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Research Article

Synthesis and Characterization of Poly(lactic-*co*-glycolic acid) Derived with *L*-Glutamic Acid and *L*-Aspartic Acid

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Abstract

Poly(lactic-*co*-glycolic acid) (PLGA) is a biodegradable and biocompatible polymer approved by the FDA and EMA, which is the most widely used in the field of health. In this study, PLGA was synthesized primarily from lactide and glycolide by polycondensation and ring-opening polymerization. Then, amino acid derivatives of PLGA were synthesized by the reaction of PLGA and amino acids in the existence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The polymers synthesized were PLGA, PLGA-*L*-glutamic acid (PLGA-G), and PLGA-*L*-aspartic acid (PLGA-A). The chemical structure of these polymers was verified by ^{1}H and ^{13}C Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and Gel Permeation Chromatography (GPC). When the ^{13}C NMR analyses of PLGA-amino acid derivatives were observed, an increase in the number of carbonyl carbons around 170 ppm was found and the structure accuracy was supported. In addition, when the FTIR analyses of PLGA-amino acid derivatives were examined, the structure was confirmed by observing the signal of the amide bond carbonyl vibration at 1700 cm^{-1}. While the typical endothermic thermogram of the PLGA-amino acid derivative structures was observed by DSC analysis, it was found that the structures were low molecular weight polymers [~5000-6000 Da] by GPC analysis.

Keywords: PLGA, amino acid, biodegradable polymer.

L-Glutamik Asit ve *L*-Aspartik Asit ile Türevlendirilen Poli(laktik-*ko*-glikolik asit)'in Sentezi ve Karakterizasyonu

Öz

Poli(laktik-*ko*-glikolik asit) sağlık alanında en çok kullanılan biyobozunur ve biyouyumlu özellikte FDA ve EMA onaylı bir polimerdir. Bu çalışmada öncelikle polikondenzasyon ve halka açılma polimerizasyonu ile laktid ve glikolidden PLGA sentezlenmiştir. Daha sonra PLGA ve amino asitlerin 1-etil-3-(3-dimetilaminopropil)karbodiimid (EDC) varlığında reaksiyonu ile PLGA'nın amino asit türevleri sentezlenmiştir. Sentezlenen polimerler PLGA, PLGA-*L*-glutamik asit (PLGA-G) ve PLGA-*L*-aspartik asit (PLGA-A)'dir. Bu polimerlerin kimyasal yapısı ^{1}H ve ^{13}C Nükleer Manyetik Rezonans (^{1}H NMR ve ^{13}C NMR), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR), Diferansiyel Taramalı Kalorimetri (DSC) ve Jel Geçirgenlik Kromatografisi (GPC) ile doğrulandı. PLGA-amino asit türevlerinin ^{13}C NMR analizleri incelendiğinde 170 ppm yakınlarında karbonil karbonlarının sayısında artış gözlenerek yapı doğruluğu desteklenmiştir. Ayrıca yine PLGA-amino asit türevlerinin FTIR analizleri incelendiğinde amid bağı karbonil titreşimine ait sinyal 1700 cm^{-1}'de gözlenerek yapı doğrulanmıştır. PLGA-aminoasit türev yapılarının DSC analizi ile tipik endotermik termogram gözlenirken, GPC analizleri ile yapıların düşük molekül ağırlıklı polimerler [~5000-6000 Da] olduğu bulunmuştur.

Anahtar Kelimeler: PLGA, amino asit, biyobozunur polimer.

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1. Introduction

Researchers have frequently investigated novel biomolecule-derived poly(lactic-*co*-glycolic acid)s (PLGAs) [1], polyurethanes (PUs) [2], and poly(ethylene glycol)s (PEGs) [3], biopolymers because of increasing attention to their biocompatibility, biodegradability superior mechanical properties, and chemical versatility. PLGA is biodegradable and biocompatible, displays different degradation times with chemical versatility has adjustable mechanical properties, and is an FDA and EMA-approved polymer [4, 5]. There are many studies where PLGA is used for new-generation drug carrier systems and biomaterials. For example, Ansari et al. have reported in their studies that the flexibility and biodegradability properties of amino acid-based polyester amides provide unique thermomechanical properties. They stated that the addition of amino acids to the structure of polyester amides increases the H-bond capacity of the structure and affects cell interaction well [6].

Zhao et al. reported which is an example of the bonding of peptide structures such as Arg-Gly-Asp tripeptide (RGD) and Tyr-Ile-Gly-Ser-Arg (YIGSR) with PLGA, indicated adjusting that the distribution, density, and bioactive regions of amino acid or peptide structures in PLGA [7]. In a different study where PLGA-mucin nanoparticles had the dual role of resistance to biofouling and, molecular recognition was reported reducing plasma protein adsorption, and subsequent dampening of complement and platelet activation [8]. It is observed that amino acid structures bonding with PLGA provide positive enhancement to biological interaction.

In the study of Takeuchi et al. reported that PLGA microparticles derived from leucine and aspartic acid significantly improved the thin particle fraction and the phagocytotic ratio of alveolar macrophages [9]. pH-sensitive nanoparticle system containing charge reversible pullulan-based (CAPL) shell and poly(β-amino ester)/PLGA structure were designed for potential novel carriers of paclitaxel (PTX) and combretastatin A4 (CA4) for combining chemotherapy and anti-angiogenesis to treat hepatocellular carcinoma (HCC) [10]. PLGA amino acid derivative structures are ideal polymer systems for micro/nanoparticular drug carriers.

Also in tissue engineering applications PLGA microparticles were combined with poly(β-amino ester) particles to create new crossbred scaffolds capable of the both release of drug and growth factor. PLGA microsphere with poly(β-amino ester) particles containing a quick-degrading porogen was observed to release two drugs while establishing a porous microarchitecture for cell ingrowth within a matrix capable of maintaining a compressive modulus applicable for soft tissue grafts [11]. Cui et al. studied that a synthetic polypeptide poly(N_{ε}-Cbz-*L*-lysine) (PZL) with PLGA having relatively high strength and osteoinductive bioglass (BG) particles, and manufactured foamy PZL/PLGA/BG composite scaffolds using a negative NaCl-templating method. The results exhibited that the composite scaffolds allowed the ingrowth of tissue and microvessels and exhibited reduced inflammation response as compared to other scaffolds after 8 weeks of implantation [12]. In summary, the PLGA amino acid composite structures demonstrated that had good comprehensive performances and would meet the needs of tissue or bone regeneration.

In this study, alternative biopolymers were produced for biomedical applications such as sustained release systems and tissue engineering by synthesizing PLGA's amino acid derivative structures.

2. Material and Methods

2.1. Chemistry

All reagents were of commercial quality and reagent-quality solvents were used without further purification. 3,6-Dimethyl-1,4-dioxane-2,5-dione (99%) (CAS number 95-96-5), 1,4-dioxane-2,5-dione (≥99%) (CAS number 502-97-6), 1-dodecanol for synthesis (CAS number 112-53-8) were obtained from Sigma-Aldrich Co. Stannous 2-ethylhexanoate (95%) (CAS number 301-10-0) was purchased from Abcr GmbH Co. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide-hydrochloride (EDC-HCl) (≥99%) (CAS number 25952-53-8) was obtained from Carl Roth GmbH+ Co. The solvents, dichloromethane (DCM) (CAS number 75-09-2) and methanol (MeOH) (CAS number 67-56-1) were supplied from Sigma-Aldrich Co. Before use DCM was stored in the presence of a 4 Å molecular sieves. The reaction was assessed by thin-layer chromatography (TLC) (Merck silica gel plates, type 60 F_{254}) and the spots were visualized using a UV lamp.

2.2. Synthesis of Poly(lactic-*co*-glycolic acid) (PLGA)

PLGA copolymer is synthesized by polycondensation and ring-opening polymerization (ROP) reaction of different ratios with lactide and glycolide monomers [13]. The reaction was carried out under a nitrogen atmosphere. To prepare [L]:[G] 75:25 PLGA by mass proportions, 0.015 mol (2.16 g) of DL-lactide, and 0.005 mol (0.58 g) of glycolide were added to a dried reaction flask. The reaction flask was sealed and purged with N_2. After solutions containing initiator and co-initiator tin(II) 2-ethylhexanoate and 1-dodecanol (0.02% over the total mass of monomers) in anhydrous toluene were added to the reaction flask and a vacuum was applied to remove the toluene. The reaction mixture was put in an inert atmosphere, placed in an oil bath at 160°C, and magnetically stirred at 400 rpm for 2.5h. After cooling to room temperature, the crude PLGA was dissolved in DCM. This PLGA solution was poured into a beaker containing anhydrous MeOH and fiber-like PLGAs were obtained for precipitation. After the solvents were removed with a rotary evaporator, the PLGA was dried for 24h at 35-40°C in a vacuum oven to prevent moisture absorption [14, 15] (Fig 1.).

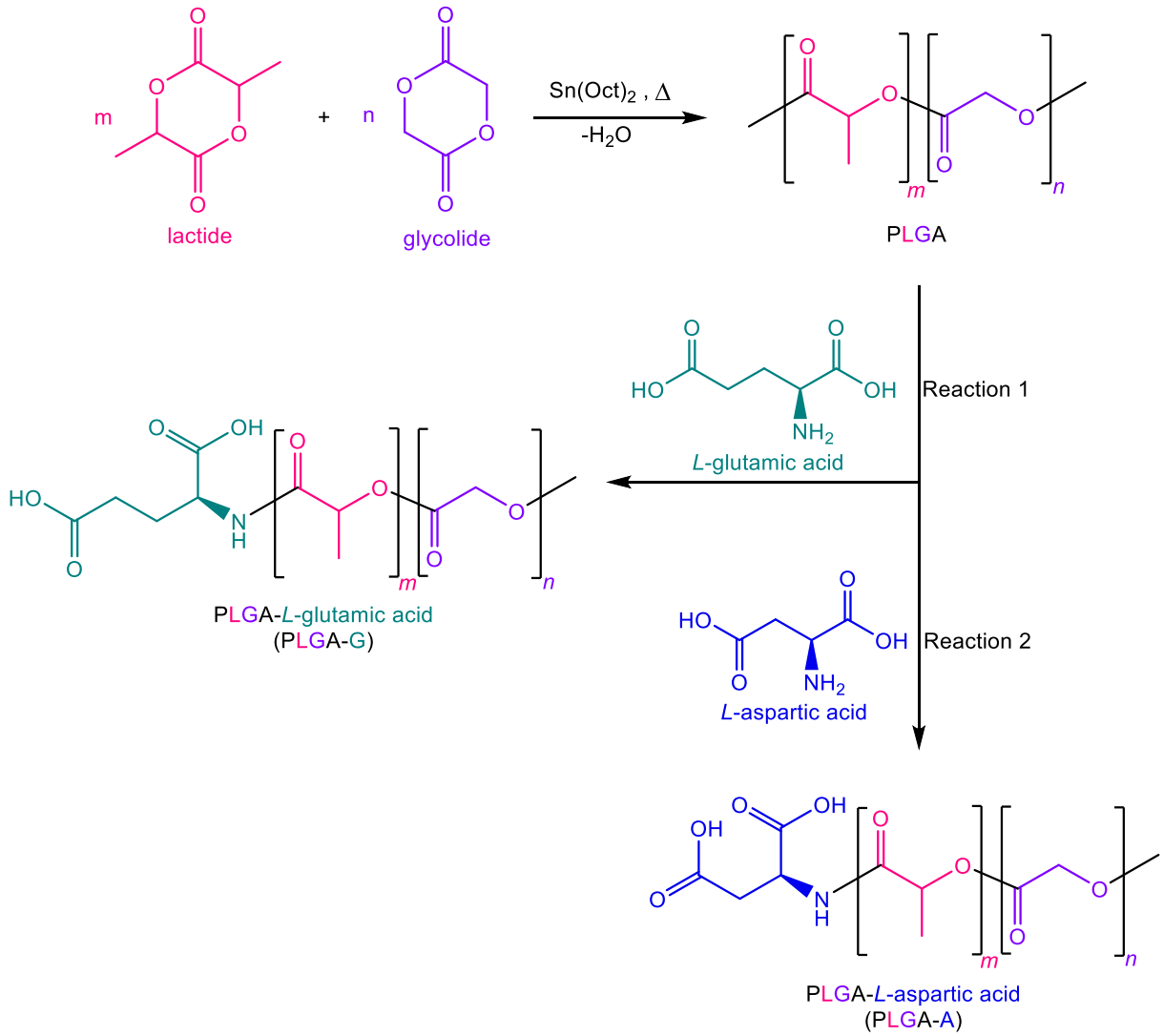

Figure 1. Schematic representation of the PLGA and PLGA derived with amino acids synthesis steps.

2.3. Synthesis of PLGA derived with amino acids

The carboxylic acid functional group of synthesized PLGA and amino functional group of amino acid reacted forming an amide bond in the presence of EDC and a suitable solvent [16]. *L*-Glutamic acid (Reaction 1) and *L*-aspartic acid (Reaction 2) were selected as amino acids to react with PLGA (Fig 1.). In a round-bottom flask equipped with a magnetic stirrer, PLGA (100 mg) dissolved in anhydrous DCM (15 mL) was reacted with EDC (48 mg). To the mixture, amino acid (10 mg) was added and stirred at room temperature for 48 h. The reaction was monitored by TLC (DCM: MeOH/9:1). When the reaction was completed, the solvent was removed by rotary evaporation and dried in a vacuum. The product was stored at 8^{o}C.

2.4. Characterizations

PLGA chemical identifications were done in terms of an explanation of the NMR and IR spectra, determination of molecular weight, and thermal properties. NMR spectra were followed on the Bruker Avance 300 MHz spectroscopy for ^{1}H NMR and ^{13}C NMR, with the chemical shifts (δs) reported in parts per million and with tetramethylsilane as an internal

standard with $CDCl_3$ as the solvent. IR spectra were acquired in reflectance mode using a Nicolet 6700 FT-IR Spectrometer from Thermo Fisher Scientific, using the ATR accessory. The spectral range was 4000–40 cm^{-1}; each spectrum was the result of 16 scans. DSC experiments were recorded on a Hitachi Exstar X-DSC7000 SII NanoTechnology Inc.). Samples were scanned over a temperature range of 25–400ºC and at a heating rate of 10ºC min^{-1} under an N_2 flow. Samples of a mass of 5 mg were used. The molecular weight determination of PLGA was studied by Gel Permeation Chromatography (METU PAL Laboratories), Malvern OmniSEC with THF column). The Universal Calibration method was used during the GPC analysis.

3. Results and Discussion

3.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

PLGA, white solid, R_f =0.51 (CH_3OH:$CHCl_3$, 9:1), ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 5.23-5.15 (-C*H*-), 4.91-4.60 (-CH_2-), 1.56 (-CH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 175.13 (*C*O), 169.38 (*C*O), 71.46 (-*C*H-), 60.78 (-*C*H_2-), 16.75 (-*C*H_3). (Fig. 2&3).

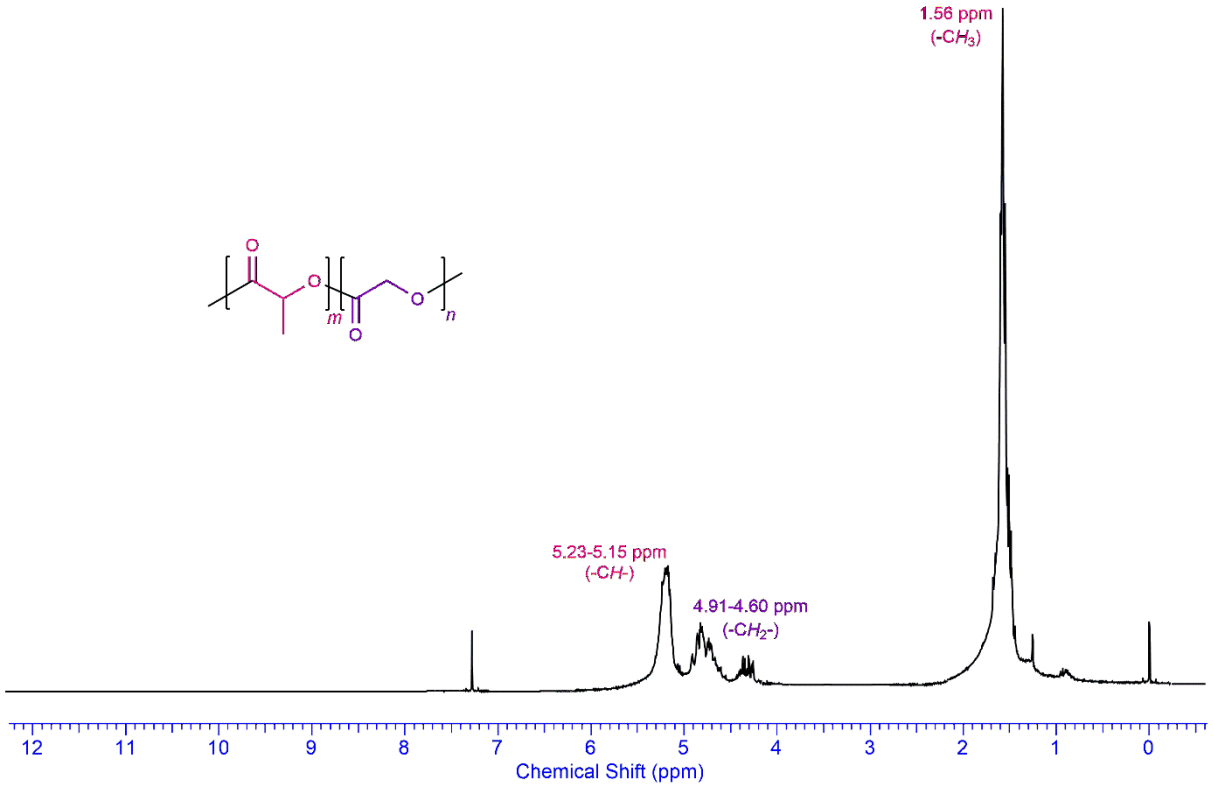

Figure 2. ^{1}H NMR spectra of PLGA.

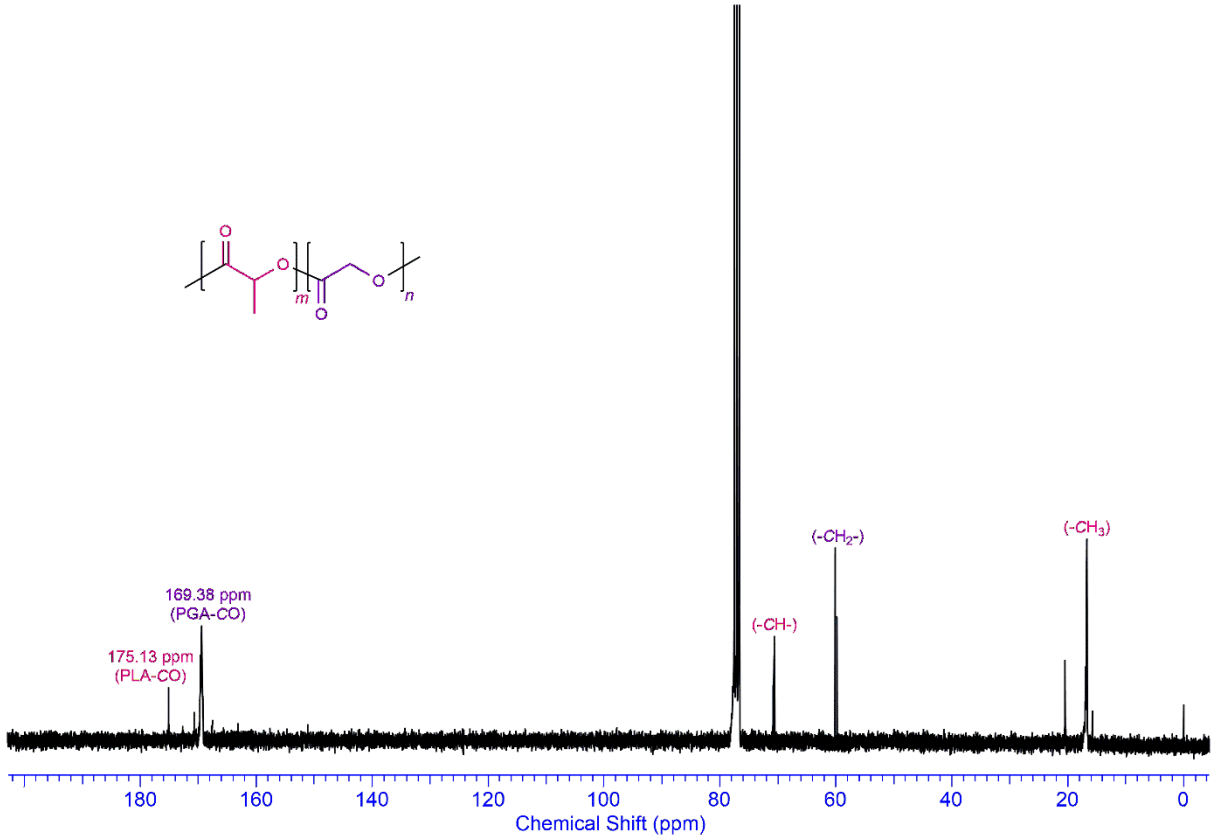

Figure 3. ^{13}C NMR spectra of PLGA.

PLGA-L-glutamic acid, white viscose solid, R_f =0.59 (CH_3OH:$CHCl_3$, 9:1), ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.74 (-O*H*), 5.24-5.14 (-C*H*-), 4.92-4.61 (-CH_2-), 3.76 (*L*-glutamic acid-C*H*-), 2.91-2.77 (*L*-glutamic acid-CH_2-), 1.57 (-CH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 175.13 (*C*O), 170.78 (*C*O), 169.33 (*C*O), 166.43 (*C*O), 69.26 (-*C*H-), 66.68 (-*C*H_2-), 60.78 (-*C*H-), 32.01 (-*C*H_2-), 26.49 (-*C*H_2-), 16.76 (-*C*H_3). (Fig. 4&5)

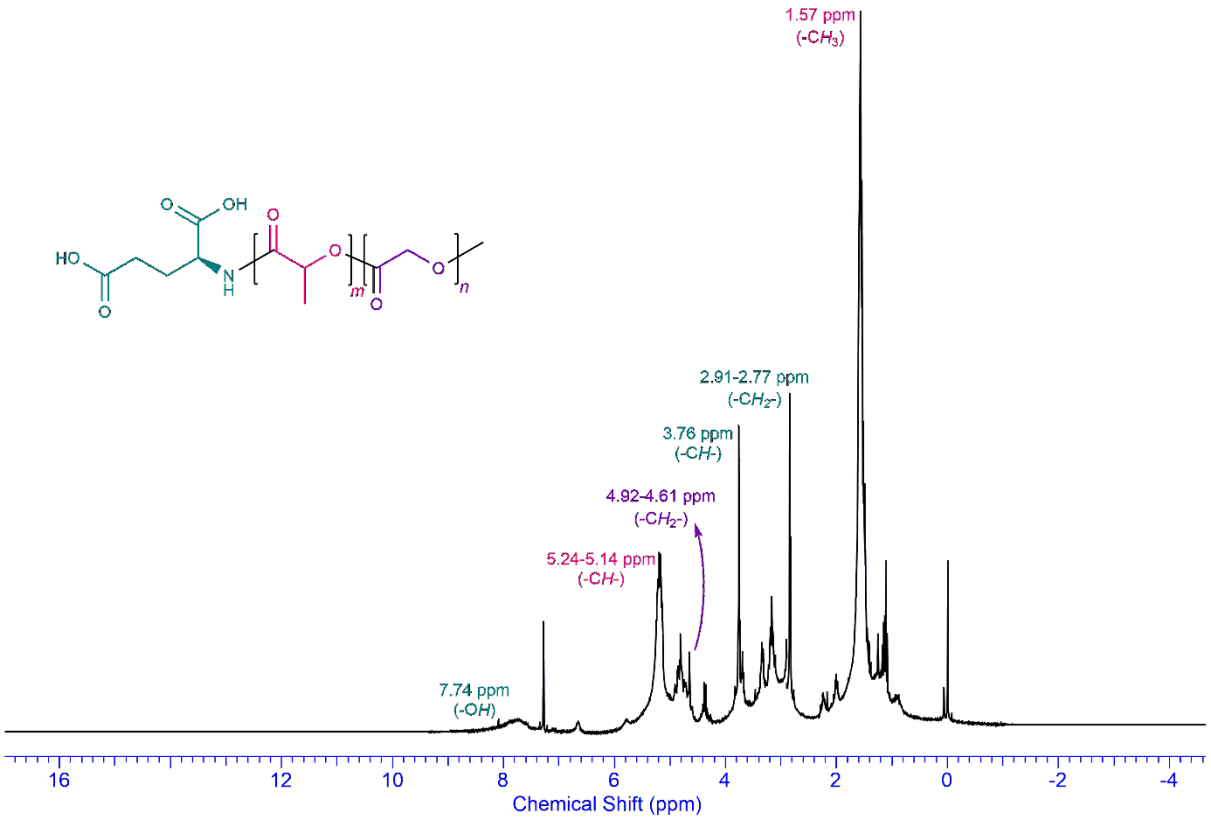

Figure 4. ^{1}H NMR spectrum of PLGA-*L*-glutamic acid.

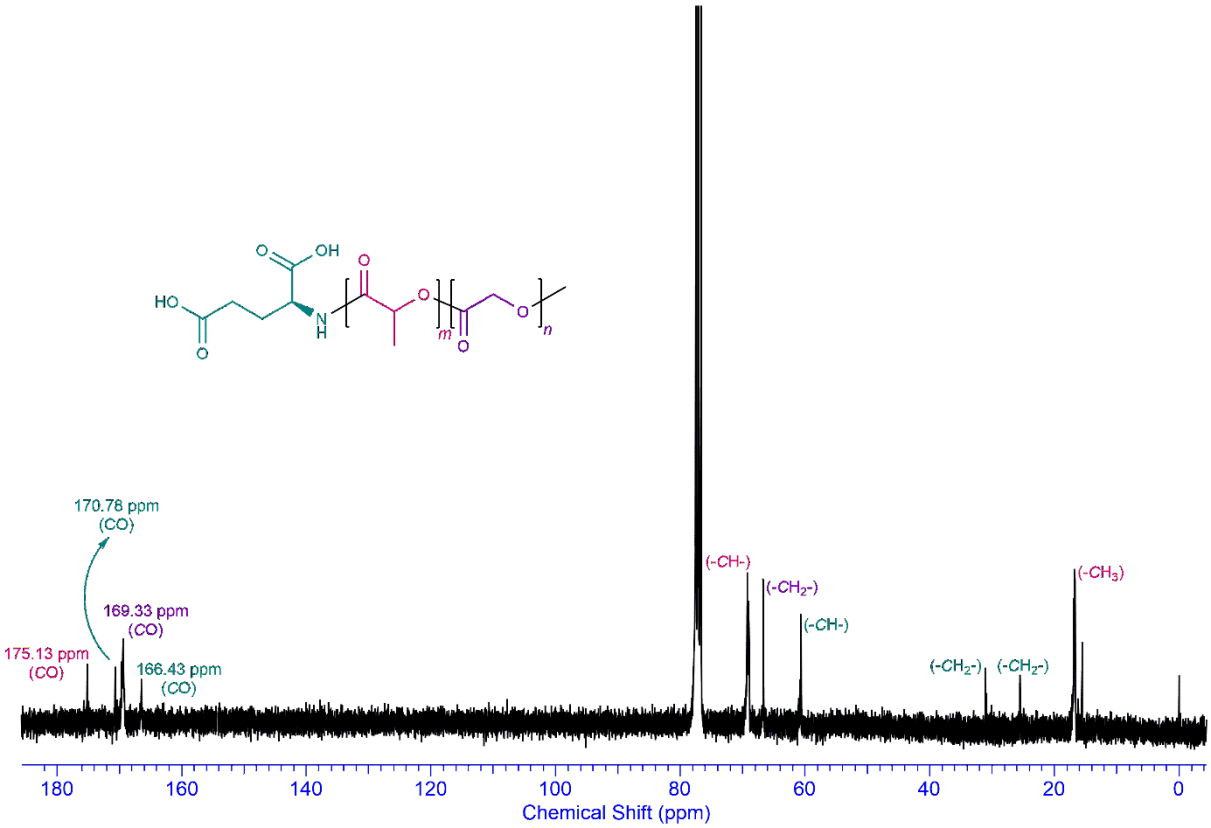

Figure 5. ^{13}C NMR spectrum of PLGA-*L*-glutamic acid.

PLGA-L-aspartic acid, white viscose solid, R_f =0.61 (CH_3OH:$CHCl_3$, 9:1), ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.72 (-O*H*), 5.22-5.14 (-C*H*-), 4.90-4.58 (-CH_2-), 4.41-4.31 (*L*-aspartic acid-C*H*-), 3.77-3.73 (*L*-aspartic acid-CH_2-), 1.56 (-CH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 175.15 (*C*O), 170.71 (*C*O), 170.37 (*C*O), 169.37 (*C*O), 69.23 (-*C*H-), 66.65 (-*C*H_2-), 52.59 (-*C*H-), 34.99 (-*C*H_2-), 16.64 (-*C*H_3). (Fig. 6&7)

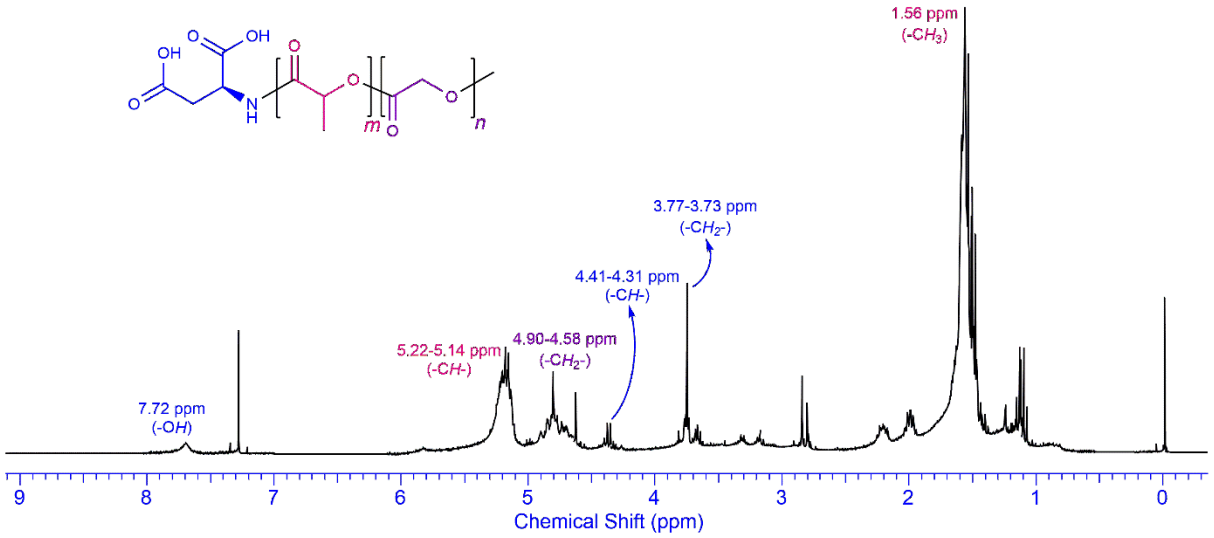

Figure 6. ^{1}H NMR spectrum of PLGA-*L*-aspartic acid.

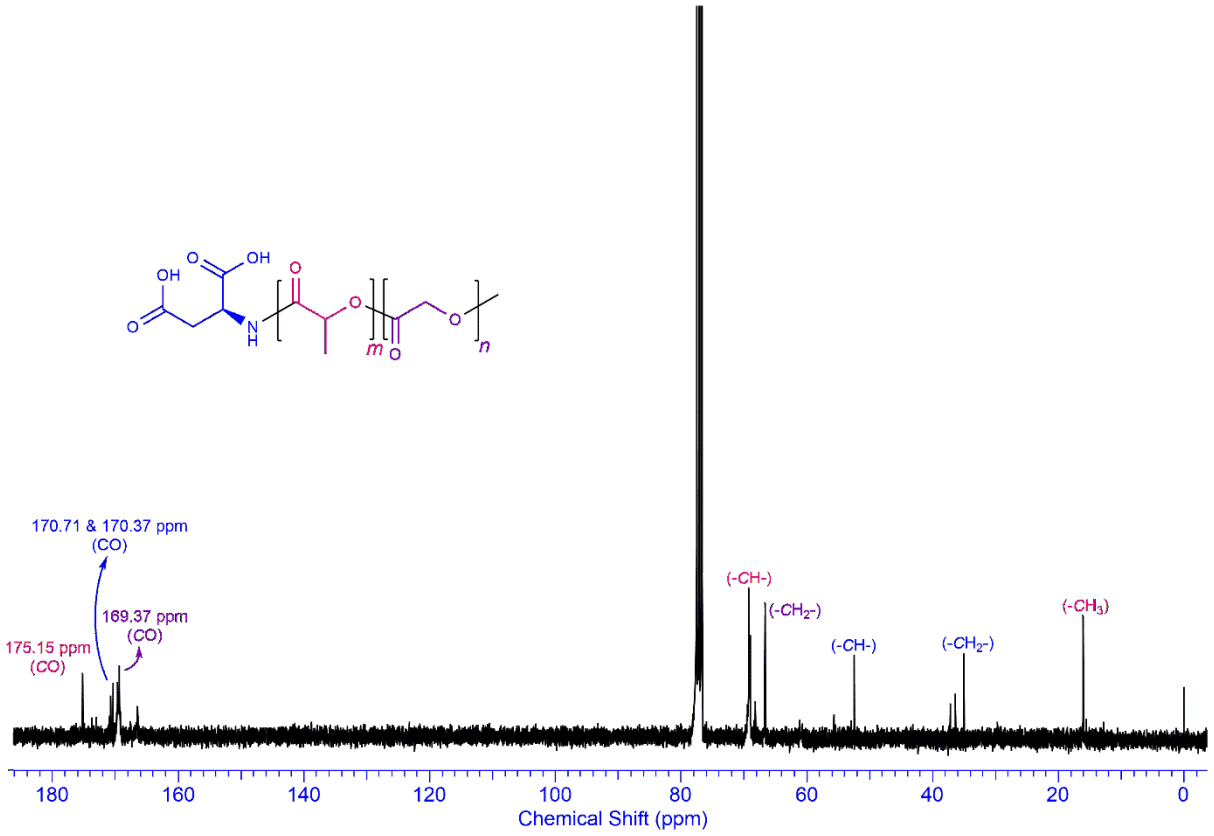

Figure 7. ^{13}C NMR spectrum of PLGA-*L*- aspartic acid.

In the ^{1}H NMR spectrum of PLGA, the C*H* proton belonging to the lactic acid chain was observed at 5.23-5.15 ppm and the CH_3 protons were also observed at 1.56 ppm, while the CH_2 protons belonging to the glycolic acid chain were observed at 4.91-4.60 ppm similarly to the literature [17]. The C*H* proton at 3.76 ppm and CH_2 protons at 2.91-2.77 ppm from the *L*-glutamic acid side observed in the ^{1}H NMR spectrum of the derivative of *L*-glutamic acid of PLGA confirm the structure. In addition, the C*H* proton at 4.41-4.31 ppm and CH_2 protons at 3.77-3.73 ppm confirm the structure of *L*-aspartic acid-derived PLGA in the ^{1}H NMR spectrum. When the ^{13}C NMR spectrum of the amino acid derivatives of PLGA were observed, their structure accuracy was supported by the characteristic carbonyl carbons at 170 ppm.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

PLGA, FT-IR (neat, cm^{-1}) 3003.58 (υCH_3), 2925.08 (υCH_3), 1750.72 (υC=O), 1445.06 (δCH_2), 1385.24 (δCH_3), 1249.21 (τCH_2), 1087.55 (υC-O-C).

PLGA-L-glutamic acid, FT-IR (neat, cm^{-1}) 3298.84 (OH), 2989.57 (υCH_3), 1746.04 (υC=O), 1700.91 (υC=O), 1449.55 (δCH_2), 1384.05 (δCH_3), 1267.51 (τCH_2), 1179.74 (υ=C-O), 1082.65 (υC-O-C).

PLGA-L-aspartic acid, FT-IR (neat, cm^{-1}) 3298.75 (OH), 2978.03 (υCH_3), 1745.99 (υC=O), 1700.84 (υC=O), 1449.31 (δCH_2), 1384.27 (δCH_3), 1267.85 (τCH_2), 1179.66 (υ=C-O), 1082.38 (υC-O-C).

For the determination of vibration, rotation, and stretching movements of IR signals of PLGA, PLGA-G, and PLGA-A structures, Xiao et al. and Anamaria T.C.R. Silva et al. works were got referenced [18, 19].

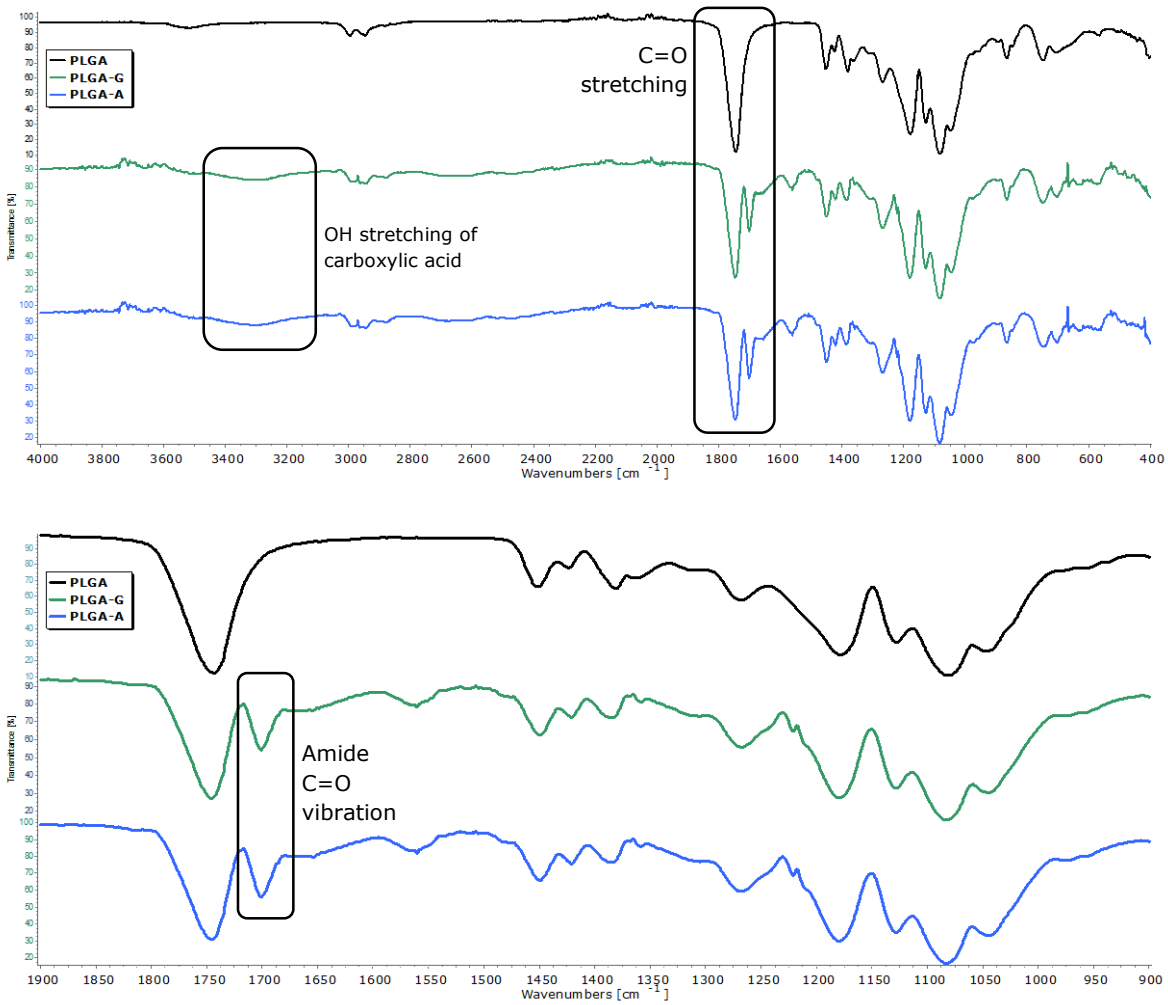

Figure 8. IR spectra of PLGA, PLGA-G, and PLGA-A.

In the IR spectra of PLGA amino acid derivative samples were observed the two typical stretching vibrations at 1745 cm^{-1} and 1700 cm^{-1} were for ester and amide carbonyl absorptions. (Fig. 8) In literature, Manoochehri et al. proved that the amide bond or conjugation of PLGA and the amino acid group HSA was formed [20]. It was noteworthy that the amide signal at 1713.4 cm^{-1} was not observed when the IR spectrum of the PLGA and HSA mixture was taken. When conjugation between PLGA and HSA occurred, the increased peak intensity in the signal belonging to the spectrum carbonyl carbon supported the structure accuracy. Similarly, for PLGA-G and PLGA-A, signals of the amide bond were observed at about 1700 cm^{-1} with a sharp peak.

For PLGA-G and PLGA-A, signals at close to 170 ppm in the ^{13}C NMR spectrums and signals at 1700 cm^{-1} in the IR spectrum indicated amino acid carbonyls and support the accuracy of the structures. In addition, the signals of all protons and carbons belonging to PLGA-G and PLGA-A structures were observed in ^{1}H NMR and ^{13}C NMR spectrum.

3.3. Thermal Analysis

The thermal transitions of the PLGA derivatives were conducted by differential scanning calorimetry (DSC).

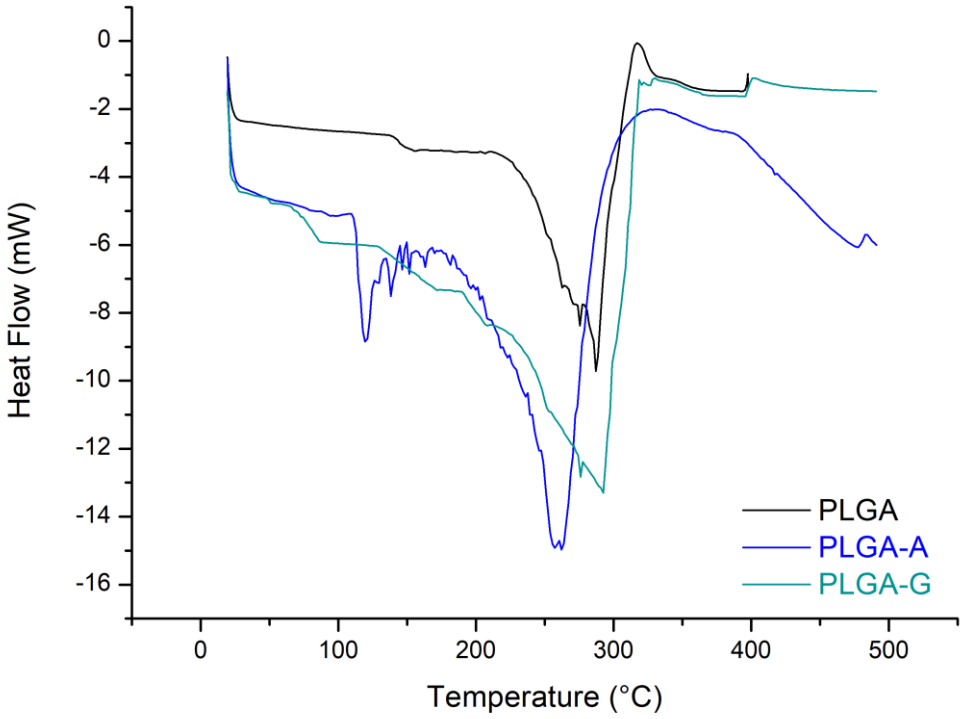

Figure 9. DSC thermograms of PLGA, PLGA-G and PLGA-A.

DSC analysis results in an order with PLGA, PLGA-G, and PLGA-A were exhibited typical endothermic peaks with an onset of melting at 288.75°C, 289.14°C, 265.49°C. (Fig. 9) Also, in Fig. 9, it was seen that the thermal behavior of PLGA and amino acid derivative PLGAs was similar. The glass transition temperature of PLGA-A and PLGA-G in both derivatives were observed differently from PLGA. It was concluded that all derivatives were amorphous and were compatible with the literature [20].

3.4. Molecular Weight Analysis

Table 1. Average molecular weights and PDI values of PLGA, PLGA-G, and PLGA-A.

Sample	*Mn (kDa)*	*Mw (kDa)*	*PDI*
PLGA	3.019	4.251	1.408
PLGA-G	3.710	5.027	1.355
PLGA-A	3.945	5.607	1.421

The compositions and properties of the molecular weight of PLGA and the derivatives were shown in **Table 1** and analyzed with GPC.

Synthesized PLGA had an average molecular weight value of 4251 g/mol, and with the presence of *L*-glutamic acid, the average molecular weight increased by 5027 g/mol, and also with the presence of *L*-aspartic acid, the average molecular weight increased by 5607 g/mol. The reason for this increase was that amino acids covalently bond with PLGA. PLGAs with lower molecular masses degrade more easily than those with higher molecular masses [20]. PLGA's amino acid derivatives could be good alternative polymers for controlled drug release systems where slow degradation is advantageous which were determined low molecular weight polymers [~5000-6000 Da] by GPC analysis [21].

4. Conclusion

It is very crucial to synthesize novel biocompatible and biodegradable derivatives of PLGA polymer, which are often needed in drug carriers and medical applications, and to complete the chemical characterizations. In this study, PLGA, PLGA-G, and PLGA-A were successfully synthesized and their chemical structure was characterized. Chemical characterizations proved the covalent bonding between amino acid and PLGA. The amide bond, indicating the formation of the PLGA-amino acid bond, was demonstrated by both ^{13}C NMR and FTIR analysis. PLGA-amino acid derivatives, which were low molecular weight [~ 5000-6000] determined by GPC analysis, were observed in DSC thermograms where thermal properties were the endothermic character.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Acknowledgments

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