ELSEVIER

Polymer 41 (2000) 5185–5193

polymer

Thermoplastic hydrogel based on hexablock copolymer composed of poly(γ-benzyl L-glutamate) and poly(ethylene oxide)

C.-S. Cho[a,*], Y.-I. Jeong[b], S.-H. Kim[c], J.-W. Nah[d], M. Kubota[e], T. Komoto[e]

[a]*Division of Biological Resources and Materials Engineering, Seoul National University, Suwon 441-744, South Korea*
[b]*Department of Polymer Engineering, Chonnam National University, Kwangju 500-757, South Korea*
[c]*College of Pharmacy, Chosun University, Kwangju 501-759, South Korea*
[d]*Department of Polymer Science and Engineering, Sunchon National University, Sunchon 540-742, South Korea*
[e]*Department of Materials Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376, Japan*

Received 11 June 1999; received in revised form 31 August 1999; accepted 14 September 1999

Abstract

Thermoplastic hydrogels based on hexablock copolymer composed of poly(γ-benzyl L-glutamate) (PBLG) as the biodegradable and hydrophobic part and poly(ethylene oxide) (PEO) as the swellable and hydrophilic part were synthesized by polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (BLG–NCA) initiated by bis[poly(ethyleneoxide)bis(amine)]. From infrared measurement in the solid state, the polypeptide block exists in the α-helical conformation, as in PBLG homopolymer. The intensity of wide-angle X-ray diffraction patterns of the block copolymers depends on the PEO content and shows basically similar reflections as the PBLG homopolymer. The morphology examined by transmission electron microscopy and differential scanning calorimetry revealed microphase-separated structure. Water contents of the copolymers are dependent on the PEO content in the copolymers, e.g. those for the GEG-2 (PEO: 66.7 mol%) and GEG-3 (PEO: 85.1 mol%) are 30.0 and 38.4 wt%, respectively, indicating characteristics of a polymeric hydrogel. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Thermoplastic hydrogel; Hexablock copolymer; Microphase-separated structure

1. Introduction

Hydrogels are one of the most promising classes of biomaterials for biomedical application because they have good biocompatibility and a large amount of equilibrium water content [1]. Especially, hydrogel is a potential candidate to incorporate polypeptide drugs in the delivery system because the drugs are mostly soluble in water [2]. But typical thermosetting hydrogels prepared by chemical crosslinking have limitations such as non-biodegradation, lack of processibility and poor mechanical strength. Recently, thermoplastic biodegradable hydrogels have been designed for biomedical applications including drug delivery systems. Casey et al. [3] synthesized non-crosslinked triblock copolymers composed of poly(ethylene oxide) (PEO) and poly(glycolic acid) (PGA). Churchill et al. [4] synthesized similar non-crosslinked amphiphilic materials consisting of PEO and poly(α-hydroxy acid). Sawhney et al.[5] reported on biodegradable hydrogels based on polymerized PEO-*co*-poly(α-hydroxy acid) acrylate macromer. But they may still have some drawbacks associated with the hydrophilic PEO component. When high molecular weight of PEOs, which are non-biodegradable and exhibit extraordinary large hydrodynamic volume, are used, they may not be eliminated in the kidney. Also, short PEO chains result in either poor mechanical property or less hydration. Therefore, such a problem may be overcome by use of PEO derivatives which are of unique structure. Recently, Li et al. reported that the star-block copolymers of PEO and lactide or lactide/glycolide show smaller hydrodynamic radii, higher swelling rates and faster degradation rates than the linear block copolymers [6]. Choi reported that star-shaped PEO-poly(L-lactic acid) (PLA) block copolymer had lower glass transition temperature and crystallinities [7].

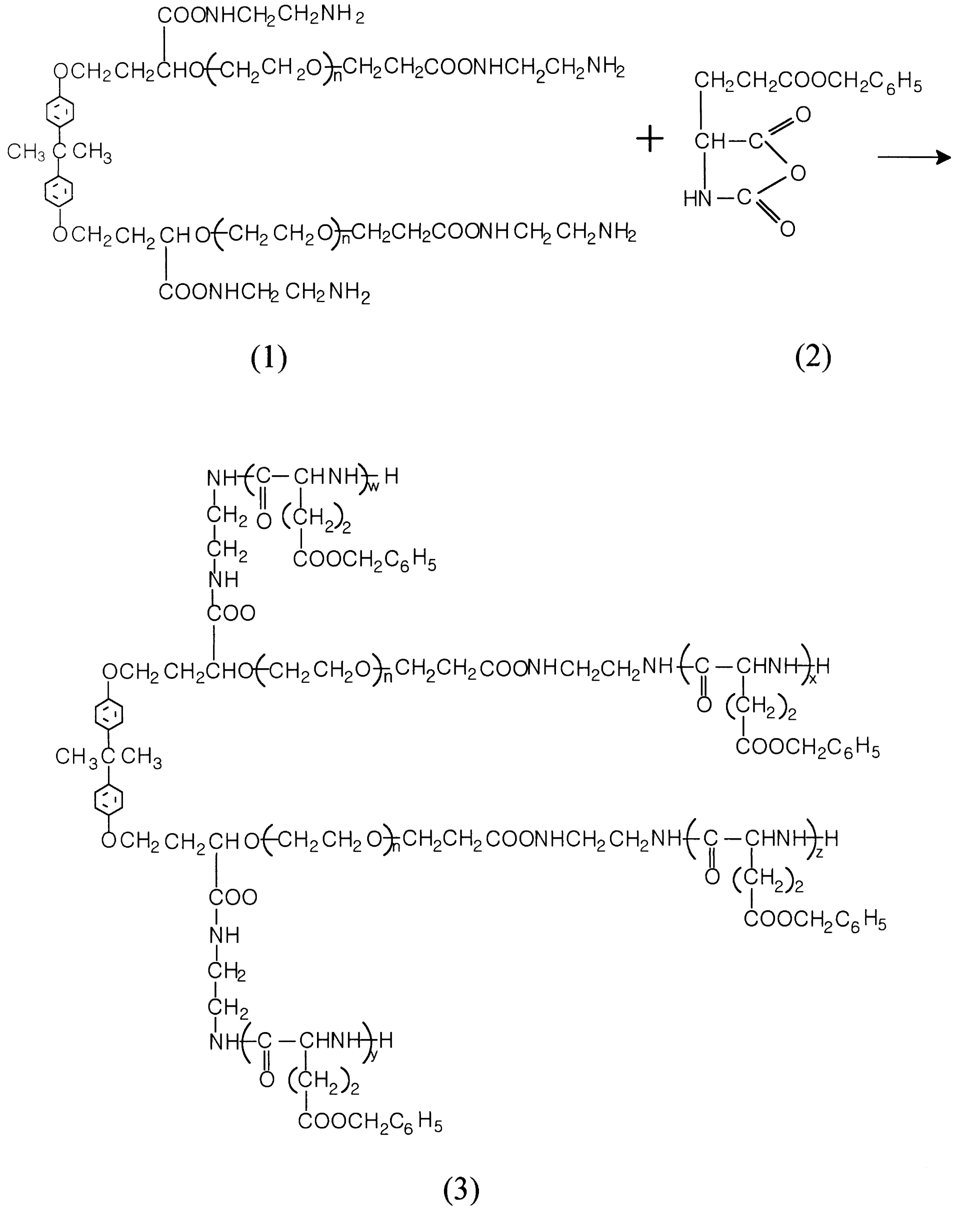
In this study, we are aiming to prepare a thermoplastic hydrogel based on hexablock copolymer composed of poly(γ-benzyl L-glutamate) (PBLG) as the hydrophobic part and PEO as the hydrophilic one. PBLG is one of synthetic polypeptides which have attracted attention for

---

[*] Corresponding author. Tel.: +82-331-290-2494; fax: +82-331-291-8697.

E-mail address: chocs@plaza.snu.ac.kr (C.-S. Cho).

PII: S0032-3861(99)00746-6



Scheme 1.

use in drug delivery matrices. PEO as the hydrogel part is a water-soluble and non-toxic polymer which can be used to control the water content in the copolymer.

2. Experimental part

2.1. Materials

Bis[poly(ethyleneoxide)bis(amine)] (BPEOBA: MW = 20,000) and γ-benzyl L-glutamate were purchased from Sigma Chem. Co. (St. Louis, MO). Triphosgene was purchased from Aldrich Chem. Co. Inc. (Milwaukee, WI). All chemicals used were of reagent or spectrometric grade. *n*-hexane and methylene dichloride were stored with 4 Å molecular sieves and used without further purification.

Table 1
Characterization of PBLG homopolymer and hexablock copolymers (molecular weight and composition were estimated by NMR measurement)

Sample	Content of monomeric units in mol%		$\bar{M}_n$
	PBLG	PEO	
PBLG	100	0	60,000
G_4E_2-1	40.3	59.7	87,300
G_4E_2-2	33.3	66.7	69,700
G_4E_2-3	14.9	85.1	37,400

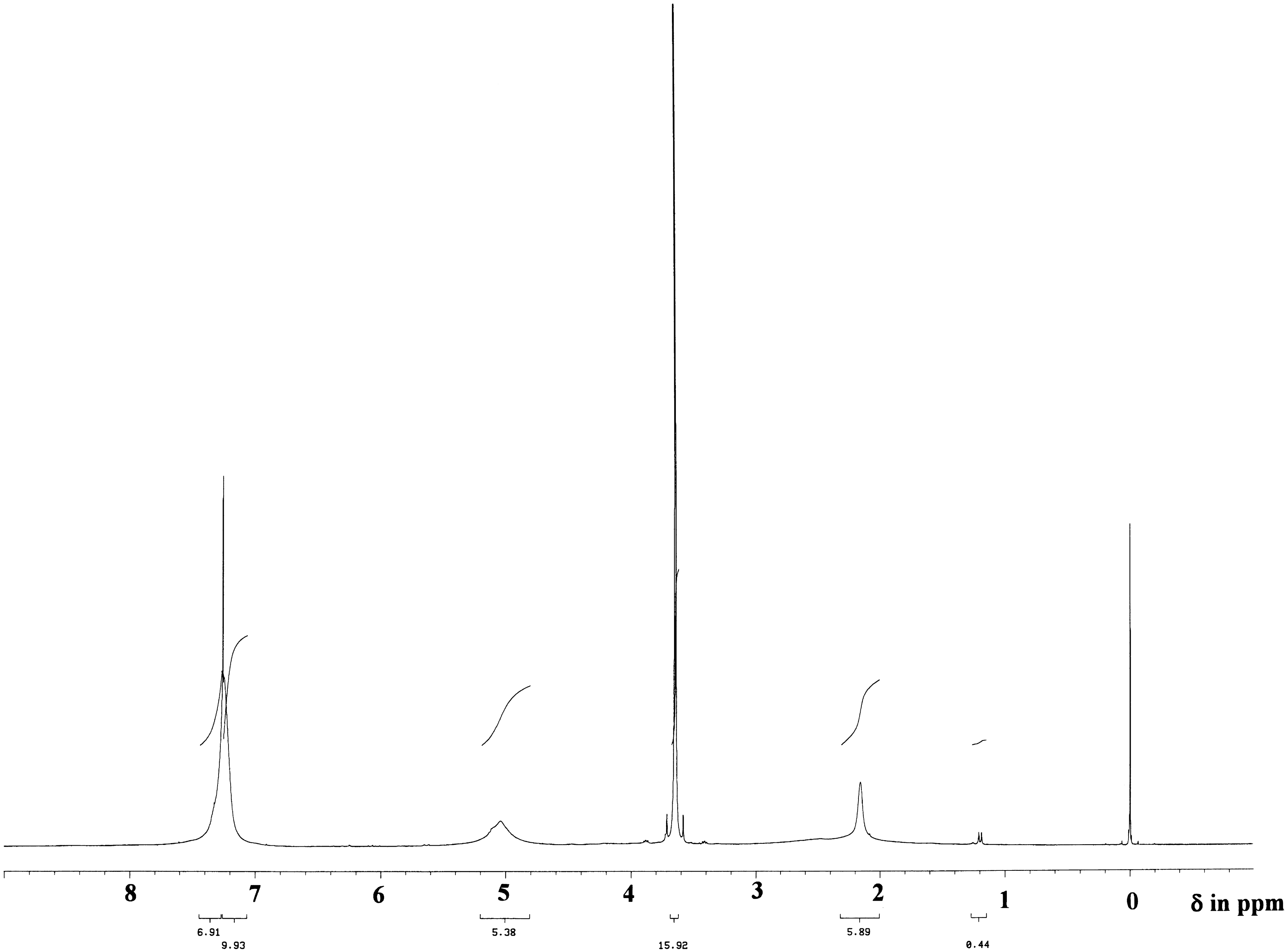


Fig. 1. ^{1}H NMR spectrum of G_4E_2-2 block copolymer in chloroform-d_1 containing 10 vol% of trifluoroacetic acid-d_1.

Table 2
Melting temperature of PEO in the copolymers

Sample	EO unit (mol%)	T_m (°C) of PEO	ΔH_f of copolymer (J/g)
PBLG	0	–	–
G_4E_2-1	59.7	45.6	9.0
G_4E_2-2	66.7	49.7	23.0
G_4E_2-3	85.1	50.2	39.6
PEO	100.0	52.5	97.5

2.2. Synthesis of hexablock copolymer

The reaction scheme is shown in Scheme 1 (synthesis of hexablock copolymer). γ-benzyl L-glutamate *N*-carboxyanhydride (BLG–NCA) was prepared by a method decribed in the literature [8]. The hexablock copolymer (3) was synthesized by a similar method to that previously reported [9–11]. Briefly, the hexablock copolymer was obtained by polymerization of BLG–NCA (2) initiated by BPEOBA (1) in methylene chloride, at a total concentration of BLG–NCA and BPEOBA of 3% (w/v), at room temperature for 72 h. The reaction mixture was poured into a large excess of diethyl ether to precipitate the hexablock copolymer. The resulting copolymer was washed with diethyl ether and then dried in vacuo. The copolymer was washed again with water to remove the unreacted PEO and dried in vacuo. The yield of these block copolymers was about 60–70%.

2.3. Measurements

2.3.1. ^{1}H NMR measurement

^{1}H NMR spectra of the copolymers were measured in a mixed solvent of $CDCL_3$ and trifluoroacetic acid (9/1; v/v) to estimate the copolymer compositions and the molecular weights of PBLG blocks, using a JEOL FX 90Q NMR spectrometer. As the number-average molecular weight (20,000) of PEO is known, one can estimate the number-average molecular weights of the PBLG blocks and the copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers, respectively [10,11].

2.3.2. IR measurement

IR spectra of solid films of the sample cast from chloroform solution were measured on a Nicolet 520P FT-IR spectrometer between 1, 800 and 400 cm^{-1}.

2.3.3. Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) and melting temperature (T_m) were measured with a PL-DSC 700 (PL Thermal Sci.) apparatus. The measurements were carried out in the range of 0–300°C at a scanning rate of 10°C/min.

2.3.4. Dynamic mechanical thermal analysis (DMTA)

The DMTA profiles of the polymers were obtained by a vibrating reed method in the form of bars using a DMTA 4 (Rheometer Sci. Inc.). The measurements were taken over a temperature range of 25–180°C at a frequency of 3 Hz and a heating rate of 2°C/min.

2.3.5. X-ray diffraction

X-ray diagrams were obtained with a Rigaku D/Max-1200 (Geigerflex) using Ni-filtered CuK$_\alpha$ radiation (35 kV, 15 mA).

2.3.6. Transmission electron microscope (TEM)

A thin film was prepared at room temperature by casting the block copolymer solution in chloroform on a carbon film coated on a copper grid for TEM observation. The specimen on the copper grid was stained by RuO_4 in the vapor phase above an aqueous RuO_4 solution containing an excess $NaIO_4$ which was prepared according to the method by Trent [12]. The staining was done in a flask equipped with a tight cap which was kept at room temperature for 30 min in a hood. Observation was done at 80 kV in a JEOL JEM 1200 EXS transmission electron microscope.

2.3.7. Water content

Dry disks (diameter: 7.1 mm and thickness: 1.1 mm) were incubated in phosphate buffered saline (PBS: 0.1 M, pH 7.4) at 37°C. At predetermined time intervals, hydrated samples were weighed after blotting the surface water with filter paper. Water contents were calculated as $[(W_s - W_d)/W_s] \times 100$, where W_s and W_d are wet weight and dry weight of the discs, respectively.

3. Results and discussion

3.1. Characterization of hexablock copolymer composed of PEO and PBLG

The block copolymers (abbreviated as G_4E_2) were prepared by polymerization of γ-BLG NCA initiated by the amine-terminated PEO in methylene chloride solution. The reaction mixture may contain unreacted amine-terminated PEO and the desired block G_4E_2 copolymers. The initiator (amine-terminated PEO) cannot be precipitated from a mixture of methylene chloride and diethyl ether, although the latter is a non-solvent for PEO. By adding diethyl ether to the reaction mixture, the G_4E_2 block copolymers precipitated were collected on a filter, while the unreacted amine-terminated PEO was removed in the filtrate.

In Table 1 are listed the content of PEO and the molecular weight of the copolymers obtained from ^{1}H NMR spectra. The copolymer composition and the molecular weight were estimated from peak intensities of the phenyl proton (7.2 ppm) signal of the PBLG block and the ethylene proton signal (3.7 ppm) of the PEO block in the NMR spectrum. Fig. 1 shows the ^{1}H NMR spectrum of the G_4E_2-2

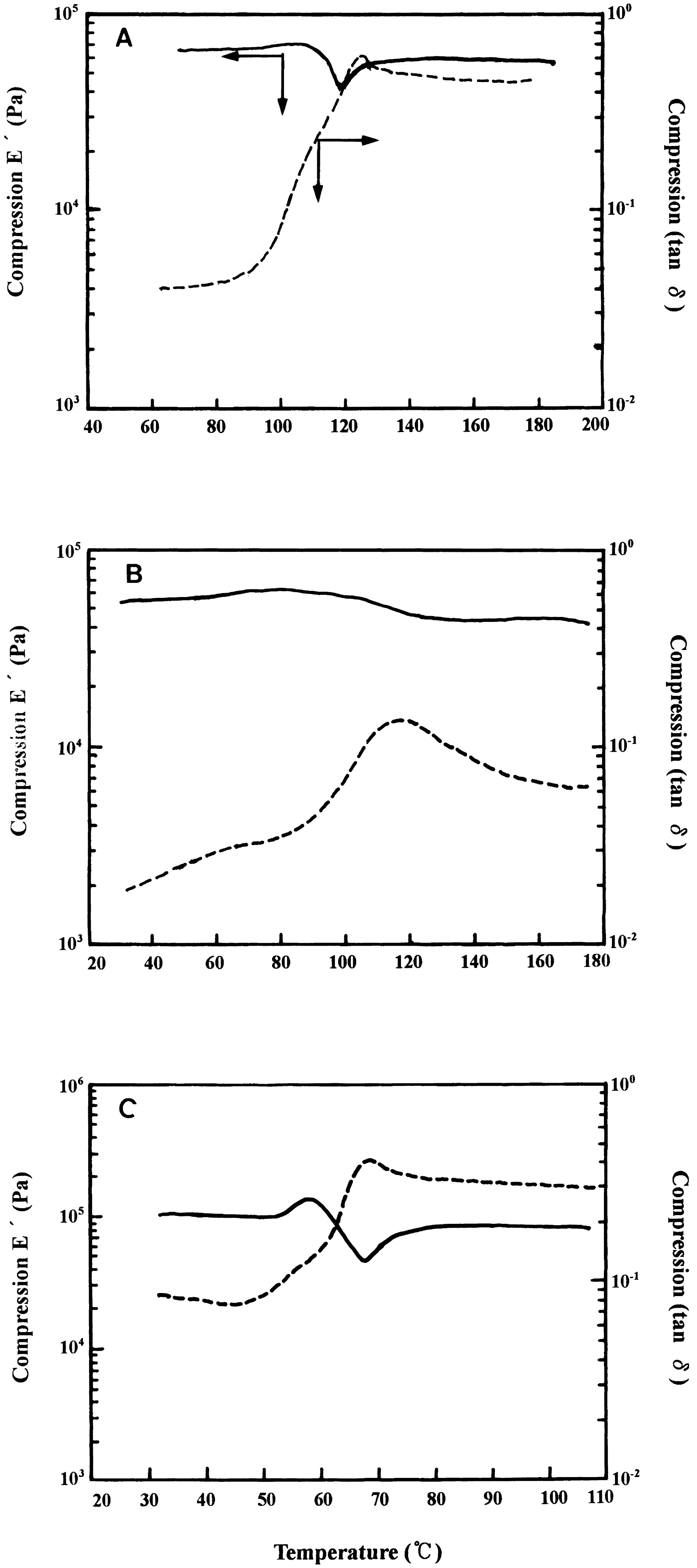


Fig. 2. DMTA profiles of (A) PBLG homopolymer; (B) G_4E_2-1 and (C) G_4E_2-3 block copolymers.

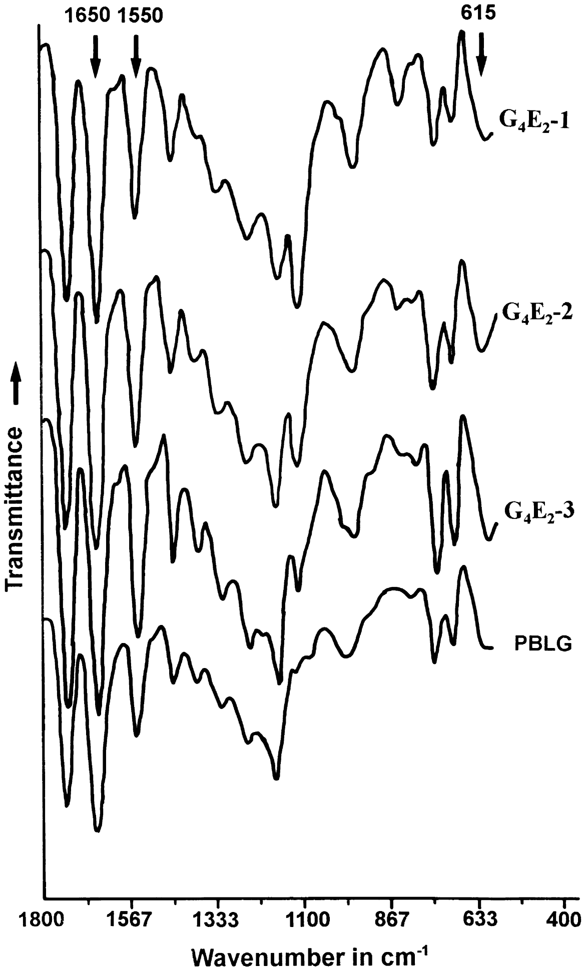


Fig. 3. IR spectra of G_4E_2 block copolymers and PBLG homopolymer cast from chloroform.

copolymer. Assuming that all the amine groups of PEO participate in the polymerization, the number-average molecular weights (M_n) of the copolymer and the PBLG block can be calculated from the copolymer composition and the molecular weight of PEO chains.

The results of DSC studies are shown in Table 2. These results indicate that the melting temperatures (T_m) of PEO in G_4E_2-1 to G_4E_2-3 ranged from 45 to 50°C, indicating that the T_ms were reduced with an increase of PBLG block in comparison with T_m of PEO itself (52.5°C). The heat of fusion for PEO is 97.5 J/g, whereas the heat of fusion of the PEO component in the copolymers is smaller. These results indicate that the crystallization of the PEO is partially prevented by the PBLG chains so that the apparent crystallinity of the PEO block in the block copolymers is nearly half of the PEO homopolymer. This is fairly consistent with the WAXD profiles shown in Fig. 4, where no crystalline reflections were observed for G_4E_2-1, although this copolymer contains 59.7 mol% of PEO. On the other hand, G_4E_2-3, which contains 14.9 mol% of PBLG, shows a WAXD reflection, though weak.

Fig. 2 shows DMTA profiles of PBLG homopolymer and G_4E_2 block copolymers at a frequency of 3 Hz. The tan δ peak against temperature is generally the α_1-peak, which is associated with the relaxation of the PBLG [16]. The tan δ peak for PBLG homopolymer, G_4E_2-1 and G_4E_2-3 block copolymer was observed at 123.7, 118.3 and 68.7°C, respectively. The resulting spectra showed significant differences in the temperature of damping at different PEO content in the copolymer. It seems to be due to the flexible chain of PEO in the copolymer. This may indicate that a large amount of PBLG chains are prevented to crystallize by the presence of the major fraction of PEO chains. This might cause a large decrease in α-1 transition temperature of PBLG chains where the disordered PBLG chains are relaxed at temperature as low as 68.7°C. Also, it may be said that the shoulder at ca. 55°C in the DMTA tan δ curve of G_4E_2-3 can be assigned to PEO melting at 52.5°C as was observed by DSC. However, the transition temperature of the tan δ maximum is much higher than T_m of PEO.

3.2. Chain conformation of the block copolymers in the solid state

Infrared (IR) spectra were measured for solid films of G_4E_2 block copolymers and PBLG homopolymer, both cast from chloroform. The spectra in the region of 1800–400 cm^{-1} are shown in Fig. 3. The amide I, II, and V bands of these G_4E_2 block copolymers appear at 1650, 1550, 615 cm^{-1}, respectively, at the same wavenumbers as for the PBLG homopolymer. These results indicate that the polypeptide block exists in the α-helical conformation, as in the PBLG homopolymer.

3.3. Wide-angle X-ray diffraction

The wide-angle X-ray diffraction patterns for copolymers and the PBLG homopolymer are shown in Fig. 4. The intensity of the diffraction patterns depended on the content of PEO in the block copolymer. The intensity of the diffraction patterns for the PBLG decreased by introducing PEO domains, indicating that the crystallinity of the PBLG was decreased by introducing PEO 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. Also, it was found that the PBLG domains in the block copolymers underwent the same structural modifications as the PBLG homopolymer [13]. Also, the intensities of the diffraction patterns for the PEO decreased by introducing PBLG domains, indicating that the crystallinity of the PEO decrease by introducing PBLG in the copolymer as the similar tendency of DSC results. The main reflections appearing at 4.6 and 3.8 Å could be identified as PEO crystals [14].

3.4. Morphology of the block copolymers in the solid state

RuO_4 staining was applied to the present copolymers where the PEO domains will be stained (reacted) as dark

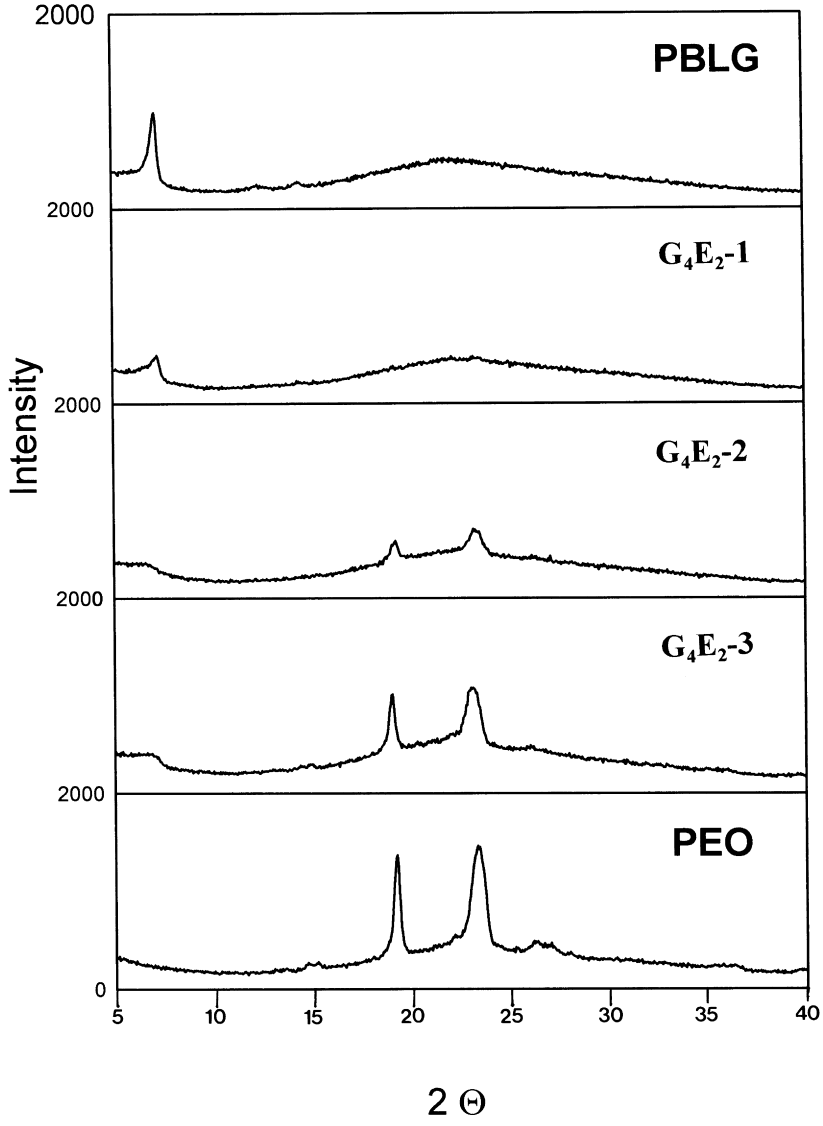


Fig. 4. Wide-angle X-ray diffraction patterns of G_4E_2 block copolymer, PBLG and PEO homopolymers cast from chloroform.

in the TEM micrographs, while the less stained PBLG domains will be seen bright. Fig. 5(a)–(c) shows TEM micrographs of thin films of the G_4E_2-1, G_4E_2-2 and G_4E_2-3 copolymers, respectively, cast from chloroform solutions onto film and stained by RuO_4.

As seen in Fig. 5(a), a homogenously dispersed microphase separation took place in case of G_4E_2-1. Bright globular PBLG microdomains with a size of ca 20–40 nm were formed, being surrounded by dark PEO domains with a thickness of ca 8–16 nm. The globular morphology may be formed by a phase separation of α-helical PBLG chains of each block copolymer and some globules are connected. This morphology may be basically simplified as a model of core/shell structure where the core is PBLG and the shell PEO. Fig. 5(b) shows a unique phase-separated morphology of G_4E_2-2, suggesting macro- and microphase separation. The thick and dark, meandered phase with a size of ca 40–100 nm may be formed by a macrophase separation. Some are dark rimmed with a thickness of 10–13 nm probably due to concentration of PEO domains. This morphology may be simplified as rod-like aggregates of PBLG domains with a dark PEO sheath. Otherwise, the microphase separated domains of G_4E_2-2 are similar to the case of G_4E_2-1. The size of globules is ca 13–21 nm which is obviously smaller than ca 20–40 nm for G_4E_2-1. Dark PEO domains have a thickness of ca 8–15 nm.

As is evident from Fig. 5(c), rod-like aggregates are dominant morphology for phase-separated G_4E_2-3. Thick and long domains of the PBLG are seen on the left part of the figure, while small and short ones on the right part. The heterogeneity in phase-separated morphology may arise from molecular fractionation during cast of the solution. Thick rods may consist of block copolymers with higher molecular weight PBLG chains, though the polydispersities of four PBLG chains of each block copolymer and the whole block copolymer molecules have not been clarified so far.

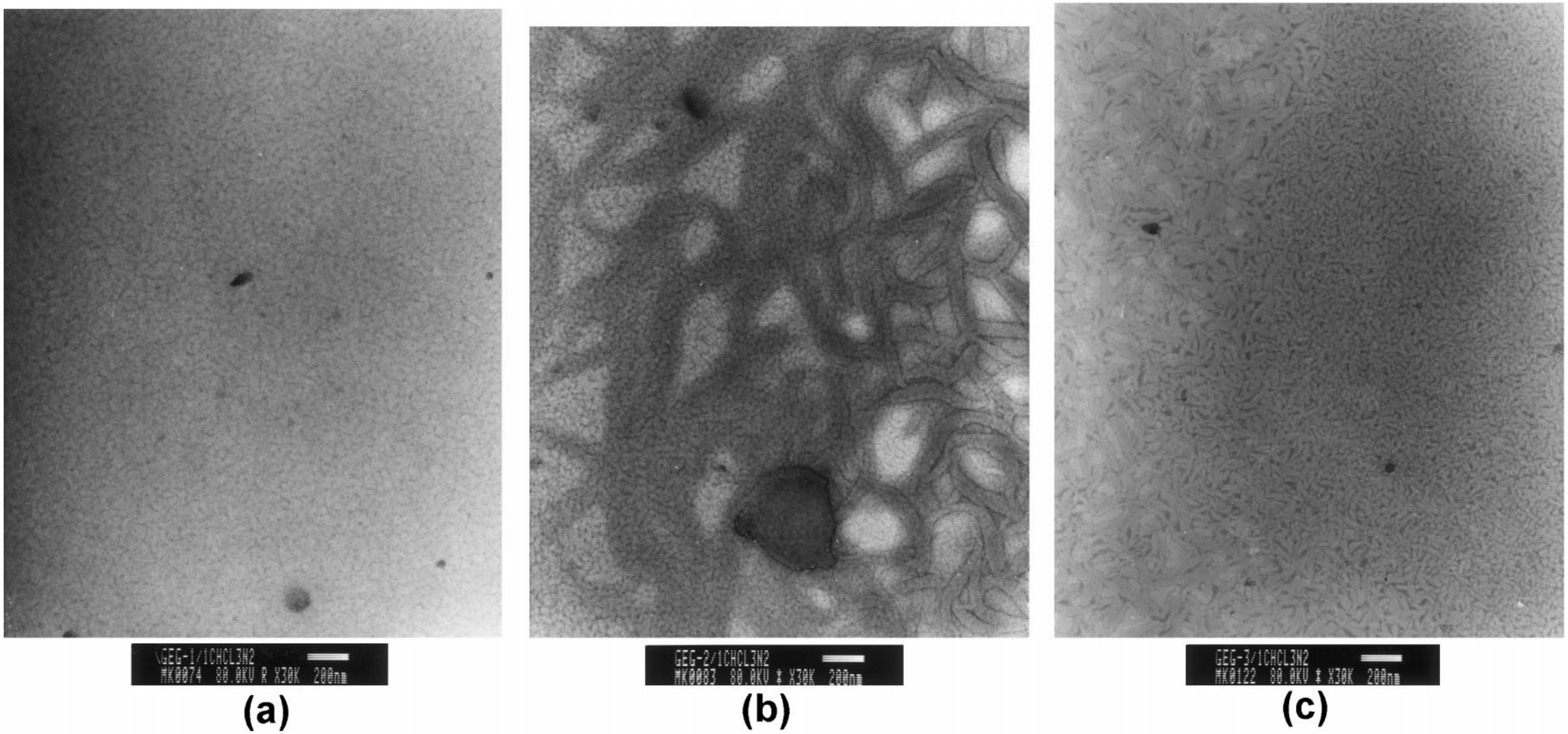


Fig. 5. Transmission electron micrographs of G_4E_2 block copolymers cast from chloroform solution and stained by RuO_4: (a) G_4E_2-1; (b) G_4E_2-2; and (c) G_4E_2-3 with mole ratio of BLG/EO of; 80.9/19.1, 40.3/59.7 and 25.4/74.6, respectively.

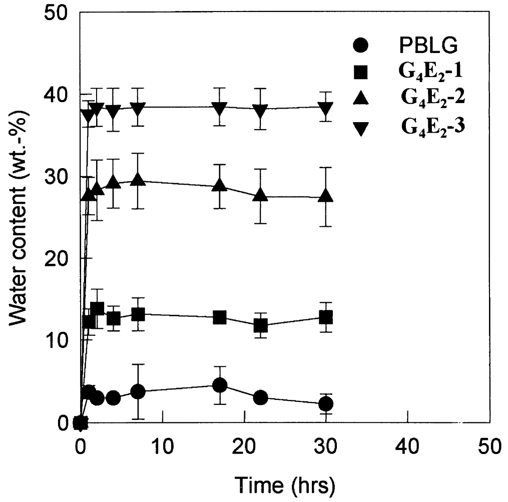


Fig. 6. Water content of PBLG homopolymer and G_4E_2 block copolymers with time.

The small rods with thickness of ca 7–13 nm and a length longer than ca 38 nm may consist of lower molecular weight PBLG chains. It can be said that the morphology of the PBLG chains changes from globule to rod with a decease in PBLG content in the copolymer composition, i.e. a decrease in PBLG chain length. It is noted that the thickness of the dark PEO domains is ca 8–14 nm, although thicker dark domains are partly seen probably due to polydispersity of the block copolymer.

It is worthy of note that the thickness of the dark PEO domains is around 10 nm independent of the kind of block copolymers, because the molecular weight of PEO is 20,000 as an initiator for all the block copolymers. This is also evidence for the dark PEO domains in the TEM micrographs.

It is of interest to compare the size of the above-mentioned microphase-separated domains with the molecular size of the block copolymer. The lengths of the PBLG chains with α-helical conformation was estimated from the molecular weights of the PEO block (20,000) and the whole copolymer molecule, as determined by NMR, assuming same PBLG chain lengths for the four blocks. As a result, the α-helical chain length of PBLG is estimated as 32.8, 15.5 and 6.2 nm for G_4E_2-1, G_4E_2-2 and G_4E_2-3, respectively. These values are compared with the size of the corresponding phase-separated PBLG domains, which are ca 20–40 nm, ca 13–21 nm and ca 7–13 nm, respectively. A rather good agreement of the calculated PBLG chain length and the size of the microphase-separated PBLG domains may indicate that the PBLG chains are aligned almost perpendicular to the thin PEO layer (domain). As for the crystallinity of PEO blocks, which has bisphenol A group at the midpoint of the PEO molecule and average MW of 20,000, i.e. average degree of polymerization of ca 454. Thus, one PEO block has an average degree of polymerization of ca 230. Taking into consideration of the PEO crystal structure that the length of the PEO chain in the extended conformation is 19.5 Å (1.95 nm) per 7 monomer units. This indicates that the extended chain length of one PEO block is calculated as ca 640 Å (64 nm). This value is an enough length for chain foldings of PEO block. On the other hand, it is obvious that the thickness of the dark part increases with increasing PEO content in the copolymer in TEM micrographs. The fact that the RuO_4 staining took place may mean that PEO is not so highly crystalline but less crystalline or in a highly mobile state in the solid state at room temperature. In fact, PEO has T_g of below 232 K (ca −41°C) [15].

3.5. Water content

Water content of G_4E_2 block copolymers against an incubation time in PBS at 37°C is shown in Fig. 6. These results show that the water content increases with increasing PEO weight fraction due to the hydrophilicity of PEO. The water contents of G_4E_2-2(PEO: 59.7 mol%) and G_4E_2-3 (PEO: 74.6 mol%) are 30.0 and 38.4 wt%, respectively, characteristic of a polymeric hydrogel.

References

[1] Park H, Park K. In: Ottenbrite RM, Huang SJ, Park K, editors. Hydrogels in bioapplications, ACS Symposium Series 627 Washington: American Chemical Society, 1996. p. 1.
[2] Huchinson FG, Furr BJA. J Controlled Release 1990;13:279.
[3] Casey DJ, Jarrett PK, Rosati L. US Patent, 4,716,203 (1987).
[4] Churchill JR, Huchinson FG. US Patent, 4,526,938 (1985).
[5] Sawhney AS, Pathak CP, Hubbel JA. Macromolecules 1993;26:581.
[6] Li Y, Kissel T. Polymer 1998;39:4421.
[7] Choi YK. PhD thesis, University of Utah, 1996.
[8] Fuller WD, Verlander MS, Goodman M. Biopolymers 1976;15:869.
[9] Cho CS, Kim SW, Sung YK, Kim KY. Makromol Chem 1988;189:1505.
[10] Cho CS, Kim SW, Komoto T. Makromol Chem 1990;191:981.
[11] Cho CS, Jo BW, Kwon JK, Komoto T. Macromol Chem Phys 1994;195:2195.
[12] Trent JS. Macromolecules 1984;17:2930.
[13] Bamford CH, Elliott A, Hanby WE. Synthetic polypeptides, New York: Academic Press, 1956 chap VIII.
[14] Takahashi Y, Tadokoro H. Macromolecules 1973;6:672.
[15] Brandrup J, Immeregut EH. Polymer handbook, 3. New York: Wiley, 1989.
[16] Kajiyama T, Kuroishi M, Takayanagi M. J Macromol Sci Phys 1975;B11:195.