

Synthesis and Degradation Behaviors of PEO/PL/PEO tri-block Copolymers[†]

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Received Jan. 4, 2002 ; Revised Mar. 21, 2002

Abstract : Poly(ethylene oxide)/polylactide/poly(ethylene oxide) (PEO/PL/PEO) tri-block copolymers, which each block is connected by ester bond, were synthesized by coupling reaction of PL with PEO in the presence of pyridine. PL/PEO/PL tri-block copolymer was synthesized by ring opening polymerization of L-lactide initiated by PEO in the presence of stannous octoate. Degradation behavior of the copolymers was investigated in a pH 7.4 phosphate buffer saline (PBS) at 37 ± 1 °C. Gel permeation chromatography (GPC) and ¹H-nuclear magnetic resonance (NMR) were used to monitor the change of mass loss, molecular weight and composition of copolymers. In hydrolytic degradation, the PEO/PL/PEO tri-block copolymer with high PEO contents affected the increase of its mass loss, and resulted in the decrease of its molecular weight as well as PEO composition. However, when PL/PEO/PL and PEO/PL/PEO tri-block copolymers had similar PEO contents, PEO/PL/PEO decreased faster in molecular weight and PEO composition than PL/PEO/PL.

Keywords : polylactide, poly(ethylene oxide), biodegradable polymer, tri-block copolymer, hydrolytic degradation.

Introduction

Aliphatic polyester is an important biomedical material due to its biodegradability and good biocompatibility. Now it is widely used as matrix for tissue engineering, surgical suture material and drug delivery carriers in the biomedical field. In particular, it is well known that aliphatic polyesters such as polylactide (PL), polyglycolide, polycaprolactone and so on are an excellent biodegradable and biocompatible polymer.¹⁻⁴ However, these polymers are hydrophobic and its degradation rate is very slow.

Poly(ethylene oxide) (PEO) is a good biomaterial which can control the degradation rate of PL. PEO has been widely used as a source of hydrophilic component because of its outstanding physicochemical and biological properties including solubility in water and in organic solvents, lack of toxicity, absence of antigenicity and immunogenicity, as well as filterability through kidney when the molar mass is below

10,000.⁵ Therefore, many biodegradable block copolymers bearing PEO have been recently studied.⁶⁻⁸ These copolymers were able to have various hydrophilic/hydrophobic and soft/hard segment ratio and thus, constitute a very attractive means to modulate the basic properties of each homopolymer. In addition, it was reported that block copolymer consisting of PL and PEO forms hydrogel in water and is able to form gel or sol by change of a temperature, pH, *etc.* so that it shows a behavior that can be used as slow releasing matrix of drug. As a result, block copolymers bearing PEO are of great interest in view of applications in drug delivery system. Recently, PEO/PL/PEO tri-block connected by urethane group has been reported.⁹ However, the block copolymer of this structure has the disadvantage that toxic diisocyanate group remains in it.^{10,11}

In this study, PEO/PL/PEO tri-block copolymers, which each block is connected by ester bond, were synthesized by coupling reaction of PL and PEO. To investigate degradation pattern of block copolymer, tri-block copolymers of various molecular weights were synthesized, and hydrolytic degradations were studied. In addition, PL/PEO/PL tri-block copolymer was synthesized to investigate difference of hydrolytic degradation pattern due to the structure of tri-block

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copolymer.

Experimental

Material. L-lactide from Purac Biochem (Holland) was purified by recrystallization from dried ethyl acetate under dry nitrogen atmosphere and thoroughly dried for 24 hrs in a vacuum at 30°C before use. Stannous octoate (Sn-oct) from Sigma (St. Louis, MO) was distilled under reduced pressure at 175°C before use. 1,6-Hexanediol was distilled under reduced pressure. Monomethoxy poly(ethylene oxide) (PEO, M_n : 0.75 k, 2 k, 5 k Aldrich, Milwaukee, WI) was dissolved in dried chloroform and then precipitated in diethyl ether, respectively. 1,4-Dioxane, methylene chloride and pyridine were thoroughly dried by distillation over calcium hydride, respectively. All other chemicals and solvents were of analytical grade and used without further purification.

Characterizations. Polymers were analyzed using a Varian 200 MHz $^1\text{H-NMR}$ in deuterated chloroform solution. Molecular weight was determined by GPC (Waters, Model 510 pump, equipped with 410 Differential Refractometer) using three micro-styragel columns (CHR2, HR4, and HR5E) from 500 Å to 4×10^6 Å connected in series. Tetrahydrofuran was eluted with a rate of 1.0 mL/min at 40°C.

Synthesis.

Synthesis of COOH Terminated PEO (PEO-COOH): PEO, succinic anhydride (SA), 4-dimethylaminopyridine (DMAP) and triethylamine (TEA) were dissolved in 1,4-dioxane and reacted for 24 hrs at room temperature under nitrogen atmosphere. 1,4-Dioxane was removed under vacuum and then the resulting product was dissolved in CCl_4 . The unreacted succinic anhydride was removed by filtering. The filtered solution was precipitated in cold ethyl ether and the precipitated PEO-COOH was dried under vacuum for more than 12 hrs.

Synthesis of COCl Terminated PEO (PEO-COCl): The PEO-COOH was completely dissolved in purified methylene chloride. Excess of thionyl chloride and two drops of dimethylformamide were added to the solution. The reaction was carried out at 60°C for 3 hrs, and the reaction solution was distilled under vacuum to remove the solvent and unreacted thionyl chloride. The product was used immediately for the coupling reaction.

Synthesis of OH Terminated Poly lactide (OH-PL-OH): L-lactide and 1,6-hexanediol were added in a glass ampoule containing a teflon-coated magnetic stirring bar. After Sn-oct was added, the ampoule was sealed under vacuum after purging three times with nitrogen at 90°C. The ampoule was heated to 130°C in an oil bath for 5 hrs with stirring. After the product was dissolved in chloroform, the polymer was precipitated by pouring the polymer solution into an excess of methanol, filtered, and dried under vacuum.

Synthesis of PEO/PL/PEO: The PEO-COCl and HO-

PL-OH were put in a flask under nitrogen atmosphere. By using an ice bath, the reaction system was maintained at a sufficiently low temperature, and then the purified pyridine was dropped slowly. The reaction system was maintained at room temperature, and the reaction was carried out for 24 hrs. After the reaction solution was precipitated in methanol, the solution was centrifuged. By repeating the procedure of the precipitation in methanol and centrifugation three times, an excess of PEO and catalyst were completely removed. The obtained product was dried under vacuum at ambient temperature for at least 12 hrs.

NMR characteristics of PEO/PL/PEO ; $^1\text{H-NMR}$ (CDCl_3) $\delta = 5.18$ (q, 2nH, (OCO(CH)OCO)), $\delta 4.25$ (t, 4H, (OCH₂(CH₂)OCO)), 4.17 (t, 4H, (CH₂(CH₂)OCO)), 3.65 (t, 8mH, (O(CH₂CH₂)O)), 3.38 (s, 6H, (CH₃)), 2.72 (t, 8H, (OOC(CH₂CH₂)COO)), 1.59 (d, 6nH, (CH₃)).

Synthesis of PL/PEO/PL: L-lactide and poly(ethylene oxide) (2 k) with two hydroxyl groups at both sides were added in a glass ampoule containing a teflon-coated magnetic stirring bar. After Sn-oct was added, the ampoule was sealed under vacuum after purging three times with nitrogen at 90°C. The ampoule was heated to 130°C in an oil bath for 5 hrs with stirring. After reaction, the ampoule was broken, the product was dissolved in chloroform, and micro-filtered through a 0.45 μm pore membrane filter. The polymer was precipitated by pouring the polymer solution into an excess of methanol, filtered, and dried under vacuum.

NMR characteristics of PL/PEO/PL ; $^1\text{H-NMR}$ (CDCl_3) $\delta = 5.18$ (q, 2nH, (OCO(CH)OCO)), 4.38 (q, 2H, (OCO(CH)OH)), 4.25 (t, 4H, (OCH₂(CH₂)OCO)), 3.65 (t, 4mH, (O(CH₂CH₂)O)), 1.59 (d, 6nH, (CH₃)).

Hydrolytic Degradation. Hydrolysis was performed using films of 10 mm \times 10 mm \times 50 μm in 25 mL of phosphate buffer saline solution (pH 7.4) at $37 \pm 1^\circ\text{C}$ for predetermined periods of time (3, 7, 15, 30 and 60 days). The buffer solution was replaced with fresh solution every week. At convenient times, samples were washed intensively with distilled water to remove residual buffer solution, and dried under reduced pressure for at least 2 days before analysis.

Results and Discussion

Synthesis and Characterization. PEO-COOH was synthesized through the ring opening reaction of the monomethoxy poly(ethylene oxide) with succinic anhydride^{12,13} (Scheme I(a)). When pyridine and CHCl_3 were used as catalyst and solvent, respectively, the ring opening reaction was inefficient. On the other hand, when DMAP and TEA were used as catalysts and 1,4-dioxane as solvent, they allowed the reaction to proceed efficiently due to the activation of succinic anhydride, and good yields of PEO-COOH were obtained. Synthesis of PEO-COCl was carried out for 3 to 4 hrs at 60°C in methylene chloride. Since the synthesized PEO-COCl has a high reactivity, it is very easy to react with

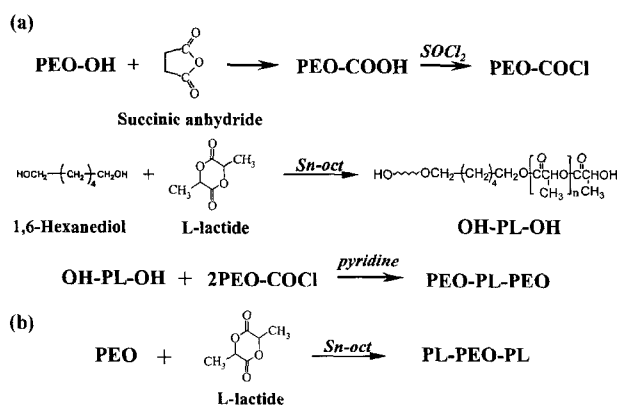
moisture in the air during storage over a long period of time. Thus it was used for next step immediately after its preparation. OH-PL-OH was synthesized by ring opening polymerization of L-lactide with 1,6-hexandiol in the presence of Sn-oct (Scheme I(a)). Polymerization was conducted in bulk with continuous stirring. Under this condition, high conversion was achieved after relatively short reaction time.^{14,15} PEO/PL/PEO copolymer was synthesized by coupling reaction of PEO-COCl with OH-PL-OH (Scheme I(a)). Pyridine which acts as both solvent and catalyst was used in the reaction. It

removes HCl which is produced during the reaction and thus plays a role in inducing the reaction toward the forward reaction. Furthermore, since addition of pyridine causes the exothermic reaction, it is gradually added with a small amount at 0.

Figure 1(a) shows the ¹H-NMR spectrum of PEO/PL/PEO tri-block copolymer. The methine proton (δ 4.35) at chain end of polylactide disappeared and the methylene proton at the chain end of PEO appeared at δ 4.25 by coupling reaction. Also, separated two methylene protons at the chain end of PEO-COCl were changed to single proton peak at δ 2.65 (Figure 1(a), F). PL/PEO/PL copolymer was synthesized by ring opening polymerization of lactide with PEO (Scheme I(b)). This reaction was widely used for synthesizing PL/PEO/PL by using PEO as an initiator. Figure 1(b) shows typical ¹H-NMR spectrum of PL/PEO/PL.

According to the ¹H-NMR analysis, it was identified that the PEO/PL/PEO tri-block copolymer was synthesized by coupling reaction of PEO-COCl and HO-PL-OH. By coupling of OH-PL-OH (3.3, 6.5, 9 and 24 k) with PEO-COCl (0.75, 2 and 5 k), PEO/PL/PEO tri-block copolymers with various molecular weights were prepared. Table I shows the molecular weight and PEO contents due to the molecular weight change of PL and PEO.

Hydrolytic Degradation Behavior. Degradation properties of the PEO/PL/PEO and PL/PEO/PL copolymers were investigated under in vitro condition (PBS, pH 7.4, 37 \pm 1 $^{\circ}$ C).



Scheme I. Synthesis route for (a) PEO_{2k}/PL_{11k}/PEO_{2k} and (b) PL_{10k}/PEO_{2k}/PL_{10k} tri-block copolymers.

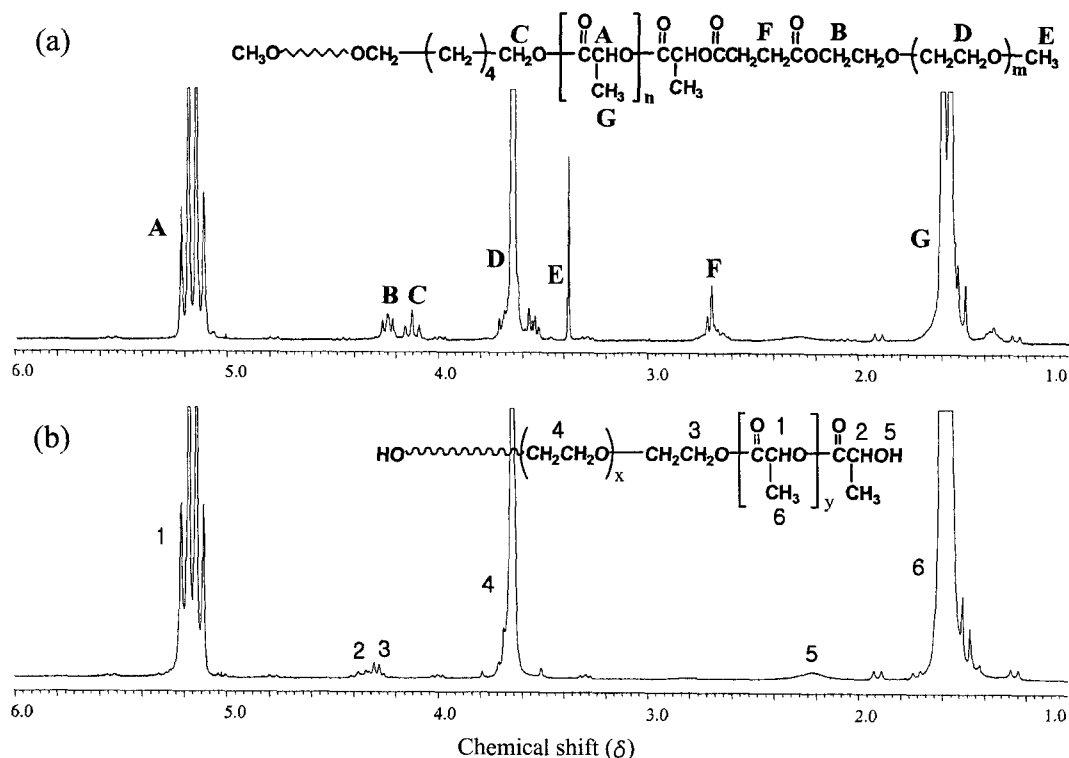


Figure 1. ¹H-NMR spectra of (a) PEO/PL/PEO and (b) PL/PEO/PL.

Table I. Molecular Weight of Poly lactides and PEO/PL/PEO

Block Length ^b	[PEO] ₀ ^c (wt%)	M _n ^a	M _w ^a	MWD ^a
PL _{3.3k}		6,030	7,660	1.27
PEO _{0.75k} /PL _{3.3k} /PEO _{0.75k}	24.4	10,640	13,030	1.22
PL _{6.5k}		10,740	15,130	1.41
PEO _{0.75k} /PL _{6.5k} /PEO _{0.75k}	16.8	14,120	18,780	1.33
PL _{9k}		13,940	19,600	1.40
PEO _{0.75k} /PL _{9k} /PEO _{0.75k}	12.8	20,190	28,066	1.39
PL _{24k}		21,200	31,320	1.48
PEO _{0.75k} /PL _{24k} /PEO _{0.75k}	5.7	24,480	35,150	1.44
PEO _{2k} /PL _{24k} /PEO _{2k}	14.2	28,600	42,460	1.48
PEO _{5k} /PL _{24k} /PEO _{5k}	29.3	29,860	44,790	1.51
PL _{5k} /PEO _{2k} /PL _{5k}	15.2	16,000	23,830	1.49

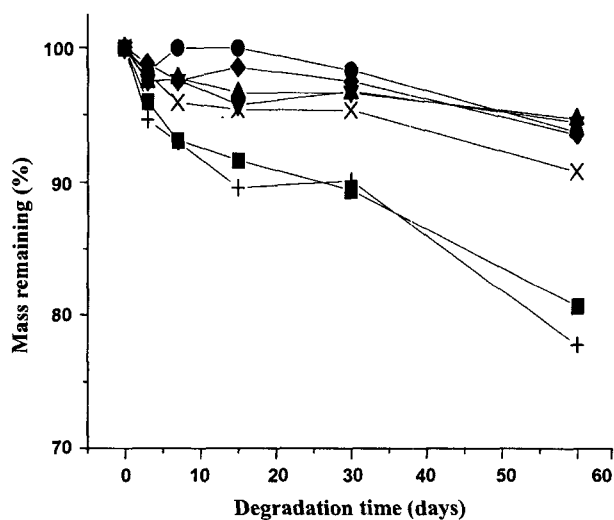
^aMeasured by GPC.^bX and Y in PEO_x and PL_y were calculated by ¹H-NMR.^cWeight percents of PEO contents in block copolymers before hydrolytic degradation.

Figure 2. Mass loss of PEO/PL/PEO and PL/PEO/PL tri-block copolymers during hydrolytic degradation in PBS, pH 7.4 at 37 °C (■ PEO_{0.75k}/PL_{3.3k}/PEO_{0.75k}; ● PEO_{0.75k}/PL_{6.5k}/PEO_{0.75k}; ▲ PEO_{0.75k}/PL_{9k}/PEO_{0.75k}; ▼ PEO_{0.75k}/PL_{24k}/PEO_{0.75k}; ◆ PEO_{2k}/PL_{24k}/PEO_{2k}; + PEO_{5k}/PL_{24k}/PEO_{5k}; × PL_{5k}/PEO_{2k}/PL_{5k}).

Despite degradation time for 60 days, the change in mass of copolymers was small (Figure 2). However, the copolymer of high PEO contents (such as PEO_{0.75k}/PL_{3.3k}/PEO_{0.75k} and PEO_{5k}/PL_{24k}/PEO_{5k}) showed higher mass loss as compared with that of low PEO contents. This result means that the copolymer with high PEO contents well absorbed water due to hydrophilic properties of PEO, and so hydrolytic degradation of copolymer fast proceeded.

From GPC analysis, remaining M_n of PEO/PL_{24k}/PEO was

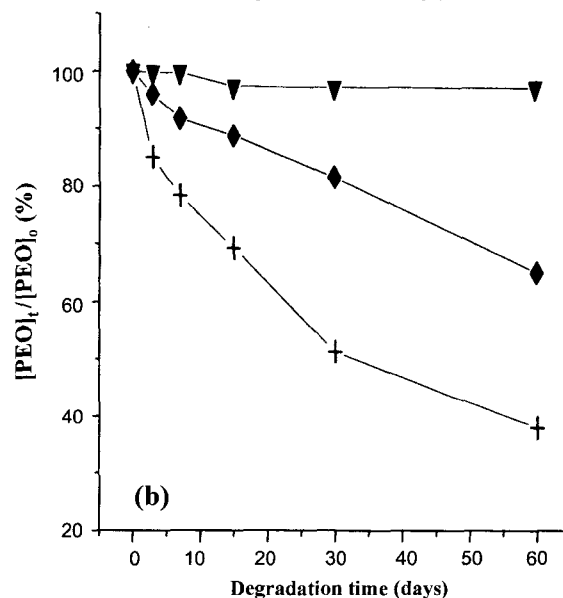
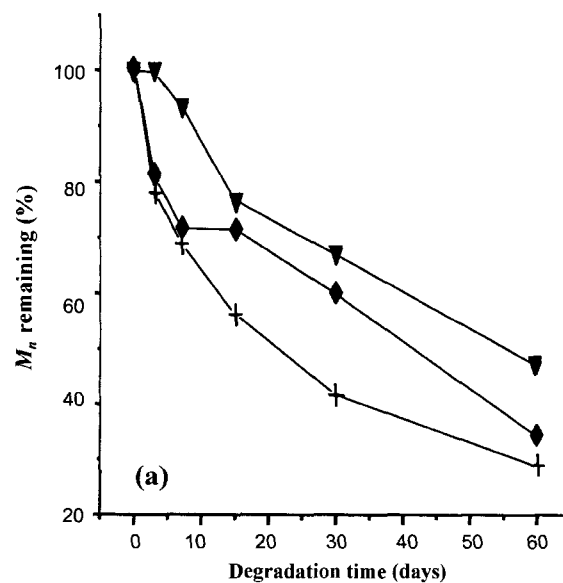


Figure 3. Molecular weight (a) and the ratio of PEO contents (b) of PEO/PL_{24k}/PEO with various molecular weights PEO during hydrolytic degradation in PBS, pH 7.4 at 37 °C ([PEO]_t: the weight percent of PEO contents after hydrolytic degradation, [PEO]₀: the weight percent of PEO contents before hydrolytic degradation, ▼ PEO_{0.75k}/PL_{24k}/PEO_{0.75k}; ◆ PEO_{2k}/PL_{24k}/PEO_{2k}; + PEO_{5k}/PL_{24k}/PEO_{5k}).

below 50% after 60 days, and degradation was increased with increasing PEO contents in copolymers (Figure 3(a)). As can be seen in Figure 3(b), the ratio of PEO contents ([PEO]_t/[PEO]₀) after hydrolytic degradation decreased as the initial PEO contents ([PEO]₀) in block copolymer increased. The decrease of the PEO contents showed the same pattern at PEO_{0.75k}/PL/PEO_{0.75k} tri-block copolymers also. As the initial PEO contents of PEO_{0.75k}/PL/PEO_{0.75k}

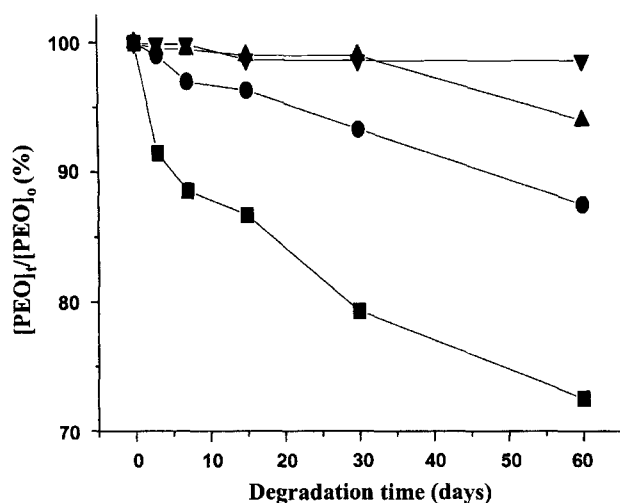


Figure 4. The ratio of PEO contents of PEO_{0.75k}/PL/PEO_{0.75k} with various molecular weights PL during hydrolytic degradation in PBS, pH 7.4 at 37 °C (■ PEO_{0.75k}/PL_{3.3k}/PEO_{0.75k}; ● PEO_{0.75k}/PL_{6.5k}/PEO_{0.75k}; ▲ PEO_{0.75k}/PL_{9k}/PEO_{0.75k}; ▼ PEO_{0.75k}/PL_{24k}/PEO_{0.75k}).

were high, the ratios of the PEO contents were remarkably decreased during hydrolytic degradation (Figure 4).

It is well known that polylactide with high molecular weight is more resistant to hydrolysis than those of low molecular weight. Comparing the molecular weight of PEO_{0.75k}/PL_{6.5k}/PEO_{0.75k} with PEO_{5k}/PL_{24k}/PEO_{5k}, former tri-block copolymer has lower molecular weight, whereas, the decrease of PEO contents is small. This means that the ratio of hydrophilic-hydrophobic contents is a very important factor in hydrolytic degradation as well as the effect of molecular weight. Li et al reported that hydrolytic degradation of PL/PEO/PL tri-block copolymer depended on the mole ratio of PEO and PL.¹⁶ The degradation results of PEO/PL/PEO tri-block copolymers also reflect the close relationship with the mole ratio of PEO and PL. The change in number average molecular weight (M_n) and PEO contents of PEO_{5k}/PL_{24k}/PEO_{5k} and PL_{5k}/PEO_{2k}/PL_{5k}, which have a similar PEO contents but a different sequence structure, was characterized by GPC and ¹H-NMR analysis. PEO_{5k}/PL_{24k}/PEO_{5k} having hydrophilic PEO in both sides was hydrolyzed faster than PL_{5k}/PEO_{2k}/PL_{5k} having hydrophobic PL in both sides. Moreover, the PEO contents of PEO_{5k}/PL_{24k}/PEO_{5k} decrease a little faster than that of PL_{5k}/PEO_{2k}/PL_{5k}. Considering that PEO_{5k}/PL_{24k}/PEO_{5k} tri-block copolymer has higher molecular weight than PL_{5k}/PEO_{2k}/PL_{5k} tri-block copolymer, PEO/PL/PEO copolymer structure easily accept hydrolytic attack than PL/PEO/PL copolymer (Figure 5).

Figure 6 illustrates the hydrolytic degradation pattern of PEO/PL/PEO tri-block copolymers in terms of their molecular weight and structure. Generally, it is well known that PEO is a hydrophilic and non-degradable polymer while PL is a hydrophobic and biodegradable polymer of which ester group

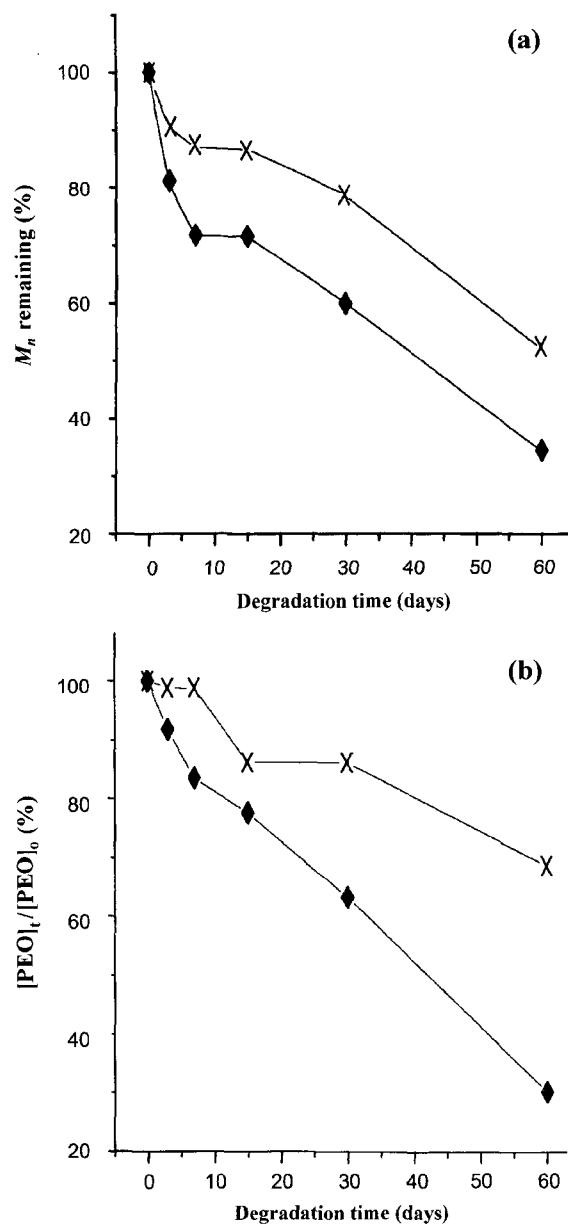


Figure 5. Molecular weight (a) and the ratio of PEO contents (b) of PEO_{2k}/PL_{24k}/PEO_{2k} and PL_{5k}/PEO_{2k}/PL_{5k} with similar PEO contents during hydrolytic degradation in PBS, pH 7.4 at 37 °C (◆ PEO_{2k}/PL_{24k}/PEO_{2k}; ✕ PL_{5k}/PEO_{2k}/PL_{5k}).

hydrolyzed during hydrolytic degradation.¹⁷⁻¹⁹ Therefore, the following mechanism could be proposed to account for degradation pattern of copolymer composed with PEO and PL. When introduced in aqueous medium, firstly the copolymer absorbs water by influence of PEO and then hydrolytic cleavage of PL blocks starts immediately at random. The PEO rich or PL poor segment among degradation products could be soluble in aqueous phase because of its increased hydrophilicity.

If PEO/PL_{24k}/PEO has hydrolytic cleavages at PL site,

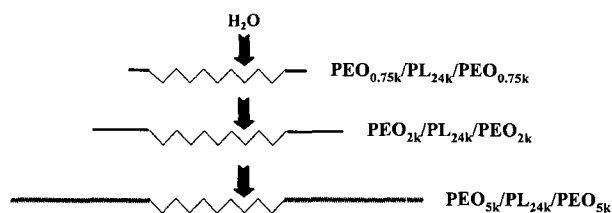


Figure 6. Schematic representation of PEO/PL_{24k}/PEO with various molecular weights of PEO during hydrolysis.

PEO/PL_{24k}/PEO copolymer of high molecular weight PEO would be hydrolyzed to be more hydrophilic product, which can be soluble in aqueous medium than that of low molecular weight PEO. As a result, PEO/PL_{24k}/PEO copolymers with high molecular weight PEO can induce penetration of water easily and form soluble products in aqueous medium faster than that of low molecular weight PEO.

In the case of PEO_{0.75k}/PL/PEO_{0.75k} copolymers with various molecular weights of PL (3.3, 6.5, 9 and 24 k), hydrolytic degradation of PEO_{0.75k}/PL/PEO_{0.75k} copolymers with low molecular weight PL is thought to be similar to that of PEO/PL_{24k}/PEO copolymers with high molecular weight PEO in terms of PEO contents. This well agrees with the degradation pattern of PEO_{0.75k}/PL/PEO_{0.75k} in Figure 4.

Conclusions

In this study, PEO/PL/PEO tri-block copolymers, which each block is connected by ester bond, were synthesized by coupling reaction of PL with PEO in the presence of pyridine. Tri-block copolymers were characterized by ¹H-NMR and GPC. PEO/PL/PEO with various molecular weights of PEO and PL blocks were prepared. In addition, PL/PEO/PL tri-block copolymer was synthesized to investigate difference of hydrolytic degradation pattern due to the structure of tri-block copolymer.

As a result of degradation study the PEO/PL/PEO tri-block copolymer with high PEO contents affected the increase of its mass loss, and resulted in the decrease of its molecular weight as well as PEO composition. However, when PL/PEO/PL and PEO/PL/PEO tri-block copolymers had similar PEO contents, PEO/PL/PEO decreased faster in

molecular weight and PEO composition than PL/PEO/PL.

Acknowledgement. This work was supported by Ministry of Commerce Industry and Energy, Grants No. 2M10561.

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