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One-pot synthesis of chitosan-g-(PEO-PLLA-PEO) via “click” chemistry and “SET-NRC” reaction

Ke Zhang[a], Pengyu Zhuang[a], Zhengke Wang[a,*], Youliang Li[a], Zhiqiang Jiang[a], Qiaoling Hu[a,*], Minying Liu[b,**], Qingxiang Zhao[b]

[a] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China
[b] School of Materials Science and Engineering, Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

For the development of biocompatible and degradable biomaterials, a kind of well-defined graft copolymer consisting of chitosan back-bone and amphiphilic PEO-PLLA-PEO branch chains was synthesized by Cu(0) catalyzed one-pot strategy combining “click” chemistry and single electron transfer-nitroxide radical coupling (SET-NRC) reaction. First, the precursors of 6-azide-*N*-phthaloyl-chitosan, TEMPO-PEO-alkyne and mPEO-PLLA-Br were designed and produced. Then, the one-pot coupling reactions between these precursors were performed in the presence of nanosized Cu and PMDETA. The efficiencies of the coupling reactions were greater than 90% determined by the FTIR and ESR spectra. The structure of graft copolymer with 43% of the grafting ratio was confirmed by the spectral analysis. This work provided a route to prepare chitosan graft copolymer.

1. Introduction

Chitosan, the partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli, 2012; Muzzarelli et al., 2012) has recently received attention as the most promising biomaterials because of its nontoxicity, good biocompatibility, biodegradability, mucoadhesive, low immunogenicity, and biological activity. Of the various possible modifications (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004), graft modification is a convenient method to overcome the insolubility of chitosan in common solvents and add new properties to the polysaccharide with minimum loss of the initial properties of the chitosan (Jayakumar, Prabaharan, Reis, & Mano, 2005). Moreover, the nonlinear structure could lead to unique properties compared with linear counterpart, such as crystallinity, mechanical property, low viscosity, and gelation behavior (Wei, Yang, Zheng, & Shen, 2009).

For the past few decades, many kinds of graft copolymer (Zohuriaan-Mehr, 2005) based on chitosan have been synthesized. Moreover, due to the pH-sensitive character of chitosan, chitosan-based graft copolymer with thermo-responsive branch chains will possess dual responsive property, and can be applied in vivo and in vitro responding to the conditions of temperature and pH. Of the many temperature-sensitive polymers, poly(*N*-isopropylacrylamide) (PNIPAAm) and some types of PEO-based amphiphilic copolymers, such as Pluronic (PEO-*b*-PPO-*b*-PEO triblock copolymer), are probably the most extensively studied. Correspondingly, chitosan-g-PNIPAAm (Chuang, Don, & Chiu, 2010) and chitosan-g-Pluronic (Yang et al., 2010) have been synthesized and studied, but their use as biomaterials is still problematic due to the undegradable property.

PLA (polylactic acid) is biocompatible and degradable material, which undergoes scission in the body to monomeric units of lactic acid as a natural intermediate for carbohydrate metabolism (Kumari, Yadav, & Yadav, 2010; Nair & Laurencin, 2007). PEO-PLA-PEO hydrogel exhibits sol–gel phase-transitions in response to external stimuli, due to the formation of reversible polymer networks caused by physical interactions. In 1997, Jeong, Bae, Lee, and Kim first synthesized PEO-PLLA-PEO copolymer by coupling mPEO-PLLA using hexamethylene diisocyanate (HMDI) as coupling agent, and studied as a sustained-release matrix for dextran. PEO-PLA-PEO has excellent stability and potential to control physical, chemical, and biological properties by tailoring of the block compositions.

Due to the structure of chitosan, there are many hydroxyl and amino groups which can be modified to generate a series of chitosan derivatives (Mourya & Inamdar, 2008), that provides an alternative method for synthesis of hybrid branched materials. Recently, Cu-catalyzed azide/alkyne “click” chemistry (Kolb, Finn, & Sharpless, 2001) invented by Sharpless has been introduced into the synthesis

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* Corresponding authors. Tel.: +86 571 8795 3726; fax: +86 571 8795 3726.
** Corresponding author. Tel.: +86 371 6778 1596; fax: +86 371 6776 3698.
E-mail addresses: wangzk@zju.edu.cn (Z.K. Wang), huql@zju.edu.cn (Q.L. Hu), lmy@zzu.edu.cn (M.Y. Liu).

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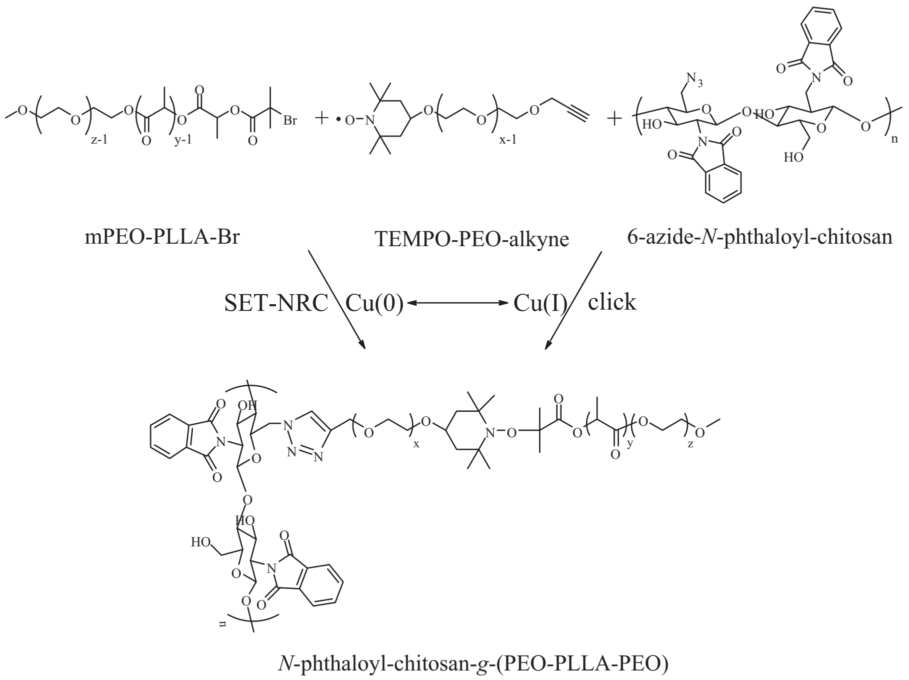

Scheme 1. Synthesis of *N*-chitosan-*g*-(PEO-PLLA-PEO) via "click" and "SET-NRC" reaction.

of well defined polymeric materials because of its high regioselectivity, quantitative yield, and mild reaction conditions (Li, Yuan, Gu, & Ren, 2010; Parrish, Breitenkamp, & Emrick, 2005). In 2008, Huang's group reported a new coupling reaction of atom transfer nitroxide radical coupling (ATNRC) reaction, in which bromine containing polymer changed to carbon radical in the presence of CuBr/ligand, then polymeric radical is immediately captured by the 2,2,6,6-tetramethyl-piperidinyl-1-oxy (TEMPO) end-functional group in another polymer chain, and then two polymer chains connect through alkoxyamine (Fu, Lin, & Huang, 2008; Fu, Wang, Lin, & Huang, 2009; Fu, Zhang, Lin, & Huang, 2009). The coupling efficiency of ATNRC was reported to be as high as 90% (Fu et al., 2008), which is similar to that of "click" chemistry. Recently, Huang et al. optimized this reaction by applying single electron-transfer (SET) mechanism (Fleischmann, Rosen, & Percec, 2010; Fu, Wang, et al., 2009; Fu, Zhang, et al., 2009; Rosen & Percec, 2008), to avoid side reactions such as cross-link and chain transfer (Jing, Wang, Zhang, & Huang, 2011). The reaction processes at ambient conditions and using Cu(0) instead of CuBr, named as single-electron-transfer nitroxide-radical-coupling reaction (SET-NRC) (Jing, Lin, Wang, & Huang, 2011). Thus graft copolymer with well-defined and complex chain architectures could be synthesized by combination "SET-NRC" and "click" reaction.

Considering the degradable and biocompatible properties of chitosan and PEO-PLLA-PEO, we report a new route for synthesis chitosan-*g*-(PEO-PLLA-PEO), a well-defined graft copolymer through the combination of "click" and "SET-NRC" reaction in this paper. The precursors of 6-azide-*N*-phthaloyl-chitosan, TEMPO-PEO-alkyne and PEO-PLLA-Br were designed and produced first, then, the target product could be prepared using one-pot method (Scheme 1). To the best of our knowledge, this is the first report of the synthesis of chitosan-*g*-(PEO-PLLA-PEO).

2. Experimental

2.1. Material

Chitosan (degree of deacetylation = 80%, determined by titration, viscosity-average molecular weight = 200 kDa, Aldrich) was dried in vacuum at 40 °C overnight before use. L-lactic (L-LA, 98%, Aldrich) were recrystallized in ethyl acetate, dried under vacuum. 3-Bromo-1-propyne (80 wt.% in toluene, Aldrich), 2-bromoisobutyryl bromide (98%, Aldrich), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%, Aldrich), *O*-phthalic anhydride (PA, Aladdin, AR), stannous octoate (CP, Sinopharm Chemical Reagent), sodium azide (NaN_3, 99%, Aldrich) and nanosized Cu powder (99.9%, 80–100 nm, Aladdin) were used as received. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxy (HTEMPO) (Sunder, Hanselmann, Frey, & Mulhaupt, 1999) and DPMK solution (Francis et al., 2003) were prepared according to literatures. All of the solvents were purchased from Sinopharm Chemical Reagent and purified before use.

2.2. Measurements

^1H NMR spectra were recorded at room temperature with a Bruker spectrometer operating at 400 MHz by using $CDCl_3$ and DMSO-d_6 as the solvents. Typically, TEMPO-PEO was measured in the present of stoichiometric $HCOONH_4$ and Pd/C (Liu, Pan, Zhang, & Huang, 2008). Chemical shifts (δ) are given in ppm using tetramethylsilane (TMS) as internal reference.

FTIR spectra were recorded on Nicolet 6700 at room temperature, the samples were prepared by the squash method with KBr.

Gel permeation chromatography (GPC) was performed on HP 1100 with Waters 2414 refractive index detector and three LP columns. THF was used as the eluent at a flow rate 1 mL/min at 35 °C. A series of narrow dispersed polystyrene were used as the standards.

Electron spin resonance (ESR) spectra of TEMPO-PEO and *N*-chitosan-*g*-(PEO-PLLA-PEO) powder were performed on a Bruker-300 spectrometer at room temperature, using 9.856 GHz microwave frequency, 2.026×10^{-1} mW microwave power, 100 kHz field modulation and 41.894 s sweep time; *g*-factor was quoted relative to the internal reference standard marker (DPPH).

Differential scanning calorimetry (DSC) thermogram registered with Diamond DSC instrument under a nitrogen flow (10 mL/min), the heating rate being 10 °C/min from −20 to 160 °C for the first scan, then cooled at −10 °C/min to −20 °C.

2.3. Synthesis of 6-azide-N-phthaloyl-chitosan

6-Azide-*N*-phthaloylated chitosan was synthesized by three steps, according to the previous literatures (Kurita, Ikeda, Shimojoh, & Yang, 2007; Wang, Li, Yu, & Zhu, 2008). Chitosan (3.0 g, 14.9 mmol of glucosamine unit) was dispersed in anhydrous DMF (135 mL), and PA (6.66 g, 44.7 mmol) was added at room temperature. After stirring at 120 °C under N_2 atmosphere for 6 h, the mixture was allowed to cool to room temperature, and the product was purified, by pouring it into ice water, filtering, extracting with methanol, and drying in vacuum at 40 °C, resulting in *N*, *O*-phthaloylated chitosan. The *O*-phthaloyl group was removed by hydrolysis in DMF containing 5% (v/v) water for 4 h, at 120 °C under N_2 atmosphere to obtain *N*-phthaloylated chitosan. The product was purified as above.

For the preparation of 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan, *N*-phthaloylated chitosan (2 g, 7.32 mmol of C6 hydroxyl) was dispersed in anhydrous pyridine (120 mL), and then *p*-toluenesulfonyl chloride (4.2 g, 22 mmol) was added. After stirring at room temperature for 48 h under N_2 atmosphere, 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan was obtained by filter and precipitation in ethyl ether, and dried at 40 °C under vacuum.

For 6-azide-*N*-phthaloyl-chitosan, 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan (2.9 g, 2.9 mmol of *p*-toluenesulfonyl) was dissolved in DMF (150 mL), and sodium azide (1.86 g, 29 mmol) was added into solution. The reaction was continued for 18 h at 80 °C. The mixture was filtered to remove the salts, and the filtrate was precipitated into ethanol. The precipitate was collected by filter, and washed again with ethanol. After drying, 6-azide-*N*-phthaloyl-chitosan was obtained.

^{1}H NMR (DMSO-d$_6$): δ (ppm) = 7.5–7.9 ($-C_6\boldsymbol{H}_4-$ of *N*-phth), 3.0–5.2 (***H***-1-***H***-6). FTIR (KBr): ν (cm^{-1}) = 3423 (O—H), 2870–2930 (C—H), 2109 ($-N_3$), 1774, 1715 ($C{=}O_{imide}$), 715 (C—H in aromatic ring).

2.4. Synthesis of bromine-terminated PEO-PLLA

Monomethyl-terminated PEO (2.50 g, $M_n = 5000$, 0.5 mmol), dried by azeotropic distillation with toluene, and *L*-LA (3.84 g, 53 mmol), stannous octoate (0.10 g, 0.25 mmol) and toluene (40 mL) were introduced into a flask. After degassing, the flask was sealed under vacuum, and the reaction was allowed to proceed at 130 °C for 48 h. The product was recovered by dissolution in dichloromethane and precipitation in ether, and dried at 40 °C in vacuum. Then the dried mPEO-PLLA (0.91 g, 0.089 mmol) was completely dissolved in anhydrous pyridine (10 mL) under stirring. The mixture was stirred and cooled to 0 °C with an ice bath. Then, 2-bromoisobutyryl bromide (27 mg, 0.27 mmol) was added to the mixture slowly. The reaction was continued for 12 h at room temperature under stirring. Bromine-terminated PEO-PLLA was purified by dissolution/precipitation thrice with methylene dichloride/ethyl ether, and drying at 40 °C in vacuum.

^{1}H NMR (CDCl$_3$): δ (ppm) = 5.2 ($-C\boldsymbol{H}-$ of PLLA), 3.4–3.8 ($-C\boldsymbol{H}_2C\boldsymbol{H}_2O-$), 3.3 ($-OC\boldsymbol{H}_3$), 1.8 ($-C(C\boldsymbol{H}_3)_2-Br$), 1.5 ($-C\boldsymbol{H}_3-$ of PLLA).

2.5. Synthesis of TEMPO-PEO-alkyne

The following procedure was adapted from Huang (Jing, Wang, et al., 2011). First, dried HTEMPO (0.519 g, 3 mmol) and solution of DPMK (2.05 mL, 0.91 mmol) were dissolved in anhydrous THF (40 mL), and then the mixture was transferred into an ampoule. In ice-bath, ethylene oxide (17 mL, 0.336 mol) was injected in the ampoule. The reaction was continued at 60 °C for 48 h. At the end of reaction, excessive methanol was used to terminate the reaction. TEMPO-PEO was purified by precipitation in ethyl ether twice, and dried at 40 °C in vacuum.

Subsequently, the hydroxyl group was substituted by nucleophilic substitution reaction of propargyl group according to following procedure: under N_2 atmosphere, TEMPO-PEO ($M_n = 5144$, 2 g, 0.40 mmol) was dissolved in THF (50 mL). Then the solution of DPMK (2.7 mL, 1.2 mmol) was added into the solution drop by drop. After stirring 1 h, propargyl bromide (0.18 mL, 2 mmol) was added drop by drop, and the reaction was continued for 24 h at room temperature. The mixture was filtered to remove the salts. TEMPO-PEO-alkyne was obtained by precipitation in ethyl ether twice, and dried at room temperature in vacuum.

^{1}H NMR (CDCl$_3$): δ (ppm) = 4.2 ($-OC\boldsymbol{H}_2C{\equiv}CH$), 3.4–3.8 ($-C\boldsymbol{H}_2C\boldsymbol{H}_2O-$), 2.4 ($-OCH_2C{\equiv}C\boldsymbol{H}$).

2.6. One-pot synthesis of N-phthaloyl-chitosan-g-(PEO-PLLA-PEO) via combination of "click" and "SET-NRC" reactions

Graft copolymer *N*-phthaloyl-chitosan-*g*-(PEO-PLA-PEO) was synthesized by one-pot strategy in following procedure: first, 6-azide-*N*-phthaloyl-chitosan (66.0 mg, 0.079 mmol of azido), TEMPO-PEO-alkyne (0.51 g, 0.10 mmol) and PEO-PLLA-Br (0.65 g, 0.10 mmol) were added into DMF (20 mL) in an ampoule. After dissolved, oxygen was removed from the system by three freeze–pump–thaw cycles. PMDETA (14.0 g, 0.080 mmol) and nanosized Cu powder (26 mg, 0.41 mmol) were quickly added, then the ampoule was re-evacuated, backfilled with N_2, and sealed. The reaction was stirred at 60 °C for 12 h, and terminated by immersing the ampoule into liquid N_2. DMF was removed under reduced pressure distillation, and the reaction mixture was diluted with THF and passed through a neutral alumina column to remove the residual copper complex. The purification of crude product was performed by dialysis for 18 h using dialysis membrane (MWCO 7000–13,000). The solution was concentrated by rotary evaporation and dried at 40 °C in vacuum to give the final product.

^{1}H NMR (DMSO-d$_6$): δ (ppm) = 7.8 ($-C\boldsymbol{H}_4-$ of *N*-phth), 5.2 ($-C\boldsymbol{H}-$ of PLLA), 4.3 (***H***-6), 3.4–3.8 ($-C\boldsymbol{H}_2-C\boldsymbol{H}_2O-$), 3.2 ($-OC\boldsymbol{H}_3$), 1.5 ($-C\boldsymbol{H}_3-$ of PLLA). ^{1}H NMR (CDCl$_3$): δ (ppm) = 7.7 ($-C\boldsymbol{H}-$ of triazole ring), 5.1 ($-C\boldsymbol{H}-$ of PLLA), 4.3 (***H***-6), 4.2 ($-O-C\boldsymbol{H}_2-C-$), 3.4–3.8 ($-C\boldsymbol{H}_2-C\boldsymbol{H}_2O-$), 3.3 ($-OC\boldsymbol{H}_3$), 1.6 ($-C\boldsymbol{H}_3-$ of PLLA), 0.9–1.3 ($-C\boldsymbol{H}_3$, $-C\boldsymbol{H}_2-$ of TEMPO). FTIR (KBr): ν (cm^{-1}) = 3303 (triazole ring), 1515 (N—O).

3. Results and discussion

3.1. Synthesis of 6-azide-N-phthaloyl-chitosan

In order to increase the solubility of chitosan, phthaloylation may be regarded as the most effective method for protection as well as solubilization to prepare various derivatives. According to relative reference (Kurita et al., 2007), chitosan reacted with PA resulting in partial *O*-phthaloylation, and the *O*-phthaloyl group could be removed by hydrolysis in DMF containing 5% (v/v) water for 4 h at 120 °C to afford *N*-phthaloyl-chitosan. The ^{1}H NMR spectra showed peaks at 7.3–7.5 ppm due to *O*-phthaloyl and at 7.5–7.9 ppm due to *N*-phthaloyl (Fig. 1). The degree of substitution of phthaloyl group was determined to be 99% by elemental analysis.

There have been several reports on preparation of chitosan containing azido group (Ifuku, Wada, Morimoto, & Saimoto, 2011). In this paper, 6-azide-*N*-phthaloyl-chitosan was obtained through the two-step modification, the tosylation and the subsequent

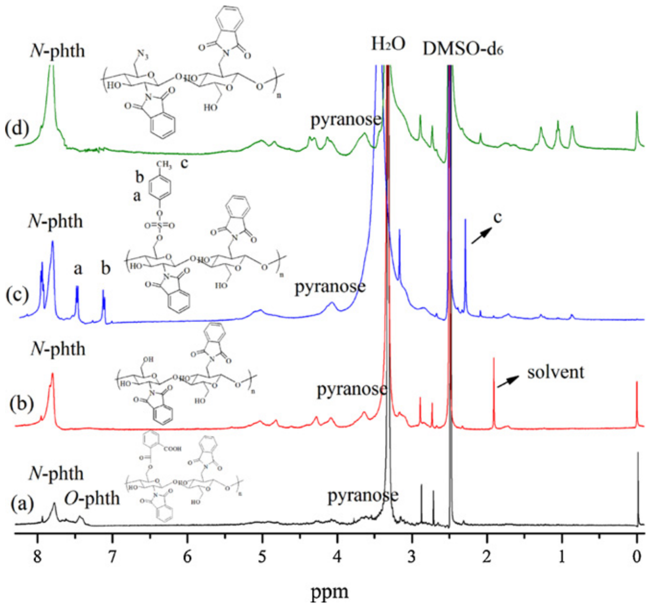

Fig. 1. ^{1}H NMR spectra of chitosan derivatives: *N*, *O*-phthaloylated chitosan (a), *N*-phthaloylated chitosan (b), 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan (c), and 6-azide-*N*-phthaloyl-chitosan (d) (solvent: DMSO-d$_6$).

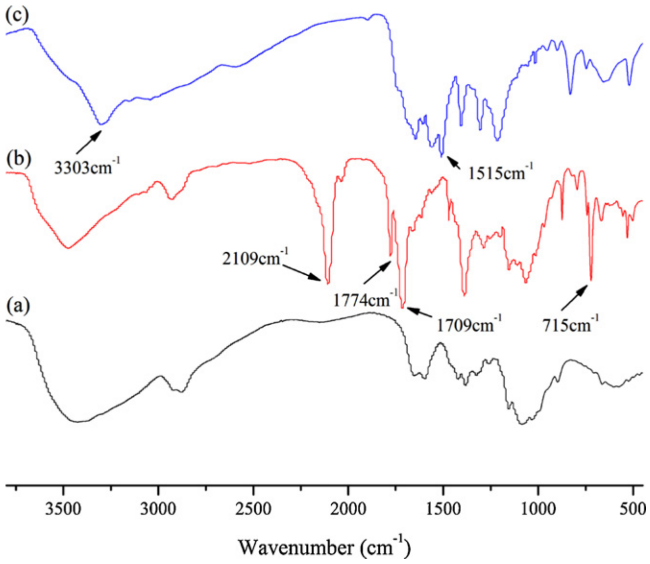

Fig. 2. FTIR spectra of chitosan (a), 6-azide-*N*-phthaloyl-chitosan (b), and chitosan-*g*-(PEO-PLLA-PEO) (c).

substitution by sodium azide of *p*-toluenesulfonyl. Although *N*-phthaloyl-chitosan was insoluble in pyridine, it became soluble after modification. The peaks at 1.06 ppm and 1.28 ppm were assigned to methylene protons ($-C\boldsymbol{H}_2N_3$) (Fig. 1d). Due to the overlapped peaks between the pyranose protons ($-\boldsymbol{H}$-1$-\boldsymbol{H}$-5$-$) and unsubstituted methylene protons ($-C\boldsymbol{H}_2OH$) in 3.2–5.2 ppm region, the precise structure and the reaction efficiency of 6-azide-*N*-phthaloyl-chitosan cannot be determined by ^{1}H NMR. Nevertheless, the information could also be confirmed through the ^{1}H NMR of 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan and FTIR spectral analysis. The peaks at 7.5 ppm, 7.1 ppm, and 2.3 ppm corresponding to *p*-toluenesulfonyl were detected, and disappeared after the subsequent substitution by sodium azide (Fig. 1d). In the FTIR spectrum of 6-azide-*N*-phthaloyl-chitosan, the azido group was evident from the presence of the significant absorption at around 2109 cm^{-1}. And the absorption peaks at 1774 cm^{-1}, 1709 cm^{-1}, and 715 cm^{-1} were assigned to the phthalimido groups and aromatic rings, respectively (Fig. 2b). Due to the steric hindrance, it was inferred that azido group was located at C6 position. Calculation of the degree of substitution of azido group on chitosan was based on the ratio between the peak integration of the protons from toluenesulfonyl and *N*-phthaloyl through ^{1}H NMR spectrum of 6-*p*-toluenesulfonyl-*N*-phthaloyl-chitosan. Thus, the concrete calculation was written as:

$$\mathrm{DS}\ \ (\%) = 0.8 \times \frac{4I_{\delta=7.1}}{2I_{\delta=7.8}} \times 100\% \qquad (1)$$

where 0.8 was the degree of deacetylation of chitosan; $I_{\delta=7.1}$ was the integral area of the protons of *p*-toluenesulfonyl at 7.1 ppm; $I_{\delta=7.8}$ was the integral area of the protons of *N*-phthaloyl. The calculational result was 43%.

3.2. Synthesis and characterization of precursors

These two precursors were synthesized by living polymerization and then modification of the end groups. In brief, mPEO-PLLA was synthesized by ring-opening polymerization of L-lactic in the presence of monomethoxy mPEO and using stannous octoate as catalyst. The peaks at 5.2 and 1.5 ppm were assigned to the methine and methyl protons of PLLA blocks, respectively, while the peaks at 3.7–3.4 ppm and 3.3 ppm were characteristic of ethylene internal and methyl terminal groups in the mPEO blocks (Fig. S1 in Supplemental date). The molecular weights that were calculated from the NMR peak integral ratio and obtained by GPC were listed in Table 1. The GPC date showed a unimodal-shaped curve, indicating all mPEO chains participated in the ring-opening polymerization of L-lactic and no homo-PLLA produced in the process (Fig. S2 in Supplemental date). Bromine-terminated mPEO-PLLA (mPEO-PLLA-Br) was synthesized through the esterification of hydroxy-terminated mPEO-PLLA with excessive 2-bromoisobutyryl bromide in the present of pyridine at room temperature. A new single peak appeared at 1.8 ppm after the esterification, which was assigned to methyl protons in isobutyl group (Fig. 3a). The integral ratio between 1.8 ppm and 3.3 ppm was 1.99 indicating the hydroxyl-terminated was substituted completely. Moreover, the molecular weight of PLA block was unchanged after esterification indicating that PLA did not degraded in pyridine; in spite of the number of repeat units of PEO block increasing, this change was attributed to

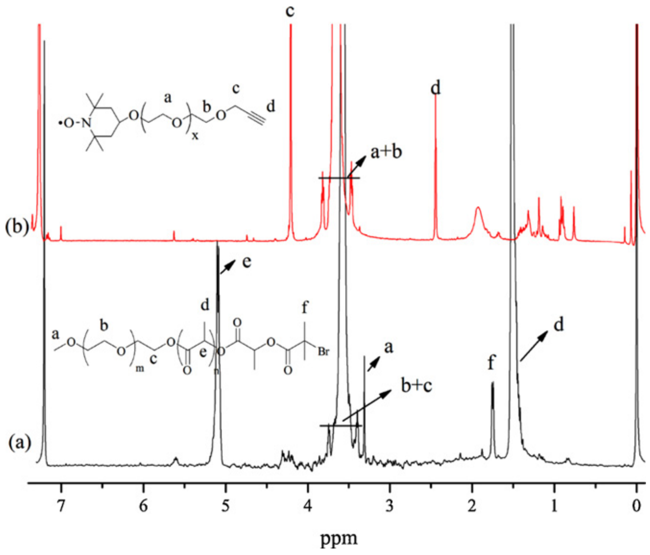

Fig. 3. ^{1}H NMR spectra of mPEO-PLLA-Br (a) and TEMPO-PEO-alkyne (b) (solvent: CDCl$_3$).

Table 1
The molecular weights of the precursors.

Sample	$M_{n,NMR}$	$M_{n,GPC}$	PDI	Composition[a]
mPEO-PLLA	9503	17,785	1.19	$mPEO_{104}$-$PLLA_{68}$
mPEO-PLLA-Br	10,482	–	–	$mPEO_{117}$-$PLLA_{72}$-Br
TEMPO-PEO	5144	7484	1.12	TEMPO-PEO_{113}
TEMPO-PEO-alkyne	5182	–	–	TEMPO-PEO_{113}-propinyl

[a] The compositions of precursors were determined by the ^{1}H NMR spectral analysis.

the integral error, because PEO block was stable in the esterification (Table 1).

HTEMPO and DPMK were used as coinitiation system for polymerization of ethylene oxide by anionic ring-opening polymerization. The molecular weight of TEMPO-PEO was 5144 by ^{1}H NMR (Fig. S3 in Supplemental date) and the molecular weight distribution was 1.12 by GPC (Fig. S2 in Supplemental date). Then alkynyl terminated TEMPO-PEO was synthesized through nucleophilic substitution reaction between hydroxyl group and propargyl bromide in the present of excessive DPMK. The ^{1}H NMR spectrum of TEMPO-PEO-alkyne shows peaks at 3.7–3.4 ppm, 2.4 and 4.2 ppm assigned to methylene protons in PEO repeating units and propargyl group respectively, which indicated the formation of TEMPO-PEO-alkyne (Fig. 3b). Due to the paramagnetism of nitroxide radicals, the protons in TEMPO cannot be detected by NMR.

3.3. *One-pot synthesis of graft copolymer*

The Cu(0) powder was oxidized to Cu^{1+} by the "SET-NRC" mechanism(Jing, Lin, et al., 2011). In "click" reaction, fresh Cu^{1+} plays an important role, which could accelerate the cycloaddition rate dramatically (Meldal & Tornøe, 2008). Therefore, it is possible to synthesize graft copolymer through the combination of "click" and "SET-NRC" reactions by one-pot process. After reaction, the residual Cu can be removed through a neutral alumina column. The feeding mPEO-PLLA-Br and TEMPO-PEO-alkyne were slightly excessive comparing with the azido group, because the excessive precursors could be easily removed from the mixture by dialysis. Fig. 4a showed the ^{1}H NMR of graft copolymer in $CDCl_3$. The new resonance signal peaks at 0.9–1.3 ppm were attributed to the TEMPO group, which meant the TEMPO radical disappeared. The signal at 5.2 and 1.5 ppm were assigned to the methine and methyl protons of PLLA blocks, respectively, while the peaks at 3.7–3.4 ppm and 3.3 ppm were characteristic of main chain methylene and methyl end group in the PEO blocks. The resonance signal at 7.7 was assigned to protons of triazole. Comparing the ^{1}H NMR spectrum of graft copolymer in $CDCl_3$, the resonance signal at 7.8 ppm was assigned to aromatic rings protons of *N*-phthaloyl-chitosan in DMSO-d_6 (Fig. 4b). The intensity of resonance signal of chitosan protons was too small, which may be resulted of the low content of chitosan chains and the poor solubility of chitosan chains with 200 kDa molecular weight. The quality was much better in that of high content of chitosan chains (Fig. S4 in Supplemental date). The molecular weight and EO/LA of branch chain were calculated by the following equation:

$$M_{n,\mathrm{branch}} = 44 \times \frac{3I_{\delta=3.7-3.4}}{4I_{\delta=3.3}} + 72 \times \frac{3I_{\delta=5.2}}{I_{\delta=3.3}} + 172 + 67 + 31 \qquad (2)$$

$$\mathrm{EO/LA} = \frac{I_{\delta=3.7-3.4}}{4I_{\delta=5.2}} \qquad (3)$$

where $I_{\delta=3.3}$, $I_{\delta=3.7-3.4}$, and $I_{\delta=5.2}$ were integration of peak at 3.3, 3.7–3.4, and 5.2 ppm, respectively. 172, 67, and 31 were the molecular weights of TEMPO, triazole ring and terminated-methoxyl group. The molecular weight of branch chain was 15,583 and the EO/LA ratio of branch chain was 3.6 calculated from the ^{1}H NMR spectra of graft copolymer, which was consistent with those of the precursors, suggesting the high efficiencies of coupling reactions.

FTIR spectrum (Fig. 2c) also confirmed the formation of chitosan graft copolymer. The new absorption bands at 3303 cm^{-1} (triazole stretching) and 1515 cm^{-1} (*N*—*O* stretching) confirmed the "click" and "SET-NRC" reaction. High efficiency of the "click" reaction was assessed by the disappearance of the azido absorption band at 2109 cm^{-1}. The ESR spectra of TEMPO and *N*-chitosan-*g*-(PEO-PLLA-PEO) were shown in Fig. 5. TEMPO radical had clean single signal, unlike triplet of other nitroxyls, due to the high concentration of radical. However, the intensity was very low in compare with that of TEMPO-PEO, by three orders of magnitude. The concentration of residual radical was about 5.1 ppm/g, which was calibrated by the signal intensity (Wang & Zhu, 2002). The concentration of

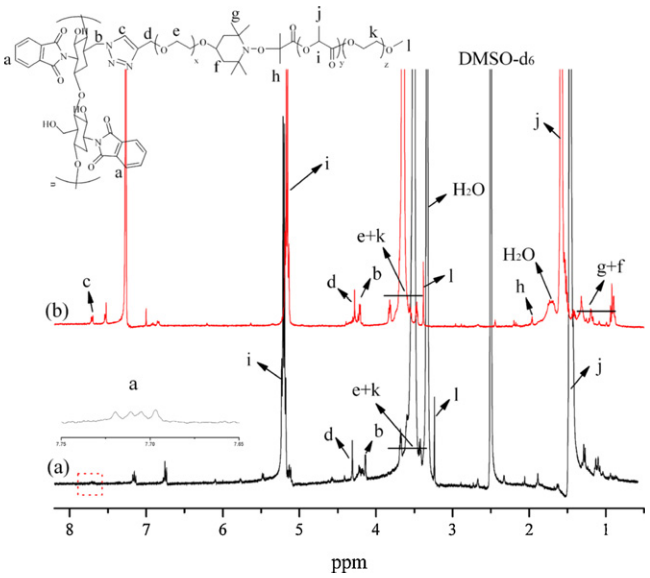

Fig. 4. ^{1}H NMR spectra of *N*-chitosan-*g*-(PEO-PLLA-PEO) in $CDCl_3$ (a) and DMSO-d_6 (b).

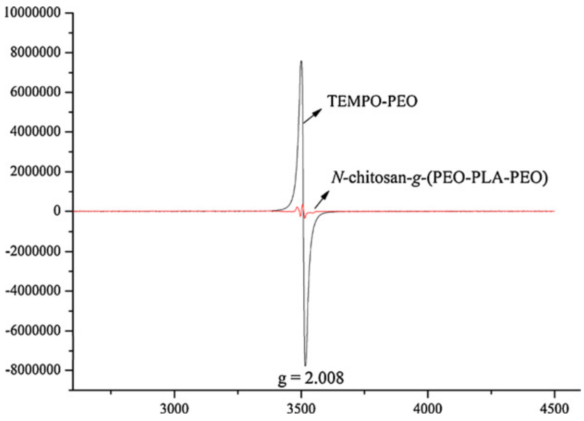

Fig. 5. ESR spectra of TEMPO-PEO and *N*-chitosan-*g*-(PEO-PLLA-PEO).

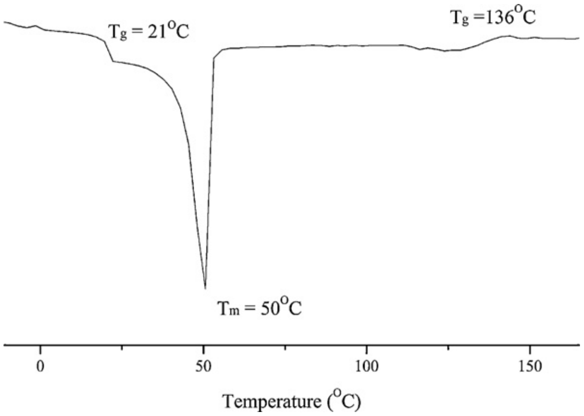

Fig. 6. DSC thermogram of *N*-chitosan-*g*-(PEO-PLLA-PEO).

residual radical can be used to calculation the efficiency of the "SET-NRC" reaction. The efficiency (%) was calculated using the equation:

$$\mathrm{EF}\ \ (\%) = \left(1 - \frac{5.1\times10^{-6}}{8.8\times10^{-5}}\right)\times 100\% \qquad (4)$$

where 8.8×10^{-5} mol/g was the theoretical concentration of radical in the reactant mixture before the coupling reaction. It was also inferred that the efficiency of "SET-NRC" reaction was about 94%. These results indicated the one-pot formation of *N*-chitosan-*g*-(PEO-PLLA-PEO) via "click" reaction and "SET-NRC" was successful.

Fig. 6 showed the DSC thermogram of *N*-chitosan-*g*-(PEO-PLLA-PEO). It exhibited a melting temperature $T_m = 50\,^\circ\mathrm{C}$, which was assigned to the PEO blocks. There were two glass transition temperatures (T_g) as shown in Fig. 6. The first transition $T_g = 21\,^\circ\mathrm{C}$ for PLA block and the second $T_g = 136\,^\circ\mathrm{C}$ for chitosan were lower than those of PLA and chitosan homopolymer (Shantha & Harding, 2002), which could be assigned to the effect of chain mobility increase for the graft copolymers. Fig. 6 exhibited no cold crystallization of PEO because the PLA block was long enough to prohibit the crystallization of PEO during the cooling process (Li, Li, El Ghzaoui, Nouailhas, & Zhuo, 2007). Evidently, the DSC thermogram provided another evidence for the successful synthesis of the graft copolymer by "click" and "SET-NRC" reactions.

4. Conclusions

N-chitosan-*g*-(PEO-PLA-PEO) was prepared for the first time. One-pot synthesis of graft copolymer via "click" chemistry and "SET-NRC" reaction was successful in the presence of Cu(0)/PDMETA system. ^{1}H NMR, FTIR, ESR, and DSC spectra proved well-defined chemical structures illustrating this one-pot strategy could be useful for designing novel chitosan materials. Further studies concerning the gelation behavior of this new graft copolymer will be reported soon.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.07.023.

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