ORIGINAL ARTICLE

Synthesis and Characterization of Novel, Biodegradable, Amphiphilic Triblock Polycaprolactone-Polyethylene Glycol-Polycaprolactone (5000-1000-5000) Copolymer for Long-acting Drug Delivery and for Biomedical Applications

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Abstract

Objective: The objective of this study was to synthesize novel, amphiphilic, triblock, biodegradable polycaprolactone-polyethylene glycol-polycaprolactone (PCL-PEG-PCL) (5000–1000–1000) copolymer through ring-opening polymerization of ε-caprolactone (CL) using PEG as an initiator for long-acting drug delivery and biomedical applications. **Materials and Methods:** The copolymers were synthesized through ring-opening polymerization of ε-CL using stannous octoate as the catalyst. The resulting copolymers were characterized by ^{1}H nuclear magnetic resonance spectroscopy (NMR), ^{13}C NMR spectroscopy to determine the composition and end group estimation, gas chromatography (GC) for residual solvent estimation, fourier-transform infrared spectroscopy (FTIR) for identification of functional groups, X-ray diffraction, thermogravimetric analysis, and differential scanning calorimetry to analyze the thermal properties. **Results and Discussion:** ^{1}H NMR spectra revealed ethylene groups at 1.35, 1.69, 2.43, and 4.06 ppm in PCL, while PEG displayed methylene (CH_2) groups at 3.8 ppm. In addition, the FTIR spectra of the PCL-PEG-PCL copolymer exhibited prominent bands at 1720cm^{-1}, indicative of carboxylic ester, C=O and 1175cm^{-1}, indicative of ether, C-O, confirming the successful synthesis of the copolymer. PEG triblock polymer end cap analysis and molar ratio of CL:PEG for synthesized triblock copolymer was found to be hydroxyl end cap and 91:09, respectively. The GC results of triblock polymer were free of dichloromethane and water content was 0.2%. The copolymer exhibited distinct peaks at diffraction angles (2θ) of 21.14°, 23.45°, 20.14°, 35.8°, and 12.15°. It demonstrates that the copolymer was presented in crystalline form. **Conclusion:** Based on the data obtained, concluded that the synthesized triblock copolymer provided an acceptable and appropriate novel polymer for long-acting drug delivery and various biomedical applications.

Key words: Є caprolactone, poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone), polycaprolactone-polyethylene glycol-polycaprolactone, polyethylene glycol, tri-block copolymer

INTRODUCTION

Poly (ε-caprolactone) (PCL) and poly (ethylene glycol) (PEG) are biocompatible and biodegradable polymers widely investigated for biomedical applications due to their unique properties such as tunable degradation rates and excellent mechanical properties. Combining these polymers into block copolymers, such as PCL-PEG-PCL, offers additional advantages including improved

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solubility, enhanced drug loading capacity, and prolonged circulation time *in vivo*. PCL-PEG-PCL copolymers are among the most widely used amphiphilic triblock copolymers.[1-5] The components include a biodegradable hydrophobic PCL block and a biocompatible hydrophilic PEG block, both of which have been authorized by the United States Food and Drug Administration (USFDA).[6] However, the properties of the resulting copolymers can be influenced by various reaction parameters, necessitating a systematic investigation to optimize the synthesis process. Structure of the PCL-PEG-PCL (5000–1000–5000) copolymer is shown in Figure 1.

A hydrophilic block of PEG and two hydrophobic blocks of polycaprolactone make up the amphiphilic triblock copolymer PCL-PEG-PCL. It is possible to create core-shell micelles, nanoparticles, and microparticles using biodegradable polyester segments and biocompatible PEGylated copolymers. The biodegradability of the PCL-PEG-PCL polymer is attributed to copolymer hydrolysis, which produces biocompatible compounds such as caproic acid, succinic acid, valeric acid, and butyric acid..[2,5,7] These compounds are quickly broken down by the body or removed from it.

The USFDA has cleared the hydrophobic polyester polymer polycaprolactone for clinical usage because of its biocompatibility and biodegradability. Because PEG and PCL are well-known FDA-approved biodegradable and biocompatible polymers, they have been employed extensively in pharmaceutical and biomedical applications. Amphiphilic PCL/PEG polymer, contingent on molecular weight and PCL/PEG hydrophobic/hydrophilic balance, can form micro/nanoparticles or thermosensitive hydrogels. For injectable drug delivery systems, the PCL-PEG-PCL copolymer is a viable option because of its low toxicity, low opsonization likelihood, easy synthesis, and strong biodegradability.[1,8,9]

The aim of the present study was to synthesize a novel, amphiphilic, biodegradable, PCL-PEG-PCL triblock polymer to prepare long-acting biodegradable copolymers with potential applications in various biomedical fields. The resulting copolymer was characterized by nuclear magnetic resonance spectroscopy (^{1}H NMR) for molecular weight determination and structural confirmation, ^{13}C NMR for end group determination, X-ray diffraction (XRD) for crystalline properties, residual solvent estimation by gas chromatography (GC), Fourier transform infrared spectroscopy (FTIR) for functional groups, moisture content by Karl Fisher (KF) titration, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) to analyze the thermal properties.

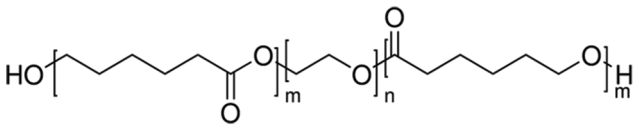

Figure 1: Chemical structure of poly (ε-caprolactone)-polyethylene glycol-Poly (ε-caprolactone)

MATERIALS AND METHODS

Materials

All of the chemicals utilized were of analytical grade and were obtained from commercial sources. € caprolactone (CL) (98% purity), stannous octoate ($Sn(Oct)_2$), polyethylene glycol with a molecular weight of 1000, diethyl ether ($(C_2H_5)_2O$), and methylene dichloride (CH_2Cl_2) were procured from Merck KGgA, Germany.

Synthesis of triblock copolymer PCL-PEG-PCL (5000-1000-1000)

The PCL-PEG-PCL (5000–1000–5000) triblock copolymer was synthesized through ring-opening polymerization of ε-CL with PEG as a starting molecule and stannous octoate as the catalyst. To initiate polymerization, 87.7 mmol of ε-CL, 1 mmol of PEG 1000, and 1 mmol of $Sn(Oct)_2$ were added to a nitrogen-filled dry round bottom flask. The reaction was carried out at 130°C under stirring for 24 h, followed by cooling to 23°C after completion of the polymerization, and then, the resulting triblock copolymer was separated by dissolving in dichloromethane and precipitating it in diethyl ether. The triblock polymer was purified by dissolving the precipitate in dichloromethane. Three purification steps were followed to remove the remained stannous octoate and contaminants. After that diethyl ether was removed by filtration and dried the triblock copolymer in vacuum-drier at ambient temperature for 24 h.[4,5,10-13] The process of triblock copolymer synthesis is represented in Figure 2. The structure, molecular weight, and functional groups of the copolymer were determined using ^{1}H-NMR (Bruker spectrometer, Ascend 500, Top Spin 3.6.4 software) and FTIR. The yield was estimated by dividing the weight of the purified PCL-PEG-PCL by the weight of starting materials utilized in the polymer preparation.

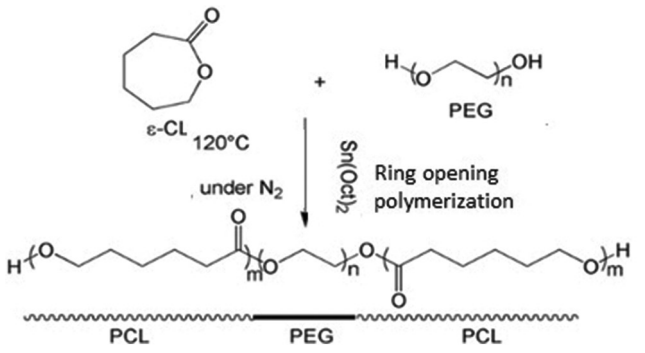

Figure 2: Schematic representation of the formation of polyethylene glycol-poly (ε-caprolactone)-polyethylene glycol triblock copolymer (5000–1000–5000)

Water content

The water content performed for the synthesized triblock copolymer. Karl Fischer titration (Volumetric titrator 915 KF Ti-touch, Metrohm) was used for measuring the water content to ensure the quality and absence of water in the synthesized polymer.[14,15] The electrometric end point was reached by titrating 20 mL of dehydrated methanol (Merck) with the KF reagent (Merck). After a careful transfer to the titration vessel, the sample was stirred for 1 min and subsequently titrated once again with the KF reagent until the typical endpoint was reached.[16]

Proton ^{1}H NMR

The copolymer was analyzed using ^{1}H NMR (Bruker Ascend 500 spectrometer, Top Spin, 3.6.4 software) operating at 400 MHz, with $CDCl_3$ as the solvent for the PCL-PEG-PCL triblock copolymer. The triblock copolymer average molecular weight and structural composition were determined by^{1}H NMR analysis.[1] The PCL-PEG-PCL triblock copolymer, CL, polyethylene glycol (PG) (CL: PG) molar ratio was determined using ^{1}H NMR method.[17-19]

^{13}C NMR, or carbon-13 NMR

An end-group analysis was performed on the PCL-PEG-PCL triblock copolymer using ^{13}C NMR (Bruker spectrometer, Ascend 500, TopSpin 3.6.4 software) at 12.5 h with 12,500 scans.[20,21]

FTIR

FTIR spectroscopy (Agilent, Agilent Cary 630 FTIR, Microlab PC software) was used to assess the structure of PCL-PEG-PCL triblock copolymer. The FT-IR scans were recorded over a range of 4000–500cm^{-1}.[22,23]

DSC

DSC conducted for synthesized PCL-PEG-PCL triblock copolymer on DSC analyzer (TA Instruments [Waters], Discovery DSC2500, Trios software). 3–5 mg of solid sample was weighed and transferred to the aluminum pan and within the temperature range of 25–350°C, dynamic scans were carried out in a nitrogen atmosphere at a continuous heating rate of 10°C/min, with a 50 mL/min flux rate.[24-26]

TGA

Thermogravimetric analysis was done for the synthesized PCL-PEG-PCL triblock copolymer using thermogravimetric analyzer (TA Instruments [Waters], Discovery TGA5500, Trios software). Approximately 5–10 mg of sample was placed on a platinum TGA pan and dynamic scans carried out in a nitrogen atmosphere at a flow rate of 30 mL/min, with a temperature range of 20–600°C and a constant heating rate of 20°C/min. Weight variations were recorded as a function of temperature throughout the dynamic scans.[27-29]

XRD

PCL-PEG-PCL triblock copolymer was analyzed using X-ray diffractometer (Powder XRD, Malvern Panalytical, Empyrean-3, high score plus version 5.1a software) to know the crystallinity. XRD was operated at 35 mA current, 40 Kv voltage, and 2°C ambient temperature using Ni filter and CuKα line (= 0.154 nm).[30,31]

Residual solvent estimation by GC

Residual dichloromethane (CH_2Cl_2) present in synthesized triblock copolymer was analyzed by GC (Agilent 7820A, Open labs 2.7 software) with FID detector and HP-5 (5% phenyl methyl polysiloxane capillary column of 30 m × 0.53 mm, 1.5 µm) column.

RESULTS AND DISCUSSION

Synthesis of PCL-PEG-PCL triblock copolymer

PCL-PEG-PCL tri-block copolymer was synthesized by ring-opening polymerization of CL in the presence of PEG 1000. The hydroxyl end group of PEG 1000 initiated the ring opening.[12] The structure and composition of the synthesized novel PCL-PEG-PCL triblock copolymer were determined. Figure 2 shows the composition and structure of triblock copolymer. ^{1}H NMR results [Figures 3-5] revealed that, PCL contained ethylene groups at 1.35, 1.69, 2.43, and 4.06 ppm, whereas PEG contained methylene (CH_2) groups at 3.8 ppm. Table 1 illustrates the features of the synthesized copolymer.

The FTIR spectra of triblock copolymer PCL-PEG-PCL indicate the existence of carboxylic ester (C=O) and ether (C-O) groups, as demonstrated by prominent and intense bands at 1720 and 1175 cm^{-1}, respectively, indicating successful copolymer synthesis [Figure 7]. The schematic of the copolymer synthesis process is depicted in Figure 2. The ^{1}H NMR peak area ratio at δ 4.2 ppm due to the presence of methylene groups adjacent to carbonyl group in PCL blocks and δ 3.8 ppm due to the presence of methylene (CH_2) groups in PEG block and from the molecular weights of CL and EO repeat units was used to calculate the CL/PEG weight ratio R in the triblock copolymer.

The methylene protons of PCL segments (CO-CH_2CH_2-CH_2-CH_2-) were responsible for peaks at 1.39, 1.66, and 2.32 ppm.[9] The 90% of yield observed for the copolymers synthesized by ring opening polymerization. The molecular

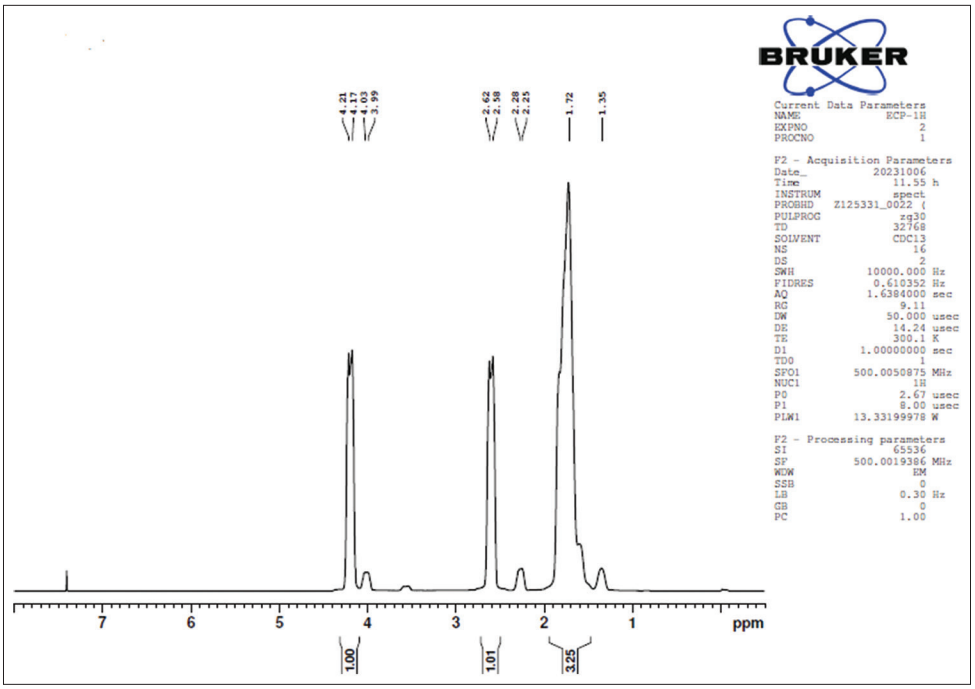

Figure 3: [1]H Nuclear magnetic resonance spectroscopy spectrum of the € caprolactone

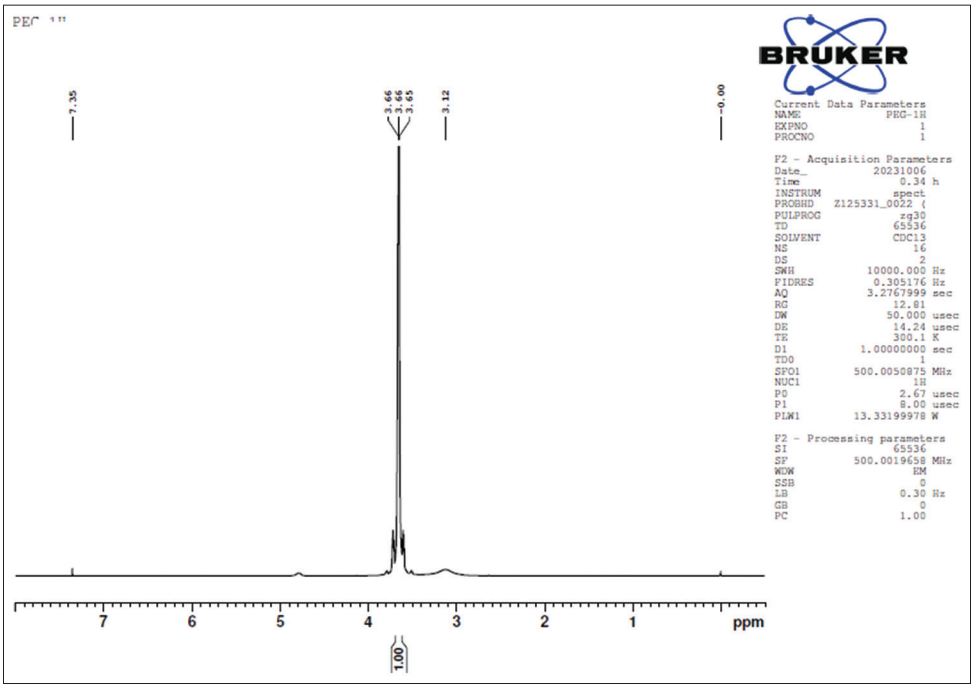

Figure 4: [1]H Nuclear magnetic resonance spectroscopy spectrum of polyethylene glycol 1000

Table 1: Molecular weight and composition of the triblock copolymer poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) (5000-1000-5000)

Synthesized polymer	M_{PEG}	PCL/PEG[a] (theoretical)	Total M_n[a] (theoretical)	PCL/PEG[b] (calculated)	Total Mn[b] (calculated)	T_M (°C)[c]	Degradation temperature
PCL-PEG-PCL polymer	1000	10:1	11,000	91:09	10,500	57.8	400°C

[a]Theoretical value was calculated according to the feed ratio, [b]Calculated using the PEG block at 3.65 ppm and the integrated area ratio of the resonance peaks from the PCL block at 4.05 pp, [c]Calculated from DSC's initial run as half of the shown tangents. PCL-PEG-PCL: Poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone)

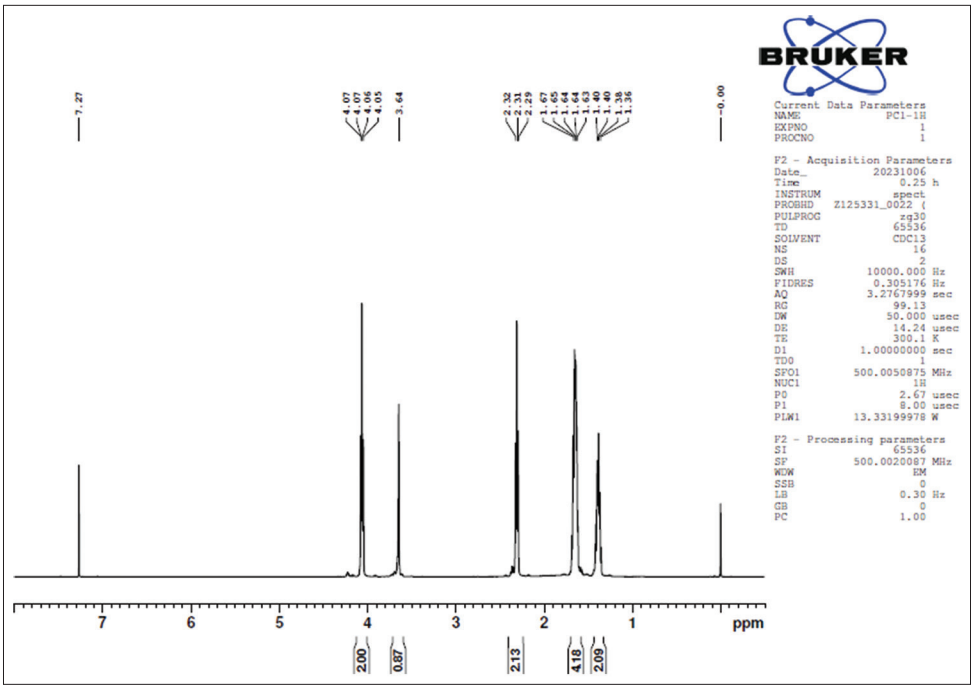

Figure 5: [1]H Nuclear magnetic resonance spectroscopy of tri-block copolymer poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000)

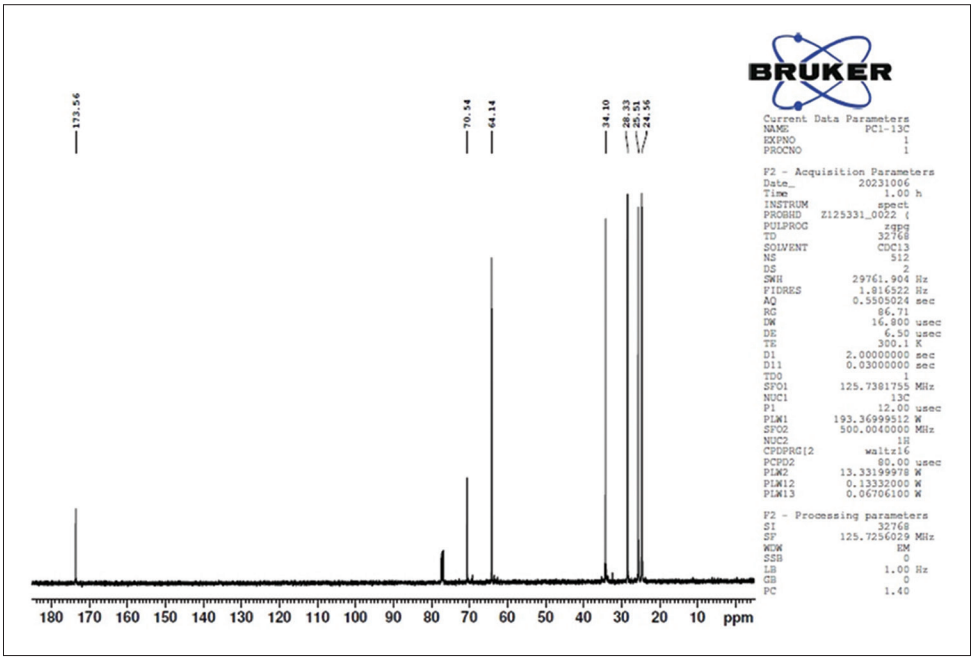

Figure 6: [13]C Nuclear magnetic resonance spectroscopy spectrum of poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000) tri-block copolymer

weight (Mn) of the copolymer was further calculated by $M_n = (1 + R) \times M_{PEG}$, where M_{PEG} was the molecular weight of PEG used and R is number of fragment present between polycaprolactone groups. [1]H-NMR was used to identify the segment of PEG and determined the molecular weight of the PCL-PEG-PCL triblock copolymer to be 10.5 KDa.[32] As shown in Table 1, the weight ratios of the synthesized copolymer CL/PEG were relatively similar to the respective feed compositions (91:09).

Effect of CL:Polyethylene glycol (PCL:PEG) ratio

PCL segments are shown peak δ 4.2 ppm due to the presence of methylene protons near the carbonyl group. In addition, methylene protons in the PCL backbone cause peaks at approximately 2.3 ppm. PEG segments often peak at δ 3.8 ppm, which corresponds to methylene protons ($-CH_2$) in the chain. Calculated the mole fractions of PEG (MP) and caprolactone (MC) using the peak integration (P) of each

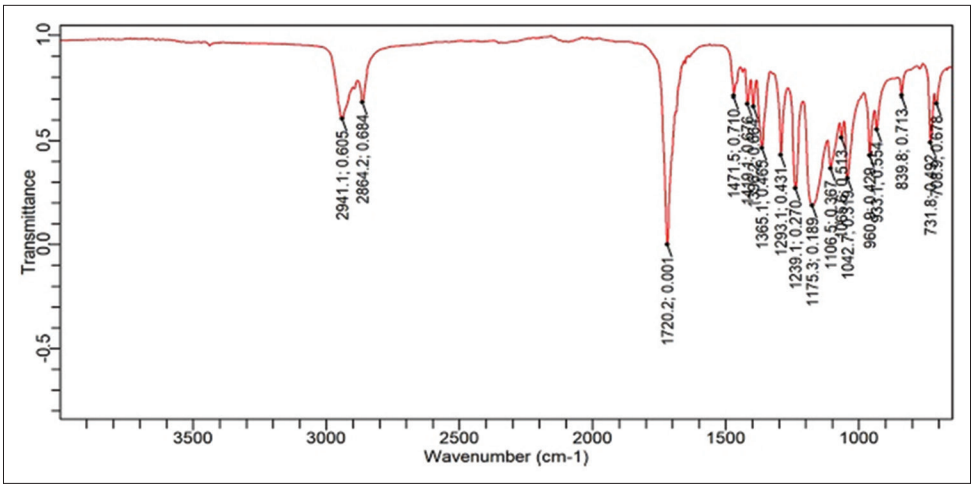

Figure 7: Fourier transform infrared spectroscopy of tri-block copolymer poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000)

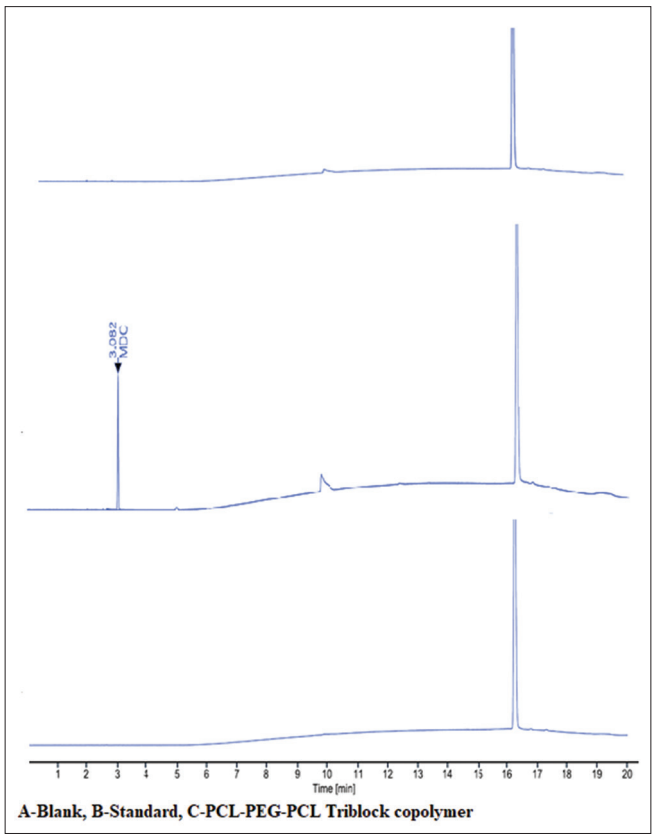

Figure 8: Gas chromatography chromatograms of tri-block copolymer poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000)

component, where "P" stands for PEG and "C" for CL. These estimations are based on the copolymerization ratio and block length. Equation 1 and Equation 2 were used to calculate the molar ratio of CL to polyethylene glycol (C:P).[33]

$$MP = (PEG/2)/[CL+(PEG/2)] \quad 1$$

$$MC = CL/\{CL+(PEG/2) \quad 2$$

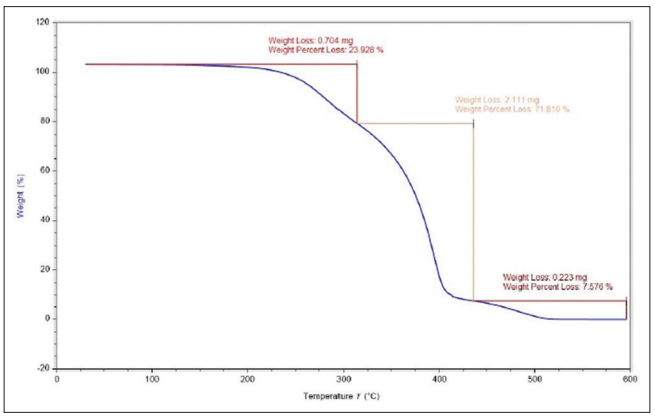

Figure 9: Thermogravimetric analysis of tri-block copolymer poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000)

Using ^{1}H NMR spectroscopy, the ratio of CL to PEG 1000 of the triblock copolymer PCL-PEG-PCL (5000–1000–5000) was determined. Figure 5 depicts the ^{1}H NMR of PCL-PEG-PCL triblock copolymer. The PEG block's oxyethylene units' methylene protons are responsible for the strong peak at 3.58 ppm. Furthermore, the methylene protons of the oxycarboxy-1, 5-pentamethylene units of methylene protons of the PCL blocks correlate with the remaining four peaks, which are located at 4.0, 2.24, 1.58, and 1.31 ppm. The PCL:PEG ratio for PCL-PEG-PCL (5000–1000–5000) polymer was discovered to be 91:09.

End cap analysis by ^{13}C NMR (Carbon-13 nuclear magnetic resonance)

^{13}C NMR spectroscopy was used for end group analysis of the PCL-PEG-PCL triblock copolymer. Figure 6 is shown peaks at δ 70 ppm due to the presence of hydroxy groups in the ^{13}C NMR spectra of triblock polymer.

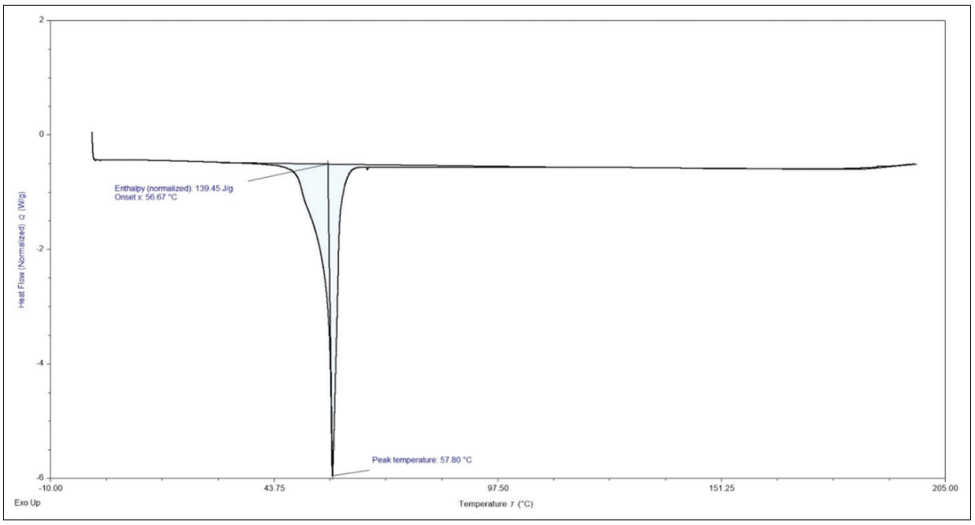

Figure 10: Differential scanning calorimetry of tri-block copolymer poly (ε-caprolactone)-polyethylene glycol-poly (ε-caprolactone) (5000–1000–5000)

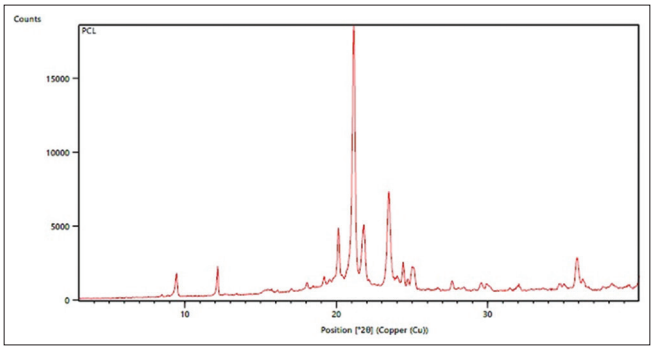

Figure 11: X-ray diffraction of triblock copolymer poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) (5000–1000–5000)

FTIR

The ring-opening polymerization of ε-CL exhibited peaks at 1720 and 1239cm^{-1} in the PCL-PEG-PCL triblock copolymer FT-IR spectra, which correspond to –C=O stretching vibrations [Figure 7]. The unique C-O-C stretching vibration of the repeated-OCH_2CH_2 units in PEG was determined at peak 1106cm^{-1}. Furthermore, signals detected at 2941 and 2864cm^{-1} were correlated with the typical absorption of C-H stretching bonds of -CH_2CH_2, comparable to those of ε-CL. These signals demonstrate the formation of the PCL-PEG-PCL triblock copolymer.[9]

The functional groups of PCL-PEG-PCL (5000–1000–5000) copolymer were investigated using FTIR spectroscopy. The FTIR spectra of the PCL-PEG-PCL copolymer are shown evidence of carboxylic ester (C=O) and ether (C-O) groups, which are represented by strong and intense bands at 1720 and 1175cm^{-1}, respectively. Furthermore, the 3436cm^{-1} absorption band was attributed to the terminal hydroxyl groups of the PCL-PEG-PCL (5000–1000–5000) triblock copolymer. Figure 7 illustrates the FTIR spectra of triblock copolymer. It was clear that PCL-PEG-PCL copolymers exhibited characteristic peaks of both PCL and PEG components.[6,34] It was clear that the PCL-PEG-PCL copolymers had distinct peaks for both PCL and PEG components.[6,34] The results obtained by FTIR are similar to what is obtained by ^{1}H NMR and ^{13}C, confirming the synthesis of the tri-block copolymer.[24]

Residual solvent estimation by GC

The dichloromethane (MDC) was used to purify the polymer. MDC is a Class II solvent, which may be considered less hazardous and provide a lower risk to human health. Figure 8 shows MDC at RT 3.08 min. While no peak for MDC was identified in the blank sample and synthesized triblock copolymer [Figure 8], the US FDA's criterion for residual acetone in pharmaceutical formulations is <600 ppm. The residual amounts of dichloromethane in PCL-PEG-PCL are substantially within the FDA's permitted limits. The synthesized novel, biodegradable, amphiphilic, triblock polymer is very safe to use for various biomedical applications.

TGA

TGA has long been a widely established polymer analysis method in polymer industry. TGA was used for evaluating a material's ability to withstand high temperatures. PEG is known to degrade at relatively low temperatures, usually between 200°C and 400°C. During this phase, a significant weight loss 23.9% corresponds to the degradation of the PEG segments. PCL tends to degrade at higher temperatures, typically above 300°C and up to around 450°C. During this phase, a significant weight loss 71.8% corresponds to the degradation of the PCL segments. The degradation of PCL segments would contribute to further

weight loss in this temperature range. The copolymer's TGA thermogram showed further a 7.5% mass loss upon heating above 450°C attributed to inorganic fillers or the carbonaceous residue from the polymer decomposition. Figure 9 displays the TGA of copolymer.

DSC

The thermal properties of the synthesized triblock copolymer were evaluated by DSC, which was showed in Figure 10. Triblock copolymer has a glass transition temperature (Tg) of 57.8°C. The thermogram of copolymer showed an endothermic peak at 57.60°C, which corresponded to the melting temperatures of PEG and PCL blocks.[35]

XRD analysis

The copolymer exhibited distinct peaks at diffraction angles (2θ) of 21.14°, 23.45°, 20.14°, 35.8°, and 12.15° [Figure 11]. It demonstrates that the copolymer was presented in crystalline form.

CONCLUSION

This study illustrates successful synthesis of a novel, amphiphilic, and biodegradable PCL-PEG-PCL (5000–1000–5000) triblock copolymer through ring-opening polymerization of ε-CL using poly (ethylene glycol) as a macroinitiator was successfully synthesized and characterized by ^{1}H NMR,^{13}C NMR, FTIR, XRD, GC, DSC, and TGA techniques. The structure of the triblock polymer was confirmed using ^{1}H and ^{13}C NMR. The CL:PG ratio of prepared triblock polymer was found to be 91:09 using ^{1}H-NMR, and ^{13}C NMR investigations which confirmed the hydroxyl end caps. The TGA curve for PCL-PEG-PCL showed multiple steps of weight loss corresponding to the degradation of PEG and PCL segments. The FT-IR, ^{1}H NMR, ^{13}C NMR, and DSC results indicated that the novel, biodegradable, triblock PCL-PEG-PCL copolymer was synthesized successfully. The GC results of triblock polymer were free of residual solvents and are very safe to use for various drug delivery systems. The unique structure and biocompatible properties of this triblock polymer make it an ideal candidate for long-acting drug delivery and biomedical solutions.

AUTHOR CONTRIBUTION STATEMENT

Rapolu Kishore: Investigation, Conceptualization, Original draft preparation. Muvvala Sudhakar: Supervision, Reviewing, and Editing of original draft.

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DECLARATION OF INTEREST

There are no possible conflicts of interest that the authors have disclosed about the research, writing, or publication of this article.

REFERENCES

1. Gou M, Zheng L, Peng X, Men K, Zheng X, Zeng S, *et al*. Poly(epsilon-caprolactone)-poly(ethylene glycol)-poly(epsilon-caprolactone) (PCL-PEG-PCL) nanoparticles for honokiol delivery *in vitro*. Int J Pharm 2009;375:170-6.
2. Tyler B, Gullotti D, Mangraviti A, Utsuki T, Brem H. Polylactic acid (PLA) controlled delivery carriers for biomedical applications. Adv Drug Deliv Rev 2016;107:163-75.
3. Feng R, Song Z, Zhai G. Preparation and *in vivo* pharmacokinetics of curcumin-loaded PCL-PEG-PCL triblock copolymeric nanoparticles. Int J Nanomedicine 2012;7:4089-98.
4. Yang Z, Peng H, Wang W, Liu T. Crystallization behavior of poly(ε-caprolactone)/layered double hydroxide nanocomposites. J Appl Polym Sci 2010;116:2658-67.
5. Kasinski A, Zielinska-Pisklak M, Kowalczyk S, Plichta A, Zgadzaj A, Oledzka E, *et al*. Synthesis and characterization of new biodegradable injectable thermosensitive smart hydrogels for 5-fluorouracil delivery. Int J Mol Sci 2021;22:8330.
6. Hu C, Chen Z, Wu S, Han Y, Wang H, Sun H, *et al*. Micelle or polymersome formation by PCL-PEG-PCL copolymers as drug delivery systems. Chin Chem Lett 2017;28:1905-9.
7. Bliley JM, Marra KG. Polymeric biomaterials as tissue scaffolds. In: Stem Cell Biology and Tissue Engineering in Dental Sciences. Amsterdam: Elsevier Inc.; 2015. p. 149-61.
8. Gao X, Kan B, Gou M, Zhang J, Guo G, Huang N, *et al*. Preparation of anti-CD40 antibody modified magnetic PCL-PEG-PCL microspheres. J Biomed Nanotechnol 2011;7:285-91.
9. Guo F, Guo D, Zhang W, Yan Q, Yang Y, Hong W, *et al*. Preparation of curcumin-loaded PCL-PEG-PCL triblock copolymeric nanoparticles by a microchannel technology. Eur J Pharm Sci 2017;99:328-36.
10. Wu Q, Li L, Wang N, Gao X, Wang B, Liu X, *et al*. Biodegradable and thermosensitive micelles inhibit ischemia-induced postoperative peritoneal adhesion. Int J Nanomedicine 2014;9:727-34.

11. Khodaverdi E, Golmohammadian A, Mohajeri SA, Zohuri G, Mirzazadeh Tekie FS, Hadizadeh F. Biodegradable *in situ* gel-forming controlled drug delivery system based on thermosensitive poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) hydrogel. ISRN Pharm 2012;2012:976879.
12. Danafar H. Preparation and characterization of PCL-PEG-PCL polymersomes for delivery of clavulanic acid. Cogent Med 2016;3:1235245.
13. Steinman NY, Bentolila NY, Domb AJ. Effect of molecular weight on gelling and viscoelastic properties of poly(caprolactone)-b-poly(ethylene glycol)-b-poly(caprolactone) (PCL-PEG-PCL) hydrogels. Polymers 2020;12:2372.
14. Schoeffski K, Hoffmann H. Karl Fischer titration: Determination of water content in pharmaceuticals. In: Pharmaceutical Sciences Encyclopedia. United States: Wiley; 2010. p. 1-14.
15. Sadikoglu H, Ozdemir M, Seker M. Freeze-drying of pharmaceutical products: Research and development needs. Dry Technol 2006;24:849-61.
16. Puthli S, Vavia P. Formulation and performance characterization of radio-sterilized "Progestin-only" microparticles intended for contraception. AAPS PharmSciTech 2009;10:443-52.
17. Liu CB, Gong CY, Huang MJ, Wang JW, Pan YF, Zhang YD, *et al*. Thermoreversible gel-sol behavior of biodegradable PCL-PEG-PCL triblock copolymer in aqueous solutions. J Biomed Mater Res B Appl Biomater 2008;84:165-75.
18. Gong CY, Shi S, Dong PW, Kan B, Gou ML, Wang XH, *et al*. Synthesis and characterization of PEG-PCL-PEG thermosensitive hydrogel. Int J Pharm 2009;365:89-99.
19. Li H, Wang X, Guo X, Wan Q, Teng Y, Liu J. Development of rapamycin-encapsulated exosome-mimetic nanoparticles-in-PLGA microspheres for treatment of hemangiomas. Biomed Pharmacother 2022;148:112737.
20. Garner J, Skidmore S, Park H, Park K, Choi S, Wang Y. Beyond Q1/Q2: The impact of manufacturing conditions and test methods on drug release from PLGA-based microparticle depot formulations. J Pharm Sci 2018;107:353-61.
21. Piao L, Dai Z, Deng M, Chen X, Jing X. Synthesis and characterization of PCL/PEG/PCL triblock copolymers by using calcium catalyst. Polymer 2003;44:2025-31.
22. Rapolu K, Sanka K, Vemula PK, Aatipamula V, Mohd AB, Diwan PV. Optimization and characterization of gastroretentive floating drug delivery system using Box-Behnken design. Drug Dev Ind Pharm 2013;39:1928-35.
23. Kesharwani P, Shadab M, Alhakamy NA, Hosny KM, Haque A. Qbd enabled azacitidine loaded liposomal nanoformulation and its *in vitro* evaluation. Polymers (Basel) 2021;13:250.
24. Dias AR, de Miranda BN, Cobas-Gomez H, Poço JG, Rubio MR, de Oliveira AM. Synthesis and characterization of amphiphilic block copolymers by transesterification for nanoparticle production. Polimeros 2019;29:e2019027.
25. Armatazaka Z, Sulaiman TN, Zulkarnain AK. Optimization and characterization of Peg-Pcl-Peg triblock copolymer as carrier of drug using full factorial design. Int J Curr Pharm Res 2019;11:65-71.
26. Ghosal K, Ghosh D, Das SK. Preparation and evaluation of naringin-loaded polycaprolactone microspheres based oral suspension using Box-Behnken design. J Mol Liq 2018;256:49-57.
27. Ramazani F, Chen W, Van Nostrum CF, Storm G, Kiessling F, Lammers T, *et al*. Strategies for encapsulation of small hydrophilic and amphiphilic drugs in PLGA microspheres: State-of-the-art and challenges. Int J Pharm 2016;499:358-67.
28. Ali AA, Niamah AK, Salih HW. Isolation, preparation and characterization of polylactic acid film reinforced with nano silica. J Phys Conf Ser 2021;2063:012028.
29. Ghose D, Patra CN, Ravi Kumar BV, Swain S, Jena BR, Choudhury P, *et al*. QbD-based formulation optimization and characterization of polymeric nanoparticles of cinacalcet hydrochloride with improved biopharmaceutical attributes. Turk J Pharm Sci 2021;18:452-64.
30. Sanka K, Bandari S, Jukanti R, Veerareddy PR. Colon-specific microparticles of piroxicam: Formulation and optimization using 32 factorial design. J Dispers Sci Technol 2011;32:1396-403.
31. Sanka K, Pragada RR, Veeraredy PR. Ph dependent colon specific ketoprofen microspheres: Screening of main effects of process and formulation variables using Plackett-Burman design. Lat Am J Pharm 2015;34:1195-204.
32. Azouz L, Dahmoune F, Rezgui F, G'Sell C. Full factorial design optimization of anti-inflammatory drug release by PCL-PEG-PCL microspheres. Mater Sci Eng C Mater Biol Appl 2016;58:412-9.
33. Mali S, Oza N. Formulation and optimization of paliperidone palmitate biodegradable injectable microspheres using Box-Behnken design. J Drug Deliv Sci Technol 2022;74:103609.
34. Tanaka K, Kanazawa T, Shibata Y, Suda Y, Fukuda T, Takashima Y, *et al*. Development of cell-penetrating peptide-modified MPEG-PCL diblock copolymeric nanoparticles for systemic gene delivery. Int J Pharm 2010;396:229-38.
35. Alami-Milani M, Zakeri-Milani P, Valizadeh H, Salehi R, Jelvehgari M. Preparation and evaluation of PCL-PEG-PCL micelles as potential nanocarriers for ocular delivery of dexamethasone. Iran J Basic Med Sci 2018;21:153-64.

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