

The effects of temperature, feed ratio, and reaction time on the properties of copolymer PLA-PEG-PLA

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Abstract:

Triblock copolymers were polymerised by the ring-opening reaction of D,L-lactide in the presence of poly(ethylene glycol) (PEG), with number-average molecular weight (M_n) of 1500 and 2050 g/mol, using Sn(Oct)₂ as a catalyst. The influences of the reaction time, the ratio of PEG and Poly(D,L-lactic acid) (PLA), and PEG types on structure and sol-gel phase transition of PLA-PEG-PLA triblock copolymers were investigated. Optimal polymerisation parameters were obtained, such as reaction time of 18 hours, a catalyst amount of 1.3%, and PEG/PLA ratio of 1/1.7, PEG ($M_n=1500$); the efficiency of the triblock synthesis was 42.3%. The properties of PLA-PEG-PLA copolymers were evaluated by analytical methods such as proton nuclear magnetic resonance ¹H-NMR spectroscopy, gel permeation chromatography (GPC), and the sol-gel state transition at varying temperature. The results show that the triblock was successfully synthesised and its hydrogel had capability of the sol-gel state transition when the temperature changed. The PLA-PEG-PLA copolymer in aqueous solution is a thermo-sensitive hydrogel that can be used for drug and protein delivery systems or triblock denaturation applications for commercial purposes.

Keywords: poly(lactide), state transition, thermo-sensitive hydrogel, triblock.

Classification numbers: 2.1, 2.3

Introduction

Thermo-responsive hydrogels have attracted extensive attention in the field of biodegradable materials because they exist as a solution at low temperature and change to a gel at the human physiological condition. This is convenient for the administration of injections [1-5]. Thermo-responsive PEG hydrogels are one of these hydrogels and are applied in drug delivery and tissue engineering thanks to the sol-gel transition in different temperature conditions and the biocompatibility of PEG [2, 6-8]. Thermo-sensitive hydrogels are synthesised based on two blocks: the hydrophilic block (PEG) and hydrophobic blocks such as poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and PLA. The amphiphilic micelles in the aqueous solution contain two parts: hydrophobic blocks form the core and the surrounding micelles are hydrophilic blocks. The sol-gel transition of triblock copolymers in aqueous solution depends on the balance between the hydrophilic/hydrophobic block and others. Gelation was obtained with high temperature due to the generation of the micelles and the hydrophobic bridge between micelles [1-3, 7-10]. These thermo-sensitive hydrogels could encapsulate some types of drugs, such as bovine serum albumin (BSA), paclitaxel, dexamethasone, thymopentine, insulin, and the like, and prolong the release time of the drugs [7, 10-13]. One thermo-sensitive hydrogel that is commercially available is pluronic. It is used for drug delivery systems, implantation, and scaffold or denaturation applications [2].

In this study, we conducted the triblock synthesis by varying the reaction time (14 hours, 18 hours, and 22 hours); the PEG/PLA ratio (1/1.7 and 1/2.2); the proportion of the catalyst Tin(II) 2-ethylhexanoate (1.3% and 1.6% catalyst); and the PEG types (PEG-2050 and PEG-1500). Finally, we optimised the polymerisation parameters for high efficiency of the triblock. The temperature-sensitive hydrogels of PLA-PEG-PLA copolymers in aqueous solution were in a

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sol state at room temperature, and they changed to a gel state when the temperature increased to human physiological temperature.

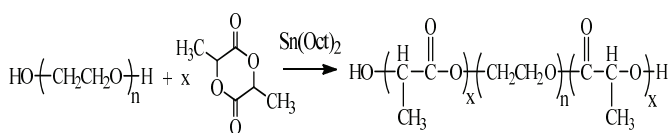
Materials and methods

Materials

D,L-Lactide (synonym: 3,6-Dimethyl-1,4-dioxane-2,5-dione), from Sigma Aldrich, is 99% pure. Poly(ethylene glycol) $M_n=1500$ Da (PEG-1500), $M_n=2050$ Da (PEG-2050), and Tin(II) 2-ethylhexanoate (SnOct_2) were purchased from Sigma Aldrich.

The PLA-PEG-PLA triblock polymerisation

The PLA-PEG-PLA triblock copolymers were formed by means of a ring-opening polymerisation of D,L-Lactide, initiated by PEG-1500 or PEG-2050, using $\text{Sn}(\text{Oct})_2$ as a catalyst (Scheme 1). The typical bulk polymerization was conducted as follows: PEG and $\text{Sn}(\text{Oct})_2$ were added to a two-neck flask and magnetically stirred at 110°C for 3-4 hours in vacuum environment to remove moisture. D,L-Lactide was added to the flask and then dried at 75°C in vacuum environment for one hour. Next, the flask was evacuated and filled with nitrogen three times to keep moisture away from the reaction. The reaction was maintained at 135°C for 14-22 hours. After reacting, the mixture was cooled to room temperature and dissolved in diethyl ether to precipitate the copolymer. After the evacuation, the purified product was collected at 45°C for 48 hours [5, 12-14].



Scheme 1. Ring-opening polymerisation of D,L-Lactide in the presence of PEG with the catalyst $\text{Sn}(\text{Oct})_2$.

Copolymer characterisation

The structures and composition of the triblock copolymers were determined by means of ^1H NMR spectroscopy. ^1H NMR spectra were recorded at room temperature using a 500 MHz spectrometer (Bruker, USA). The internal standard was tetramethylsilane (TMS) and deuteriochloroform (CDCl_3) was used as the solvent.

The M_n and the polydispersity index (PDI) of the triblock copolymers were determined by GPC (PL-GPC 50 Plus, Agilent Technology, USA). The mobile solvent was chloroform with the flow rate of 1.0 ml/min (30°C , PEG as standard).

Sol-gel phase transition measurement

The sol (flow)-gel (no flow) phase transition of the triblock copolymer in the aqueous solution was determined using the inverting vials method (with 4 ml tightly screw capped vials with a 10 mm inner diameter). The sol (flow) or gel (no flow) condition was determined in 1 minute. Briefly, each sample at a given concentration was absolutely dissolved in phosphate-buffered saline (PBS) (10 mM, pH 7.4) at 0°C overnight and was continuously stabilised at 5°C for 4 hours. The vials were then placed in a water-bath and were heated from 15 to 80°C at the step $2^\circ\text{C}/\text{time}$. The sol-gel transition temperature was determined by inverting the vial after maintaining it at a constant temperature for 10 min [1, 6, 14].

Results

Characteristics of the PLA-PEG-PLA copolymer using ^1H NMR

The PLA-PEG-PLA triblock copolymers were analysed using ^1H NMR spectra, as displayed in Fig. 1. The shift of 4.267 ppm (3) and 5.162 ppm (4) was assigned to the methine hydrogen ($-\text{CH}-\text{CO}$) and ($-\text{CH}-\text{COO}$) of the lactide unit, respectively. The presence of the methyl hydrogen ($-\text{CH}_3$) of the D,L-lactide unit was observed at $\delta=1.533$ ppm (1). The methylene hydrogen ($-\text{CH}_2-$) of PEG was recorded at $\delta=3.504$ ppm (2).

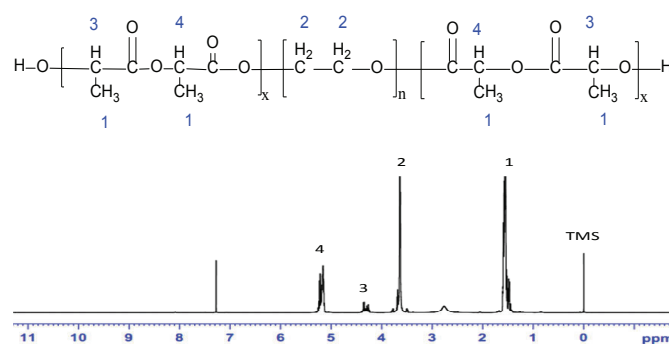


Fig. 1. ^1H NMR spectrum (CDCl_3) of the copolymer.

Effect of the reaction time on triblock structure and sol-gel transition of the hydrogel

The chain length of triblock 2050-14h (reaction time of 14 hours), $M_n=4448$ g/mol, was shorter than the triblock chain 2050-22h (reaction time of 22 hours), $M_n=4826$ g/mol. In addition, when the reaction time was 18 hours, M_n (4844 g/mol) of copolymer 2050-18h was higher than that of triblock 2050-22h (Table 1). Increasing the reaction time from 18 hours to 22 hours not only reduced the length of copolymer (by 18 g/mol) but also slightly

increased the PDI, from 1.18 to 1.19. The PEG/PLA ratio decreased from 1/1.36 to 1/1.35 when the reaction time was prolonged for four more hours (from 18h to 22h).

Table 1. Characteristics of PLA-PEG-PLA copolymer using H¹NMR and GPC.

Sample name	PLA-PEG-PLA M _n ^a	M _n ^a	PDI ^b (M _n /M _w)	PEG/PLA (wt/wt) ^a
2050-14h	1199-2050-1199	4448	1.21	1/1.17
2050-18h	1397-2050-1397	4844	1.18	1/1.36
2050-22h	1388-2050-1388	4826	1.19	1/1.35

a: determined by H¹NMR; b: determined by GPC.

The sol (flow) - gel (no flow) transition and the precipitation (separate two phase, including an aqueous phase and polymer in the bottom of vials) of the copolymer PLA-PEG-PLA in PBS are displayed in Fig. 2.

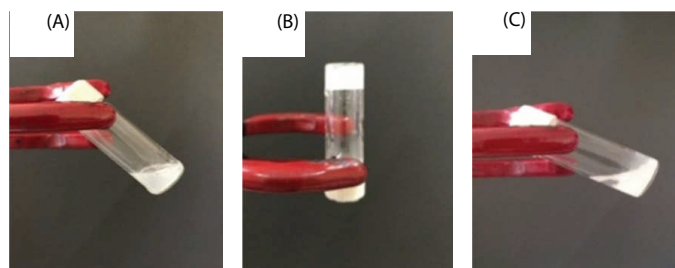


Fig. 2. Sol-gel transition of hydrogel PLA-PEG-PLA in PBS (A) sol, (B) gel, and (C) precipitation.

The sol-gel phase diagram of the copolymer 2050-18h in PBS shows that the hydrogels which were created from 20% and 30% triblock concentration were in a sol state below 38°C. The hydrogel of the 35% copolymer appeared as a gel state at about body temperature and was changed to precipitation state at 74°C. In addition, the 2050-14h hydrogel occurred in a sol state at all given concentrations, from 25% to 40% copolymer (Fig. 3). Therefore, the optimum reaction time was 18 hours; this was retained for the next experiments.

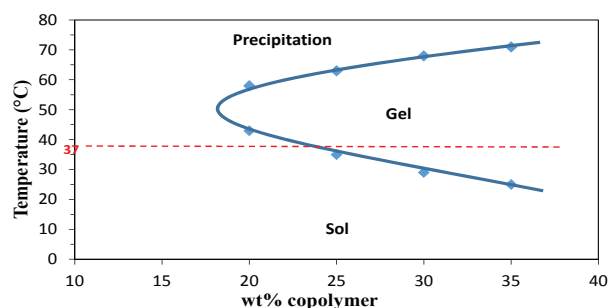


Fig. 3. Sol-gel phase diagram of hydrogel PLA-PEG-PLA (2050-18h) in PBS.

Effect of the PEG/PLA ratio and PEG types on the triblock structure and sol-gel transition of hydrogel

When the PEG/PLA ratio increased from 1/1.7 to 1/2.2, the M_n of triblock 2050-1.7 changed from 4844 Da to 5654 Da (M_n triblock 2050-2.2), especially, the length of PLA chain added to 405 Da. However, the reaction efficiency of triblock 2050-2.2 was reduced to 38.9%. In the case of PEG 1500, the triblock's M_n and reaction efficiency (36.5%) were lower than that of triblock 2050-2.2 (38.9%). The PEG/PLA ratio of triblock 1500-2.2 (1.90) was higher than that of triblock 2050-2.2 (1.76) although their designed PEG/PLA ratios were similar. In addition, the PDI of triblock 2050-2.2 (PDI=1.161) was the lowest while its M_n was the biggest (Table 2).

Table 2. The reaction efficiency, M_n, and PEG/PLA ratios of the PLA-PEG-PLA copolymer using PEG-2050 and PEG-1500.

Sample	PLA-PEG-PLA (M _n) ^a	M _n ^a	PDI ^b M _n /M _w	PEG/PLA ^a (wt/wt)	Reaction efficiency
2050-1.7	1397-2050-1397	4844	1.184	1/1.36	42.3%
2050-2.2	1802-2050-1802	5654	1.161	1/1.76	38.9%
1500-2.2	1424-1800-1424	4348	1.181	1/1.90	38.5%

a: determined by H¹NMR; b: determined by GPC.

According to Fig. 4, the gel phase area of hydrogel using triblock 2050-2.2 was broader than that of hydrogel 2050-1.7, and the gel temperature range was lower than that of the lactide ratio, at 1.7. The critical gel temperature (CGT) of hydrogel triblock 2050-2.2 was lower than the hydrogel 2050-1.7. Furthermore, the hydrogel triblock 2050-1.7 could not convert from sol to gel at 37°C although the CGT of 2050-2.2 could convert from sol to gel. At 30% and 35% copolymer concentration, the hydrogel of triblock 2050-2.2 was converted from sol to gel phase at 37°C and separated to two phases (precipitation) at 72°C.

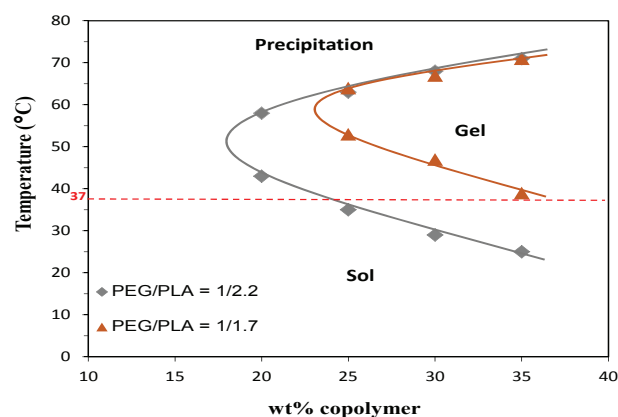


Fig. 4. The sol-gel phase diagram of the PLA-PEG-PLA hydrogel with a comparable ratio of PEG/PLA, 1/2.2 and 1/1.7 (PEG 2050, reaction time: 18 hrs).

As illustrated in Fig. 5, the hydrogel of 1500-2.2 triblock was in a gel state at 25-35%wt copolymer concentration at a low temperature (from 17 to 25°C). In the case of the 2050-2.2 hydrogel (20-35%wt triblock in PBS solution), the gel state was obtained at a higher temperature, more than 25°C for the 35% copolymer, and more than 42°C for the 20% copolymer. At lower copolymer concentration, at 25%wt, the hydrogel of triblock 1500-2.2 was separated to sedimentation at 37°C. Moreover, the ability sol-gel phase transition of triblock 2050-2.2 was better than that of triblock 1500-2.2 and, as a result, the gel area was broadened.

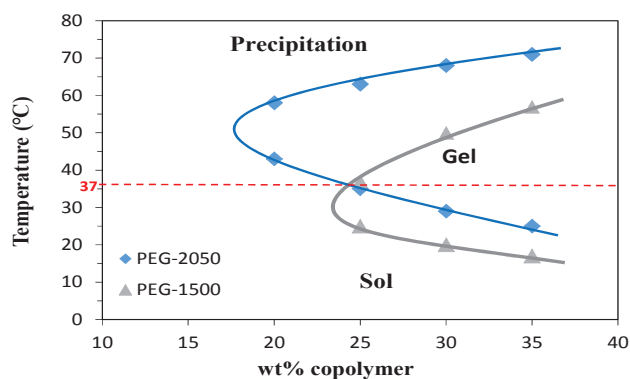


Fig. 5. The sol-gel phase diagram of PLA-PEG-PLA hydrogel with different PEG types, $M_n=1500$ and 2050 g/mol (reaction time: 18h, PEG/PLA ratio: 1/1.2).

Discussion

We succeeded in synthesizing triblock PLA-PEG-PLA using D,L-lactide, PEG and $\text{Sn}(\text{Oct})_2$ catalyst. The $^1\text{H-NMR}$ spectrum showed the specific peak (3) and peak (4) of methine hydrogen (CH-CO) and (CH-COO). The PLA-PEG-PLA hydrogel underwent the sol-gel transition at determined temperatures and copolymer concentrations. Furthermore, we investigated the effect of reaction time, PEG/PLA ratios, and PEG types on the properties and the sol-gel transition of the hydrogel of triblock. Time influenced the number of D,L-Lactides reacting with PEG, in the presence of $\text{Sn}(\text{Oct})_2$ catalyst. The reason for this is that the length of the triblock prevented lactides from attaching on the PEG backbone and generated the branch on the main polymer chain [5, 8, 10, 12, 14]. Moreover, with a short reaction time (14 hours), D,L-Lactide could not add to the PEG backbone more effectively. When reaction time was increased, the number of D,L-Lactides attached to PLA-PEG-PLA increased. The reaction temperature caused the differences in the PEG/PLA ratio and the length of PLA blocks [8]. In the hydrogel triblock structure, PLA blocks were hydrophobic and PEG blocks were hydrophilic, similar to the experiment based on the hydrophobic PLGA or PCL block, therefore the micelles

and bridging micelles were formed in aqueous solution. At a high temperature, the number of micelles and bridging micelles increased, so that the hydrogel changed from a sol state to gel. With continuously increasing temperature, the thermo-vibration of H_2O molecules was strong and caused the precipitation of the hydrogel PLA-PEG-PLA copolymer [12, 15, 16]. When the reaction time was less than 14 hours, the length of PLA block was shortened, leading to a decrease in the hydrophobicity of the hydrogel copolymer. As a result, the sample 2050-14h occurred in a sol state at all given concentrations and temperature.

In the second part of the survey, we changed the PEG/PLA ratio, increasing it from 1/1.7 to 1/2.2, and the triblock was synthesized with a low polydispersity index. However, the length of triblock caused the lack of space to lactide to connect with the PEG block and reduced the reaction efficiency. Moreover, the hydrogel of triblock 2050-2.2 had a gel phase area broader than that of triblock 2050-1.7 due to development of the length of hydrophobic block (PLA) in the copolymer structure. The reason for this phenomenon is that the establishment of bridging connections between micelles and high number of micelles in the triblock 2050-2.2 hydrogel structure [16]. Increasing the amount of lactide for polymerisation, resulted in the M_n increasing and PDI decreasing. This proved that the PEG/PLA ratio was 1/2.2 - the triblock product had fewer impurities and had chemical homogeneity. On the other hand, reaction efficiency decreased as the lactide ratio increased to 2.2 because the polymer chain entanglements of the long triblock were formed, leading to the PLA block being difficult to connect with the PEG block. The amount of lactide for polymerisation was increased, the M_n increased and PDI decreased. In addition, the higher lactide ratio increased sublimation and reduced reaction efficiency.

Finally, we changed the molecular weight of PEG block (M_n 2050 g/mol and 1500 g/mol) and fixed the designed PEG/PLA ratio. Triblock PLA-PEG(1500)-PLA had a PEG/PLA ratio that was greater than that of triblock PLA-PEG(2050)-PLA. The reason for this is that the small molecular chains of PEG were effectively added to PLA or lactide. The hydrogel 1500-2.2 triblock had a lower CGT than the hydrogel 2050-2.2 triblock, with the consequences that it became a gel and could not be injected at room temperature (25-32°C). The length of the hydrophilic block (PEG) influenced the balance of the hydrophobic (PLA) and hydrophilic parts (PEG) in the hydrogel structure. When the hydrophobic part was larger the hydrophilic part, the ability to form micelles in an aqueous solution was easier [12, 15]. At low M_n of PEG (1500 g/mol) the micelles were formed more easily than with PEG (2050 g/mol), so that the gel state of hydrogel triblock 1500-2.2 was obtained at a

lower temperature than that of hydrogel triblock 2050-2.2. The sol-gel transition area depended on the total molecular weight of the block copolymer. This effect related to the amphiphilic copolymers with low molecular weight more easily forming micelles than those with high molecular weight [15].

Conclusions

In this study, the influences on the phase diagrams of the reaction times, the PEG/PLA ratio, and the PEG types were investigated. The optimal reaction time was 18 to 22 hours for the synthesis of triblock PLA-PEG-PLA. A lower critical gel concentration was found when we increased PLA/PEG ratios. The sol-gel transition diagrams were shifted to higher temperatures by increasing the molecular weight of the triblock without changing the composition of the copolymers. The results of this study suggest that PLA-PEG block copolymers can be the next step in the development of polymeric drug delivery.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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