-*O*-succinate-*N*-phthaloyl-chitosan and the synthetic polymers.

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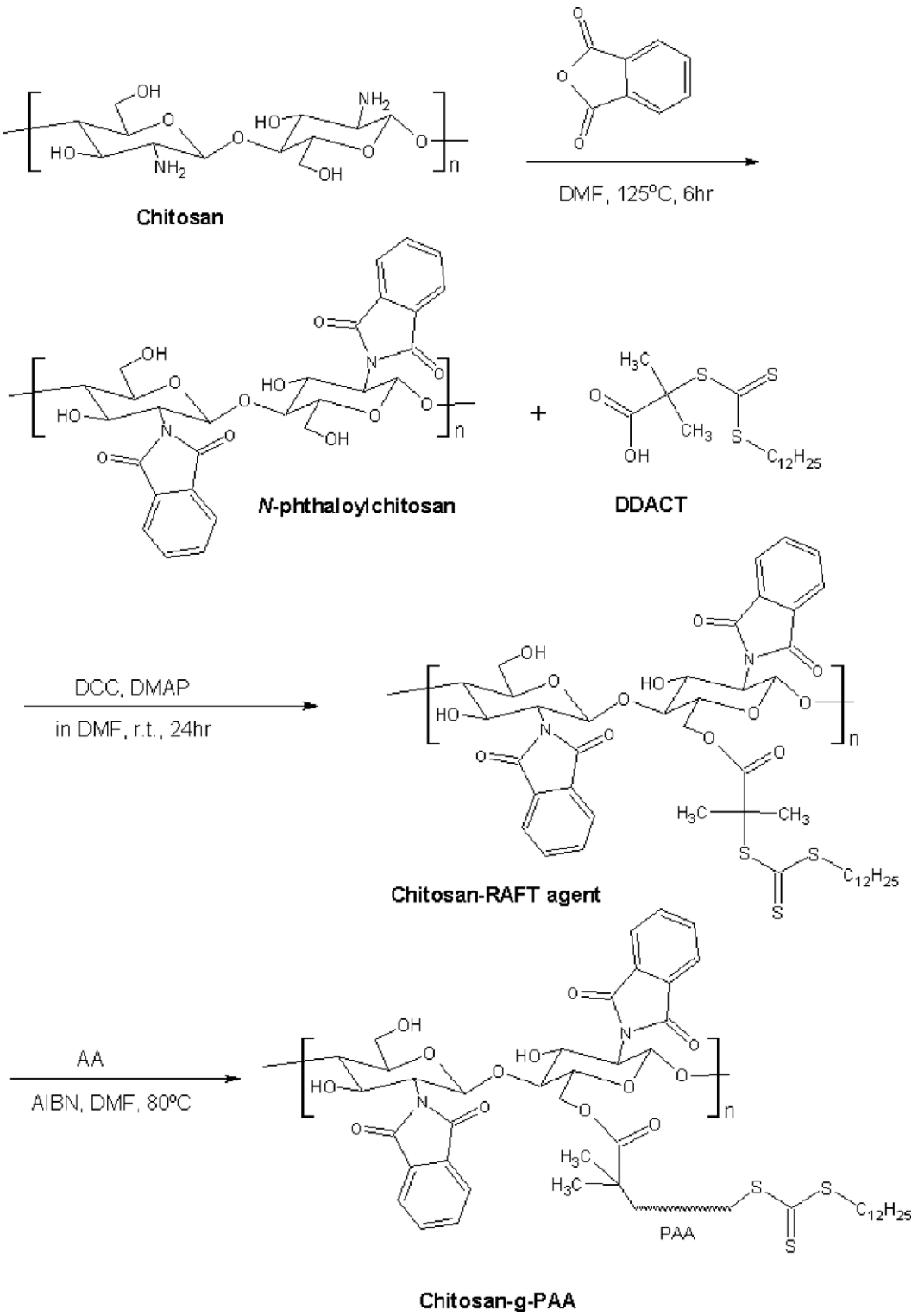

Scheme 1. Schematic representation for the modifications and graft polymerization of chitosan.

On the other hand, controlled/living free radical polymerizations have attracted more and more attention in the past few decades due to their ability to synthesize polymers with well-controlled molecular weights and well-defined structures under relatively simple and convenient experimental conditions (Sebenik, 1998). Some prominent techniques have developed, such as living free radical nitroxide-mediated polymerization (NMP) (Georges, Veregin, Kazmaier, & Hamer, 1993; Hawker, Bosman, & Harth, 2001; Keoshkerian, Georges, & Boilsboissier, 1995), atom transfer radical polymerization (ATRP) (Kato, Kamigaito, Sawamoto, & Higashimura,

1995; Matyjaszewski & Xia, 2001; Wang & Matyjaszewski, 1995) and reversible addition-fragmentation chain transfer (RAFT)(Chiefari et al., 1998; Le, Moad, Rizzardo, & Thang, 1998) process. These techniques could potentially provide new ways to utilize this abundant natural polymer. It would enable a wide variety of molecular designs to afford novel types of tailored hybrid materials composed of natural polysaccharides and synthetic polymers. However, until now, these techniques are seldom used in the modification of chitosan. Using ATRP technique, Hudson et al.(El Tahlawy & Hudson, 2003) once reported the synthesis of a well-defined chitosan graft poly(methoxy polyethyleneglycol methacrylate), and Malmstrom et al. (Lindqvist & Malmstrom, 2006) performed surface modification of various solid polysaccharide substrates by grafting methylacrylate (MA) and styrene. However, for the ATRP process, the polymers obtained are contaminated with metal ions that are difficult to completely get rid of (Destarac, Bessiere, & Boutevin, 1998), which may be a serious problem to the use of chitosan derivatives in biomedical applications. In contrast, RAFT polymerization is developed as a versatile "living" radical technique, which involves a fast, reversible chain-transfer process of a thiocarbonylthio compound reacting with propagating chain radicals. The process is no metal contamination, and is applicable to a wide range of monomers (most monomers polymerized by free radical methods) and reaction conditions (Chiefari et al., 1998).

In this paper, we first report the controlled graft modification of chitosan via RAFT polymerization. The chitosan was modified with *S*-1-dodecyl-*S*'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DDACT) to serve as RAFT agent, and then the graft polymerization of acrylic acid was performed at 80 °C. The reactions are listed in Scheme 1. Acrylic acid (AA) was selected as the monomer for the graft polymerization, because the corresponding copolymer will have the pH-sensitivity and stimulating response, which may be used as degradable matrix for drug delivery applications. The resultant copolymers were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared spectrometer (FT-IR), X-ray powder diffractometer (XRD), high performance particle sizer (HPPS), and transmission electron microscopy (TEM).

2. Experimental procedures

2.1. Materials and reagents

Chitosan (degree of deacetylation = 95.2%, determined by element analysis, average molecular weight = 200 K) was purchased from Qingdao Haoda Biochemical Co., Shandong, China. *N,N*-Dimethyl formamide (DMF) was distilled under reduced pressure from calcium hydride and stored over molecular sieves (4A). AA was obtained from China National Pharmaceutical Group Corporation and was distilled under reduced pressure before polymerization. *S*-1-dodecyl-*S*'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DDACT) was synthesized according to the related reference (Lai, Filla, & Shea, 2002). *N*-Phthaloylchitosan was prepared according to the reported procedure (Kurita et al., 2002), the degree of substitution of phthaloyl groups was determined by elemental analysis. 1,3-Dicyclohexylcarbodiimide (DCC, 99%) and 4-(*N,N*-dimethylamino) pyridine (DMAP, 99%) were purchased from Alfa Asear China (Tianjin) Co., Ltd. and were used as received. All other chemical reagents were of analytical grade and used without further purification.

2.2. Synthesis of Chitosan-RAFT Agent

N-Phthaloylchitosan (0.292 g, 1.0 mmol repeating unit) was dissolved in dry DMF (30 mL), and reacted with DDACT (0.370 g, 1.0 mmol) in the presence of DCC (0.205 g, 1.0 mmol) and DMAP (0.015 g, 0.12 mmol) for 48 h at room temperature. The resulting mixture was poured into ice water. The precipitate was collected on a filter, and washed completely by Soxhlet's extraction with acetone and dried to give a yellow powder (0.502 g, 78% yield). ^{1}H NMR (d_6-DMSO), δ: 7.2–8.0 (–C_6H_5), 2.8–5.2 (–CH– and –CH_2– of chitosan), 1.8–1.9 (–CH_3), 1.0–1.1 (–$C_{12}H_{25}$).

2.3. Preparation of graft copolymers

A mixture of chitosan-RAFT agent (0.0468 g, 0.05 mmol trithio groups) and dry DMF (5 mL) was stirred magnetically under argon atmosphere. After dissolving completely, AIBN (0.0032 g, 0.02 mmol) and AA (1.0 g) were added. The polymerization proceeded at 80 °C for a predetermined time. After the polymerization, the reaction mixture was precipitated in 10-fold benzene, and then filtered. The crude copolymer was dried in vacuum oven at 60 °C, and the monomer conversion (Conv.%) was calculated based on Eq. (1):

$$\text{Conv.}\% = (W_P - W_0)/W_M \times 100\% \quad (1)$$

where, W_P, W_0 and W_M stand for the weights of the crude copolymer, chitosan-RAFT agent and monomer, respectively.

The crude copolymer was then made free from homopolymer by exhaustive extraction with hot deionized water and ethanol for 48 h. Finally, the copolymer was dried in a vacuum oven at 60 °C to constant weight. The graft content (G%) was calculated as follows:

$$G\% = (W_g - W_0)/W_0^* 100\% \quad (2)$$

where, W_g and W_0 are weights of graft copolymer and chitosan-RAFT agent, respectively.

On the other hand, the extraction solution was condensed by evaporation, and then precipitated by added into 10-fold acetone. So the PAA homopolymers were collected by filtration, and dried in a vacuum oven at 60 °C.

2.4. Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian INVOA-400 instrument with DMSO-d_6 as the solvent. Fourier transform infrared spectrometer (FT-IR) spectra were recorded on a Varian-1000 at room temperature, and the samples were ground with KBr crystals and the mixture was then pressed into a pellet for IR measurement. The *Z*-average size and the polydispersity index (PDI) of the micelles were measured by Malvern HPP 5001 high performance particle sizer (HPPS). X-ray powder diffraction diagrams were recorded on a Panalytical X'Pert-Pro MPD X-ray diffractometer (XRD). Elemental analyses were performed using a HP5988A elemental analyzer. The molecular weights of the homopolymers in solution was measured by gel permeation chromatography (GPC) on a Waters 515 GPC equipped with Ultrahydrogel 250, 500 columns using pululan as the calibration standard, and water solution (pH 7.0) of 0.2 M Na_2HPO_4–NaH_2PO_4 as the eluent at a flow rate of 1.0 mL/min. Transmission electron microscopy (TEM) images were taken with a FEI Tecnai G20 electron microscope, using an accelerating voltage of 160 kV.

3. Results and discussion

In order to increase the solubility in organic solvents, as shown in Scheme 1, chitosan was first reacted with phthalic anhydride in dry DMF according to related reference (Kurita et al., 2002; Liu, Li, Fang, & Chen, 2005). The degree of substitution of phthaloyl groups was determined to be 0.98 by elemental analysis. On the other hand, DDACT was also prepared according to the reported procedure (Lai et al., 2002). Then *N*-phthaloylchitosan was reacted with DDACT in the presence of DCC and DMAP to serve as RAFT agent (Scheme 1). According to the integration value of characteristic peaks in ^{1}H NMR spectrum, there is a trithio-group concentration of 1.06 mmol/g for chitosan-RAFT agent, which means that there is almost one trithio group in two repeating units of chitosan.

The controlled polymerization of AA was once carried out under ^{60}Co γ-ray irradiation in the presence of dibenzyl trithiocarbonate (Hong, You, Bai, Pan, & Borjihan, 2001), and under ultraviolet radiation in the presence of *S*,*S*-bis(α,α'-dimethyl-α''-acetic acid) trithiocarbonate(Muthukrishnan et al., 2007). Last year Huang et al. prepared graft copolymer of chitosan and PAA by grafting acrylic acid onto maleoylchitosan using potassium persulfate as an initiator in aqueous medium(Huang, Jin, Jin, & Fang, 2006), but the polymerization is uncontrolled. In this study, the graft polymerization of acrylic acid were performed at 80 °C using the ratio between chitosan-RAFT agent and AIBN of 2.5 mol/mol and [AIBN] = 0.004 mol/L. The graft content is an important parameter of graft copolymerization reaction, which indicates the percent of synthetic polymer in the graft copolymer. It can be seen from Fig. 1 that the graft content could reach 60% within

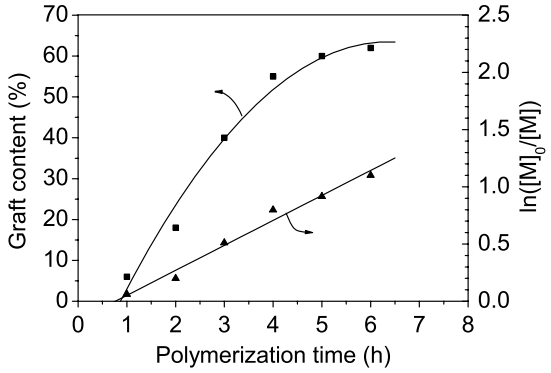

Fig. 1. Graft content of poly(acrylic acid) onto chitosan and ln([*M*]$_0$/[*M*]) as functions of polymerization time for the graft polymerization.

5 h though there was an inhibition of about 45 min in the graft copolymerization. In addition, the kinetic curve for the graft polymerization is a straight line (Fig. 1), which indicates that the graft polymerization is a first-order reaction with respect to monomer concentration.

On the other hand, in order to characterize the grafted PAA, an attempt was once made to cleave the grafted chains from chitosan, unfortunately it was failed. According to RAFT process, it is known that the growing tethered chains generated on the surface are in a dynamic equilibrium with untethered chains in the solution phase (Barner, Zwaneveld, Perera, Pham, & Davis, 2002). Therefore, free polymers formed in the solution could be used as an indicator for the molecular weight and polydispersity index of grafted polymers (Barner et al., 2002; Wang, Neoh, & Kang, 2006). Fig. 2 shows the molecular weight and polydispersity index of the PAA homopolymers from the reaction mixture. The results show the polydispersity is almost independent of the polymerization time, while the molecular weight increases with the polymerization time. All the evidences indicate that the graft polymerization was well controlled by the RAFT process.

The copolymer structures were characterized by ^{1}H NMR spectra and a typical ^{1}H NMR spectrum of chitosan-*g*-PAA is shown in Fig. 3. Except for the characteristic peaks for *N*-phthaloylchitosan, the characteristic reso-

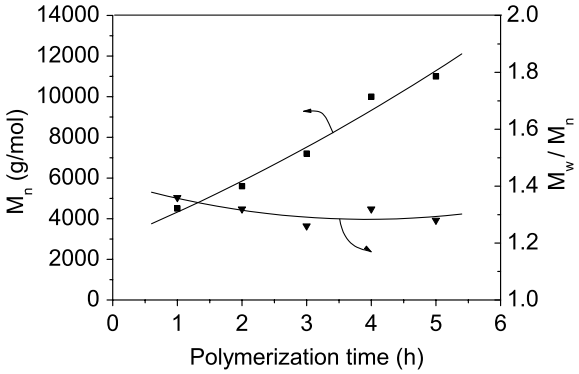

Fig. 2. Dependence of molecular weight and polydispersity index of PAA homopolymers from the mixture on the polymerization time.

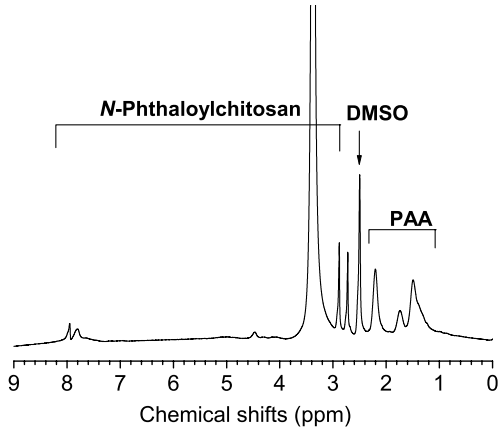

Fig. 3. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of chitosan-*g*-PAA ($t = 4$ h).

nances of PAA were detected at $\delta = 1.0$–2.4 ppm, which confirms the structure of chitosan-*g*-PAA. Fig. 4 shows the FT-IR spectra of original chitosan, *N*-phthaloylchitosan, chitosan-RAFT agent and graft copolymer. In comparison with the original chitosan (a, Fig. 4), the characteristic peaks at 1725.2 cm^{-1} (carbonyl anhydride) and 719.9 cm^{-1} (phenyl ring) appeared for *N*-phthaloylchitosan (b, Fig. 4). Chitosan-RAFT agent (c, Fig. 4) showed stronger absorbance at 2800–3000 cm^{-1} for C–H (of $C_{12}H_{25}$) than *N*-phthaloylchitosan (b, Fig. 4). For chitosan-*g*-PAA (d, Fig. 4), the characteristic peaks at 3100–3500 cm^{-1} and 900–1000 cm^{-1} should be ascribed to the absorbance of O–H of carboxyl group, and characteristic peak was enhanced at 1724 cm^{-1} belonging to carbonyl stretching band (C=O), compared to chitosan–RAFT agent (c, Fig. 4). These evidences further demonstrated the successful synthesis of the graft copolymer.

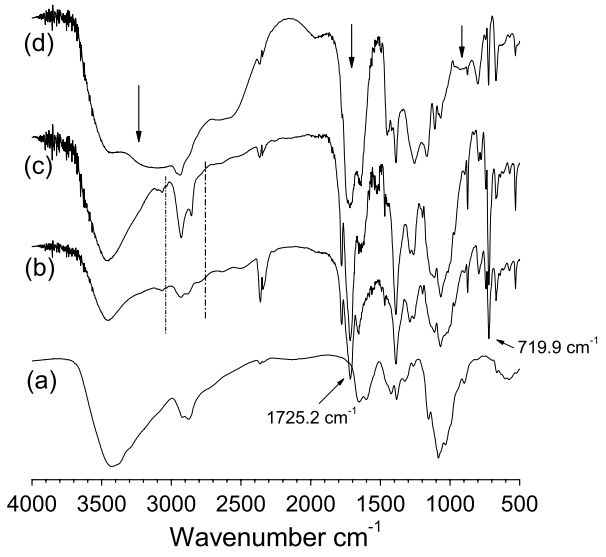

Fig. 4. IR spectra of (a) original chitosan, (b) *N*-phthaloylchitosan, (c) chitosan-RAFT agent, and (d) chitosan-*g*-PAA ($t = 4$ h).

The X-ray powder diffraction patterns of original chitosan and chitosan-*g*-PAA ($t = 4$ h) were detected as illustrated in Fig. 5. It was known that the diffraction pattern of original chitosan shows the characteristic peaks at $2\theta = 11^\circ$ and 20° (a, Fig. 5). Whereas, for chitosan-*g*-PAA, there is a broad peak in the $2\theta = 10$–35° region correspondingly (b, Fig. 5), which indicates a remarkable decrease in crystallinity in the graft copolymer. This may be ascribed to the introduction of bulky pendent chains of grafted PAA in chitosan matrix. The good conjugation of PAA with chitosan markedly disordered the pure chitosan at a molecular level, leading to the low crystallization of the copolymer.

Recently more and more effort is devoted to controlling structure and function on the nanometer scale, and the role of well-defined polymeric materials with controlled size, polydispersity and functional group is critical. The nanometer size and size distribution of the self-assembly of chitosan-*g*-PAA in ethanol medium were characterized by HPPS, and a typical result was represented in Fig. 6. A unimodal size distribution with the polydispersity index of 0.040 for chitosan-*g*-PAA is observed, suggesting that the size distribution of the synthesized chitosan-*g*-PAA in ethanol is very narrow. It is known that the graft copolymer is different from block copolymer in the structure, so the self-assembly of the graft copolymer may be different from that of block copolymer. The morphologies of graft copolymers were not only associated with the polydispersity of the copolymer, but also with the grafting density (Shen, Yu, & Huang, 2005). In this study, although the polydispersity of chitosan is unknown, the narrow polydispersity of the self-assembly may be associated with the grafting density and the ''well-defined'' PAAs with controlled molecular weight onto chitosan from RAFT polymerization.

Typical TEM of chitosan-*g*-PAA ($t = 5$ h) cast from dilute ethanol dispersion is shown in Fig. 7. Fig. 7 shows chitosan-*g*-PAA is roughly 80 nm in size with some aggregation. Huang et al. reported that ethyl cellulose-*g*-PAA copolymers were self-assembled to micelles or particles with diameters of 5 and 100 nm in water (pH 10) with a single molecule and with many chains, respectively (Kang

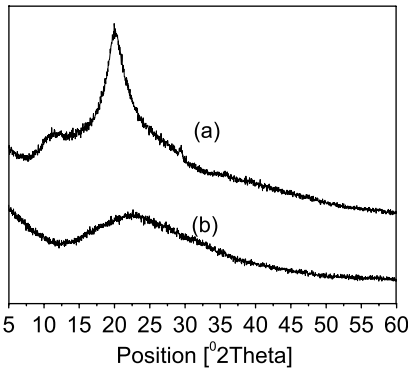

Fig. 5. X-ray diffraction patterns of (a) chitosan and (b) chitosan-*g*-PAA ($t = 4$ h).

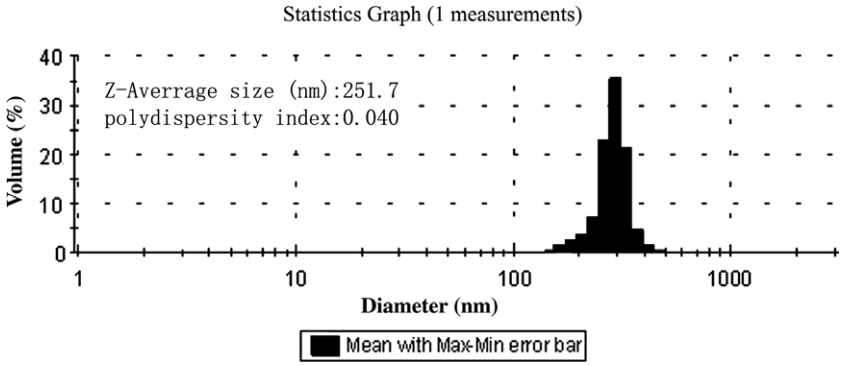

Fig. 6. Size distribution of the self-assembly of chitosan-*g*-PAA ($t = 4$ h) in ethanol.

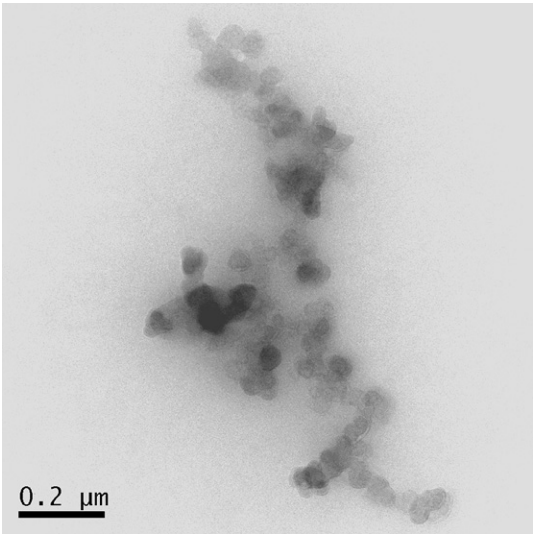

Fig. 7. TEM image of chitosan-*g*-PAA ($t = 5$ h) ethanol dispersion.

et al., 2006). In the case of chitosan-*g*-PAA ($t = 5$ h), the aggregates of the nanoparticles observed in the TEM images are likely due to the chitosan chain entanglement in the sample preparation for TEM analysis.

4. Conclusion

The controlled graft modification of chitosan has first been achieved by RAFT polymerization using chitosan-RAFT agent. The chitosan was successively modified with phthalic anhydride, and with *S*-1-dodecyl-*S*'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DDACT) to serve as RAFT agent, and then the graft polymerization of acrylic acid was performed at 80 °C. The results indicate that the graft polymerization is a RAFT process. ^{1}H NMR and FT-IR spectra demonstrate the successful synthesis of the graft copolymer, and the X-ray powder diffraction patterns show the low crystallization of the copolymer. The size distribution of the self-assembly of chitosan-*g*-PAA in ethanol is very narrow, which may be associated with the grafting density and the ''well-defined'' PAAs onto chitosan from RAFT polymerization. TEM shows chitosan-*g*-PAA in dilute ethanol dispersions is roughly 80 nm in size with some aggregation. This work provides a new method to prepare chitosan grafting copolymers with controlled molecular weights and ''well-defined'' structures such as random copolymers, block polymers, and star-shaped polymers. Further extensive work is underway in our lab.

Acknowledgments

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