Synthesis and Characterization of Amphiphilic Biodegradable Poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid) by Direct Polycondensation

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Received 10 May 2007; accepted 19 September 2007
DOI 10.1002/app.27549
Published online 5 December 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid) (PGLG), an amphiphilic biodegradable copolymer, was synthesized by simply heating a mixture of L-glutamic acid (Glu), DL-lactic acid, and glycolic acid with the present of stannous chloride. The unique branched architecture comprising of glutarimide unit, polyester unit, and polyamide unit was confirmed by NMR spectrum. The PGLG was soluble in many organic solvents and aqueous solution of sodium hydroxide (pH ≥ 9.0). The thermal properties were evaluated using thermogravimetric analysis and differential scanning calorimetry. Molecular weights were determined by ^{1}H NMR end-group analysis and GPC, respectively, and the results indicated that the higher Glu content resulted in a decrease of the molecular weight. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3638–3643, 2008

Key words: biodegradable polymer; direct polycondensation; amphiphilic copolymers; polyesteramides

INTRODUCTION

Polylactide or polylactic acid (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) have been utilized as bioabsorbable materials in the medical and pharmaceutical fields because of their biodegradable and biocompatible properties.[1–3] However, the application scope of PLA (or PLGA) is limited because it lacks highly reactive groups for easy chemical modification, such as hydroxyl, amino, carboxyl, etc. Furthermore, PLA is too hydrophobic, and the degradation rate is too slow. For example, biomedical applications, such as DDS or tissue engineering, require materials with a variety of hydrophobicities/hydrophilicities to improve the affinity with various kinds of drugs or tissues.

Polyamino acid (PAA), such as poly(γ-glutamic acid), on the other hand, is a typical hydrophilic biodegradable polymers.[4,5] However, in some cases, PAA is too hydrophilic. They are insoluble in organic solvents and do not have thermoplastic properties. One promising approach that combines the advantages of both PLA and PAA derivatives is to make amphiphilic copolymers comprised of both segments, preferably in a blocky form. There have been some studies on preparing and applying the copolymers of lactic acid (LA) with glutamic acid.[6–12] Most of the synthetic processes require the preparation and polymerization of morpholine-2,5-dione derivatives, or synthesized from poly(γ-glutamic acid) and poly(lactide) via a coupling reaction. In the case of the amino acid–hydroxyl acid copolymerization, however, the amino or carboxyl group of amino acid must be protected to avoid unfavorable side reactions during the monomer synthesis and subsequent polymerization. Unfortunately, the protection–deprotection process is too complicated to perform on an industrial scale economically.

The process would be simplified if polyesteramide was synthesized from amino acid and hydroxyl acid by direct melt polycondensation. Shinoda et al. reported a simple synthetic method for preparation a novel type of amphiphilic biodegradable poly(aspartic acid-*co*-lactic acid) copolymer from L-aspartic acid and L-lactide without additional catalysts or solvents. The method is facile and ''environmentally friendly,'' but not most economical for the use of lactide.[13] So far there were no report about synthesis of poly(glutamic acid-*co*-lactic acid) copolymer via polycondensation.

In this study, we report a simple synthetic method for preparing a novel type of amphiphilic biodegradable polyesteramide copolymers from hydroxyl acid

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Contract grant sponsor: Ministry of Science and Technology of China; contract grant number: 2005CCA0600.
Contract grant sponsor: Key Laboratory of Eco-Environment-Related Polymer Materials (Northwest Normal University), Ministry of Education of China.

Journal of Applied Polymer Science, Vol. 107, 3638–3643 (2008)

TABLE I
Synthesis of Poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid)

		Hydroxyl acid (mol)	
Run no.	Glutamic acid (mol)	Lactic acid	Glycolic acid
PGLG-1	0.01	0.03	0.03
PGLG-2	0.02	0.03	0.03
PGLG-3	0.03	0.03	0.03

(D,L-lactic acid and glycolic acid) and L-amino acid (L-glutamic acid) via direct polycondensation. The main aim of this work was obtain amphiphilic biodegradable copolymers by cheap and simple method. The structural analysis, solubility, and thermal properties were discussed.

EXPERIMENTAL

Materials

L-Glutamic acid (Glu) (>99%) was purchased from Sinopharm Chemical Reagent Co., (ShangHai, China). Glycolic acid (GA) (>98 wt %) was obtained from YiXing GaoJing Chemical corp. (JiangSu, China). D,L-lactic acid (D,L-LA) 85 ~ 90 wt % aqueous solution was got from TianJin Chemical corp. (TianJin, China). All other chemicals were analytical grade and used as received without further purification.

Measurements

Both ^{13}C NMR and ^{1}H NMR spectra were taken at a temperature of 300 K on a Mercury-400BB NMR spectrometer (VARIAN, USA). All polymer samples were dissolved in deuterated dimethyl sulfoxide ([D_6]DMSO) with tetramethylsilane as an internal standard. Chemical shifts (in ppm) were referenced relative to dimethyl sulfoxide at 39.50 and 2.50 ppm in ^{13}C NMR and ^{1}H NMR spectrum, respectively. The number-average molecular weight ($\overline{M}_n$) and weight-average molecular weight ($\overline{M}_w$) were determined with respect to polystyrene standards by gel penetration chromatogram (GPC) on a GPCV2000 machine (Waters, USA) at 30°C, THF was used as the mobile phase (flow rate = 1.0 mL/min). Poly(-styrene) standard samples were used for calibration. Thermogravimetric analyses (TG) were measured by Perkin-Elmer (USA) TGA 7 Thermogravimetric analyzer. Differential scanning calorimetry (DSC) was taken using a Perkin-Elmer 7 Series thermal analysis system in the range of −50 to 400°C at heating rates of 10 K/min.

Synthesis of the poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid)

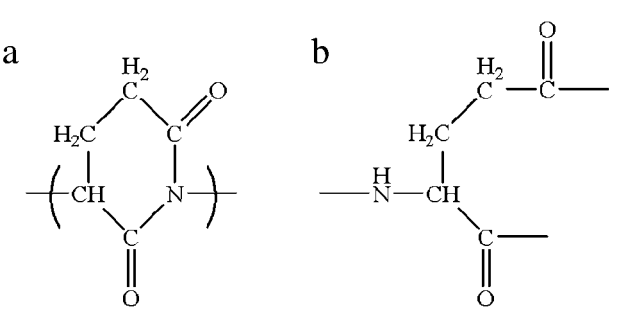
In a typical polymerization, glutamic acid, 85 ~ 90 wt % D,L-lactic acid aqueous solution, glycolic acid, and stannous chloride (0.3 wt % of total amount of the reactants) were added to a 100-mL three-necked flask equipped with a mechanical stirrer and vacuum pump system. The flask was deoxygenated by degassing and back-filling with nitrogen and then immersed in an oil bath at 180°C under a nitrogen flow, with stirring. The insoluble white amino acid powder was dissolved and the reaction mixture turned into a clear solution. The pressure of the reaction system was gradually reduced to 1300 Pa and kept this pressure for more than 10 h, the reaction solution became light brown and viscous. Then the reaction flask was taken out of the oil bath and cooled. The resulting yellowish brown transparent solid was dissolved in 50 mL of DMF, and the insoluble white particle (unreacted amino acid) was removed by filtration. The filtrate was precipitated into 400 mL of distilled water (pH 5.8) with stirring. The yellowish white polymer powder was collected and dried at room temperature under reduced pressure (1300 Pa).

Synthetic routes to the poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid) (PGLG) copolymers with the variation in dosage of materials are represented in Table I.

RESULTS AND DISCUSSION

Structure of the copolymer

L-Glutamic acid has three functional groups: amino group and two carboxyl groups. Carboxyl of glutamic acid can react with not only hydroxyl, but also amino group, so that the poly(L-glutamic acid) units in copolymers have possibly two kinds of structure units: glutarimide segments (as shown in Scheme 1a), the ring-opened units (Scheme 1b). The amide units in PGLG are possibly due to the connection points of PLA, PGA, glutarimide segments, the ring-opened units (glutamic acid unit or glutamate ester unit), and the branching sites. To investigate the copolymer structure, NMR spectra of PGLG-3 which was obtained from L-glutamic acid, lactic acid, and glycolic acid with the feed molar ratio of 1 : 1 : 1 were recorded (Figs. 1 and 2).



Scheme 1 The chain units of poly(L-glutamic acid) in the copolymers.

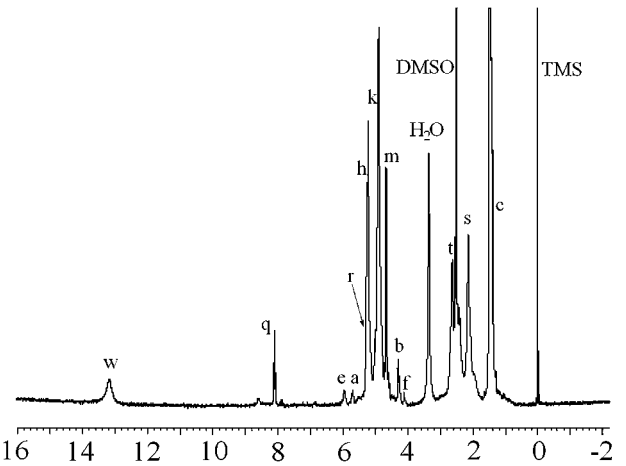


Figure 1 ^{1}H NMR spectrum of poly(glutamic acid-*co*-glycolic acid-*co*-lactic acid).

In the ^{1}H NMR spectrum, five major resonances corresponding to the methyl, methylene, and methine groups of the poly(lactic acid), poly(glycolic acid), and poly(L-glutamic acid) segments were observed at 1.47 ppm (c), 2.14 ppm (s), 2.41 $\sim$ 2.63 ppm (t), 4.90 ppm (k), and 5.22 ppm (h), respectively. In Figure 1, the assignments of the rest of small resonances, a, b, e, f, were consistent with the NMR study on PLGA oligomer of literature: e and a were corresponding to the terminal hydroxyl of PLA and PGA segments, respectively; b was corresponding to the terminal PLA methine group; f was corresponding to the terminal PGA methylene group.[14–17] The multiplet between 8.2 and 8.8 ppm were corresponding to the amide protons. The broad resonances at 13 ppm were assigned to the terminal carboxyl protons. The broad resonance at 4.6 ppm was assigned to the methine protons of the poly(L-glutamic acid) segment adjacent to an amide NH group. Moreover, the methine proton (r) of glutarimide (5.2 ppm) most likely overlaps with the large resonance of PLA methine (Scheme 2).

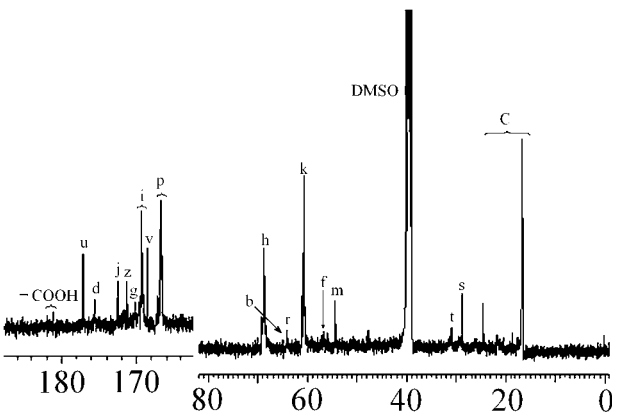
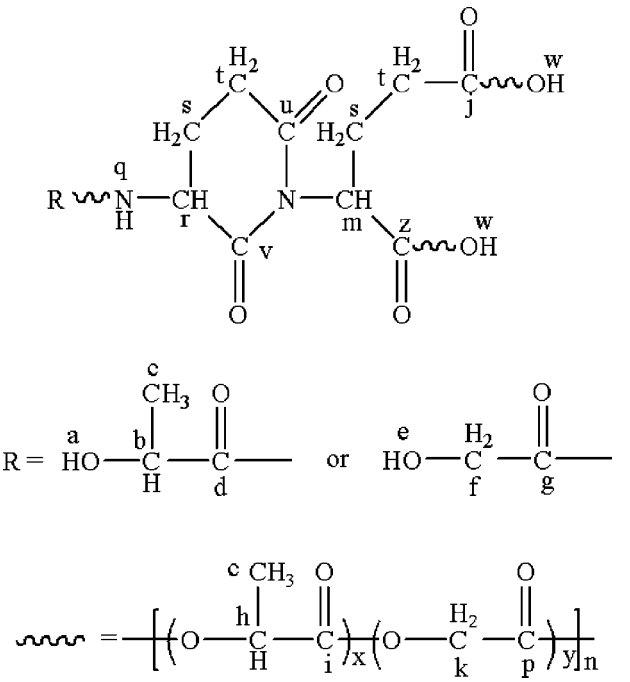


Figure 2 ^{13}C NMR spectra of poly(glutamic acid-*co*-glycolic acid-*co*-lactic acid).



Scheme 2 The various structural units of PGLG copolymers.

The ^{13}C NMR spectrum of PGLG in DMSO is shown in Figure 2, and the peaks corresponding to various carbons in PGLG have been assigned in Figure 2. The carbonyl carbons were observed in the 165–185 ppm region. Resonance at 169.5 ppm (i) and 166.8 ppm (p) corresponding to the carbonyl carbons, respectively, in PLGA chains was found as a sharp and simple peak. Since the carbonyl carbon resonance is sensitive to the copolymer sequences,[18,19] this result implies that the PGLG has blocky PLGA segments.

To assign the signals of PGLG copolymer more accurately, the HMQC spectra were recorded. The HMQC spectra of PGLG in Figure 3 showed the cor-

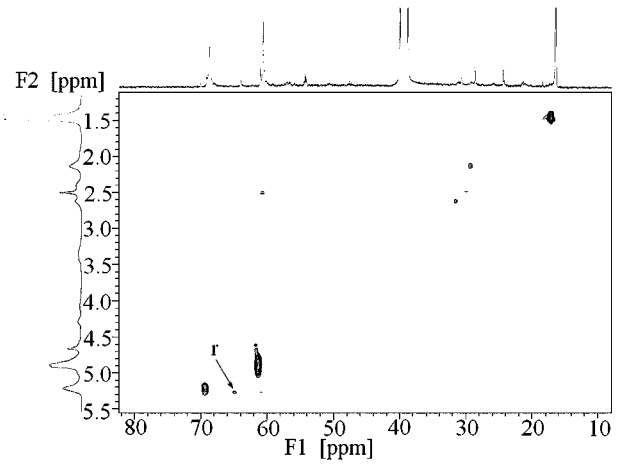


Figure 3 The HMQC spectra of poly(glutamic acid-*co*-glycolic acid-*co*-lactic acid).

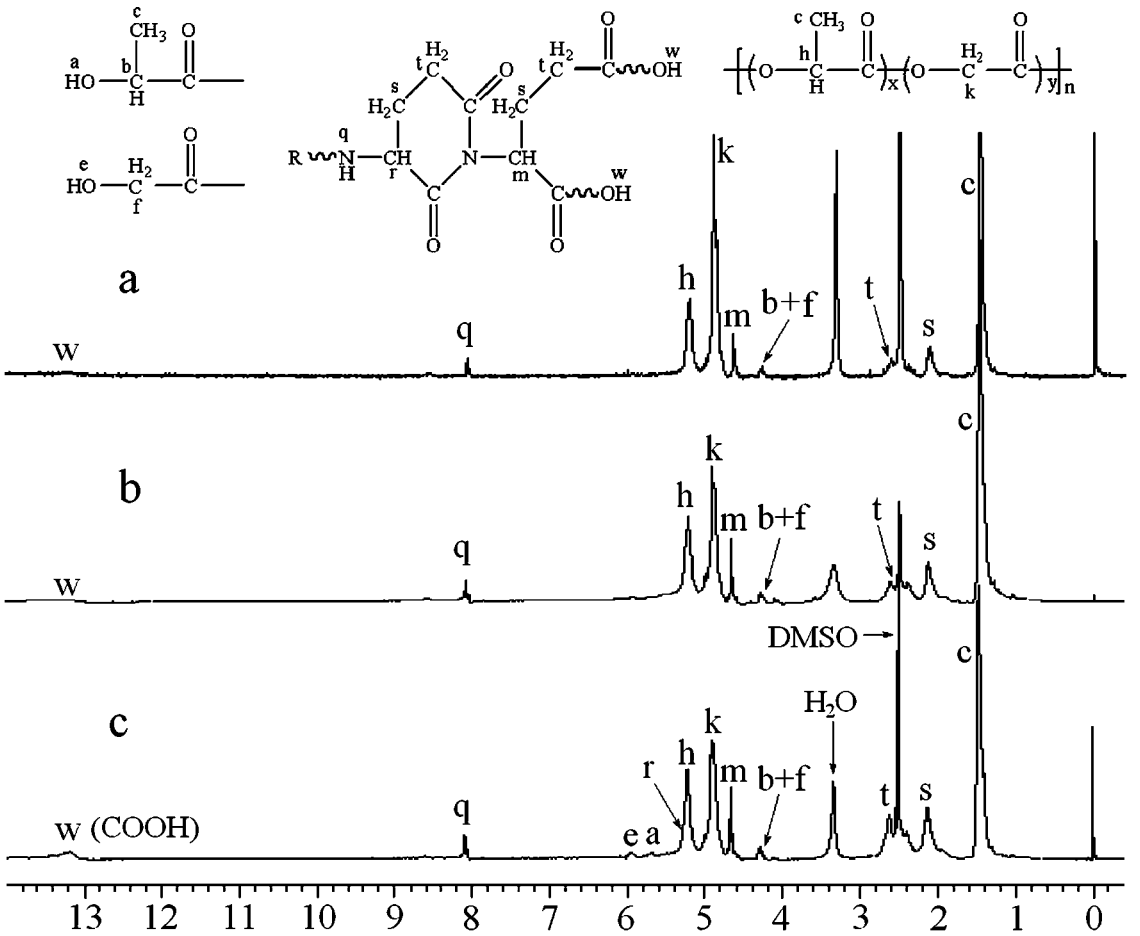


Figure 4 ^{1}H NMR spectra of PGLG copoloymers with different feed ratios. (a) PGLG-1, (b) PGLG-2, and (c) PGLG-3.

relation peak of each carbon and its directly attached proton. From Figure 3, we found that the resonance of methine carbon (*r*) of glutarimide unit was at 63.9 ppm and that of its directly attached proton was at 5.2 ppm. It overlaps with the large resonance of PLA methine proton undoubtedly. The result was consistent with the ^{1}H NMR analysis relating to Figure 1.

To clarify the differences in the chemical structure between different feed ratios clearly, the ^{1}H NMR spectra of PGLG copoloymers with different feed ratios are shown in Figure 4.

From Figure 4, we can see that there exist only little differences in ^{1}H NMR spectra of PGLG copoloymers between three feed ratios. The resonance of terminal carboxyl (w) was intensified with increasing of the feed ratio of Glu. However, for a single copolymer molecule, it only has one terminal hydroxyl group, that is to say, signal of terminal PGA methylene (f) and terminal PLA methine (b) were not greatly changed with increasing feed ratio of Glu. Moreover, in ^{1}H NMR spectra of PGLG-1 and PGLG-2, the intensity ratio of methyl (*c*) to methine (*h*) resonance is 3:1 approximately, but in ^{1}H NMR spectrum of PGLG-3, the value of intensity ratio is 2.6. According to the intensity ratio analysis of methyl (c) and methine (h) resonances of PLA units, the resonance of the methine of glutarimide appeared in PGLG-3, and that did not appear in PGLG-1 and PGLG-2. The results indicated that the branched degrees increased and formed glutarimide in PGLG copolymers with increasing of the feed ratio of Glu.

Properties of the copolymer

The characteristics of the copolymers derived from glutamic acid, lactic acid, and glycolic acid are summarized in Table II. The composition and $\overline{M}_n$ of the copolymer (LA/GA/Glu) of PGLG was confirmed by the ^{1}H NMR spectroscopy (Fig. 4).

In Table II, the average molecular weight of PGLG determined by NMR increased with increasing of the contents of poly(glutamic acid) units, but GPC gave contrarily changes to the average molecular weight. Because of the lower hydrodynamic volume of branched (dendritic, star and hyperbranched, etc.) polymers than that of linear analogs, the measured molecular weights of these types of copolymers by

TABLE II
Characterization of PGLG

Run no.	(LA/GA/Glu)[a]	$\overline{M}_n$ of copolymer[b] ($\times 10^3$)	$\overline{M}_n$ [c] ($\times 10^3$)	$\overline{M}_w$ [c] ($\times 10^3$)	$\overline{M}_w/\overline{M}_n$	Ratio of glutarimide[d] (mol %)	Unreacted Glu[e] (mol %/feed)
PGLG-1	3.0/3.1/1.0	5.9	1.9	2.1	1.10	0	0
PGLG-2	3.0/2.9/1.46	7.8	1.7	1.8	1.05	0	4
PGLG-3	3.0/2.9/2.94	9.2	1.7	1.8	1.05	5	6

[a] ''LA/GA/Glu'' stands for the composition of the copolymer determined by ^{1}H NMR spectroscopy([D_6]DMSO).
[b] Absolute number-average molecular weights, determined by ^{1}H NMR end-group analysis.
[c] Determined by GPC.
[d] The molar of glutarimide units in copolymer chain/all poly(glutamic acid) units in copolymer (determined by ^{1}H-NMR).
[e] Determined by weighed [the crude products was dissolved in DMF, and the insoluble white particle (unreacted glutamic acid) was removed by filtration].

GPC were lower than those of the actual.[20] Therefore, the results suggested that the branched degrees of PGLG increased with increasing of feed ratio of Glu. Moreover, the branched copolymers showed an apparently lower melting viscosity than linear analogs, so it was more easily to extract the water from the reaction system. Based on the above reasons, the average molecular weight of PGLG increased with increasing of the contents of poly(glutamic acid) units in copolymers, but GPC gave contrarily changes. However, both unreacted glutamic acid and the ratio of glutarimide unit in the resultant increased with increasing of the feed ratio of Glu.

PLGA is a kind of lipophilic polymer, but the PGLG is an amphiphilic polymer. It is a particularly important function of the amphiphilic properties to various applications. In particular, amphiphilic polymers materials capable of forming hydrogels remain an active area of research because of applications in drug delivery, tissue engineering, and biomedical devices.[21,22] So the solubility is an important index to the copolymers. The solubility of the copolymers was shown in Table III.

Learned from Table III, we can know that the copolymers are soluble not only in some organic solvents but also in alkaline aqueous solution, while not soluble in acidic aqueous solution, which is mainly affected by the terminal carboxyl. In alkaline aqueous solution, the terminal carboxyl becomes salt, the hydrophilicity of the copolymers increase and then the copolymers become soluble. However, it is contrary to the copolymers in acidic aqueous solution, and they become insoluble.

The thermal stability (thermolysis temperature, T_d) and glass transition temperature (T_g) of the copolymers have been investigated by thermogravimetric analysis (TGA) and DSC measurements, respectively. The results were shown in Table IV.

The TGA data clearly demonstrates that the copolymers are stable under thermal condition up to 200°C. At the same time, the DSC data show that the melt points of these copolymers are lower. These properties show great advantages in materials for DDS or controlled release use. The low melt viscosity enables melt-blending of PGLG with drugs at relatively low temperatures without the detrimental effects arising by heating. It is a more biologically friendly approach than the conventional emulsion methodusing halogenated solvents (e.g., methylene chloride).[23] The amorphous nature of the copolymer may provide for the homogeneous and high loading of drugs. The appropriately high T_g is necessary for its handling and preservation.

TABLE III
Solubility of Poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid) in Different Solvents

Solvent	PGLG-1	PGLG-2	PGLG-3
CH_3CH_2OH	--	--	--
THF	√	√	√
$CO(CH_3)_2$	√	√	*
$CHCl_3$	√	*	*
DMF	*	*	√
DMSO	*	*	√
CH_3CN	√	*	*
H_2O	--	--	--
H_2O (pH = 5)	--	--	--
H_2O (pH = 9)	√	√	√

(√) Soluble; (*) partially soluble; (--) insoluble.

CONCLUSIONS

A novel type of amphiphilic biodegradable polymer, PGLG, was synthesized from glutamic acid, lactic acid, and glycolic acid by direct polycondensation with the absence of solvents. PGLG has a unique

TABLE IV
Thermal Properties of Poly(glutamic acid-*co*-lactic acid-*co*-glycolic acid) (PGLG)

T (°C)	PGLG-1	PGLG-2	PGLG-3
T_d	231.3	246.6	258.2
T_g	53.08	51.88	61.70

branched structure comprised of polyglutarimide segments, the ring-opened poly(glutamic acid) segments and polyester segments. PGLG is a low sticky amorphous polymer ($T_g > 40^\circ$C) and will melt at relatively low temperatures with a low melt viscosity. PGLG was soluble in most common organic solvents and was easily converted to a hydrophilic copolymer in alkaline aqueous solution.

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