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Fabrication and Characterization of Poly(L-lactic acid) Gels Induced by Fibrous Complex Crystallization with Solvents

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Abstract

Complex-crystal induced gelation of poly(L-lactic acid) (PLLA) solutions was studied for a series of solvents, including *N,N*-dimethylformamide (DMF). By cooling the solutions prepared at elevated temperatures, PLLA gels were produced in solvents that induced complex crystals (ϵ -crystals) with PLLA. Fibrous structure of PLLA in the gel with DMF was observed by polarizing optical microscopy, field emission electron microscopy, and atomic force microscopy. Upon heating, the crystal form of PLLA in

the DMF gel changed from ϵ -crystal to α -crystal, the major crystal form in common untreated PLLA films, but the morphology and high elastic modulus of the gel remained until the α -crystal dissolved at higher temperature. In addition, a solvent exchanging method was developed, which allowed PLLA gels to be prepared in other useful solvents that do not induce ϵ -crystals without losing the morphology and mechanical properties.

1. Introduction

Poly (L-lactic acid) (PLLA) has been attracting industrial interest as an environmentally friendly polymer because it can be produced by plants, and the amount of carbon dioxide needed for its synthesis is relatively small [1,2]. This characteristic has stimulated not only industrial research of PLLA, but also basic studies on its physical and chemical properties. Crystallization behavior is one of the important physical properties for polymer materials and dominates their mechanical properties. PLLA is a semi-crystalline polymer, and its complicated crystallization behavior has been studied intensely. PLLA films without any special treatments form α -crystals [3-7], but stretched or stroked ones forms β -crystals [8,9], and epitaxial crystallization in hexamethylbenzene induces γ -crystals [10]. Crystals with structures slightly different from that of α -crystals, which are called α' [11-13] and α'' -crystals [14] are also prepared by annealing below 120°C and under high pressurized carbon dioxide, respectively.

Recently, Marubayashi et al. [15,16] discovered a new crystal form of PLLA called as “ ϵ -crystals” in PLLA films which were immersed or exposed to the vapor of some particular solvents such as *N,N*-dimethylformamide (DMF), cyclopentanone (CPO),

γ -butyrolactone (GBL), 1,3-dioxolane (DOL), and tetrahydrofuran (THF). Based on the precise X-ray analysis, they concluded that PLLA and the solvents formed complexes in ϵ -crystals. Tonelli et al. [17-19] investigated inclusion compounds formed by PLLA and some low molecular weight compounds such as urea and cyclodextrins. There have been a few reports of the formation of complex crystals with some polymers and specific solvents. Guenet, Daniel, and their coworkers characterized the complex crystals of syndiotactic polystyrene with benzene, [20] toluene, [21] chloroform, [21] 1,2-dichloroethane, [22] 1-chloropropane [22] , and naphthalene [23] by X-ray diffraction and infrared spectroscopy. They also found that syndiotactic polystyrene was gelled in these solvents.

Gels are widely used as soft materials encountered in daily life like foods, as structural materials for buildings, and as functional materials for medical purposes. Because PLLA can be decomposed into L-lactic acid and eliminated from the body by the common metabolic pathway and thus has no toxicity, it can be used safely in living bodies, and thus has enjoyed increasing usage in applications such as sutures and in bone fixation. Some fundamental research on the formation of PLLA gels has been carried out. Ma et al. [24] dissolved PLLA into THF, and prepared PLLA gels induced by nano-sized fibers formed during the phase separation processes. They reported the morphologies of the gels prepared under different conditions, such as PLLA concentration, gelation temperature, and gelation time. Shao et al. [25] also observed the nano-sized fibers formed in the early stage of the gelation for a gel prepared by a procedure similar to that proposed by Ma et al. [24]

In our previous letters, [26,27] we succeeded in forming PLLA gels by simply dissolving PLLA in solvents which were reported by Marubayashi et al. to induce the

formation of ϵ -crystals (ϵ -crystallization) [15,16], and cooling the solutions to room temperature.

In this report, the results of polarizing optical microscopy (POM), field emission scanning electron microscopy (FE-SEM), and atomic force microscopy (AFM) are shown to elucidate more detailed morphology of PLLA in DMF. The change of the morphology, crystal form, and rheological behavior upon heating the gel is also investigated. The microstructures of PLLA and DMF are studied by infrared spectroscopy and dielectric measurements. It is shown that gels with high heat resistivity and low toxicity could be prepared by exchanging DMF with less harmful solvents after gel formation. The gelation mechanism and the fibrous structure of PLLA in the gels are also discussed based on the obtained experimental data.

2. Experimental

2.1 Sample preparation

A product of Polysciences (catalog number 21512) was mainly used in this study, and its weight-average molecular weight and melting temperature were reported to be 7×10^5 and 179°C by the supplier. PLLA sample with lower molecular weight was used to prepare only the THF solution because THF was not a good enough solvent to dissolve PLLA with high molecular weight. A product of Polymer Source (sample number P6691-LA) was purchased for this purpose. The weight-average molecular weight and polydispersity index were reported to be 5.30×10^4 , and 1.38 by the supplier. Five organic solvents which are reported to induce PLLA films to form ϵ -crystals by Marubayashi et al. [15] and three organic solvents; *N,N*-dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), and acetic anhydride (ACA) were purchased from Wako

Pure Chemical Industries, Tokyo Chemical Industry, Kanto Chemical, or Sigma Aldrich.

Water purified with a Direct-Q 3 UV system (Millipore) was used in this study.

PLLA samples were dissolved in solvents on a hot plate/stirrer heated at different temperatures, depending on the solvents as shown in Table 1. The temperatures of the solutions on the hot plate/stirrer were measured with an infrared thermo camera (Testo, 875-2i). After cooling the solutions, some solutions formed gels. In this study, samples which did not flow for a day by inverting their solutions in the cylinder-shaped sample bottles with a diameter of 17 mm were considered to be gelated.

solvent	solution temperature when dissolved	crystal form	gelation
DMF	113°C	ϵ	gelated
CPO	104°C	ϵ	gelated
GBL	113°C	ϵ (crystalized < 5°C) α (crystalized > 5°C)	Gelated (crystalized < 5°C) precipitated (crystalized > 5°C)
DOL	61°C	not measured for gels , but ϵ for films	gelated (crystalized < -18°C) solution (crystalized > -18°C)
THF	61°C	not measured for gels , but ϵ for films	gelated
DMSO	120°C	α	precipitated
DMA	113°C	α	precipitated

ACA	113°C	α	precipitated
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Figure 1. Crystal forms and gelation behavior of PLLA in various solvents. The weight fraction of PLLA in the solutions was fixed to be 5 wt%. All the experiments in this table were conducted for the sample with molecular weight of 7×10^5 , except the sample with molecular weight of 5.30×10^4 was used for the experiments using THF.

The solvents of DMF gels were exchanged with other solvents as follows. A DMF solution of PLLA was poured into a breakable container, and cooled in a freezer controlled at -18°C for gelation. The container was broken and taken apart to remove the gel, and the gel was immersed in another solvent. If the gel remained without collapsing, the gel was immersed in the solvent overnight, and the solvent was changed to fresh solvent three times. Infrared spectroscopy for the gels after the solvent exchange was carried out to verify the substitution of solvents.

Because gels prepared by dissolving 5 wt% of PLLA with molecular weight of 7×10^5 in DMF were mainly used for the experiments, the gels prepared by this procedure will be discussed in the following sections unless other gels are specifically mentioned.

2.2 Measurements

X-ray diffraction (XRD) was carried out with a RINT-2200 and Mini Flex 300 of Rigaku. The current and voltage of the filament in the RINT-2200 were 40 mA and 40 kV. The width of the receiving slit in the RINT-2200 was 0.80 mm. The current and

voltage of the filament in the Mini Flex 300 were 10 mA and 30 kV. The widths of the divergence, scattering, and receiving slits in Mini Flex 300 were 1.25 mm, 1.25 mm, and 0.3 mm, respectively. The X-ray source of both of the two instruments was CuK α radiation with wavelength of 0.154 nm. The scan speed was fixed to be 2°/min. When the sample temperature needed to be controlled, the sample was cooled in a refrigerator controlled at 5°C or heated on a hot stage (type MHS-2000) with a digital thermo controller (type ES100-P) purchased from Imoto Machinery, and the measurements were carried out immediately. To observe exactly the same portion of a gel sample before and after exchanging the solvent from DMF to water, an excess amount of water was dropped onto the sample and oozed solvent was wiped away after the measurement of the DMF gel. Dropping water and wiping away the oozed solvent was repeated three times to ensure the solvent exchange.

Polarizing optical microscopy (POM) was carried out with a Labophotal-pol microscope (Nikon) equipped with the same hot stage used for XRD to control the sample temperature. The samples were put on a glass plate and pressed with a cover glass. The heating rate of the sample was fixed to be 1°C/min. Because it was difficult to measure the sample temperatures directly during the POM observations, they were estimated from the temperature of the hot stage displayed at its controller as follows. A gel sample similar to the sample for the POM was prepared, and a thermocouple was attached to this sample. The temperature was calibrated using the relation between the temperatures displayed in the hot stage and that of the thermocouple. To observe the exactly same portion of a gel sample before and after exchanging the solvent from DMF to water, the solvent was exchanged by the same procedure used for XRD. The intensity of light source was kept constant when changing temperature or solvents. The

concentration of the solutions used for POM was fixed at 5 wt%.

Field emission scanning electron microscopy (FE-SEM) was carried out with a JSM-7001F microscope (JEOL). The solvent of the DMF gel was exchanged with water that was subsequently removed by freeze-drying, and the surface of the sample was coated with gold at a thickness of about 5 nm, for the observation of the structure of the DMF gel. The precipitate of PLLA in DMA was collected, dried in vacuo, and the surface was coated by the same procedure used for the DMF gel. The concentration of the solutions used for FE-SEM was 5 wt%.

Atomic force microscopy (AFM) was carried out with a Nanoscope IIIa Microscope (Veeco) equipped with a multi-mode controller using a tapping mode. ACT type tips which were pyramidal-shaped non-coated silicon tips and purchased from App Nano were used in this study. The spring constant, frequency, length, width, thickness, and radius of the curvature reported by the supplier were 37 N/m, 300 kHz, 125 μm , 30 μm , 4 μm , and 6 nm, respectively. The scan rate was approximately 2 $\mu\text{m/s}$. PLLA (0.5 wt%) was dissolved in DMF or DMA on a hot plate/stirrer heated to 130°C. Approximately 2 μl of the solution was dropped onto a mica plate with diameter of 9.9 mm purchased from Ted Pella, and dried before the measurements. The mica plates were freshly cleaved just before dropping the solutions.

Viscoelastic measurements were performed with a PZ-Rheo NDS-1000 (TAISEI). The thickness of the samples and measurement frequency were fixed to be 1 mm and 1.023 Hz. The sample temperature was controlled with a hot stage installed in the viscometer when measurements were carried out below 100°C. For measurements above 100°C, the temperature was controlled with another hot stage which was also used for XRD and POM, because the hot stage installed in the viscometer could not heat

above 100°C. The sample temperature was monitored with a thermocouple. The heating rates were 0.75°C/min for DMF gel, 3.0°C/min for the hydrogel, and 1.6°C/min for the glycerin gel.

Fourier transform infrared spectroscopy (FT-IR) was carried out with an FT/IR-4100 (JASCO). A type DX2010 diamond cell (Sumitomo Electric Hardmetal) was used for the measurements for gel samples and neat DMF. The resolution and scan number were 2 cm⁻¹ and 32, respectively. To investigate the FT-IR spectra of the gel sample after heating at the temperature inducing the change of crystal forms, the gel sample was spread on a glass plate, and put on the hot plate/stirrer at 45°C for 30 sec. The FT-IR spectrum for PLLA without any solvents was measured for PLLA film prepared by dropping a DOL solution containing 1wt% of PLLA on a silicon wafer. After drying the DOL in the air, the film was put on a hot plate/stirrer and heated to 180°C for 3 min to remove DOL completely. The absence of DOL was confirmed by the disappearance of the peak of DOL at 3000 cm⁻¹.

Dielectric measurements of the DMF gel and DMA solution were carried out by using a PNA network analyzer (E8364C, Agilent) with a slim form probe (85070E) in the frequency range of 20 MHz-50 GHz. The dielectric experiments for the DMF gel were performed at 2°C in a cold bath of ice-water mixture. The experiments for DMA solution were carried out at 110°C in an oil bath on a hot stage. The sample temperatures were monitored with a K-type thermocouple.

3. Results and discussion

3.1 Morphology and gelation behavior of PLLA in various solvents

Figure 1 shows the XRD patterns of PLLA in various solvents. Marubayashi et al.

[15,16] reported that PLLA films exposed to DMF or GBL formed ϵ -crystals, but not DMA. While the XRD patterns of PLLA in DMA and GBL cooled at 20°C were the typical patterns of α -crystals of PLLA, those in DMF and GBL cooled at 5°C were very similar to the patterns for PLLA films forming ϵ -crystals in the paper of Marubayashi et al. [15,16] The Miller indices of α - and ϵ -crystals shown in Figure 1 were assigned according to the previous reports by Bouapao et al. [28] and Marubayashi et al. [15,16], respectively.

Figure 1. X-ray diffraction patterns of PLLA in DMF, GBL, and DMA cooled at the temperature shown in the figure for crystallization.

Table 1 summarizes the crystal forms and gelation behavior of PLLA in various solvents. DMF, CPO, GBL, DOL and THF are solvents which Marubayashi et al. [15,16] reported to induce the ϵ -crystallization of PLLA films, and DMSO, DMA, and ACA are not. The DMF, CPO and THF solutions of PLLA were gelled when they were

cooled at room temperature, 5°C, and -18°C, respectively. The GBL solutions cooled at 5°C and -18°C were gelated, and their XRD patterns indicated the formation of ϵ -crystals, while the GBL solution cooled at 20°C were not gelated, and the XRD pattern for the precipitate was the typical patterns for α -crystals. We could not measure the XRD pattern of PLLA in DOL because the DOL solution was gelated only below -18°C, and became a solution above -18°C. However, because Marubayashi et al. [15,16] reported that PLLA film exposed to DOL formed ϵ -crystals, DOL also have the ability to induce ϵ -crystallization. In the three solvents which were reported not to form ϵ -crystals, no gels were formed by the procedure mentioned in the experimental section. These experimental data suggest that ϵ -crystals promote the gelation of PLLA.

The POM images for PLLA gel prepared from a DMF gel and those for PLLA precipitates prepared from a DMA solution are shown in Figure 2. The images for PLLA in DMF clearly indicate the existence of spherulites and a fibrous structure. Crystals were observed in the images for PLLA in DMA, but they formed not a fibrous, but a spherulitic structure. The morphology of PLLA in DMF and DMA was also revealed by FE-SEM as shown in Figure 3. While a network of sub-micron fibers of PLLA was observed in the freeze-dried sample of the DMF gel, PLLA in the precipitate collected from the DMA solution formed spherical particles with wrinkles. The morphologies of PLLA in DMF and DMA solutions with much lower concentration were investigated by AFM. While PLLA in DMF formed entangled fibers in Figure 4, spherical particles of PLLA in DMA were observed in Figure 5. The images of POM, FE-SEM, and AFM support the formation of sub-micron fibers entangled in DMF, and spherical particles with wrinkles in DMA. This difference of the morphology can affect the gelation behavior of PLLA in different solvents.

Figure 2. Polarizing optical micrographs of PLLA in DMF and DMA at room temperature.

Figure 3. Field emission electron micrographs of PLLA in DMF and DMA.

Figure 4. Atomic force micrographs of PLLA in DMF. (a) and (c) are height images, and (b) and (d) are phase images.

Figure 5. Atomic force micrographs of PLLA in DMA. (a) and (c) are height images, and (b) and (d) are phase images.

3.2 Temperature dependence of the morphology and viscoelastic behavior of the DMF gel

To investigate the temperature dependence of the morphology and mechanical properties of the PLLA gels, and understand the influence of the morphology on the mechanical properties, POM, XRD, and viscoelastic measurements were carried out at various temperatures. Figure 6, which is a rearranged figure of Figure 3 in reference 27, summarizes POM images taken for the same position of the DMF gel by heating at 1°C/min. The intensity of the birefringence decreased at 27-42°C, increased at 42-46°C, decreased again above 46°C, and finally disappeared at 69°C. It should be noted that the shapes and positions of the spherulites were exactly same before and after the decrease of the birefringence around 40°C. If the crystals which formed below 40°C had completely melted and PLLA had crystalized again above 45°C, the shapes and positions of the spherulites above 45°C would have been different from those below 40°C. The results in Figure 6 indicate that the spherulites did not melt even when the birefringence became very weak around 40°C. The disappearance of the birefringence at 69°C was caused by the disappearance of PLLA crystals by dissolving into DMF.

Figure 6. Polarizing optical micrographs of PLLA in DMF at various temperatures.

The crystal form of PLLA during the decrease of the birefringence around 40°C was investigated by the XRD patterns shown in Figure 7. The XRD patterns of PLLA in DMF gel at 34 and 46°C appear typical patterns of ϵ - and α -crystals, respectively. Peaks assigned as those of ϵ - and α -crystals coexist in the pattern at 44°C, and this behavior well agrees with the results obtained in Figure 6 that the crystals never melted during the decrease of the birefringence around 40°C. It should be noted that Marubayashi et al.

[16] also reported that the coexistence of α - and ϵ -crystals in PLLA films during the desorption of solvents inducing ϵ -crystallization and exchange of the guest molecules. Because it was clarified that the optical sign of the spherulites of ϵ -crystals was opposite with that of α -crystals by the POM using a sensitive color plate in Figure 15, the decrease of birefringence around 40°C can be explained by the offset of the birefringence of ϵ - and α -crystals.

Figure 7. X-ray diffraction patterns of PLLA in DMF at various temperatures.

Viscoelastic measurements were carried out for the DMF gel to investigate the influence of the change of PLLA crystals on the viscoelastic behavior of the gel. G' and G'' in Figure 8 represent the storage modulus and loss modulus, respectively. G' decreased slightly at 45°C, and drastically at 65°C where the sample began to flow. The temperatures of 45 and 65°C are the temperatures at which ϵ -crystals changed to

α -crystals, and the temperatures at which the birefringence disappeared by dissolving of PLLA to DMF, respectively.

Figure 8. Viscoelastic behavior of PLLA in DMF measured by heating.

Combining the experimental results of POM, XRD, and viscoelastic measurements, the temperature dependence of the structure and viscoelasticity can be summarized as follows. By heating the DMF gel, ϵ -crystals changed to α -crystals around 45°C. Both α - and ϵ -crystals coexisted at this temperature, and the fibrous morphology remained unchanged, but the birefringence became very weak because of the opposite signs of birefringence of α - and ϵ -crystals. The slight decrease of the storage modulus of the gel at 45°C might be caused by the change of the crystal form. All the crystals in the gel dissolved and the storage modulus decreased drastically above 65°C, for there remained no structure to prevent the solvent from flowing.

3.3 Microstructures of PLLA and DMF in the gel

To further understand the interaction and conformation of PLLA and DMF in the gels, infrared spectroscopy and dielectric measurements were carried out for the samples that could form complex crystals, as well as those with no capability of forming complexes. Our investigation was focused on the infrared bands around 1760 cm^{-1} and 1680 cm^{-1} which were assigned as carbonyl peaks of PLLA and DMF, respectively. The carbonyl peaks of PLLA in the gels shown in Figure 9 were sharper than that of PLLA film without solvents. Meaurio et al. [29,30] divided the carbonyl peak of the α -crystals of PLLA into four peaks; gauche-gauche interphase around 1776 cm^{-1} , trans-gauche interphase around 1767 cm^{-1} , gauche-trans crystalline and interphase around 1759 cm^{-1} , trans-trans interphase around 1749 cm^{-1} . Because the crystal form of PLLA in our gels was not α -crystal but ϵ -crystal, and we have not assigned detailed infrared peaks in ϵ -crystals, the peaks could not be separated. However, the change of the carbonyl peaks of PLLA suggests the decrease of gauche-gauche, trans-gauche, and trans-trans interphases by forming the gel. The carbonyl peaks of DMF were also slightly sharpened and shifted to smaller wavenumber by forming the gels. DMF molecules without PLLA form dimers [31] and the change of their carbonyl groups of DMF in the gel suggests the dissociation of the dimers and formation of other structures induced by interaction with PLLA. Although other peaks of DMF showed no significant change, some other peaks of PLLA [32-36] were slightly sharpened, but their peak wavenumbers were not changed by forming the gels.

Figure 9. Infrared spectra of the gels formed by PLLA and DMF with various concentrations.

Figure 10 shows the difference of the infrared spectra of PLLA gel (0.5 wt%) before and after heating at 45°C, at which the ϵ -crystals changed to α -crystals. The peak of the carbonyl groups in DMF changed to a peak similar to that of neat DMF. This behavior clearly indicates the existence of some interaction between DMF and PLLA when PLLA forms ϵ -crystals, and supports the formation of complex crystals in the DMF gel. The peak of the carbonyl groups in PLLA shifts slightly to larger wavenumber upon heating, which suggests structural change of the carbonyl groups induced by the dissociation of the complex crystals with DMF.

Figure 10. Infrared spectra of the PLLA gels (0.5 wt%) before and after heating the gel at 45°C.

High frequency dielectric measurements were performed to the DMF gel, DMA solution as well as the corresponding neat solvents to investigate the influence of solvent on the complex crystal formation. Figure 11 (a) compares the dielectric spectra of the DMF gel containing 5 wt% of PLLA and that of the neat DMF, where the real (ϵ') and imaginary (ϵ'') parts of permittivity are shown as a function of frequency. The main dielectric relaxation in the frequency window is due to the rotational motion of the DMF molecule. The low-frequency plateau value of ϵ' defines the dielectric constant of the samples. Figure 11 (a) reveals that the dielectric constant of the DMF gel as well as the peak value of ϵ'' are about 11% and 16% lower than those of the neat DMF, respectively. In contrast, no such significant difference was detected for the DMA solution and the neat DMA, as shown in Figure 11 (b). When DMF molecules are bound to PLLA chains by forming the complex crystals, it is expected that these molecules will “disappear” from the dielectric spectrum. The significant decrease of ϵ' and ϵ'' in the

DMF gel supports the existence of strong interaction between DMF and PLLA in the gel.

Figure 11. Dielectric spectra of (a) the DMF in the PLLA gel and solvent without PLLA, and (b) the DMA in the PLLA solution and solvent without PLLA.

3.4 Morphology and viscoelastic behavior of the gel prepared by solvent exchange

Figures 6-8 indicate that once a network of fibers of ϵ -crystals is formed, the gel does not flow even after the ϵ -crystals change to α -crystals unless α -crystals are dissolved out. This result suggests the possibility that the solvent of the gel is exchangeable without collapsing the gel after a network of fibers of ϵ -crystals is formed. Because the number of solvents that can induce ϵ -crystallization is limited, this solvent exchanging method is extremely useful in the sense that it substantially broadens the choice of solvent for making PLLA gels. Table 2 summarizes the results of trying to change the solvent of the DMF gel to another solvent by the procedure described in the

experimental section. Hansen solubility parameters of the solvents [37] and the distance between the parameter of PLLA [2] and that of solvents are also tabulated to investigate the relation between the affinity of the solvents to PLLA and exchangeability of the solvents. The dispersion, polar, and hydrogen bond parts of the Hansen solubility parameters were designated as δ_D , δ_P , and δ_H . A smaller difference between the Hansen solubility parameters of a solvent and that of PLLA suggests that the solvent has a higher affinity for PLLA. The states of the gels after exchanging the solvents were classified as opaque gels, transparent gels, and collapsed gels. Photographs of some typical gels are shown in Figure 12 as examples. Although the gels tended to collapse or became transparent by replacing DMF by solvents with small differences of Hansen solubility parameters, and to remain opaque by replacing DMF by solvents with large differences of Hansen solubility parameters, there were many exceptions to this tendency. The affinity of a solvent to PLLA which is predicted by Hansen solubility parameters can be one of the factors determining the exchangeability, but it can be concluded that the affinity of a solvent to PLLA is not the primary factor and the sizes and shapes of solvents which were reported to be ones of the factors to determine the formation of ϵ -crystals [15] might also influence the exchangeability. The dominating factor for the exchangeability will be investigated in our future studies.

solvent	$\delta_D /$ MPa ^{1/2}	$\delta_P /$ MPa ^{1/2}	$\delta_H /$ MPa ^{1/2}	distance / MPa ^{1/2}	after exchanging solvents
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CPO	17.9	11.9	5.2	2.6	collapsed
nitrobenzene	20.0	8.6	4.1	3.6	transparent
DOL	18.1	6.6	9.3	4.8	collapsed
DMA	16.8	11.5	10.2	5.8	transparent
THF	16.8	5.7	8.0	5.9	transparent
acetone	15.5	10.4	7.0	6.3	collapsed
GBL	19.0	16.6	7.4	6.9	transparent
ACA	16.0	11.7	10.2	6.9	opaque
DMSO	18.4	16.4	10.2	7.7	collapsed
methyl salicylate	18.1	8.0	13.9	8.2	collapsed
1,4-dioxane	19.0	1.8	7.4	8.3	collapsed
propylene carbonate	20.0	18.0	4.1	8.8	transparent
toluene	18.0	1.4	2.0	9.5	collapsed
cyclohexanol	17.4	4.1	13.5	9.8	collapsed
2-buthanol	15.8	5.7	14.5	11.0	opaque
acetic acid	14.5	8.0	13.5	11.3	opaque
1-buthanol	16.0	5.7	15.8	11.9	opaque
2-propanol	15.8	5.2	16.4	12.7	opaque
1-propanol	16.0	6.1	17.4	13.1	opaque
ethanol	15.8	8.8	19.4	14.6	opaque
ethylene glycol	17.0	11.0	26.0	20.3	transparent
formamide	17.2	26.2	19.0	21.0	transparent
glycerin	17.4	12.1	29.3	23.5	transparent
water	15.5	16.0	42.3	37.3	opaque

Tabel 2. Results of solvent exchange and Hansen solubility parameters.

Figure 12. Photographs of typical three gels after the solvent exchange; (a) an opaque gel (exchanged to water), (b) a transparent gel (exchanged to glycerin), and (c) a collapsed gel (exchanged to toluene)

The crystal form and morphology of PLLA before and after changing the solvent from DMF to water are presented in Figures 13 and 14, respectively. While the XRD patterns in Figure 13 clearly indicate that the ϵ -crystals in the DMF gel changed to α -crystals, the morphology of the crystals in Figure 14 showed no significant changes upon exchanging the solvent. Although POM images after exchanging the solvent were slightly darker due to the increased turbidity of the gel, the shapes and positions of spherulites after exchanging the solvent were exactly same as those before exchanging the solvent. As shown in Figures 6 and 7, the ϵ -crystals changed to α -crystals, but no significant change of the morphology was observed in the DMF gel by heating it above 45°C. The changes of the morphology and crystal form of PLLA which were induced by the solvent exchange were similar to those induced by heating. POM images taken with a sensitive color plate indicate that the sign of the spherulites before exchanging the

solvent was positive, and that after exchanging the solvent was negative. As mentioned before, while α - and ϵ -crystals coexisted in the DMF gel around 45°C, the birefringence became very weak. This behavior can be explained by the opposite signs of the spherulites of α - and ϵ -crystals shown in Figure 14.

Figure 13. X-ray diffraction patterns of PLLA before and after exchanging the solvent from DMF to water.

Figure 14. Polarizing optical micrographs of the PLLA gels before and after exchanging the solvent from DMF to water. The black or white bars in the images indicate 100 μm .

Figure 15 (a) shows the viscoelastic behavior of the gels prepared by exchanging DMF to water. While G' of the DMF gel before the solvent exchange slightly decreased at 45°C, and the sample began to flow at 65°C due to the change of crystal form and dissolution of the crystals, respectively G' of the gel after exchanging the solvent to water showed no significant decrease and did not flow even at 80°C. It is reasonable that G' of the gel after the solvent exchange did not decrease at 45°C because its XRD pattern clearly indicated that PLLA formed α -crystals even at room temperature. Table 2 shows that the solubility of water to PLLA is much poorer than that of DMF, and the poor solubility of water might be the reason why the gel after the solvent exchange remained gel at 80°C.

Figure 15. Viscoelastic behaviors of PLLA gels of which solvent was exchanged from DMF to (a) water, and (b) glycerin.

Because it was difficult to measure the viscoelastic behavior of the gels after exchanging DMF to water without drying the solvent above 80°C, the viscoelastic behavior of the gel prepared by exchanging DMF to glycerin, which has high boiling temperature and poor solubility to PLLA, was measured as shown in Figure 15 (b). G' of the gel decreased drastically at 140°C which was about 75°C higher than the temperature at which the gel before the solvent exchanging began to flow. Although residual water in glycerin can decrease the melting temperature of the gel because of the hydrolytic degradation, it is clear that the gel prepared by exchanging DMF to glycerin has significantly higher melting temperature than the gel without solvent exchange.

The results obtained in Table 2 and Figures 13-15 indicate that the solvent of the gel of PLLA and DMF can be exchanged to some other solvents without collapsing the gel once a network of fibers of PLLA is formed. Although the number of the solvents inducing ϵ -crystallization is limited, PLLA gels can be prepared easily in various solvents by forming gels in solvents that induce ϵ -crystallization and subsequently exchanging the solvents.

4. Conclusions

A new method of preparing PLLA gels has been demonstrated: by dissolving PLLA at elevated temperatures in solvents that can induce ϵ -crystallization, and subsequently cooling the solutions, PLLA gels could be produced. The ability of forming complex crystals with PLLA has been shown to be critical for the formation of gels. The same procedure cannot produce PLLA gels when solvents (e.g. DMA) that do not induce ϵ -crystals are used. The images obtained using POM, FE-SEM, and AFM indicate that

PLLA form a network of fibers in DMF while globular particles are observed in DMA. The results of FT-IR and dielectric measurements indicate that the interaction between PLLA and DMF changes the mobility and distribution of DMF molecules in the gels, while no significant change can be observed in the DMA solution.

In addition, the thermal stability of the PLLA gels has been investigated. It is shown that ϵ -crystals transform into α -crystals upon heating, but no morphological changes could be observed because the two types of crystals coexist during the crystal form change. The gel begins to flow only after the melting of the α -crystals at higher temperature.

Lastly, it has also been demonstrated that the solvent of the gel of PLLA formed with DMF could be exchanged with some other solvents such as water by simply immersing the gel into the new solvents, for the ϵ -crystals can change to α -crystals without losing the morphology during the solvent exchange. The melting temperature of the gel increases after the exchange, probably because of the poorer solubility of PLLA in water. This new solvent exchange method allows the preparation of PLLA gels in solvents that do not induce ϵ -crystallization and can serve as a new general strategy for producing PLLA gels for various applications.

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7. References

- [1] Tsuji H, Polyesters III: Application and Commercial Products in Biopolymers: Biology, Chemistry, Biotechnology, Applications, Doi Y, Steinbüchel A, editors. Wiley-VCH, 2002.
- [2] Auras R, Lim L-T, Selke S E M, Tsuji H, Poly(lactic acid), John Wiley & Sons, 2010.
- [3] De Santis P, Kovacs A J, Biopolymers 1968:6:299-306.
- [4] Hoogsteen W, Postema A R, Pennings A J, Ten Brinke G, Zugenmaier P, Macromolecules 1990:23:634-42.
- [5] Aleman C, Lotz B, Puiggali J, Macromolecules 2001:34:4795-801.
- [6] Sasaki S, Asakura T, Macromolecules 2003:36:8385-90.
- [7] Wasanasuk K, Tashiro K, Hanesaka M, Ohara T, Kurihara K, Kuroki R, Tamada T, Ozeki T, Kanamoto T, Macromolecules 2011:44:6441-52.
- [8] Eling B, Gogolewski S, Pennings A J, Polymer 1982:23:1587-93.
- [9] Puiggali J, Ikada Y, Tsuji H, Cartier L, Okihara T, Lotz B, Polymer 2000:41:8921-30.
- [10] Cartier L, Okihara T, Ikada Y, Tsuji H, Puiggali J, Lotz B, Polymer 2000:41:8909-19.
- [11] Zhang J, Duan Y, Sato H, Tsuji H, Noda I, Yan S, Ozaki Y, Macromolecules 2005: 38:8012-21.
- [12] Zhang J, Tashiro K, Domb A J, Tsuji H, Macromol. Symp. 2006:242:274-8.
- [13] Wasanasuk K, Tashiro K, Polymer 2011:52:6097-109.

- [14] Marubayashi H, Akaishi S, Akasaka S, Asai S, Sumita M, *Macromolecules* 2008;41:9192-203.
- [15] Marubayashi H, Asai S, Sumita M, *Macromolecules* 2012;45:1384-97.
- [16] Marubayashi H, Asai S, Sumita M, *J. Phys. Chem. B* 2013;117:385-97.
- [17] Tonelli A E, *Polymer* 1994;35:573-9.
- [18] Rusa C C, Wei M, Shuai X, Bullions T A, Wang X, Rusa M, Uyar T, Tonelli A E, *J. Polym. Sci. Part B Polym. Phys.* 2004;42:4207-24.
- [19] Tonelli A E, *Macromol. Symp.* 2003;203:71-87.
- [20] Daniel C, Deluca M D, Guenet, J-M, Brûlet A, Menelle, A, *Polymer* 1996;37:1273-80.
- [21] Daniel C, Menelle A, Brûlet A, Guenet J-M. *Polymer* 1997;38:4193-99.
- [22] Daniel C, Guerra, G, Musto P, *Macromolecules* 2002;35:2243-51.
- [23] Malik S, Rochas C, Guenet J M, *Macromolecules* 2005;38:4888-93.
- [24] Ma P X, Zhang R, *J. Biomed. Mater. Res.* 1999;46:60-72.
- [25] Shao J, Chen C, Wang Y, Chen X, Du C, *React. Funct. Polym.* 2012;72:765-72.
- [26] Matsuda Y, Fukatsu A, Tasaka S, *Chem. Lett.* 2012;41:1420-1.
- [27] Matsuda Y, Fukatsu A, Tasaka S *Chem. Lett.* 2013;42:1046-7.
- [28] Bouapao L, Tsuji H, Tashiro K, Zhang J, Hanesaka M, *Polymer* 2009;50:4007-17.
- [29] Meaurio E, Zuza E, López-Rodríguez N, Sarasua J R, *J. Phys. Chem. B* 2006;110:5790-800.
- [30] Meaurio E, Martines de Arenaza I, Lizundia E, Sarasua J R, *Macromolecules* 2009;42:5717-27.
- [31] Ohtaki H, Itoh S, Yamaguchi T, Ishiguro S, Rode B M, *Bull. Chem. Soc. Jpn.* 1983;56:3406-9.

- [32] Jarmelo S, Marques D A S, Simoes P N, Carvalho R A, Batista C M S G, Araujo-Andrade C, Gil M H, Fausto R, J. Phys. Chem. B 2012;116:9-21.
- [33] Auras R, Harte B, Selke S, Macromol. Biosci. 2004;4:835-64.
- [34] Kister G, Cassanas G, Vert M, Polymer 1998;39:267-73.
- [35] Kister G, Cassanas G, Vert M, Pauvert B, Térol A, J. Raman Spectrosc. 1995;26:307-11.
- [36] Garlotta D J. Polymer Environ. 2001;9:63-84.
- [37] Hansen M H, Hansen Solubility Parameters A User's Handbook, CRC Press, 2007.

ϵ -crystal

α -crystal