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Preparation of a thermosensitive and biodegradable microgel via polymerization of macromonomers based on diacrylated Pluronic/oligoester copolymers

Wen Zhu, Biaobing Wang, Ying Zhang, Jiandong Ding *

Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

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Abstract

A novel biodegradable and thermosensitive hydrogel microparticle was prepared via suspension polymerization of a kind of block copolymer macromonomers. According to the molecular design, the macromonomer is composed of a thermosensitive triblock copolymer poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) and two oligomers of biodegradable polyester such as oligo(lactic acid) or oligo(ε-caprolactone), and end-capped with acryloyl groups. Microgels were obtained by inverse suspension polymerization of the macromonomer aqueous droplets initiated by a redox initiator. Thermosensitivity and in vitro biodegradation of the resultant microgels were confirmed. The gel microparticles in an aqueous solution were swollen at low temperature and shrunken at high temperature (human body temperature). Degradation rate could be adjusted by controlling the composition and the degree of polymerization of oligoester. Thus, the microgels exhibit combinatory and tunable properties.

Keywords: Hydrogel; Biodegradable; Thermosensitive

1. Introduction

Polymeric hydrogels are a kind of quite unique soft matter and wet materials. They exhibit wide application in various fields such as in drug controlled release [1–5]. Biodegradable hydrogels with tunable degradation rate are especially suitable for potential biomedical materials. Compared with other biodegradable materials such as poly(D,L-lactide-*co*-glycolide) (PLGA) [6,7], hydrogels more closely resemble natural living tissues due to their high water contents and rubbery consistency.

Some hydrogels respond to environmental stimuli strikingly [8–16] and thus exhibit "intelligence". Recently, the intelligent hydrogels have been rather "hot" in studies of drug delivery system etc. [2,8,9,16]. There are many different physical forms of hydrogels such as microgel, bulk gel etc., among which, microparticles exhibit better injectability.

Although there are many reports so far merely about biodegradable polymers, intelligent hydrogels or microparticles, the reports of biodegradable and intelligent hydrogels in the microparticle form are relatively rare. A well-designed macromonomer has been reported by

* Corresponding author. Tel.: +86 21 6564 3506; fax: +86 21 6564 0293.
E-mail address: jdding1@fudan.edu.cn (J. Ding).

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Hubbell et al. [17,18] to prepare a biocompatible hydrogel with tunable degradation rate, where the central part is polyethylene glycol (PEG). The hydrogel has also been tried in encapsulation of osteoinductive growth factors [19] and DNA [20]. These works revealed a novel and wonderful class of biodegradable biomaterial. However, the hydrogels are not intelligent, and just bulk hydrogels instead of gel microparticles have been reported [18]. Another biodegradable and injectable bulk hydrogel instead of gel microparticles was investigated by Kim et al. [8].

In the present study, we combined a well-designed macromonomer and inverse suspension polymerization to prepare a microgel which exhibits both biodegradability and thermosensitivity. Redox agents were used in the initiation system of polymerization. The macromonomer is designed as in Fig. 1. The central part of the macromonomers is a triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) with the symmetrical structure of PEO–PPO–PEO commercially called Pluronic. The Pluronic is then copolymerized with oligo(hydroxy acid) such as oligo(lactic acid) (oligoLA) and oligo(ε-caprolactone) (oligoCL) capped with polymerizable double bonds.

Pluronic is a well-known thermosensitive triblock copolymer. The chains of PEO–PPO–PEO in hydrogels undergo phase transition when the temperature is changed. Although the biodegradable component and the cap groups in our macromonomers are hydrophobic, the resultant polymeric network is still a hydrogel due to the Pluronic central part. The basic originality of our work is the combination of thermosensitivity and biodegradability into one molecule or molecular network by molecular design. The combination aims to prepare a novel drug delivery carrier. In this paper, the synthesis of the macromonomers, preparation of the microgels, swelling behavior of the resultant microgels at different temperatures and in vitro biodegradability were reported.

2. Experimental

2.1. Materials

Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer (Pluronic F127, EO_{99}–PO_{65}–EO_{99}) was purchased from Sigma Chemical

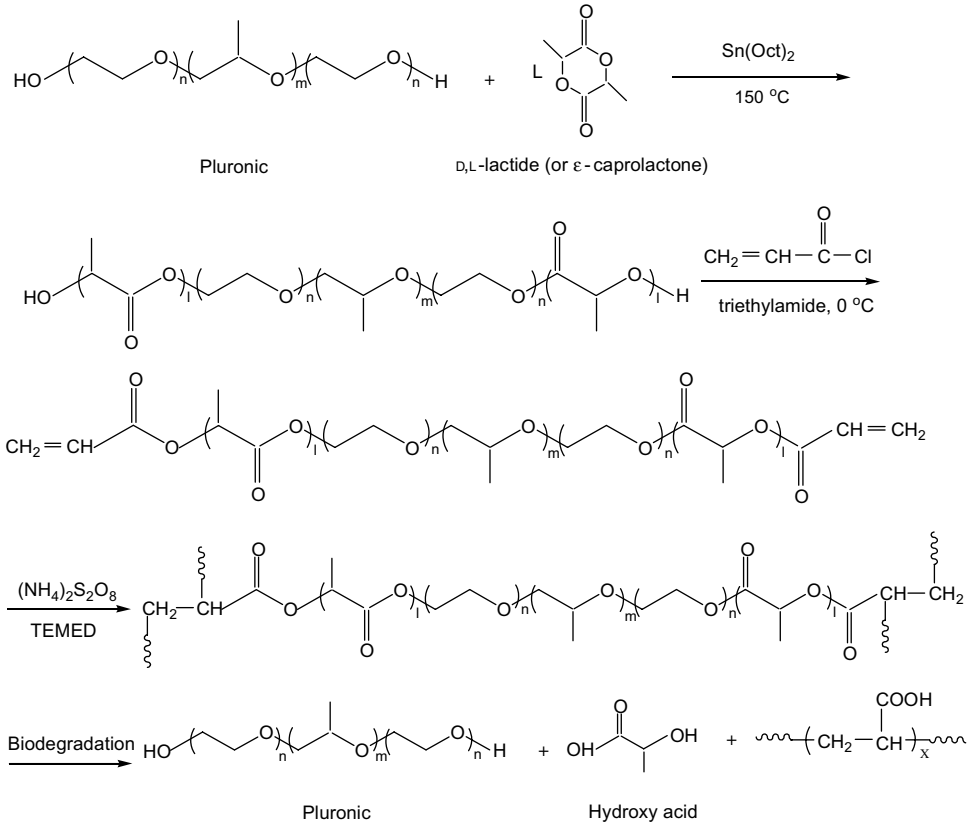

Fig. 1. Reaction schemes for the synthesis of macromonomers of Pluronic/oligoLA or Pluronic/oligoCL, chemically-crosslink reaction, and their degradation in aqueous solution.

Corporation and used without further treatment. Stannous octoate was obtained from Aldrich Chemical Co. (USA). *N*,*N*,*N*′,*N*′-Tetramethylethylene-diamine (TEMED) (99%) was purchased from Merck Chemical Co. (Germany). The D,L-lactide and ε-caprolactone were obtained from Purac Co. (France) and Aldrich Chemical Co. (USA), respectively. D,L-Lactide was recrystallized from ethyl acetate, and ε-caprolactone was distilled at 97 °C under 10 mm Hg with CaH_2. Span-60 was obtained from Shanghai Chemical Co. (Shanghai). Acryloyl chloride was the product of the reaction between benzoyl chloride and acrylic acid. We collected the distilled component at temperature from 60 °C to 90 °C, then from 72 °C to 76 °C. Ammonium persulfate (APS) was obtained from Aijian Chemical Co. (Shanghai) and recrystallized from distilled water. Phosphate-buffer saline (PBS: pH 7.2, 8.5 g/l of NaCl, 0.2 g/l of KCl, 2.85 g/l of $Na_2HPO_4 \cdot 12H_2O$, 0.27 g/l of KH_2PO_4 in deionized water) was used as degradation medium. All other chemicals used were of reagent grade and were used without further purification.

2.2. Synthesis and characterization of Pluronic/oligo(hydroxy acid) macromonomer

Ampoule tubes were treated with 10% (v/v) solution of trimethylchlorosilane in toluene, washed with acetone and followed by drying. Pluronic/oligo(hydroxy acid) block copolymers were synthesized by ring opening polymerization of hydroxy acid cyclic monomers in the presence of Pluronic F127 using stannous octoate as a catalyst. Twenty grams F127 and 1.83 g D,L-lactide were added in an 80 ml dried ampoule tube connected with a vacuum joint, and the reaction mixture was preheated to the melting state in order to mix thoroughly. Subsequently the mixture was cooled to room temperature. One milliliter catalyst solution (12.4 mg of stannous octoate dissolved in 1 ml toluene) was added into the tube. The tube was degassed under vacuum at 60 °C for 4 h and filled with argon. Next the tube was sealed off and placed in an oil bath at 150 °C for 24 h, and was subsequently cooled to room temperature. After reaction, the ampoule tube was crushed and the resultant copolymer was dissolved in dichloromethane, precipitated in anhydrous ether, filtered, washed several times with anhydrous ether, and dried in vacuum at room temperature (yield 93%). Other copolymers were synthesized by varying the feed conditions of cyclic monomers and Pluronic F127.

The degree of polymerization (DP) of oligoester was determined by 500-MHz proton NMR spectra (Bruker, DMX500 spectrometer). Spectra were recorded in $CDCl_3$, and tetramethylsilane (TMS) was used as internal standard. The block lengths of oligoester were calculated from the following equations [21]:

$$L_{PCL} = L_{PPO} \times \frac{3(\text{Peak intensity of } CH_2 \text{ group of PCL})}{2(\text{Peak intensity of } CH_3 \text{ group of PPO in Pluronic})} \Big/ 2 \tag{1a}$$

$$L_{PLA} = L_{PPO} \times \frac{3(\text{Peak intensity of } CH_3 \text{ group of PLA})}{3(\text{Peak intensity of } CH_3 \text{ group of PPO in Pluronic})} \Big/ 2 \tag{1b}$$

Here, L_{PPO} denotes DP of PPO block in Pluronic and reads 65 for Pluronic F127. Since the copolymer has one oligo(hydroxy acid) block at each end of Pluronic, the calculated value for the DP of each oligoester block should be divided by 2.

Dichloromethane and triethylamine were dried over with a 3A molecular sieve. Fifteen grams of the dried Pluronic/oligo(lactic acid) copolymer was dissolved in 120 ml dichloromethane in a 250 ml round-bottom flask. 0.67 ml of triethylamine was added to the flask. The flask was cooled to 0 °C in an ice bath, followed by dropwise adding 0.60 ml solution of acryloyl chloride in 10 ml dichloromethane. The reaction mixture was stirred for 12 h at 0 °C and another 12 h at room temperature under argon. Finally, the insoluble triethanolamine salt was filtered. The filtrate was precipitated in a large excess of anhydrous ether, filtered again, washed several times with anhydrous ether, and dried in vacuum at room temperature (yield 90%). This Pluronic macromonomer is termed as F127-LA_8-DA, which indicates that the di-acrylate-terminated macromonomer and the end groups of Pluronic F127 segment extended with oligo(lactic acid) with eight lactoyl repeats per hydroxyl end group according to the feed ratio. Other macromonomers were synthesized through copolymerization of Pluronic F127 and the different cyclic monomers.

The structure of Pluronic/oligo(hydroxy acid) macromonomers was confirmed by Fourier transform infrared spectroscopy (FT-IR spectra, Nicolet Magna-550). For FT-IR analysis, KBr tablets were prepared by dissolving the sample in dichloromethane and evaporating the solvent under the light.

2.3. Preparation of microgels

4.8 g span-60 was dissolved in 100 ml *n*-heptane in a 250 ml four-neck round-bottom flask with refluxed condenser pipe at 60 °C under nitrogen. Five grams 20% w/w solution of the macromonomer in deionized water with ammonium persulfate (26 mM) and 20 μl TEMED was added into flask dropwise. The reaction mixture was stirred at 600 rpm or other indicated stirring speed at 70 °C for 1.5 h under nitrogen, and immediately filtered with standard sieve, washed with acetone and deionized water several times, then rinsed in plenty of deionized water for 2 days at the refrigerator temperature to remove the unreacted macromonomer and the redox agent. The resultant microparticles were dried to constant weight under vacuum with P_2O_5 as desiccant.

Microgels were observed by a Zeiss Axiovert-200 optical microscope and recorded using a digital camera (Sony DSC-S75). Average size and size distribution of microgels were measured with a LS230 Particles Size Analyzer, a light scattering apparatus.

2.4. Swelling behavior of microgels

Swelling behavior of microgels was measured following the method reported by Zhang et al. in studies of poly(*N*-isopropylacrylamide)/dextran-allyl isocyanate hydrogel particles [11]. In the swelling experiment of microgels, the dried microgels of about 20 mg were placed into a cylindrical tube. One milliliter of distilled water was added to soak the microgels. After sealing the tube with a lid to avoid the distilled water volatilizing, the tube was put into the water bath at a predetermined temperature. After sufficient time for achieving swelling equilibrium, the tube was centrifuged quickly and the supernatant water was removed. The swollen microgels were collected and weighed quickly. The swelling ratio (Q) was calculated with the following equation:

$$Q = \frac{W_2 - W_1}{W_1} \tag{2}$$

where W_1 is the weight of the dried microgels, W_2 is that of the swollen microgels at the predetermined time. Swelling ratio was measured in triplicate.

2.5. In vitro degradation of microgels

About 25 mg dried microgels were loaded in dialyzer (12,000 MW cutoff). The dialyzer was kept in 20 ml PBS solution at 37 °C. PBS solution was changed every other day. Weight loss was monitored gravimetrically at some time intervals.

2.6. Thermosensitivity of microgels

For temperature-sensitivity measurements, an Olympus BX51 microscope with heating and freezing stage (THMS600) connected with a temperature controller (LTS350) was used. In order to enhance the optical contrast, microgels were dyed with a 0.5% (w/w) solution of methylene blue in deionized water for 10 min, and then were put into water in a glass vessel. The vessel was placed on the heating and freezing stage, and kept at the predetermined temperature for 5 min. The range of temperature was from 4 °C to 37 °C with a heating rate of 1 °C /min. The images of the microgels at different temperatures were captured by a CCD camera (TK-C1381EG). The volume shrinkage ratio of microgels (S) is defined as the following equation:

$$S = (1 - V/V_0) \cdot 100\% \tag{3}$$

where V is the volume of a microgel at the indicated temperature, V_0 is the volume of the microgel at 4 °C. The volume is estimated by the measured diameter.

3. Results and discussion

3.1. Synthesis of Pluronic/oligo(hydroxy acid) macromonomer

Pluronic/oligo(hydroxy acid) copolymer was synthesized by ring-opening polymerization of cyclic monomer such as lactide and ε-caprolactone in the presence of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers using stannous octoate as a transesterification catalyst. The reaction route of the copolymerization of Pluronic and cyclic monomer is shown in Fig. 1. The hydroxyl groups at each end of Pluronic chains initiate the ring-opening copolymerization of cyclic monomer through the acyl-oxygen cleavage. The propagation reaction was completed by stepwise addition of cyclic monomer to the hydroxyl groups of Pluronic chains [22]. Fig. 2 exhibits a typical ^{1}H NMR spectra of Pluronic F127/oligoLA and Pluronic F127/oligoCL block copolymer and the corresponding macromonomers. CH_2 (~1.6 ppm) groups are attributed from oligo(ε-caprolactone), and CH_2 (~3.63 ppm) from PEO block in Pluronic. In the spectrum of F127/oligoLA block copolymer, CH_3 (~1.5 ppm) belongs to oligo(lactic acid). Compared with F127/oligoester copolymer, three weak peaks appeared from ~5.7 ppm to ~6.5 ppm which attribute to the proton of –CH=CH_2 group. The NMR experiments confirmed that F127/oligoester copolymers were end-capped with the acryloyl group. Sometimes, two extra peaks might appear at ~1.4 ppm and ~3.1 ppm in the spectra of macromonomers, which may be attributed from the protons of remaining triethylamine. The DP

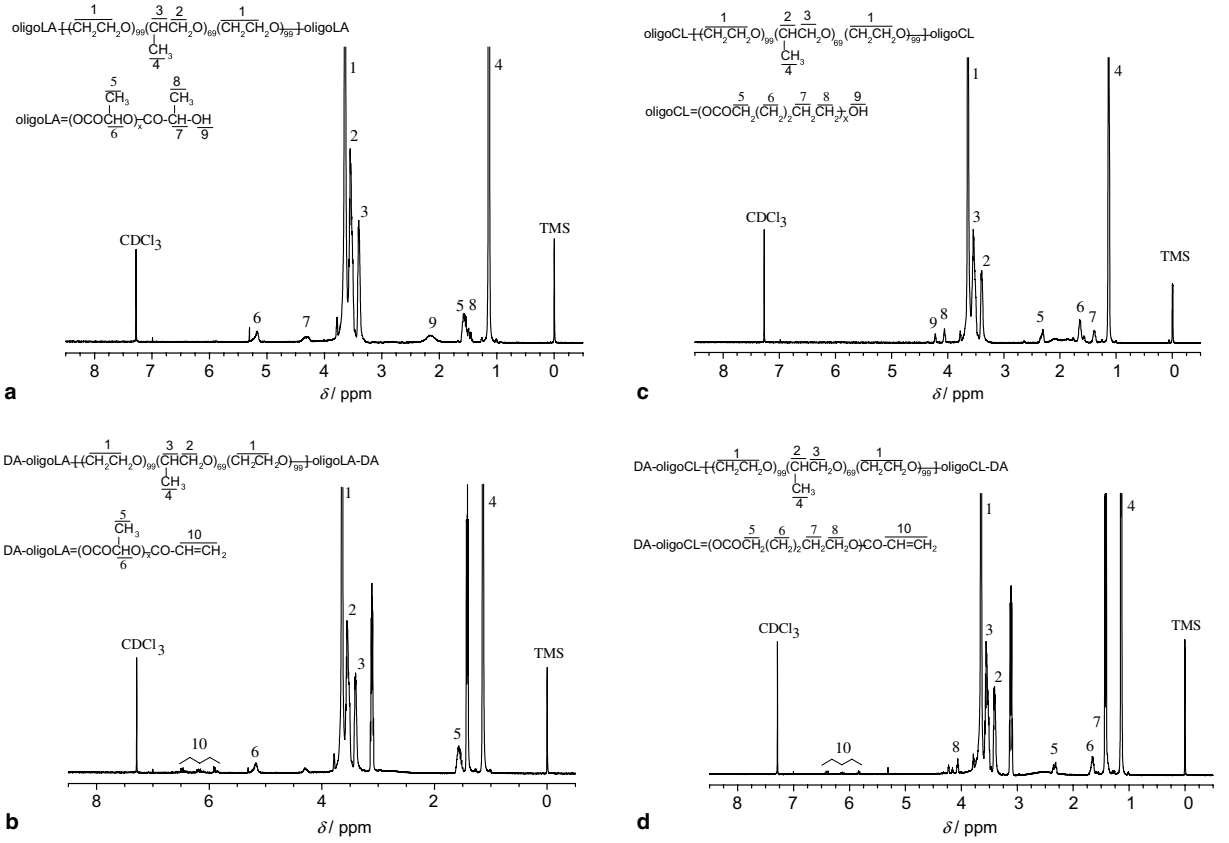

Fig. 2. ^{1}H NMR spectra of Pluronic F127/oligoLA copolymer (a), Pluronic F127/oligoLA macromonomer (b), and Pluronic F127/oligoCL copolymer (c), Pluronic F127/oligoCL macromonomer (d).

Table 1
Synthesis and composition of Pluronic F127/oligoester macromonomers

Sample	Feed molar ratio (Pluronic/Lactide or Pluronic/(Caprolactone/2))	DP of each oligoester block (determined by ^{1}H NMR)	Macromonomer yield (%)
F127-LA_4-DA	1:4	3.8	75
F127-LA_8-DA	1:8	7.9	70
F127-CL_4-DA	1:4	4.1	72
F127-CL_8-DA	1:8	7.8	80
F127-CL_{12}-DA	1:12	11.2	82
F127-CL_{16}-DA	1:16	14.8	81

of oligoester depends on the ratio of cyclic monomer molar concentration to Pluronic concentration. Characteristic results of some synthesized Pluronic F127/oligo-(hydroxy acid) diacrylated macromonomers are shown in Table 1.

FT-IR spectra of Pluronic F127, a typical copolymer of F127/oligo(ε-caprolactone) and the corresponding macromonomer are shown in Fig. 3. The FT-IR spectrum of Pluronic F127 presented an absorption band at 3500 cm^{-1} due to the terminal hydroxyl group. This band disappeared in the F127/oligo(ε-caprolactone) macromonomer due to acrylation. A new and strong carbonyl band was seen at 1735 cm^{-1} in the FT-IR spectra of F127/oligoCL and the macromonomer, which confirms the formation of block copolymer of Pluronic and oligoester.

The terminal hydroxyl groups in the Pluronic/oligoester block copolymer were subsequently converted to acrylic groups by reaction with acryloyl chloride. The number of acrylic groups on the Pluronic/oligoester

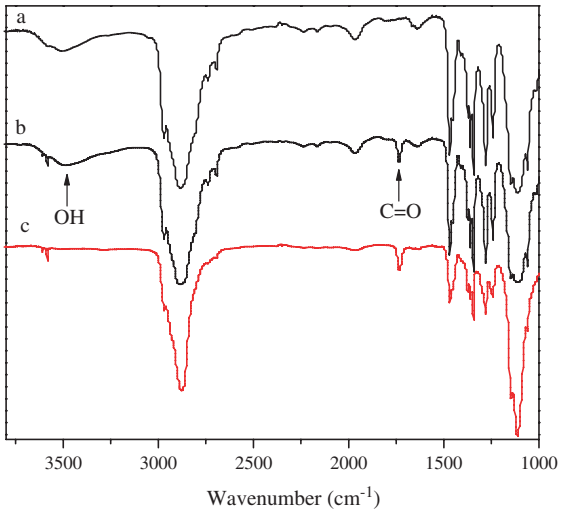

Fig. 3. FT-IR spectra of F127 (a), F127-CL_4 copolymer (b) and F127-CL_4-DA macromonomer (c).

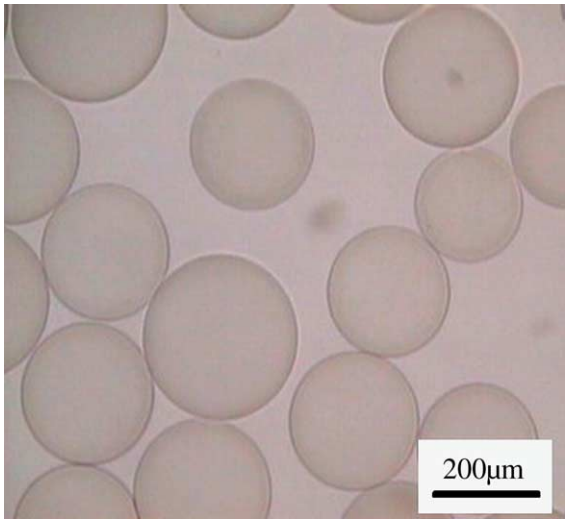

Fig. 4. Optical images of the microgels prepared from F127-LA_4-DA macromonomer.

block copolymer is expected to be 2 since Pluronic has two hydroxyl groups per molecular chain. Thus, the concentrated macromonomer aqueous solution can form a cross-linked three-dimensional network under free-radical polymerization.

3.2. Preparation of microgels

We prepared the microgels by the inverse suspension polymerization method. In our experiments a redox initiation system was used to trigger free-radical polymerization in an aqueous solution. The cross-linking reaction scheme is also shown in Fig. 1.

The macromonomer aqueous solution with APS as an initiator and TEMED as an accelerator was added dropwise into *n*-heptane, while Span-60 was used as an emulsifier. The macromonomer aqueous solution was separated into microdroplets under stirring condition, and thus a W/O suspension was formed. Macromonomers dissolved in the aqueous solution underwent chemical cross-linking under the reaction conditions. Thus, the microdroplets were converted into microgels with three-dimensional chemical network. Fig. 4 shows the morphology of some microgels in deionized water. The resultant microgels after polymerization have spherical shapes with smooth surface.

To further investigate the particle size distribution, microgels were measured by dynamic light scattering. Fig. 5 shows a typical size distribution of microgels prepared from the F127-CL_8-DA macromonomers. Those microgels with the particle diameter of 340 μm contribute the dominant volume fraction. The sizes of the microgels prepared at different stirring rates are listed in Table 2. It is quite reasonable that the average diameter of the microgels is dependant upon stirring rate. The average size is decreased with stirring speed increased.

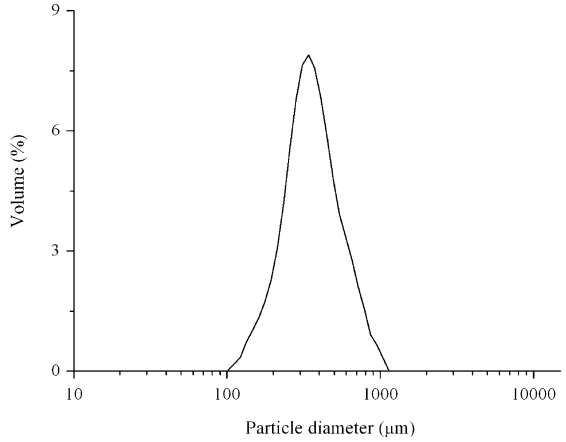

Fig. 5. A size distribution profile of microgels prepared from F127-CL_8-DA macromonomer determined by dynamic light scattering.

Table 2
Average size and size distribution of microgels prepared from inverse suspension polymerization of F127-CL_8-DA macromonomer at different stirring rates (accelerator concentration, 4 μl/g; reaction temperature, 70 °C)

Stirring speed (rpm)	Average size (10^2 μm)	S.D. (10^2 μm)
450	6.1	2.4
600	2.5	1.8
750	2.1	1.6

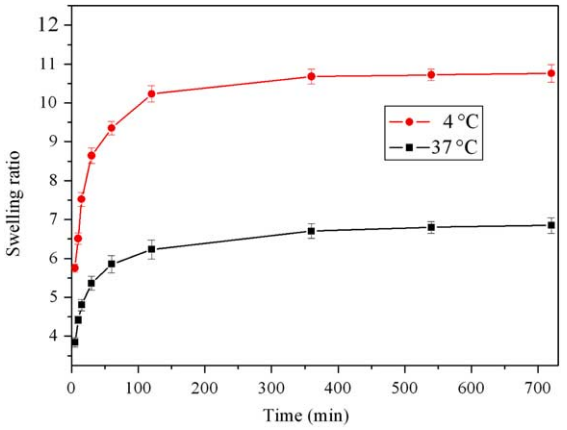

Fig. 6. Swelling behavior of dried microgels prepared from F127-LA_8-DA macromonomer in distilled water at 4 °C and 37 °C, respectively. Indicated values are mean ± S.D. of three experiments.

3.3. Swelling kinetics of microgels

Swelling behavior of dried microgels is presented in Fig. 6. At the early stage of immersion in distilled water, the dried microgels were quickly swollen due to very fast water absorption. After 12 h microgels exhibited the state of swelling equilibrium. So, a typical swelling profile of hydrogel has been reproduced. In addition, the swelling at 4 °C is much faster than that at 37 °C. The PPO block in Pluronic F127 is less hydrophobic at low temperature, which accounts for the difference of swelling behaviors at the two temperatures.

3.4. Volume phase transition of microgels

In the present studies, an amphiphilic ABA-type triblock copolymer such as Pluronic was used as the central block polymer to prepare microgels. Pluronic possesses negative temperature sensitivity with low critical solution temperature (LCST) in aqueous solution, and has been widely used for drug delivery systems due to its ability to micellization and gellation with temperature and concentration changes [23]. If the polymer chains are covalently cross-linked, the hydrogel may show volume phase transition. Fig. 7 exhibits the influence of temperature on microgel size. As is seen from the images, at 4 °C the diameters of microgels are in the range from 260 μm to 330 μm, while at 37 °C the diameters of microgels are decreased to the range from 200 μm to 240 μm. Fig. 8 indicates that the volume of the microgel at 37 °C is contracted to 36% of the volume at 4 °C and the volume shrinking is remarkable from 15 °C to 20 °C. The phase transition temperature is just between the normal refrigerator temperature and human body temperature. When the temperature is raised over

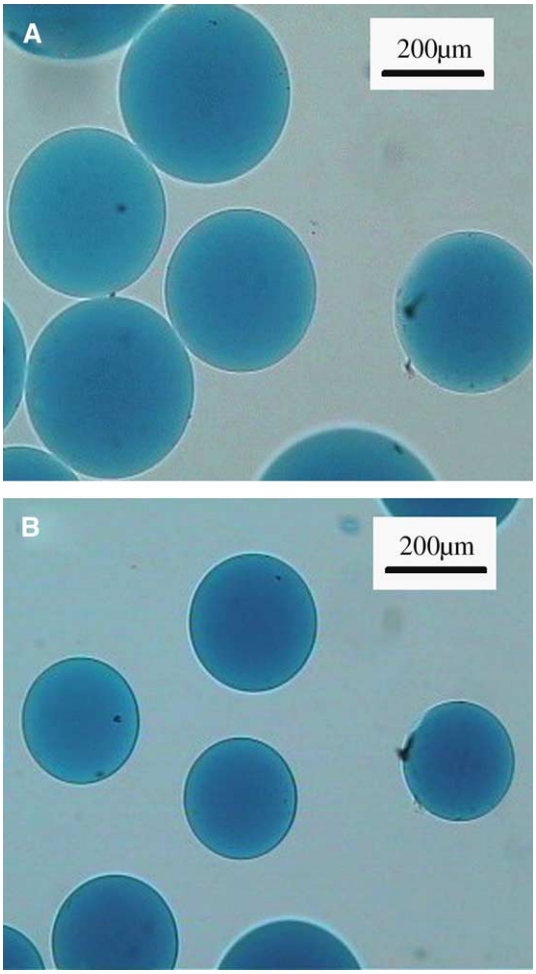

Fig. 7. Microgels prepared from F127-CL_8-DA macromonomer in deionized water at (A) 4 °C and (B) 37 °C.

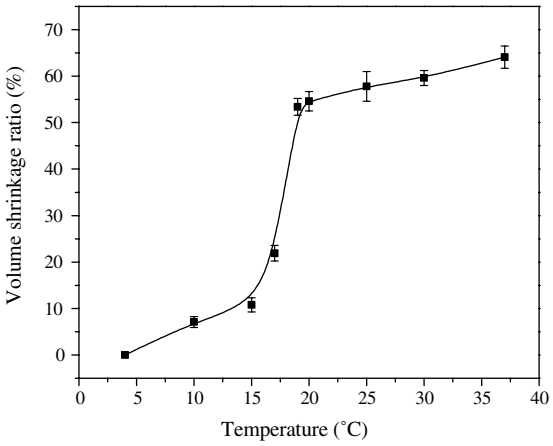

Fig. 8. The volume shrinkage ratio of a microgel as a function of temperature.

the phase transition temperature, the increased hydrophobicity of the PPO block in the Pluronic component leads to further aggregation of amphiphilic blockcopolymer micelles, and a further physical gellation happens in the chemical hydrogel, which accounts for the shrinkage of hydrogel with temperature. The hydrophobic oligoesters might also contribute to micellization. The detailed micro-structure in the resultant hydrogels will be investigated in the future. Such equilibrium-volume change with temperature is also consistent with the above swelling-kinetics observation shown in Fig. 6.

3.5. In vitro degradation of microgels

The degradation of these microgels might be subjected to hydrolysis of the oligoester extending the central polymer. The most widely investigated biodegradable synthetic polymer, poly(hydroxy esters) have been used in medical applications including drug controlled release and tissue engineering [24,25]. In general, degradable rates depend on many factors including the type and component of polyester, molecular weight, molecular weight distribution, and crystallinity etc. [25] and also the macroscopic geometry [24].

In the present study, the oligoesters, which were copolymerized with Pluronic F127, were used as the biodegradable moiety in the cross-linked network of microgels. It has been indicated that the degradation of a bulk hydrogel from PEG-containing macromonomers depends on the cross-linking density and the type of polyester [18]. So does the degradation of our microgels from thermosensitive Pluronic-containing macromonomers.

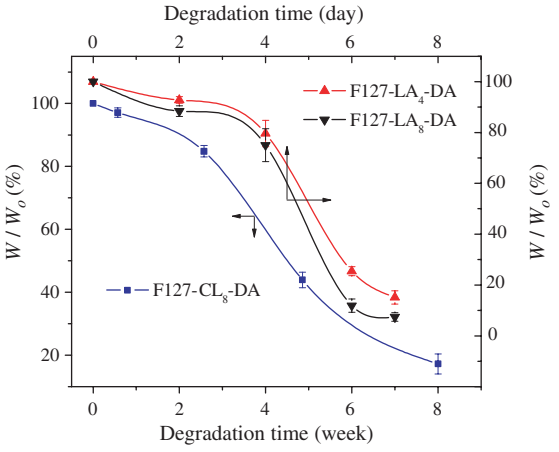

Fig. 9. Weight of the microgels made of marked macromonomers as a function of degradation time. Degradation was performed under PBS at pH 7.2 and 37 °C. W denotes the weight of microgels at the marked degradation time, while W_0, the initial weight. Indicated values are mean ± S.D. of at least three experiments.

As seen from Fig. 1, the hydrolysis is caused at each end of the cross-linked polymer chain, and the degradation products are presumably Pluronic, hydroxy acid, and oligomeric acrylic acid. Besides the cross-linking density, biodegradable rates of this kind of microgels could be adjusted by the type and the length of hydrolytically susceptible oligoesters.

Fig. 9 shows the degradation behavior of microgels achieved by altering the length and the type of oligoesters. It is reasonable that microgels made from the shorter oligoester blocks present relatively slow degradation due to less chance of hydrolysis of oligoester blocks. Longer time is required for degradation of a microgel containing oligo(ε-caprolactone) (in units of week) than oligo(lactic acid) (in units of day), which is attributed to the more hydrophobic property of oligo(ε-caprolactone) than oligo(lactic acid). Microgels made from the macromonomer of F127-CL_8-DA spent 56 days to degrade up to 83%, while those made from the macromer of F127-LA_8-DA degraded almost completely within 7 days. So, alternation of the oligoester composition is more effective to tailor the degradation rate of the microgels.

4. Conclusions

In this work, a biodegradable and thermosensitive microgel has been prepared by combination of the macromonomer synthesis technique and inverse suspension polymerization. The macromonomer has an amphiphilic central part of triblock copolymer, extended with oligo(hydroxy acid) and terminated with acryloyl groups. The macromonomer aqueous solution was suspended in an organic solvent and microgels with three-dimensional cross-linked network were formed after free-radical polymerization triggered by the redox initiation system with APS as an initiator and TEMED as an accelerator. FT-IR and ^{1}H NMR analysis demonstrated the synthesized products, namely, Pluronic/oligo(hydroxy acid) block copolymers and the eventual macromonomers.

Microgels obtained via suspension polymerization of the macromonomers were confirmed to be biodegradable. Degradation rates of microgels are tunable by adjusting both the length and the composition of the oligoester blocks. So, it is possible to tailor the degradation rate in a larger extent to meet diverse requirements. Microgels exhibit the larger swelling ratio at 4 °C in contrast to that at 37 °C. The volume phase transition temperature is between the refrigerator temperature and the human body temperature. These properties might be very beneficial for some special applications such as in drug release.

In recent years many researches have focused on using microparticles [26–29] and nanoparticles

[21,30,31] as drug carriers. This paper affords a novel biodegradable and intelligent microgel. Based on the above results, these biodegradable and thermosensitive microgels are suggested as potential new controlled release carriers of drug especially of protein drug. Swollen in aqueous solution at low temperature and shrunken at high temperature, these microgels might be employed to entrap drugs at the refrigerator temperature *after* preparation of drug carriers in the form of microparticles and thus not to take any risk to contact organic solvent and high temperature during drug encapsulation. The encapsulated protein drug might be controlled released at high temperature (human body temperature). It is worthwhile to indicate that Pluronic and poly(hydroxy ester) have been approved by FDA as biomaterials used in human body. Studies on the loading of drug into microgels are underway in our lab and will be published in another paper.

Acknowledgments

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