Thermoplastic Hydrogel Based on Pentablock Copolymer Consisting of Poly(γ-benzyl L-glutamate) and Poloxamer

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ABSTRACT: A thermoplastic hydrogel based on a pentablock copolymer composed of poly(γ-benzyl L-glutamate) (PBLG) and poloxamer was synthesized by polymerization of BLG *N*-carboxyanhydride, which was initiated by diamine-terminated groups located at the ends of poly(ethylene oxide) (PEO) chains of the poloxamer, to attain a new pH- and temperature-sensitive hydrogel for drug delivery systems. Circular dichroism measurements in solution and IR measurements in the solid state revealed that the polypeptide block existed in the α-helical conformation, as in the PBLG homopolymer. The intensity of the wide-angle X-ray diffraction patterns of the polymers depended on the poloxamer content in the copolymer and showed basically similar reflections to the PBLG homopolymer. The melting temperature (T_m) of the poloxamer in the copolymer was reduced with an increase of the PBLG block in comparison with the T_m of the poloxamer, which is indicative of a thermoplastic property. The water contents of the copolymers were dependent on the poloxamer content in the copolymers, for example, those for the GPG-2 (48.7 mol % poloxamer) and GPG-1 (57.5 mol % poloxamer) copolymers were 31 and 41 wt %, respectively, indicating characteristics of a polymeric hydrogel. J Appl Polym Sci 88: 2649–2656, 2003

Key words: thermoplastic hydrogel; pentablock copolymer; poloxamer

INTRODUCTION

Hydrogels as hydrophilic materials are one of the most promising classes of biomaterials for biomedical applications because they have good biocompatibility and a large amount of equilibrium water content.[1] They are one of the potential candidates to incorporate polypeptide drugs in delivery systems because the drugs are mostly soluble in water.[2] However, typical thermosetting hydrogels obtained by chemical crosslinking have limitations such as lack of processability, poor mechanical strength, and lack of biodegradability. Recently, thermoplastic biodegradable hydrogels have been designed for drug delivery systems because they have the combined characteristics of hydrogels, thermoplastics, and biodegradation, and they have lower glass-transition temperatures and crystallinities.[3] Physically crosslinked triblock copolymers composed of poly(ethylene oxide) (PEO) and poly(glycolic acid) were synthesized by Casey et al.[4] Churchill and Hutchinson also synthesized similar amphiphilic materials consisting of PEO and poly(α-hydroxy acid).[5] Recently, Li and Kissel reported that the star-block copolymers of PEO and lactide or lactide/glycolide showed smaller hydrodynamic radii, higher swelling rates, and faster degradation rates than the linear block copolymers.[6]

In a previous study,[7] thermoplastic hydrogels were prepared based on hexablock copolymers composed of poly(γ-benzyl L-glutamate) (PBLG) as the hydrophobic part and PEO as the hydrophilic part. It was found that the morphology of the copolymer revealed a microphase-separated structure and the water contents of the copolymer were over 30 wt %, an indication of a polymeric hydrogel.

In this study, we investigated another thermoplastic hydrogel based on pentablock copolymers composed of PBLG as the hydrophobic part and poloxamer as the hydrophilic part. PBLG as the biodegradable part is one of synthetic polypeptides that have attracted attention in a drug delivery matrix. Poloxamer as the hydrogel part is a water-soluble polymer that can be used to control the water content in the copolymer. In addition, poloxamer has been used for drug delivery systems because of rapid reversible sol–gel transition behavior with respect to the temperature and concentration.[8] The copolymer is expected to be a promising candidate material for a new pH- and temperature-sensitive hydrogel after debenzylation of PBLG in the copolymer, because poly(L-glutamic acid) and poloxamer show pH-sensitive and temperature-sensitive behaviors, respectively.

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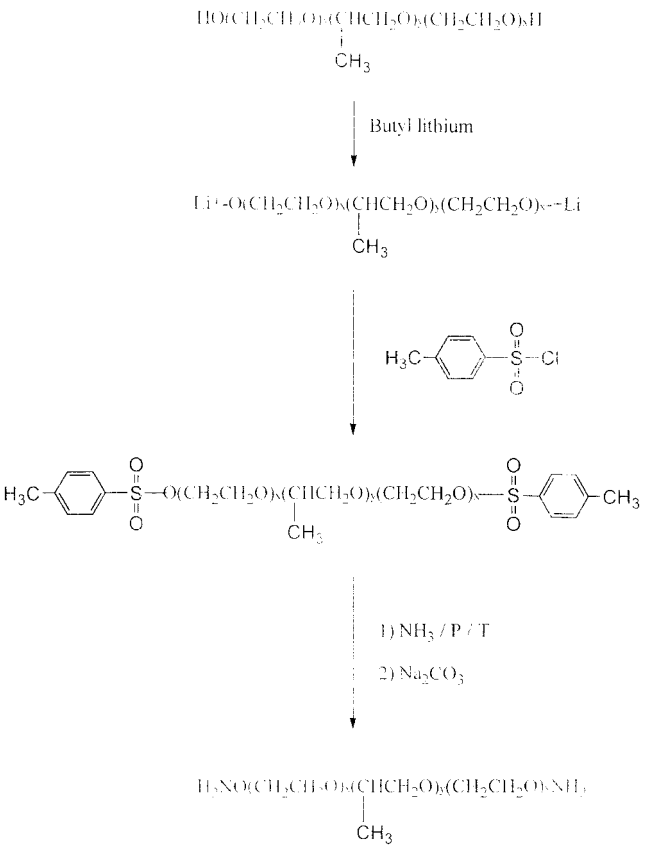


Scheme 1 A synthetic scheme of the diamine-terminated poloxamer.

EXPERIMENTAL

Materials

The poloxamer (poloxamer 407, composed of EO and propylene oxide, PO, according to the manufacturer as $EO_{97}PO_{69}EO_{97}$, weight-average molecular weight = 12,500 g/mol, 70 wt % EO) and BLG were purchased from Sigma (St. Louis, MO). The poloxamer was dried by azeotropic distillation with benzene prior to use. Phosgene was kindly supplied by Korea Fine Chemical Co., Ltd. (Yeosu, Korea).

Preparation of diamine-terminated poloxamer (DATP)

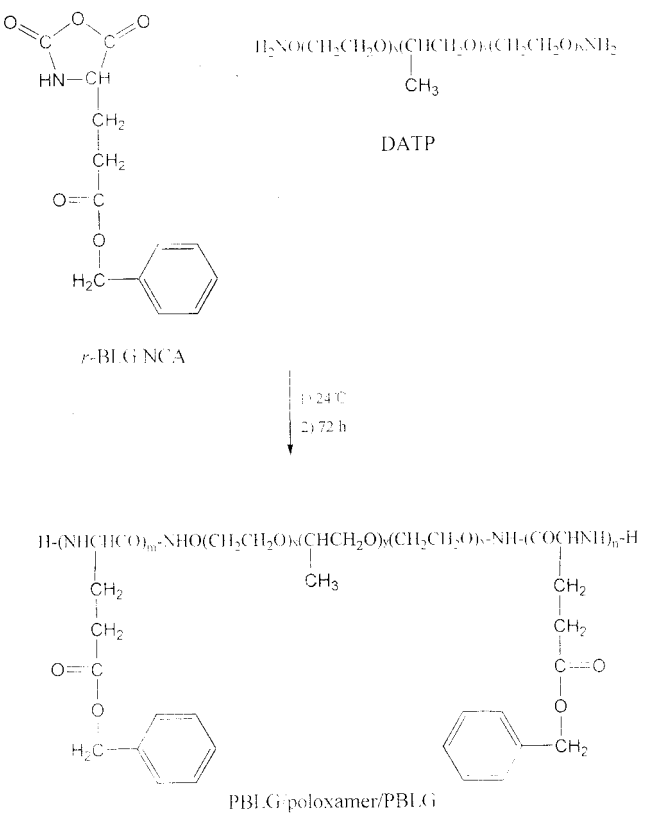
The terminal hydroxyl groups in the poloxamer were converted into amino groups by a modification of the method of Loccufier et al.[9] The methods are the following three steps.

Tosylation of poloxamer

An quantity of 51.75 g (4.14 m*M*) of previously dried poloxamer was dissolved in 600 mL of dry benzene in a 1000-mL round-bottom flask. Then 3.6 mL of a 2.5*M* solution (9 m*M*) of butyllithium in *n*-hexane was added while vigorously stirring. To this was added a solution of 2.4 g (12.6 m*M*) of tosyl chloride in 50 mL of dry benzene. The solution was stirred overnight under a nitrogen atmosphere and evaporated, and 500 mL of dry methylene chloride was added. The precipitated lithium chloride was removed by filtratio, the filtrate was evaporated, and 800 mL of diethyl ether was added with vigorous stirring. The precipitate was dried *in vacuo*. The obtained solid was redissolved in 150 mL of dry ethanol and cooled to −15°C. The precipitate was isolated by filtration using filter paper and dried *in vacuo* (89 wt % yield).

Preparation of ammonium *p*-toluene sulfonate of poloxamer

Forty grams of tosylated poloxamer (3.12 m*M*) were dissolved in 250 mL of a 30% aqueous ammonia solution. The solution was heated at 135°C in a high-pressure vessel for 7 h. After cooling, the solution was evaporated under reduced pressure. The residue was redissolved in 200 mL of water and freeze-dried. The product was redissolved in 300 mL of dry ethanol and evaporated under a vacuum again, and the oily residue was poured into diethyl ether. The precipitate was collected by filtration and dried to constant weight (79 wt % yield).



Scheme 2 A synthetic scheme of the PBLG/poloxamer/PBLG block copolymer.

TABLE I
Characteristics of Prepared Polymers

Samples	Monomeric unit (mol %) Poloxamer (PPO + PEO mol %)	PBLG	M_n
GPG-1	57.5	42.5	55,000
GPG-2	48.7	51.3	73,100
GPG-3	43.0	57.0	88,900
PBLG	0	100	226,000

The contents of monomeric units in the polymers were estimated by ^{1}H-NMR measurements.

Preparation of DATP

Ammonium *p*-toluenesulfonate of poloxamer (28.5 g, 2.22 m*M*) was added into 500 mL of a 1*M* sodium carbonate solution and stirred overnight. The solution was freeze-dried and 500 mL of benzene was added to the residue. The insolubles in the benzene were removed by filtration, and the solution was evaporated under reduced pressure. The oily residue was poured into 1000 mL of *n*-hexane, and the precipitate was collected by filtration and dried. The solid was redissolved in 300 mL of dry ethanol and cooled to −18°C. The product recrystallized from ethanol was filtered and dried. The conversion of poloxamer into DATP as determined by the titration method was 74.5 wt %. The reaction procedures are shown in Scheme 1.

Preparation of BLG *N*-carboxyanhydride (BLG NCA)

The BLG NCA was prepared by the direct phosgenation of BLG according to the modification method proposed by Fuller et al.[10]

Preparation of PBLG homopolymer

The homopolymer was obtained by polymerization of BLG NCA initiated by triethylamine in methylene chloride with a monomer to initiator ratio of 50.

Preparation of PBLG/poloxamer/PBLG pentablock copolymer

The pentablock copolymer was prepared by polymerization of BLG NCA that was initiated by DATP in methylene chloride at a total 3% (w/v) concentration

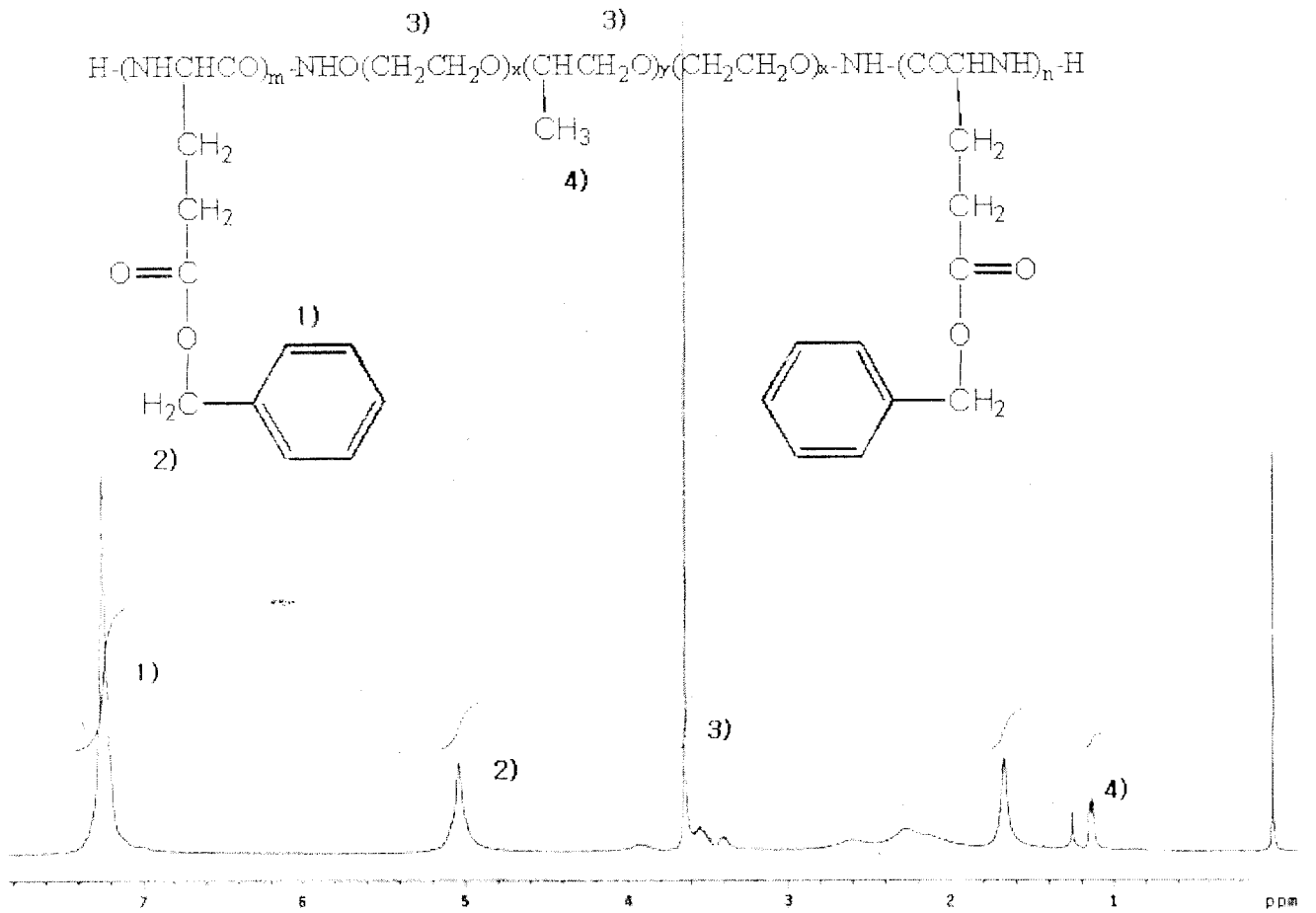


Figure 1 The ^{1}H-NMR spectrum of the GPG-1 block copolymer.

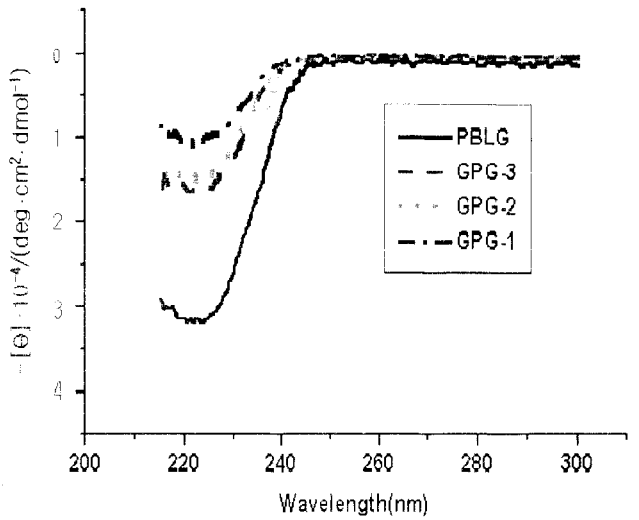


Figure 2 The CD spectra of PBLG/poloxamer/PBLG block copolymers and PBLG homopolymer in 1,2-dichloroethane.

of BLG NCA and DATP at 25°C for 72 h by a method reported previously.[11] When the C═O stretching absorptions of BLG NCA at 1785 and 1860 cm^{-1} in the IR spectrum disappeared in the course of polymerization, the resulting mixture was poured into a large excess of diethyl ether to precipitate the polymer. The product was washed several times with diethyl ether and dried *in vacuo*. The copolymer was washed again with water to remove the unreacted DATP and dried *in vacuo*. The synthetic scheme is shown in Scheme 2. The yield of these block copolymers was about 60–70 wt %.

Measurements

^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra of the polymers were measured to estimate the composition and molecular weight of the pentablock copolymers using a Varian 300 NMR spectrometer in deuterated chloroform. Because the number-average molecular weight of the PEO and PPO blocks of the poloxamer are known from the manufacturer, the number-average molecular weights of the PBLG blocks and the pentablock copolymer composition can be estimated from the peak intensities in the spectrum assigned to both polymers.[12,13]

TABLE II
Negative Ellipticity at 222 nm ($-[\theta]_{222}$) of PBLG Homopolymer and PBLG/Poloxamer/PBLG Block Copolymers in 1,2-Dichloroethane at 25°C

Polymer	L (mol %)	$-[\theta]_{222}$	$[\theta]^c_{222}/[\theta]^h_{222}$
GPG-1	42.5	11.180	0.35
GPG-2	51.3	15,590	0.48
GPG-3	57.0	17,100	0.53
PBLG	100	32,400	1.00

L, the content of PBLG units in the block copolymers estimated from the ^{1}H-NMR spectra; $[\theta]^c_{222}$, the ellipticity of the block copolymers; $[\theta]^h_{222}$, the ellipticity of the PBLG homopolymer.

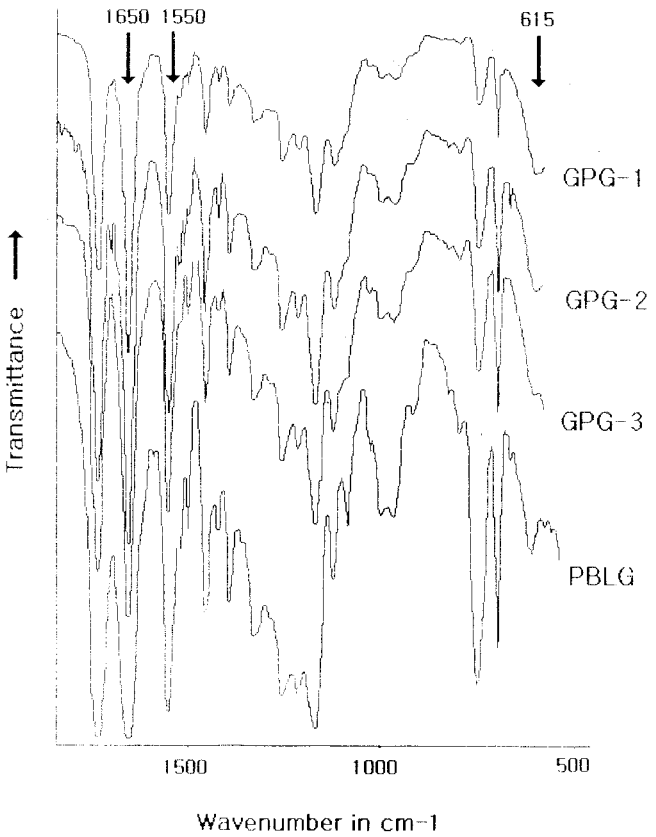


Figure 3 The IR spectra of PBLG/poloxamer/PBLG block copolymers and PBLG homopolymer.

Circular dichroism (CD)

The CD spectra of the PBLG homopolymer and pentablock copolymers were recorded on a Jasco J-500A spectropolarimeter equipped with a quartz cell having a path length of 1 mm at 25°C.

IR spectroscopy

The IR spectra of solid films of samples cast from chloroform solution were obtained with a Mattson

TABLE III
Melting temperature (T_m) and Heat of Fusion (ΔH_f) of PBLG, Poloxamer, and Block Copolymers

Polymers	Poloxamer (PEO + PPO mol %)	Poloxamer T_m (°C)	Copolymer ΔH_f (J/g)
Poloxamer	100	63.0	109.6
GPG-1	57.5	52.7	13.5
GPG-2	48.7	51.1	10.6
GPG-3	43.0	49.2	6.0
PBLG	0	NO	—

NO, not observed.

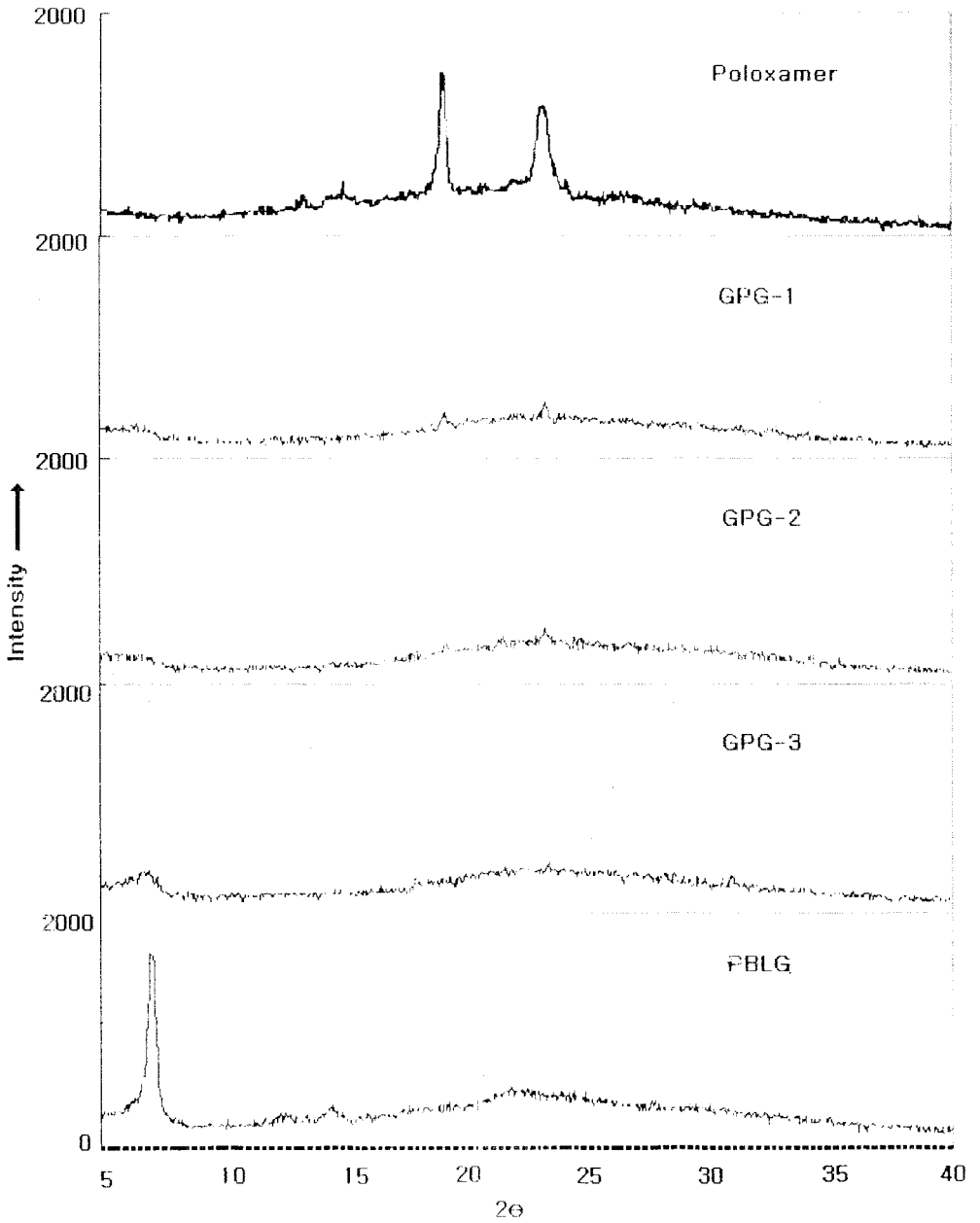


Figure 4 Wide-angle X-ray diffraction patterns of PBLG/poloxamer/PBLG block copolymers, poloxamer, and PBLG homopolymers cast from chloroform.

Genesis 2 FTIR spectrometer operating between 4000 and 500 cm^{-1}.

X-ray diffraction

Wide-angle X-ray diffraction (WAXD) of samples cast from chloroform solution was performed with a Rigaku Geigerflex using Ni-filtered CuK_α radiation.

Differential scanning calorimetry

The T_m values of the polymers were measured with a Mettler DSC-30 differential scanning calorimeter with a TC-11 microprocessor at a scan speed of 10°C/min.

Dielectric measurements

Dielectric experiments were carried out using a dielectric thermal analyzer (DETA 500, Reometric Scientific, UK). Measurements were performed at a frequency of 20 Hz in a temperature range of 30–200°C at a heating rate of 2°C/min. Samples were molded in a disk shape (33-mm diameter and thickness) from 50 to 100 μm in size. The permittivity (ϵ') and tan δ of each sample were obtained as a function of temperature.

Water content

The dried polymer disks (20-mm diameter, 2-mm thickness) were immersed in distilled water and kept

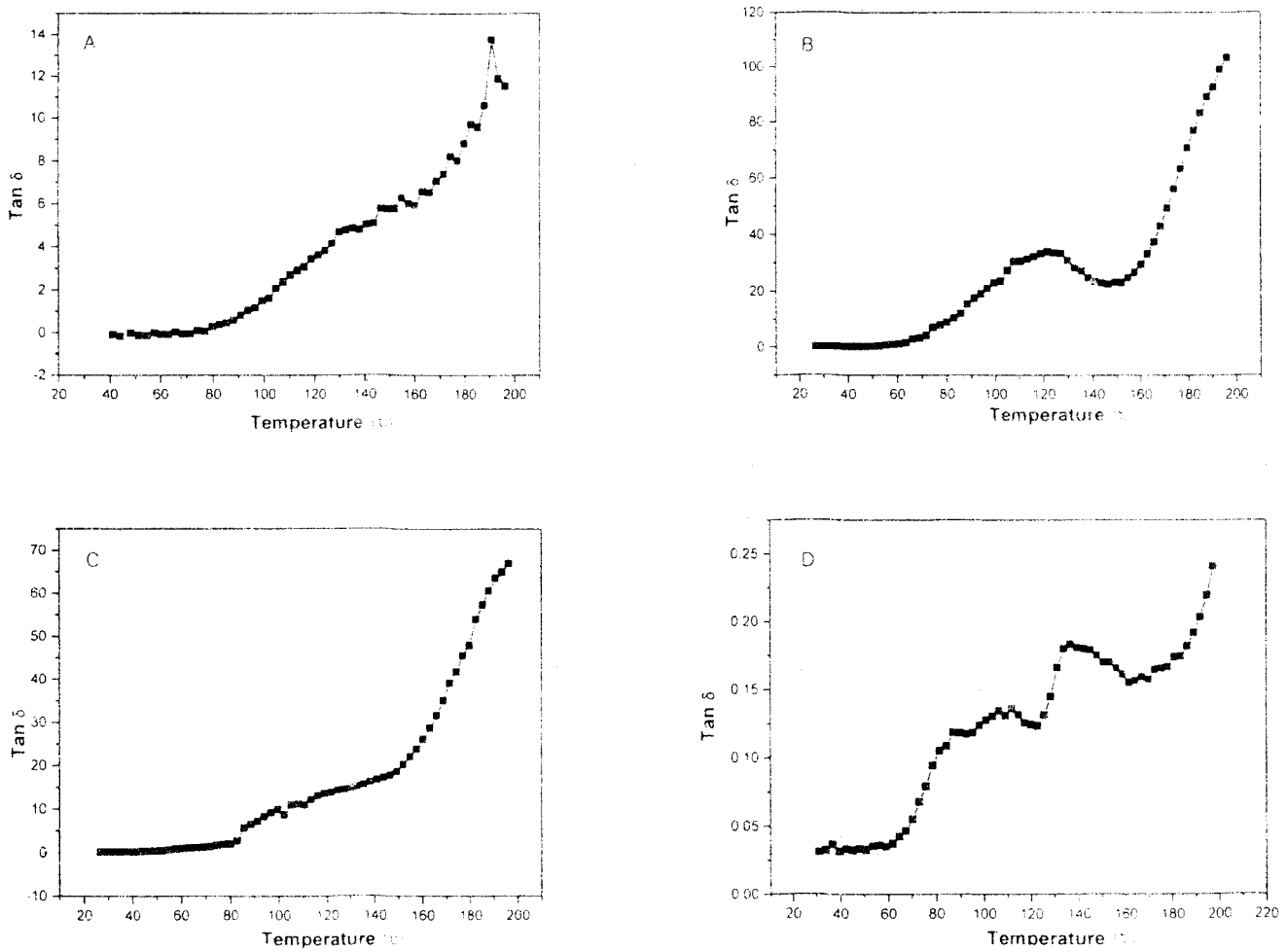


Figure 5 Dielectric thermal analyzer profiles of (A) PBLG homopolymer and (B) GPG-1, (C) GPG-2, and (D) GPG-3 block copolymers.

at 37°C. After a specific time, the samples were weighed after blotting the surface water with filter paper. The water contents were calculated as $(W_s - W_d)/W_s \times 100$, where W_s and W_d were the wet and dry weights of the disks, respectively.

RESULTS AND DISCUSSION

Characterization of pentablock copolymers

The pentablock copolymers (GPG) were prepared by polymerization of BLG NCA with DATP as the polymeric initiator in methylene chloride solution as shown in Scheme 2. The reaction mixture may contain some unreacted DATP. The unreacted DATP that was soluble in water was removed by washing the products with water several times. It is assumed that the polymerization mechanism is the primary-amine mechanism in which the amine groups of DATP undergo nucleophilic addition to the C-5 carboxyl group of the NCA, as suggested by Fuller et al.[10]

Table I lists the composition and molecular weights of the polymers obtained from the ^{1}H-NMR spectra. The copolymer composition and the molecular weights were estimated from the peak intensities of the phenyl proton signal (7.2 ppm) of the PBLG blocks and the methyl proton signal (1.08 ppm) of the PPO blocks of the poloxamer in the spectrum. Figure 1 shows the ^{1}H-NMR spectrum of the pentablock copolymer GPG-1. Assuming that all the amine groups of DATP participate in the polymerization, the number-average molecular weights of the copolymers can be calculated from the copolymer composition and the molecular weight of the DATP chains. Also, it is possible that heterogeneity in the block chain lengths of PBLG may hold in the copolymers.

Chain conformation of pentablock copolymers in solution state

Figure 2 shows the CD spectra of the pentablock copolymers and PBLG homopolymer, which all have a

trough of $[\theta]$ at about 222 nm ($[\theta]_{222}$), indicating the existence of an α-helical conformation[14] at different levels. The other troughs at 208 nm that are typical of α helices could not be recorded because of the high absorption by the 1,2-dichloroethane (EDC) solvent that was used. Table II shows the $[\theta]_{222}$ experimental data for samples in EDC solution at room temperature. The ratios of the $[\theta]_{222}$ values of the block copolymers to that of PBLG homopolymer, $[\theta]^c_{222}/[\theta]^h_{222}$, are shown in Table II. The helical contents of the block copolymers are in good agreement with the content of PBLG in the block copolymer chain, as evaluated from ^{1}H-NMR measurements.

Structure of pentablock copolymers in solid state

The IR spectra of the solid films of the copolymers and PBLG homopolymer cast from chloroform are shown in Figure 3. The amide I, II, and V bands of the copolymers appear at 1650, 1550, and 615 cm^{-1}, respectively, at the same wavenumbers as for the PBLG homopolymer.

Thermal properties of pentablock copolymers

Table III shows the T_m of the poloxamer in the copolymers. It ranged from 49.2 to 52.7°C, indicating that the T_m values of the poloxamer were lowered with an increase of the PBLG block in comparison with the T_m of the poloxamer itself (63.0°C). The heat of fusion for the poloxamer was 109.6 J/g, whereas the heat of fusion of the poloxamer component in the copolymer was smaller. These results indicated that the PBLG component in the copolymer prevented crystallization of the poloxamer chains of the copolymer. This result is fairly consistent with the WAXD profiles shown in Figure 4, where no crystalline reflections were observed for GPG-3, although this copolymer contains 43.0 mol % poloxamer. However, the exact T_m of the PBLG in the copolymer was not observed because of the occurrence of thermal degradation of PBLG near the T_m of the PBLG.

Figure 5 shows DETA profiles of the PBLG homopolymer and GPG block copolymers at a frequency of 20 Hz. The tan δ peak against the temperature is generally the α_1 peak, which is associated with the relaxation of the PBLG.[15] The tan δ peak for the PBLG homopolymer and GPG-1, GPG-2, and GPG-3 block copolymers was observed at 130.1, 120.2, 109.2, and 92.1°C, respectively. The resulting spectra showed significant differences in the temperature of damping with different poloxamers in the copolymer. This seems to be due to the flexible chain of the poloxamer in the copolymer. This may indicate that PBLG chains are prevented from crystallizing by the presence of poloxamer chains.

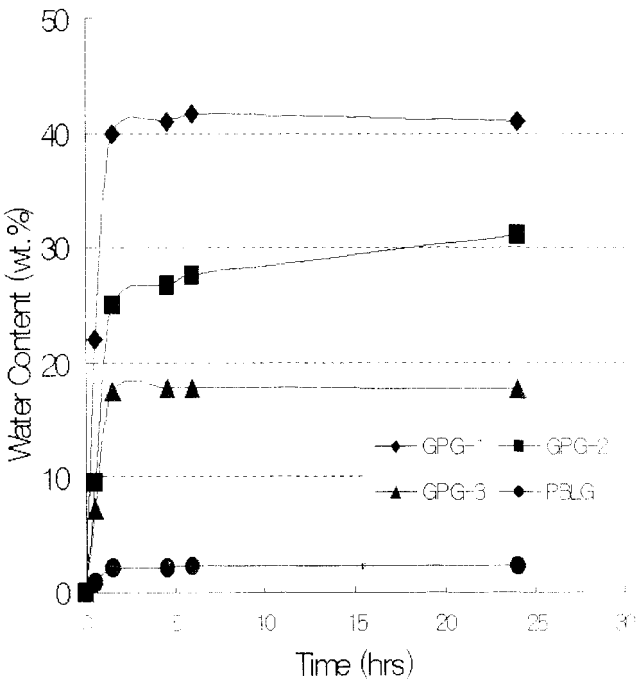


Figure 6 The water content of the copolymers and PBLG homopolymer as a function of time.

WAXD

The WAXD curves for the copolymers, poloxamer, and PBLG homopolymer are shown in Figure 4. The intensity of the diffraction patterns depended on the content of poloxamer in the block copolymer. The intensity of the diffraction patterns for the PBLG was decreased by introducing poloxamer domains, indicating that the crystallinity of the PBLG was decreased by increasing the poloxamer content in the block copolymer. The first main reflection corresponding to an intermolecular spacing of the α-helical chains is 12.5 Å, as for the film cast from chloroform. It was also found that the PBLG domains in the block copolymers underwent the same structural modifications as the PBLG homopolymer.[16] The intensity of the reflections appearing at 4.6 and 3.8 Å could be identified as PEO crystals,[17] and they also decreased with an increase of PBLG in the copolymer.

Water content

The block copolymers are expected to swell in an aqueous environment due to the hydrophilic nature of the poloxamer segments. The sample disks were prepared by casting the sample polymers from chloroform. The water content for the PBLG homopolymer and the block copolymers as a function of time is presented in Figure 6. The results showed that the water content increased with an increasing poloxamer weight fraction because of the hydrophilicity of the poloxamer, indicating that the magnitude of water

content was controlled by the hydrophilic poloxamer content. The water contents of GPG-1 and GPG-2 are 41 and 31 wt %, respectively, which is characteristic of polymeric hydrogels. This result clearly shows that the block copolymers swell in water and their equilibrium water contents are considerably higher than that determined for the PBLG homopolymer (2.3 wt %). For these samples, the time required to reach an equilibrium degree of swelling was approximately 1 h.

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