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폴리펩티드-의약 전달체 및 폴리펩티드 공중합체의 합성 및 물성에 관한 연구 (I) L-Lactic Acid와 L-Glutamic Acid 공중합체의 합성 및 그의 물성*

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Studies on Synthesis of Block Copolymers Containing Polyester and Polypeptide for Drug Delivery System

I. Synthesis and Characterization of Copolymer of L-Lactic Acid and L-Glutamic Acid

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요 약. 의약 전달체에 사용되는 생체분해성 고분자로서 L-lactic acid와 L-glutamic acid가 각기 다른 조성비로 이루어진 공중합체를 합성하였다. Poly(L-lactide)는 zinc oxide를 이용하여 합성하였으며, poly(L-lactide) 말단에 3-amino-1-propanol을 도입시킨 다음 이미 합성된 γ-benzyl-L-glutamate-N-carboxyanhydride(γ-BLG-NCA)를 개환중합시켜서 block copoly(L-lactide-γ-benzyl-L-glutamate)를 합성하였다. NMR로써 L-lactic acid와 γ-BLG-NCA가 서로 일정한 비율로 이루어진 공중합체가 합성되었음을 확인하였으며, 생성된 공중합체들의 열적성질은 시차주사열량계법 및 열무게 측정법으로 조사하였다.

ABSTRACT. As a possible biocompatible and biodegrable polymer skeleton for drug delivery system, block copolymers of L-lactic acid and L-glutamic acid with different composition were synthesized and characterized. Poly (L-lactide) was prepared by polymerization of L-lactide with zine oxide at 130 °C for 72 hrs. 3-Amino-1-propanol was introduced to poly (L-lactide) by an ester linkage in order to initiate polymerization. Polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride (γ-BLG-NCA) utilizing the amino group of modified poly (L-lactide) as an initiator gave rise to the block copoly (L-lactide-γ-benzyl-L-glutamate). The NMR study of resulting block copolymers showed that the composition of L-lactic acid and γ-benzyl-L- glutamate in block copolymers was depended on the weight ratio of poly (L-lactide) and γ-BLG-NCA. The thermal properties of the resulting block copolymers were determined by the differential scanning calorimetry and by the thermogravimetry.

INTRODUCTION

For the last two decades, studies for the application of polymer in biological system have made remarkable progress.[1] Numerous biomedical polymers [2,3] have been synthesized and modified by various reaserchers in order to apply them in medical and pharmaceutical area. During our continuing efforts[4,5] to develop biocompatible,

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biodegradable, and non-toxic polymer/drug conjugate (drug delivery system), we were interested in synthesis of a block copolymer of L-lactic acid and L-glutamic acid. Inspite of well known poly (L-lactic acid), poly (L-glutamic acid) and its analogous, a block copolymer of L-lactic acid and L-glutamic acid has not been reported yet. The target polymer was expected to provide a polymer-backbone for drug delivery system since it would have biocompatibility and necessary sites for drug attachment. Our strategy for the synthesis of the target polymer was based upon the initial polymerization of L-lactide to which 3-amino-1-propanol was introduced to initiate polymerization of -benzyl-L-glutamate-N- carboxyanhydride. Based on this plan, synthesis and characterization of a block copolymer of L-lactic acid and L-glutamic acid were presented.

EXPERIMENTAL

Materials

All reagents and solvents were reagent grade and used without further purification unless specified otherwise.

Instruments

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken at 60 MHz (Hitech) Chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane (δ 0.00) as an internal standard. Infrared (IR) spectra were recorded on a Nicolet 5-MX as a KBr pellet. Melting points were determined by Thiele apparatus and were uncorrected. Molecular weight were determined by Ostwald viscometer. Thermal propeties were obtained by the Differential Scanning Calorimetry (Perkin Elmer DSC-4) and the Thermogravimetry (Perkin-Elmer TGS-2).

Synthesis

γ-Benzyl-L-Glutamate (γ-BLG) 2. To a solution of concentrated sulfuric acid (31.7 m*l*, 0.600 mol) in water (24.8 m*l*) was added L-glutamic acid (88.2g, 0.600 m*l*) followed by the addition of benzyl alcohol (69.2 m*l*, 0.660 mol). The reaction mixture was stirred at 70 °C until it was sticky and transparent. The solvent was removed *in vacuo* and the residue was neutralized by the addition of water (140 m*l*) and 1.2 N sodium hydroxide solution (300 m*l*, 0.360 mol). The product was collected by filteration and washed with water. Recrystallization from 5% ethyl alcohol in water afforded 40.0g (31.5%) of γ-benzyl-L-glutamate as a colorless powder.; mp. 169.0~170.0, IR (KBr) 3400 (N-H), 1750 (C=O), 1050~1150 (C-O-C), 780~800 cm^{-} (aromatic ring); NMR ($CDCl_3$) 7.50 (C_6H_5, 5H), 4.90 ($-OCH_2C_6H_5$, 2H), 3.90 ($-NHCOCH$, 1H), 2.40 ($-CH_2CO-$,2H), 1.60 ($-CH-CH_2$, -2H).

γ-Benzyl-L-Glutamate-N-Carboxyanhydride (γ-BLG-NCA) 3. To a solution of γ-benzyl-L-glutamate (2.00g, 8.44 mmol) in dry tetrahydrofuran (30.0 m*l*) under nitrogen was added diphosgene (1.67 m*l*, 16.99 mmol) in small portion. The reaction mixture was heated at 40 °C for 2 hrs. After the suspension became clear yellowish solution, it was standed at -30 °C for overnight and then the solvent was removed by aspirator (r.t, 15 mmHg). The product was solidified by the addition of *n*-hexane. Recrystallization from *n*-hexane afforded 1.82g (91.0%) of γ-benzyl-L-glutamate-N-carboxyanhydride as a colorless powder. ; mp. 93.0~94.0 °C, IR (KBr) 3400 (N-H), 1750 (C=O), 780~850 (aromatic ring), 936 cm^{-1} (ring peak); NMR ($CDCl_3$) 7.20 (C_6H_5, 5H), 5.00 ($-O-CH_2-C_6H_5$, 2H).

Polymerization of L-Lactide 5.

a) Poly (L-Lactide) by stannous octoate; To a L-lactide (10.0g, 69.4 mmol) under nitrogen was added stannous octoate (0.50 m*l*, 96 μmol) in toluene using by syringe. The mixture was heated for 5 min under the reduced pressure in order to remove toluene and then stirred at 130 °C for 72 hrs. The product was solidified by the addition of methanol as a nonsolvent. Recrystallization from

methanol-chloroform (1:1) afforded 8.70g of poly (L-lactide).; IR (KBr) 3300 (-OH), 1760 (-C=O), 1050~1150 cm^{-1} (C-O-C); NMR ($CDCl_3$) 1.70 (-CH_3, 3H), 5.00~5.20 (-CH, 1H).

b) Poly (L-lactide) by zinc oxide; To L-lactide (10.0g, 69.4 mmol) was added zinc oxide (0.001g, 120 μmol) without solvent. The mixture was allowed to react at 130 °C under nitrogen. The product was solidified by the addition of methanol as a nonsolvent. Recrystallization from methanol-chloroform (1:1) afforded 8.00g of poly (L-lactide); IR (KBr) 3300 (-OH), 1760 (-C=O), 1050~1150 cm^{-1} (C-O-C); NMR ($CDCl_3$) 1.70 (CH_3, 3H), 5.00~5.20 (-CH, 1H).

Block Copoly (L-Lactide-γ-Benzyl-L-Glutamate 8.

a) Introducing the 3-amino-1-propanol to the terminal of poly (L-lactide); To a solution of poly (L-lactide) (1.00g) in 20.0 m*l* of benzene was added thionyl chloride (1.50 m*l*, 20.7 mmol). The above mixture was heated at 40 °C for 30 min. The unreacted thionyl chloride and solvent were removed *in vacuo*. To a 3-amino-1-propanol (28.1 *l*, 300 mol) was added concentrated hydrochloric acid (75 μl, 900 mol) and stirred at room temperature for 30 min to use the next step. The above 3-amino-1-propanol was added to the resulting solution and stirred at room temperature for 2 hrs. The crude product was soldified by the addition of methanol and used directly to the next step without purification.

b) Block Copoly (L-Lactide-γ-Benzyl-L-Glutamate); To a solution of γ-BLG-NCA (1.00g, 3.80 mmol) in 20.0 m*l* of benzene was added the above product (0.200g, 2.22 mmol). After 72 hrs at 40 °C the mixture became gel state. The product was solidified by the addition of methanol as a nonsolvent. Recrystallization from methanol-chloroform (1:1) afforded 1.02g (85.0%) as a colorless powder.; IR (KBr) 3400 (N-H), 1780 (C=O), 1650 (amide I), 1560 (amide II) 1050~1150 (C-O-C), 936 cm^{-1} (aromatic ring); NMR (DMSO-d_6) 1.70 (-CH_3, 3H) 1.80~2.30 (-CH_2, 4H), 3.50 (-NH-CH-CO, 1H), 4.90~5.20 (C_6H_5 CH_2-, -CH-, 3H) 7.20 (-C_6H).

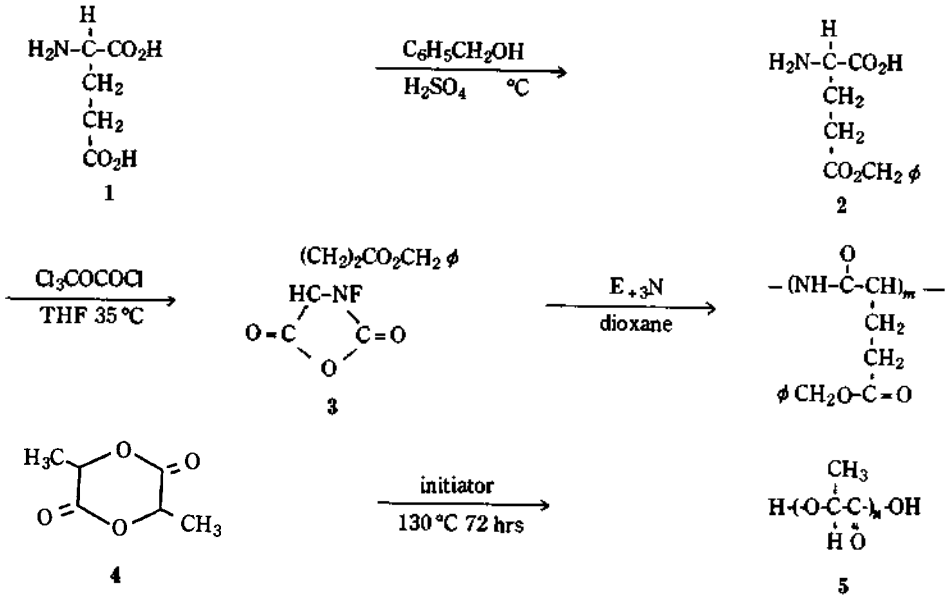
Block Copoly (L-Lactide-L-Glutamic acid) 9. To a solution of polymer 8 (500 mg) in 20.0 m*l* dichloromethane was added 10.0% Pd/C (400 mg) at room temperature. The above mixture was shaked at 60 psi for 48 hrs using Parr Hydrogenator. Addition of ethanol followed by filtration with celite gave rise to colorless filtrate. Condensation of the filtrate *in vacuo* afforded 50 mg (10.0%) of polymer 9 as a solid.; IR (KBr) 3400 (N-H), 1780 (C=O), 1650 (amide I), 1560 (amide II), and 1050~1150 cm^{-1} (C-O-C); NMR (DMSO-d_6) 1.70 (CH_3-, 3H), 1.8~2.30 (CH_2-CH_2, 4H), 3.50 (-NH-CH-CO, 1H), and 4.90~5.20 (C_6H_5 CH_2-, -CH-, 3H).

Poly (γ-Benzyl-L-Glutamate) 10. To a stirred solution of γ-BLG-NCA (1.00g, 3.80 mmol in 20.0 m*l* of dioxane was added triethylamine (0.116 m*l*, 0.084 mol). The mixture was heated at 25 °C for 72 hrs. The product was solidified by the addition of ethanol as a nonsolvent. Recrystallization from chloroform-ethanol (1:1) afforded 0.800g of poly (γ-benzyl-L-glutamate) as a colorless solid; IR (KBr) 3400 (N-H), 1750 (C=O), 780~850 cm^{-1} (aromatic ring).

RESULTS AND DISCUSSION

Synthesis

Our approach to the synthesis of the polymer 8 was depicted in *Scheme* 1 and 2. We began with preparation of the compound 3 according to the known procedure.[6~8] Protection of glutamic acid as a benzyl ester followed by treatment with diphosgene gave rise to the compound 3 in 91.0% yield. The IR spectrum of the compound 3 displayed an absorption at 936 cm^{-1} (ring peak) for N-carboxyanhydride. In spite of difficulty arising from handling diphosgene, the compound 3 was obtained in gram scale. With an ample amount of



Scheme 1

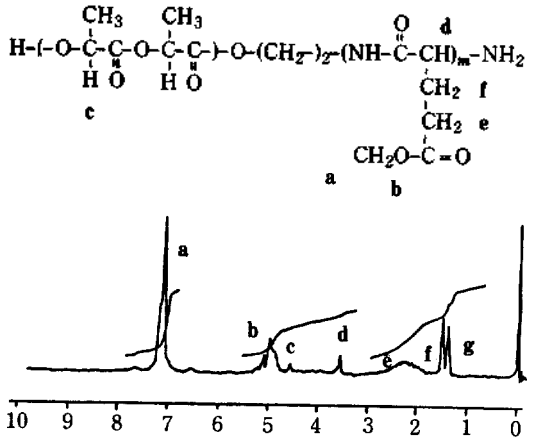
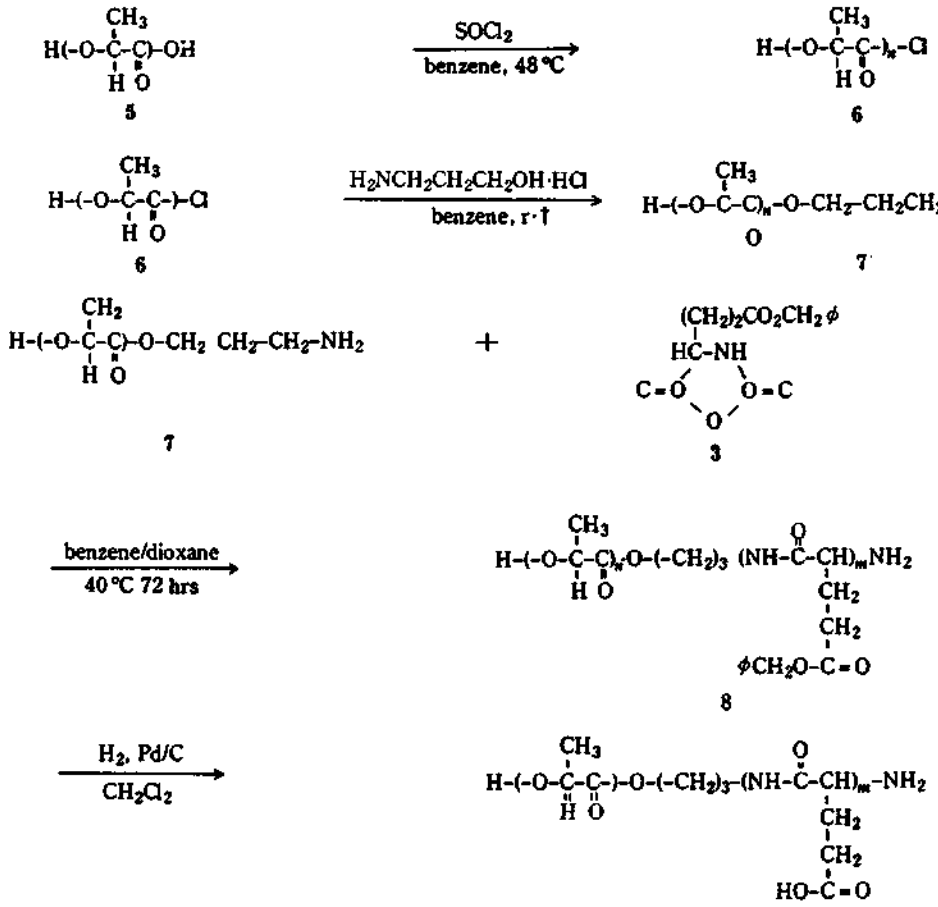


Fig. 1. ^{1}H NMR spectrum of block copoly(L lactide benzyl glutamate).

the compound 3 in hand, poly (L-lactide) 5 was prepared by polymerization of L-lactide, 4. Because of its low toxicity[9~11] stannous octoate was the first choice among other initiators[12,13] for polymerzation. Polymerization of the polymer 4 using stannous octoate as an initiator provided the polymer 5 as a colorless solid. Synthesis of the compound 5 was confirmed by disappearance an absorption at 935 cm^{-1} (ring peak of compound 4) in IR spectrum. The weight average molecular weight ($\overline{M}$w) of the polymer 5 determined by the dilute viscosity method[14] was $5,500 < \overline{M}w < 6,000$.

The polymer 5 was further treated by the reaction sequences according to *Scheme* 2, but did not give any product except recovery of starting material. After considerable time consuming, we discovered that the polymer 5 prepared by zinc oxide indeed gave rise to the target polymer 8. We assumed that these different reactivities of the polymer 5 obtained from stannous octoate and zinc oxide[15] was derived from different polymerization mechanism[16~20] but were not conclusive. This interesting result will be reported in detail later. Treatment of the compound 4 using zinc oxide as an initiator provide the polymer 5 as a colorless solid. The IR spectrum showed that an absorption of ring peak at 935 cm^{-1} was completely disappeared. The molecular weight ($\overline{M}$w) of the polymer 5 determined by the dilute viscosity method was $4,500 < \overline{M}w < 5,000$.

In order to introduce a primary amino group as an initiator [21,22] for polymerization of the compound 3, the polymer 5 was treated with thionyl



Scheme 2

chloride followed by addition of 3-amino-1-propanol. Amino group in 3-amino-1-propanol was protected as a ammonium salt by treatment with hydrochloric acid to prevent it from forming an amide bond. Synthesis of the target polymer 8 was finally realized by reaction of 1.00g of poly (L-lactide) 7 and 0.200g of γ-BLG-NCA 3 at 40 °C for 72 hrs. The IR spectrum of the polymer 8 displayed absorption at 1650 (amide I), 1560 (amide II), 1780 (-C=O), and 936 cm^{-1} (aromatic ring). The NMR spectrum of the polymer 8 revealed peaks at 1.70 ($-CH_3$, 3H), 3.50 (-NH-CH-CO, 1H), and 7.20 ($-C_6H_5$, 5H) (Fig. 1). Interpretation of integration peaks showed that the mole ratio of each repeating unit in polymer 8 was 1:1=L-lactic acid: γ-benzyl-L-glutamate. When we changed weight ratios of poly (L-lactide) 7 and γ-BLG-NCA 3 from 1:5 to 1:10 and 1:2:5, the mole ratios of the resulting polymer 8 were changed from 1:1 (L-lactic acid: γ-BLG-NCA) to 1:2 and 2:1, repectively (*Table* 1). From these unexpected results, we could synthesis block copoly (L-lactide-γ-benzyl-L-glutamate) with various composition in order to adjust biocompatibility and the number of sites for drug attachement.

The polymer 8 was further subjected to debenzylation condition in order to regenerate carboxyl groups as sites for drug attachment. To our surprise, debenzylation of the polymer 8 was not an easy task. It was found that the debenzylation polymer 8 was reluctant nonionic conditions-H_2/Pd/C, iodotrimethylsilane thiol/$Br_3 \cdot Et_2O$- and

Table 1. Composition of polymer 8 calculated from integration of ^1H NMR

Weight Ratio		Mole Ratio	
Poly(L-lactide) 7	γ-BLG NCA 3	L-lactic acid	γ-Benzyl-L-Glutamate 2
1	5	1	1
1	10	1	2
1	2.5	2	1

Table 2. Enthalpy of melting calculated from DSC

Polymers	L-lactic acid : γ-benzyl-L-glutamate in mole ratio	ΔH_m (cal/g)
Poly(L lactide) 7	1:0	5:41
Block copoly(L-lactide-γ-benzyl L-glutamate) 8	2:1	1.41
	1:1	1.20
	1:2	0.80
Poly(γ-benzyl L glutamate) 10	0:1	0.00

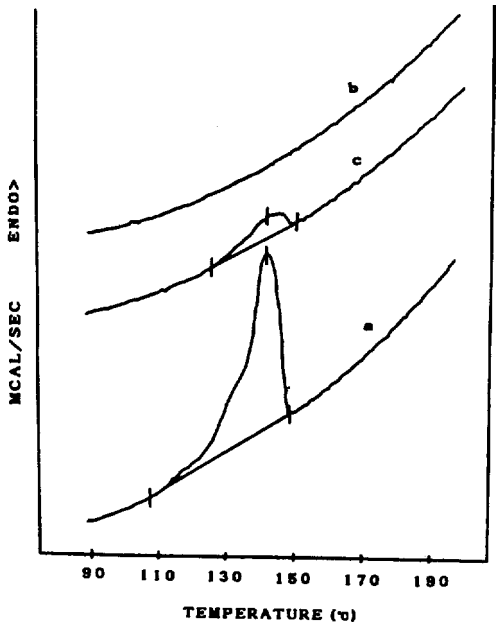


Fig. 2. DSC curves of poly(L lactide) (a), poly(benzyl L glutamate) (b), and block copoly(L lactide benzyl L glutamate) (c).

sensitive to ionic condition. After numerous attempts, treatment of the polymer 8 with hydrogen and Pd/C under harsh condition gave rise to the

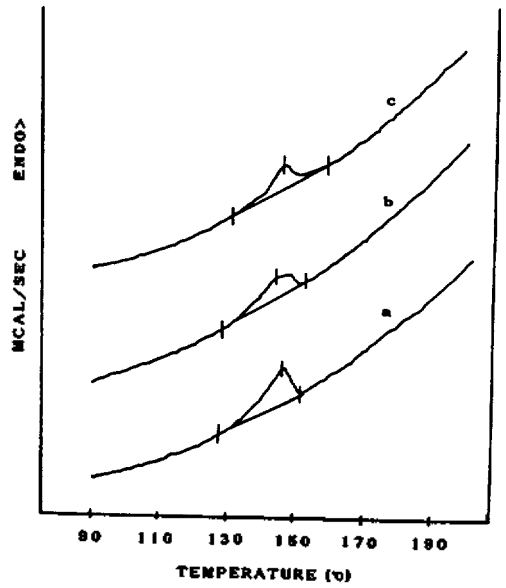


Fig. 3. DSC curves of block copolymers of L lactic acid and benzyl L glutamate in mole ratio: (a) 2:1, (b) 1:1, and (c) 1:2.

block copoly (L-lactide-L-glutamic acid) in various yields. The NMR study of the polymer 9 revealed that a peak at aromatic region (δ 7.20, $-C_6H_5$) was disappeared but not completely. We believed that difficulties in hydrogenolysis were arisen from the conformation of the polymer 8. Efforts will be emphasized to optimize the reaction conditions for this step. The polymer 9, which had a site for drug attachment, should be further studied as a viable backbone polymer for drug delivery system. Investigation for utilization of the polymer 9 in polymer/drug conjugate and for biocompatibility will be continued in our laboratory.

Characterization

The DSC[23] curves of the poly (L-lactide) 5, the poly (γ-benzyl-L-glutamate) 10, and the polymer 8 were shown in *Fig.* 2 and 3, respectively. The enthalpies of their melting (ΔH_m) were changed according to the amount of L-lactic acid in each polymer (*Table* 2). It revealed that new polymers containing L-lactic acid were synthesized. The range for the degradation temperature and the residual

Table 3. Range of degradation temperature and residual weight

Polymers	Range of Degradation Temperature		Residual weight (%) (at 385 °C)	D_{max} (°C)
	Initial point (°C)	Final point (°C)		
Poly(L-lactide) 7	270	370	2.30	364
Block copoly(L-lactide γ-benzyl-L-glutamate) 8	270	385	19.2	316
Poly(γ-benzyl-L-glutamate) 10	290	385	16.0	334

D_{max}: maximum decomposition temperature.

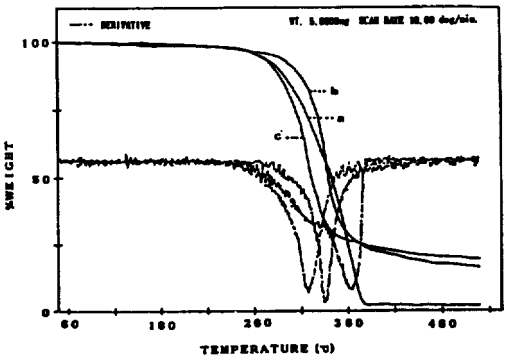


Fig. 4. TG curves of poly(L lactide) (a), poly(benzyl L glutamate) (b), and block copoly(L lactide benzyl L glutamate) (c).

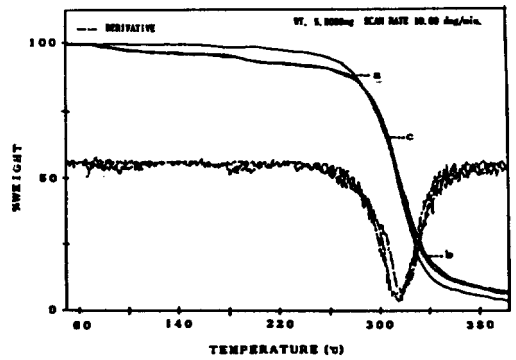


Fig. 5. TG curves of block copolymers of L lactic acid and benzyl L glutamate in mole ratio: (a) 2:1, (b) 1:1, and (c) 1:2.

weight of the polymer 5,10 and 8 measured for TG[24-26] curves (*Fig.* 4) were shown in *Table* 3. The polymer 8 was degraded from the initial point (270 °C) of the polymer 5 to the final point (385 °C) of the polymer 10. The value of residual weight of the polymer 8 (19.2%) was close to the summation of those of the polymer 5 (2.30%) and the polymer 10 (16.0%). TG curves of the polymer 8 with different composition displayed that the value of residual weight was increased when the amount of γ-benzyl-L-glutamate in polymers were increased (*Fig.* 5). From these data, we concluded that new block copolymers of L-lactic acid and γ-benzyl-L-glutamate with different composition were synthesized.

CONCLUSION

The block copolymers of L-lactic acid and L-glutamic acid with different composition were synthesized. Their composition was controlled by weight ratio of γ-BLG-NCA to poly (L-lactide) during polymerization. Physical properties including thermal properties of them were characterized and discussed. Study of their application as a skeleton for drug delivery system is currently under investigation.

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REFERENCES

1. R. L. Kronenthal, in "Polymers in Medicine and

Surgery", Polymer Science and Technology, Vol.8, 119–137 (R. L. Lronethal, Z. Oser, E. Martin, Eds), Plenum Press, New York (1975).
2. D. L. Wise, in "Biopolymeric Controlled Release System", Vol.1, 3–28 (D. L. Wise, Ed.), CRC Press, Boca Raton, Florida (1985).
3. S. W. Kim, R. V. Pertersen and J. Feijen, in "Drug Design", Vol.10, 193–250 (E. J. Ariens, Ed.), Academic Press New York (1980).
4. S. W. Jang, J. H. Kim, K. Y. Kim, Y. K. Sung, and C. S. Cho, *Polymer* (Korea), Vol.**12**, No.5, 455–459 (1988).
5. J. Y. Kim, Y. J. Kim, and Y. K. Sung, *Yeon Ku Non* jip, Vol.**17**, 175–194, Dongguk University (1988).
6. R. W. Woody, *J. Polym. Sci. Macromol.* **12**, 181 (1977).
7. Y. Kato, N. Umemoto, Y. Kayama, H. Fukushima, Y. Takeda, T. Hara, and Y. Tsukada, *J. Med. Chem.* **7**, 1602 (1984).
8. W. D. Faller, M. S. Verlander, and M. Goodman, *Biopolymers*, **15**, 1869 (1976).
9. D. K. Gilding and A. M. Reed, *Polymer*, **20**, 1458–1464 (1979).
10. A. Kafrawy, F. V. Mattei and S. W. Shalaby, assignors to Ethicon, *U.S. Patent.* **4**, 470, 297 (1974).
11. Anon. (FDA), Fed. Regist. 40(121), C (23 Jnu 1975) (CA 83: 112493h).
12. R. G. Sinclair, Gynn, U. S. Natl. Tech. Intorm. Seru., AD Report No. 748, 410 (1972).
13. B. Eling, S. Gogolewski and A. J. Penning, *Polymer* **23**, 1587 (1982).
14. A. Schindler and D. Happer, *J. Polym. Sci. Polym. Chem.* **17**, 2593 (1979).
15. M. Goodman and M. D. Alagni, *J. Polym. Sci. Polym. Let.* **5**, 515 (1967).
16. Mhala and J. P. Mishra, *Ind. J. Chem.*, **8**, 243 (1970).
17. S. Fririch, D. Gerther, and A. Zlikha, *Eur. Polym. J.* **10**, 439 (1974).
18. F. E. Kohn, J. G. Van Ommen, and J. Feifen, *Eur. Polym. J.*, Vol.**19**, 1018 (1980).
19. R. Kricheldorf, T. Mang, and J. M. Jonte, *Makromolecules*, **17**, 2173 (1984).
20. H. R. Kricheldorf, J. M. Jonte, and M. Berl, *Mackromol. Chem. Suppl.*, **12**, 25 (1985).
21. M. Mutter, *Tetrahedron Lett.*, **31**, 2839 (1978).
22. K. Kugo, A. Ohji, T. Uno, and J. Nishino, *Polym. J.*, **19**, 375 (1987).
23. W. W. Wendlant, "Thermal Methods and Analysis", 2nd Ed, John Wiley, and Sons Inc., New York , N.Y. (1974).
24. E. S. Freedman and B. Carroll, *J. Polym. Sci., Part C*, **6**, 183 (1963).
25. H. E. Kissinger, *Anal. Chem.*, **21**, 1702 (1957).
26. T. Ozawa, *Bull. Chem. Soc. Jap.*, **38**, 1881 (1965).