Gelation Behavior Study of Thermogelling Poly(ε-Caprolactone-*co*-1, 4, 8-Trioxa[4, 6]Spiro-9- Undecanone)-Poly(Ethylene Glycol)-Poly(ε-Caprolactone-*co*-1, 4, 8-Trioxa[4, 6]Spiro-9-Undecanone)

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ABSTRACT

Poly(CL-*co*-TOSUO)-PEG-poly(CL-*co*-TOSUO) (PCT-PEG-PCT) has been successfully synthesized via ring opening polymerization. By adjusting initial monomer feeding ratio, TOSUO molar content in the hydrophobic block increases from 0% to 20% while the repeat units of the blocks remain the same. Analysis on the copolymers shows that TOSUO units disrupt crystal structure in the hydrophobic PCL block, and thus reduces its crystallinity and subsequently the hydrophobicity of the copolymer. As a result, properties of the copolymer aqueous thermogels are affected. Introduction of TOSUO component improves the sol stability and reduces the gelation temperature below 37 ºC, suggesting its suitability to potential applications in injectable drug delivery. The study also indicates that one is able to tune the gel properties, such as gelation temperature, solution stability and gel mechanical stiffness, by de-novo designing copolymer chemistry to meet different application demands.

Keywords: injectable thermogel, copolyester, sol-gel transition, crystallinity, rheology

1 INTRODUCTION

Injectable thermosensitive hydrogel is well known to exhibit as liquid in vitro and transform into hydrogel in situ at 37 ˚C when injected into the body. This gel maintains its solid structure by physical intanglement of polymer chains. This kind of physically cross-linked hydrogel is considered as biologically safe due to elimination of chemical reactions during gelation. The injection implantation method can also be combined with minimal invasive technology to avoid surgical trauma, and therefore thermosensitive hydrogel has attracted increasing attention in the areas of material development and medical applications [1-3].

Thermosensitive hydrogel, consisting of hydrophilic polyethylene glycol (PEG) and hydrophobic biodegradable polyesters, such as PLA, PCL, PGA, and PLGA, etc., are well studied for its assembly and hydrogel properties [3, 4]. When in aqueous environment, the hydrophobic blocks of these copolymers are collapsed to form assembled core while the hydrophilic PEG segments swell in water. The assembled polymeric structures then physically entangle and ultimately form an interconnected, crosslinked 3D structure. This thermosensitive hydrogel has found wide applications including wound healing, drug controlled release, and tissue engineering scaffolds thanks to their excellent self-assembly performance, biodegradability and biocompatibility [5, 6].

However, there are still issues with these polyether ester hydrogels. Thermosensitive hydrogels with components of PLA and PLGA polyesters can generate acidic products resulted from biodegradation [7]. While PCL-based hydrogels, owing to strong crystallinity of PCL chains, they have poor sol-gel stability, causing spontaneous crystalline gel before injection. Other drawbacks include narrow gel window, high gel stiffness and low gel tunability [7, 8].

Effort has been made in our group to modify the crystallinity of PCL segment by replacing ε-CL with modified monomers with pendent cyclic ketals substituted ε-CL, TOSUO. Previous studies have shown that addition of TOSUO to the hydrophobic PCL segments will reduce the crystallinity of the hydrophobic core and thus affect the gelation behavior of the copolymer aqueous solutions and the mechanical properties of corresponding thermosensitive hydrogels. This paper focuses on synthesis of thermogelling block copolymers with hydrophobic PCT blocks with functional monomer TOSUO and ε-CL and hydrophilic PEG, and gelation properties of correspondent hydrogels. In the course of this research, block copolymers with controlled block composition are obtained by synthesis design to study self-assembly activity of their micelle solution and affecting factors of their gelation behavior. In the end, the relationship between chemical composition and gelation behavior of this copolymer hydrogel is established. Based on this study, the release behaviors of the protein-loaded thermosensitive hydrogels and their biocompatibility studies as macromolecular drug carriers are proposed for future study.

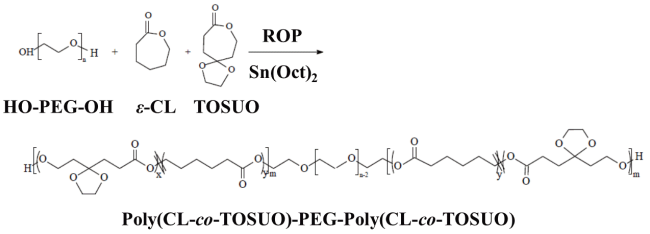
	PCT-PEG-PCT Structure	M_n	PDI	TOSUO/PCT [mol%]	Gel temperature [20wt%]
A619	$(CL)_{16}$-$(EG)_{34}$-$(CL)_{16}$	4995	1.31	0%	42 ~ 60 ˚C
B619	$(CL)_{14.6}(TOSUO)_{1.4}$-$(EG)_{34}$-$(CL)_{14.6}(TOSUO)_{1.4}$	5012	1.18	8.8%	40 ~ 53 ˚C
C619	$(CL)_{13.8}(TOSUO)_{2.2}$-$(EG)_{34}$-$(CL)_{13.8}(TOSUO)_{2.2}$	5128	1.3	13.8%	35 ~ 46 ˚C
D619	$(CL)_{13.2}(TOSUO)_{2.8}$-$(EG)_{34}$-$(CL)_{13.2}(TOSUO)_{2.8}$	5234	1.22	17.5%	30 ~ 44 ˚C
E619	$(CL)_{12.7}(TOSUO)_{3.3}$-$(EG)_{34}$-$(CL)_{12.7}(TOSUO)_{3.3}$	5380	1.29	20.6%	25 ~ 35 ˚C

Table 1: Structure, composition and gel temperatures of PCT-PEG-PCT copolymers.

2 EXPERIMENTAL

2.1 Material Synthesis

TOSUO was synthesized via Baeyer-Villiger oxidation reported in the literatures following Scheme 1 [9].



Scheme 1: Synthesis schematic of PCT-PEG-PCT

A series of triblock copolymers PCT-PEG-PCT were synthezied via ring-opening polymerization of ε-CL and TOSUO monomer mixture using PEG (M_n = 1500) as macro-initiator. Anhydrous PEG macro-initiator was first dissolved in toluene with presence of ε-CL and TOSUO followed with addition of catalyst stannous octoate. The reaction was carried out at 120 ˚C for 16 hrs under vaccum. The product was first precipitated in diethyl ether and then re-dissolved in methylene chloride for second precipitation. The final PCT-PEG-PCT polymer was filtered and dried under vacuum.

2.2 Polymer Micelle Solution

The polymer micelle solution was prepared by solvent-exchange method. PCT-PEG-PCT copolymer was first dissolved in acetone. Then the solution was slowly added in DI water in dropwise. The aqueous solution was achieved after acetone was evaporated under vigorous stirring. The final polymer aqueous solution was stored at 4 ˚C.

2.3 Characterization Methods

Molecular weight and weight distribution of the synthesized copolymers were determined by gel permeantion chromatography (GPC) on Breeze 2 HPLC System, Waters. The polymer chemical structure was evaluated by FTIR (Bruker Vertex 70, Germany) with a resolution of 4 cm^{-1} in the range of 400 ~ 4000 cm^{-1} and by 300 MHz NMR (Bruker, Germany) in $CDCl_3$ solution. The melting and cooling behavior of copolymer powders under nitrogen were recorded by DSC 204 F1 Phoenix (NETZSCH, Germany). In order to analyze the isothermal crystallization of the copolymers, the samples of 5~10 mg were heated from 25 ˚C to 80 ˚C and then was quenched to -20 ˚C to eliminate their thermal history. Then the sample temperature was raised from -20 ˚C to 80 ˚C at a rate of 10 ˚C/min and its isothermal behavior was recorded.

The gelation temperature, gelation time at 37 ˚C as well as gel strength were determined by a Fluids Rheometer (Malvem, Kinexus Pro). The polymer aqueous solution of 20 wt% was kept below 4 ˚C before was placed between a 2˚ core-plate with a diameter of 60 mm and a gap of 0.07 mm for temperature sweep and time sweep. For temperature sweep, the solution was evaluated at heating rate of 1 ˚C/min under controlled stress of 0.01 Pa and frequency of 1 Hz. The storage modulus (G') and loss modulus (G") were plotted with temperature in the range of 15~55 ˚C. The gelation time of polymer aqueous solution at 37 ˚C was determined by a single frequency (1 Hz) and constant shear (1%) measurement. When the storage modulus (G') is larger than the loss modulus (G"), we consider the gel is formed.

3 RESULTS AND DISCUSSION

3.1 Copolymers with TOSUO Contents

Copolymers PCT-PET-PCT with varied TOSOU contents were successfully synthesized. The molecular weight, PDI and copolymer chemistries are listed in Table 1. The polymerization was controlled in such a way that the repeat units of hydrophobic PCT blocks of the copolymers remained the same (about 16 repeat units) while the TOSUO to CL molar percentage was tuned by feeding different ratio of TOSUO to ε-CL monomers. Since all polymers were synthesized using the same PEG macro-initiators, the PEG blocks of all copolymers remain the same.

IR, ^{1}H NMR and GPC were employed to confirm the chemical structures and analyze the composition of copolymers. The characteristic peaks of functional groups, including ether stretching absorption at 1105 cm^{-1}, carbonyl stretching absorption at 1732 cm^{-1}, alkane stretching absorption at 2875 cm^{-1} and 2939 cm^{-1} as well as hydroxide stretching absorption at 3448 cm^{-1}, were detected by IR. The ^{1}H NMR spectra also confirms the successful polymerization of copolymer with ε-CL and TOSUO hybrid block. The peak at 4.20 ppm (H_i) indicated the establishment of chemical links between hydroxyl group in PEG and carbonyl group. Moreover, the peaks at 3.92 ppm (H_a) and 2.02 ppm (H_e) attributed to TOSUO were observed in copolymer PCT-PEG-PCT but not in the spectrum of PCL-PEG-PCL. The GPC analyzed the molecular weight and polydispersity index of the copolymers and the results were summarized in Table 1. The PDIs of the copolymers are in the range of 1.2 to 1.3, indicating controlled structures.

The molar weights in PCT block were determined by characteristic peaks in ^{1}H NMR spectra. Since the PEG molecular weight is known as 1500 g/mol, the molar contents of TOSUO and ε-CL components were calculated by comparing integral peak areas of 3.92 ppm (H_a) of TOSUO and 1.63 ppm (H_c) of ε-CL to peak 3.63 ppm (H_b) of PEG. The total repeat units in PCT of all copolymers were 16 based on ^{1}H NMR spectra. The molar content of TOSUO in PCT blocks was then calculated and it ranged from 0 to 30% as summarized in Table 1. In conclusion, we have successfully synthesized PCT-PEG-PCT copolymers with controlled structures. The PCT blocks can be conveniently tuned by changing the feeding quantities of the monomers of TOSUO and ε-CL. This series copolymers share the same molar block lengths in hydrophilic (PEG) and hydrophobic segments but with different amounts of hydrophilic TOSUO pendants in PCT hydrophobic segments.

3.2 Crystallinity of PCT

When hydrophilic TOSUO molecules are incorporated in hydrophobic PCL, the crystallinity of PCT segments is affected. DSC has been employed to verify the effects of TOSUO content in PCT blocks on copolymers crystalline and thermal properties.

As shown in Figure 1, the DSC thermograms of copolymers PCT-PET-PCT clearly demonstrate the impact of TOSUO content on reducing melting temperature of copolymers. Two melting peaks are shown in the range of 45 ~ 55 ˚C for PCL-PEG-PCL (A619), which are attributed to the melting of the as-formed PCL crystals (at 50 ~ 55 ˚C) and the recrystallized PCL domains (at 45 ~ 50 ˚C). This double-peak characteristic of PCL is because PEG will interrupt crystalline domains of PCL, causing reduction in melting temperature.

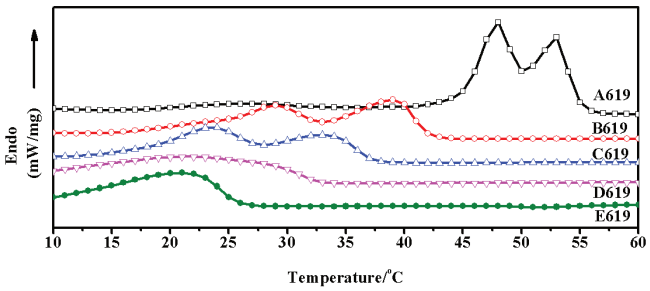


Figure 1: DSC heating thermograms of copolymers.

The melting temperatures of copolymer PCT-PEG-PCT shift to lower temperature ranges and the enthalpy decreases with increase in TOSUO components in the hydrophobic blocks. The double-melting-peak characteristics is no longer observed when TOSUO content in PCT is over 17.5% and only a single and broad melting peak is shown for D619 and E619. This DSC measurement indicates a less regular crystalline structure in PCT block with increase in TOSUO content. The DSC measurement indicates a less regular crystalline structure in PCT block with increase in TOSUO content. This is because the hydrophilic TOSUO pendant molecules are able to disrupt the crystaline structure of the hydrophobic PCL blocks. Simultaneously, the hydrophobicity of the PCT blocks are also reduced with TOSUO content.

3.3 Sol-Gel Stability and Gelation

Copolymer aqueous solutions were prepared by solvent-exchange method detailed in Experimental Section and the sol-stability of the polymer aqueous solutions were monitored since the stable solutions were prepared.

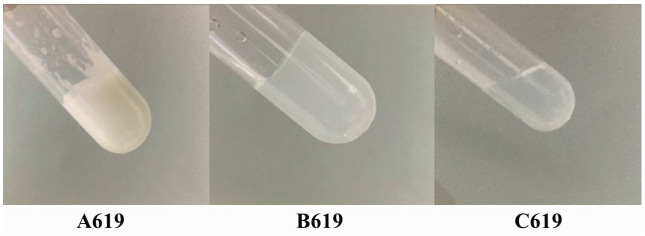


Figure 2: Copolymer aqueous solution (20 wt%) stability after 48 hours at 4 °C.

It was noticeable that PCL-PEG-PCL 20 wt% aqueous solution became cloudy in less than 1 hour and eventually completely solidified after 24 hours even when it was stored at 4 ˚C. This phenomenon reveals that the PCL-PEG-PCL finished gelation relatively easily at 4 °C. It will be difficult to store this material at sol state in regular refrigeration. On the other hand, PCT-PEG-PCT aqueous solutions presented a much improved sol stability. As shown in Figure 2, the aqueous solutions of copolymer PCT-PEG-PCT B619 and C619 remained in sol state after 48 hrs at 4 ˚C. However B619 appeared more turbid than

C619 solution, suggesting enhanced gelation compared to C619. Among all three solutions, A619 has demonstrated the stronger crystallinity in its hydrophobic domain, while C619 has the least crystallnity. Apparently higher the crystallinity in hydrophobic segments, the lower the solution stability.

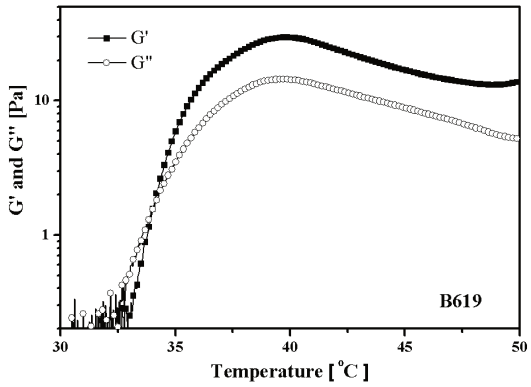


Figure 3: Rheological measurement of B619 aqueous solution (20 wt%) with temperature.

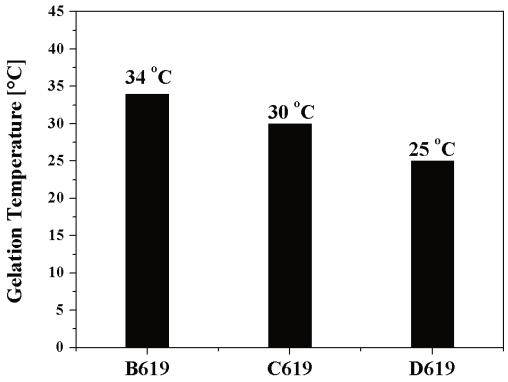


Figure 4: Gelation temperatures of copolymer aqueous solutions (20 wt%) measured by rheology under temperature sweep.

This trend in sol-gel transition has been reexamined by rheological measurement of copolymer aqueous solutions under temperature sweep. The change in viscoelastic property of the solution will occur when a three dimensional network is established. The sol-gel transition is generally considered to be completed when the value of storage modulus (G') surpasses that of loss modulus (G"). A representative rheological measurement of shear moduli of PCT-PEG-PCT B619 aqueous solutions (20 wt%) with temperature is presented in Figure 3. The measurement confirms the gelation temperature for B619 is at 34 °C, significantly higher than 4 °C. This result matches what is observed at sol stablity analysis.

Rheological measurement also reveals the gelation temperature for each PCT-PEG-PCT copolymers is determined by its chemistry. Figure 4 presents the change in gelation temperature with copolymer. It appears the gelation temperature was steadily shifted to lower point with TOSUO content and D619 has the lowest gelation temperature at 25 ˚C among three copolymers presented. It is worth noting that the gelation temperatures for all PCT-PEG-PCT copolymer solutions are below body temperature (37 ˚C). This property is preferred for medical applications that requires *in situ* injection for drug release, gene delivery or tissue engineering. Based on these testing results, we conclude that the TOSUO molar content is critical to achieve a thermosensitive hydrogel with desired performance. The solution stability can be improved and sol-gel-sol transition temperature can be reduced for PCT-PEG-PCT solutions when the crystallinity in hydrophobic segments is compromised.

4 CONCLUSION

A series PCT-PEG-PCT copolymers were successfully synthesized by linking TOSUO components into PCL segments via a ring-opening polymerization. DSC analysis confirms the hydrophobic PCT blocks have lower crystalline structure with TOSUO incorporation. The change in crystallinity in PCT segments is critical to achieve preferred solution stability and gelation temperature. In order to make a thermogel with a better stability and lower sol-gel changing temperature, a polymer with higher TOSUO molar weight is recommended. This thermogelling system with improved solution stability and tunable gelation properties will possibly find wide applications in areas of drug delivery, gene delivery and tissue engineering.

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