

Thermo-Responsible Sol-Gel Transition of Bioabsorbable Hydrogels Consisting of Enantiomeric A-B-A Tri-Block Copolymers of Poly(lactic acid) (A) and Poly(oxypropylene-co-oxyethylene) (B)†

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For the purpose of controlling gelation rate of the above suspension, the A-B-A triblock copolymers system comprising PLLA-PN-PLLA and PDLA-PN-PDLA was synthesized using PN (Pluronic®) instead of PEG. Since PN is a non-ionic macromolecular surfactant that can aggregate into micelles in aqueous solution in itself, not only the stereocomplexation of PLLA and PDLA segments but also the phase change behaviour of PN are expected to contribute to the sol-gel transition of the mixed suspension of enantiomeric copolymers to realize a sharp sol-gel change. Based on the results of these studies, we will discuss the changes in hydrophilic/hydrophobic balance in these copolymers that gives rise to the controlled gelation time and gel strength.

Keywords: Gelation, Triblock copolymer, Stereocomplexation, Sol-gel transition, Enantiomeric copolymer.

INTRODUCTION

Various plastic materials made of synthetic polymers are widely used nowadays^{1,2}. The increased consumption of these plastic materials has currently resulted in enormous increase of waste and the environmental contamination by the waste plastics is no longer neglected. This situation has enlightened an expectation to develop environmentally friendly or biodegradable polymers that can be degraded by the action of microorganisms without polluting the environment.

On the other hand, the materials used for recovering human body and organisms have been named “bio-materials” and constituted an important field in the current material science. Many biomaterials, particularly, those used as implants inside the human body, have been made from biodegradable polymers that can be eliminated without repeated surgery. Polymer gels have also been applied to biomedical field to bring remarkable advances in the medical disciplines³. Their applications involve cell culture, tissue engineering, drug delivery system (DDS), medical sensor and so on, for which the bio-compatibility, bio-functionality and safety of the gels are extremely important as well as the physicochemical properties⁴⁻⁶. These polymer gels are also expected to have biodegradability by adopting the biodegradable polymers as the gel networks. Among the biodegradable polymers

developed both for biomedical and plastic uses, poly(lactides) (PLA) have been attracting the highest interest because of their balanced mechanical and cost performances.

Recently, the novel thermo-responsive hydrogel formation was discovered in an enantiomeric mixture of the A-B-A type triblock copolymers, poly(L-lactide)-b-poly(ethylene glycol)-b-poly(L-lactide) (PLLA-PEG-PLLA) and poly(D-lactide)-b-poly(ethylene glycol)-b-poly(D-lactide) (PDLA-PEG-PDLA)⁷. The gel formation was driven by the stereocomplexation between the PLLA and PDLA segments⁸. With this system we developed a novel injectable vehicle for cell therapy. The rate of gelation, however, was rather slow and the gel strength was also slightly low for use as cell-holding scaffold.

In order to improve the gelation rate, we synthesize both PLLA-PN-PLLA and PDLA-PN-PDLA with Pluronic® F-68 (PN) in which a PPG segment is flanked with PEG block sequences to form an A-B-A type block copolymer⁹⁻¹². The reasons for selecting PN as the block segment are following. (1) PN with a number average molecular weight (Mn) of 8,400 is one of the very few synthetic polymers approved by the US Food and Drug Administration (FDA) as a safe pharmaceutical surfactant. (2) PN has a hydrophobic domain, which drives aggregation of itself into micelles in aqueous solution and exhibits the thermo-responsive character. Namely, PN has been utilized in drug delivery by manipulating the phase change of

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its aqueous solution that form a free-flowing sol at low temperature and a gel at body temperature¹³⁻¹⁶.

Here, we synthesize PLLA-PN-PLLA and PDLA-PN-PDLA block copolymers by the ordinary ring-opening polymerization of lactides in the presence of PN (Pluronic®F-68)⁹. Each of these enantiomeric copolymers is suspended in aqueous media. Both suspensions are then mixed together and the sol-gel behaviour of the mixed suspension is studied and compared with the PLA-PEG-PLA system previously studied. The present mixed suspension with a different block structure is promising for use as an injectable material because of the fast gelation rate.

EXPERIMENTAL

L- and D-lactides were supplied by Musashino Chemical Research Institute Corp (Tokyo). Pluronic®F-68 (PN) comprising oxyethylene (EO) and oxypropylene (PO) blocks in a unit ratio of 8:2 (Mn = 8,400) was supplied by ADEKA Co., Ltd., Tokyo. It was thoroughly dried at reduced pressure before use. Tin octoate (Sn(Oct)₂) was purchased from Aldrich. It was purified by distillation under high vacuum and dissolved in distilled toluene to have a concentration of 0.2 g/mL.

500 MHz ¹H NMR spectra were measured on a Bruker ARX-500 spectrometer. Deuterated chloroform (CDCl₃) containing 0.03 vol % tetramethyl silane (TMS) as the internal reference was used as the solvent.

The number-average (Mn) and weight-average (Mw) molecular weights and polydispersity index in Mw/Mn of polymer samples were determined by gel permeation chromatography (GPC). The analyzer was composed of a Shodex GPC SYSTEM-21, a Shodex RI-71S detector, a ERC-3415α and a SIC480 data station. A set of two identical columns of a fixed pore size (500 Å; 10 mm × 250 mm; Jordi Associates, Bellingham, MA) was used. The mobile phase was tetrahydrofuran (THF) flowing at a rate of 1 mL/min at 35 °C. The molecular weight was calibrated with polystyrene standards from 500 to 100,000 Da.

Differential scanning calorimetry (DSC) was carried out on a Shimadzu DSC-50 thermal analyzer under a nitrogen flow of 20 mL/min at a heating rate of 10 °C/min. The sample was heated from 20 to 200 °C.

Rheological behaviour of suspensions was measured on a rheometer (Rheogel-E4000, UBM Co. Ltd., Japan). A portion of a suspension was quickly transferred in a slit (slit size: 13 mm × 10 mm × 0.25 mm) installed on the rheometer, which was operated by the slit-shear measuring mode at a frequency of 10 Hz.

Dynamic light scattering (DLS) was conducted on an Otsuka Electronics DLS-7100 instrument utilizing He-Ne laser and the data were analyzed according to the cumulant method.

Copolymerization of L- and D- lactides with PN:

Typically, 7 g of PN was placed in a round-bottomed, three-necked flask equipped with a mechanical stirrer. The flask was immersed in an oil bath and evacuated by a vacuum pump for several hours in order to thoroughly dry up the PN and then filled with nitrogen gas. A prescribed amount of L-lactide (or D-lactide) was added and the flask was heated at 90 °C in order to homogenize the mixture. Then a certain amount of

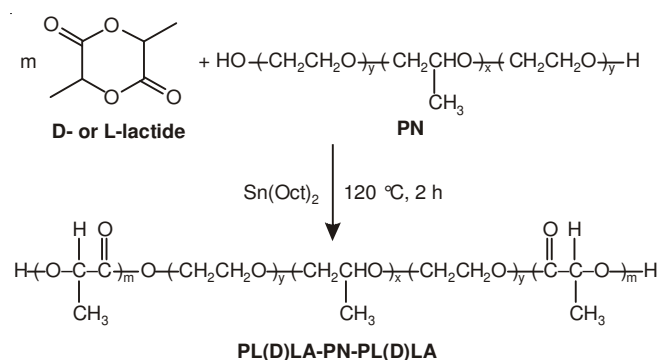
toluene solution of Sn(Oct)₂ (10 mol % relative to the hydroxyl end group of PN) was added and the flask was evacuated again for several minutes to remove the toluene at 110 °C and filled with nitrogen gas. The mixture was heated at 120 °C with stirring for 2 h. After the reaction had finished, the product was dissolved in a small volume of dichloromethane and poured into a 10-fold volume of diethyl ether. The precipitates were isolated by filtration and dried *in vacuo*. All the block copolymers were obtained as white powders in high yields. ¹H NMR (500 MHz, in CDCl₃): δ = 1.17 (CH₃ for PO), 1.57 (CH₃ for LA), 3.3-3.5 (CH₂CH for PO), 3.5-3.6 (CH₂CH for PO), 3.6-3.7 (CH₂CH₂ for EO), 4.3 (CH₂CH₂ for EO bonded with LA) and 4.9-5.1 (CH for LA), where LA, EO and PO denote the lactate units in PLA, the oxyethylene unit in PN and the oxypropylene unit in PN, respectively.

Preparation of aqueous suspensions of the block copolymers: Each of the above block copolymers (0.05 g) was dissolved in THF (0.5 mL) and added into a saline (1 mL) at 5 °C with ultrasonic wave applied. Then, THF was thoroughly evaporated from the resultant suspension under reduced pressure at 10 °C. The polymer concentration in the finally obtained aqueous suspension was adjusted to 2-10 wt/wt %. Subsequently, the aqueous suspensions of PLLA-PN-PLLA and PDLA-PN-PDLA were mixed in 1:1 ratio and warmed up to 37 °C to induce spontaneous gelation.

RESULTS AND DISCUSSION

Synthesis of PLLA-PN-PLLA and PDLA-PN-PDLA:

We prepared the A-B-A type triblock copolymers, PLLA-PN-PLLA and PDLA-PN-PDLA, by the ring-opening copolymerization of L- and D- lactides in the presence of PN (Scheme-I). Fig. 1 shows the ¹H NMR spectrum of the PLLA-PN-PLLA obtained. The PLA/PN ratios of the copolymer was determined from the integral ratio of the PO (1.17 ppm) and LA (4.9-5.1 ppm) and their Mn was further calculated by the following equation:



Scheme-I: Ring-opening copolymerization of enantiomeric lactides in the presence of PN to obtain PLA-PN-PLA

$$M_n = M_n^{\text{PN}} + 72/58 \times 3 \times M_n^{\text{PO}} \times (\text{lactate/oxypropylene}) \quad (1)$$

where 58 and 72 are the MW of oxypropylene and lactate units, respectively. The multiplier 3 corresponds to the three protons of the methyl groups of PO and Mn(PN) and Mn(PO) are 8400 and 1750, respectively. Table-1 summarizes the results

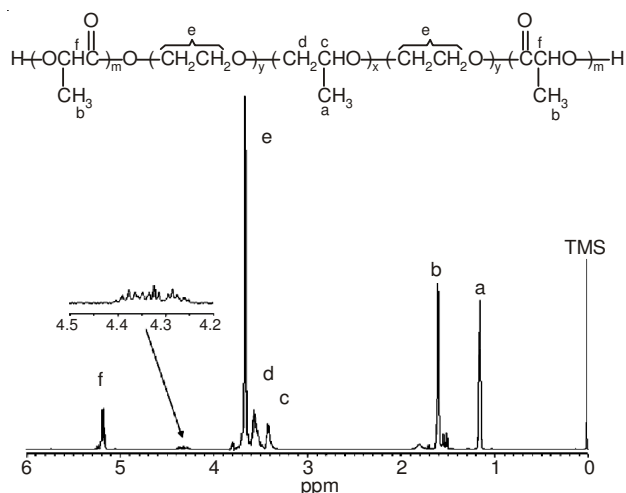


Fig. 1. A typical 500 MHz ^1H NMR spectrum of PLLA-PN-PLLA in CDCl_3

of synthesis of the two enantiomeric triblock copolymers. The PLA/PN compositions were almost identical with the lactide to PN ratios in feed in both series, suggesting that the lactide polymerization was induced efficiently. The isolated yields of the both copolymers were around 85 % owing to the slight loss in the reprecipitation process. Their molecular weight distribution was maintained narrow in each case. The copolymer samples are coded 1L: PLLA-PN-PLLA (900-8400-900) and 1D: PDLA-PN-PDLA (900-8400-900) according to the enantiomeric structure of PLA as noted in Table-1.

Fig. 2 shows the GPC curves of the triblock copolymers 1L and 1D as compared with the curve of PN (Pluronic[®]F-68; $M_n = 8400$). The GPC curves of the copolymers exhibited no peak corresponding to the starting PN, supporting the effective formation of the copolymers.

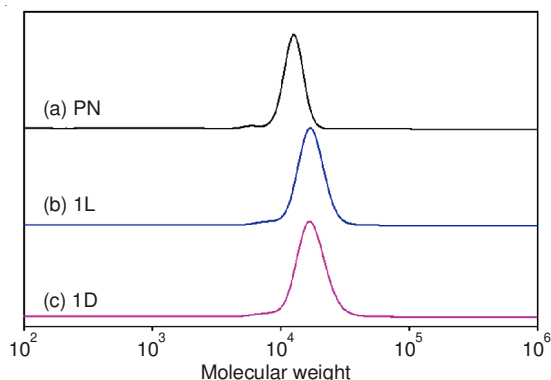


Fig. 2. GPC curves of (a) the starting Pluronic[®]F-68 (PN) and the triblock copolymers (b) 1L and (c) 1D

Fig. 3 shows the DSC thermograms of the copolymers 1L and 1D as well as the starting PN. PN exhibited a T_m at

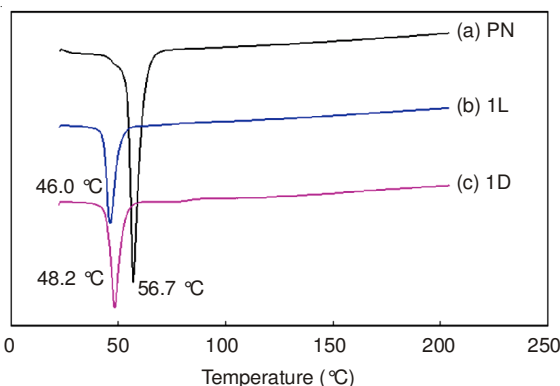


Fig. 3. DSC thermograms of (a) the starting PN and the triblock copolymers (b) 1L and (c) 1D

56.7 °C with a melting enthalpy of $\Delta H_m = 114.1$ J/g. T_m and ΔH_m of 1L decreased to 46.0 °C and 47.1 J/g, respectively. In the case of 1D, T_m and ΔH_m also decreased to 48.2 °C and 59.2 J/g. These data confirmed that the crystallization of PN was interfered with the PLLA blocks. No melting behaviour of the PLA blocks was observed in the DSC curves probably because their crystallization was interfered with the PN blocks.

Sol-gel transition behaviour of the aqueous suspensions of PLLA-PN-PLLA and PDLA-PN-PDLA: Aqueous suspensions of 1L and 1D were prepared at different polymer concentrations and mixed to study the sol-gel transition of their mixed suspension. For the DLS analysis, a dilute aqueous suspension of each of 1L and 1D was prepared at a concentration of 1.0 wt. %. Table-2 shows the average size of the micelle particles formed in each of the suspensions. The average diameter was 30 nm for both block copolymers.

TABLE-2
AVERAGE PARTICLE SIZES OF THE PLA-PN-PLA SUSPENSIONS AS DETERMINED BY DLS

Copolymer	Diameter (nm)
PLLA-PN-PLLA	35.3 ± 10.6^a
PDLA-PN-PDLA	29.7 ± 8.2^b

^aCoexisting with the particle aggregates (185.8 ± 53.7 nm).
^bCoexisting with the particle aggregates (150.4 ± 43.8 nm).

Fig. 4 shows the typical photographs of the mixed suspension of 1L and 1D (total 5 wt. %) and a single suspension of 1L (5 wt. %) at room (25 °C) and body temperatures (37 °C). The mixed suspension was fluid at 25 °C (a) and turned to turbid gel on heating to 37 °C (b). On the other hand, the single suspension remained fluid even at 37 °C (d). These results clearly demonstrate the temperature-dependent sol-to-gel transition of the mixed suspension.

Fig. 5 shows the sol-gel transition profile with respect to the micelle concentration and temperature for the mixed

TABLE-1
SYNTHESIS OF THE ENANTIOMERIC TRIBLOCK COPOLYMERS PLA-PN-PLA BY RING-OPENING POLYMERIZATION OF L- AND D-LACTIDES IN THE PRESENCE OF PN

Polymer Sample	Structure	Feed ratio (w/s)		Yield (%)	PLA-PN-PLA			
		LA/PN (w/w)	LA/EO/PO (unit ratio)		PLA/PN (w/w)	LA/EO/PO ^{*1} (unit ratio)	M_n^{*1} (Da)	M_w/M_n^{*2}
1L	PLLA-PN-PLLA	25/75	25/59/16	84	18/82	18/65/17	10150	1.08
1D	PDLA-PN-PDLA	25/75	25/59/16	86	18/82	18/65/17	10150	1.1

^{*1}Determined by ^1H NMR, ^{*2}Determined by GPC.

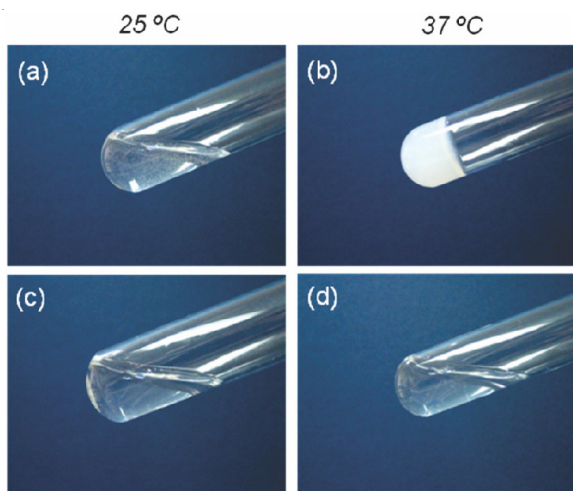


Fig. 4. Phase changes of a mixed suspension of 1L and 1D at (a) 25 °C and (b) 37 °C as compared with those of an aqueous suspension of 1L at (c) 25 °C and (d) 37 °C

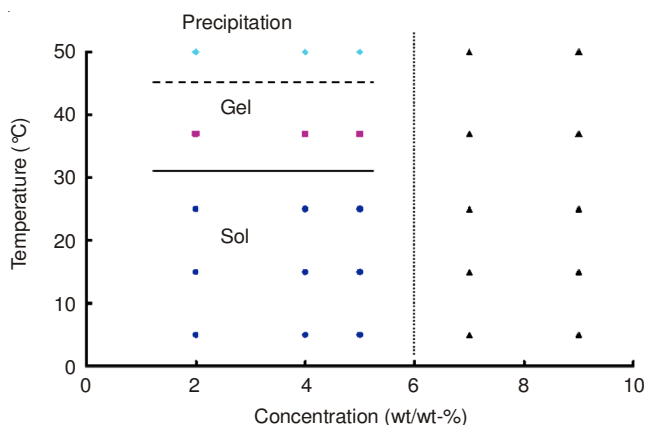


Fig. 5. Sol-gel transition profile of the mixed suspensions of 1L : PLLA-PN-PLLA (900-8400-900) and 1D : PDLA-PN-PDLA (900-8400-900) in saline

suspension of 1L and 1D in saline. The suspensions with higher polymer concentration were too viscous and were not able to mix with each other. At concentration ranging from 2 to 5 wt/wt %, the sol-to-gel transition was induced around the body temperature. In these mixed suspensions the soft gel state formed around the body temperature was converted into a polymer precipitated state above 40 °C with the aqueous medium separated. This may be caused by the dehydration of PN chain which is known to be driven by the LCST phenomenon of PN. Based on this profile, we used the 5 wt/wt % mixed suspension in saline for further study. It was characterized by the short gelation time, being as short as 30 s at 37 °C whom determined by the test tube inverting method. The fast gelation of this system is interesting when compared with the slow gelation of the PLA-PEG-PLA system.

Fig. 6 shows the time-dependent rheological change with the gelation of the above mixed suspension (5 wt/wt %) at 37 °C. It was shown that the storage modulus (G') quickly rose up to 0.8 kPa, which is similar or even higher compared to that of the PLA-PEG-PLA system. Since G' became higher than G'' ($G' > G''$) at the start of the measurement, the gelation was initiated at the mixing stage as in the PLA-PEG-PLA system.

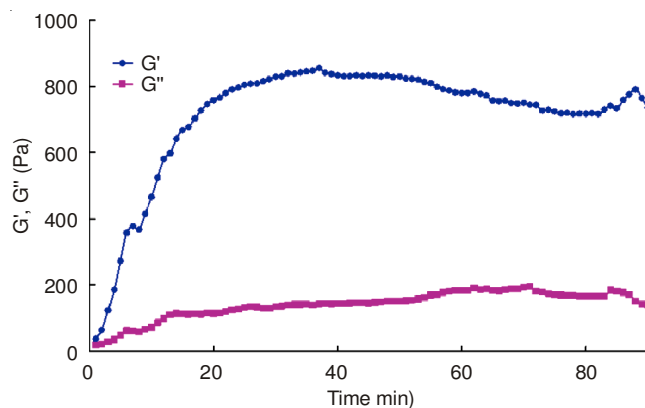


Fig. 6. Rheological changes of a mixed suspension (5 wt/wt %) of 1L and 1D in saline at 37 °C as a function of time

Fig. 7 shows the temperature-dependent rheological change of the same mixed suspension (5 wt/wt %). An abrupt increase in G' was observed from 30 °C by the sol-to-gel transition, followed by a decrease in G' above 40 °C by the gel-to-precipitate transition. This behaviour was completely different from the PLA-PEG-PLA systems, in which G' did not decrease with the temperature increase. We think that the gelation mechanism of the present PLA-PN-PLA system may not directly driven by the stereocomplexation of the PLLA and PDLA block segments although the enantiomeric interaction of PLLA and PDLA segments may influence the hydrophobic/hydrophilic balance of PN to induce the chain dehydration at lower temperature. The precipitation behaviour may be driven by the strong stereocomplexation to make the phase change irreversible.

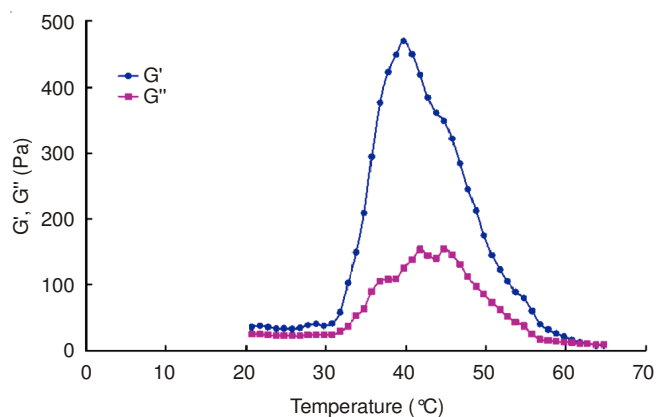


Fig. 7. Rheological changes of a mixed suspension (5 wt/wt %) of 1L and 1D in saline with a heating rate of 1 °C/min

Conclusion

The enantiomeric triblock copolymers, PLLA-PN-PLLA and PDLA-PN-PDLA, were prepared by ring-opening polymerization of L- and D-lactides in the presence of PN. The aqueous suspension of each of the copolymers was prepared and to prepare a mixed suspension of the enantiomeric block copolymers. The mixed suspension was found to occur the sol-gel transition at the body temperature (37 °C). It was characterized by the short gelation time as short as 30 s at 37 °C. Thus, it was suggested that this system is promising for use as the scaffold cell injection.

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