HEALTH SCIENCES QUARTERLY

Volume: 5
Issue: 1
2025
E-ISSN: 2791-6022
https://journals.gen.tr/jsp

Received: 2024-11-29
Accepted: 2024-12-31

ORIGINAL ARTICLE

Synthesis, characterization and cytotoxicity analyzes of novel AB-Type Amphiphilic Block Copolymers

Murat Mısır

Department of Chemical Engineering, Faculty of Engineering and Architecture, Kırşehir Ahi Evran University. Kırşehir / Türkiye

Abstract

AB-type novel amphiphilic poly(L-lactide)-*block*-(*N*-isopropylacrylamide) (PLLA-*b*-PNIPAM) and poly(L-lactide)-*block*-(*N*-vinyl-pyrrolidone) (PLLA-*b*-PNVP), diblock copolymers were synthesized through the combined use of ring-opening polymerization (ROP) and controlled/living radical polymerization (CRP) techniques. (PLLA-*b*-PNIPAM) block copolymer was prepared via combination of ROP and atom transfer radical polymerization (ATRP) using the novel PLLA-based ATRP macroinitiator. (PLLA-*b*-PNVP) block copolymer was synthesized via combination of ROP and reversible addition-fragmentation chain transfer (RAFT) polymerization using the PLLA-based RAFT macro chain transfer agent (CTA). For this purpose, at first 2,4-difluorobenzyl alcohol **(1)** was used to initiate the ROP of (L-LA) using tin(II) 2-ethylhexanoate Sn(Oct)2 as a catalyst at 120 °C for synthesis of PLLA-OH **(2)**. Secondly, bromoester end-functionalized PLLA-based ATRP macroinitiator **(3)** was synthesized by esterification of hydroxyl end group of **(2)**. The first block copolymer, (PLLA-*b*-PNIPAM) **(5)**, was synthesized by ATRP of NIPAM using **(3)** in presence of copper(I) chloride/tris[2-(dimethylamino)ethyl]amine (CuCl/Me6TREN) as catalyst system in DMF/water at 25 °C. For the synthesis step of second block copolymer, at first PLLA macro chain transfer agent (CTA) **(4)** was then synthesized via substitution reaction of **(3)** with potassium ethyl xanthogenate (KEX) and finally PLLA-*b*-PNVP **(6)** diblock copolymer was prepared via RAFT polymerization of NVP using **(4)**. The molecular structures of novel polymers (**2-6**) were elucidated by spectroscopic (FTIR and 1H NMR) methods. In the application phase of this study, the effectiveness of copolymers was examined on cervical cancer cells. Cytotoxicity effects were evaluated in vitro on HeLa cell lines.

Keywords: AB-type amphiphilic block copolymer, ROP, RAFT polymerization, cytotoxicity

Citation: Mısır M. Synthesis, characterization and cytotoxicity analyzes of novel AB-Type Amphiphilic Block Copolymers. Health Sci Q. 2025;5(1):51-63. https://doi.org/10.26900/hsq.2622

Corresponding Author:
Murat Mısır
Email: murat.misir@ahievran.edu.tr

Introduction

The preparation of block copolymers with amphiphilic character consisting of a biodegradable hydrophobic portion and a hydrophilic portion by combination of ROP controlled/living radical polymerization (CRP) techniques has made a great contribution to the studies in biomaterial and nanomedicine. ROP is one of the polymerization methods that can be performed depending on the variations of catalyst/initiator system and the monomer in the syntheses of several aliphatic polyesters [1]. Polyesters such as poly(lactide) (PLA) and poly(ε-caprolactone) (PCL) used for controlled drug delivery studies were generally prepared via ROP as the most chosen technique [2,3]. Among biodegradable polyesters, PLA is a bio-based and bio-compatible polymer used in many applications such as biomedicine, agriculture, packaging etc. not only because of its renewability, biocompatibility, and excellent process ability, but also because it decomposes to H2O and CO2 when degraded, forming non-toxic and non-carcinogenic products. [4,5]. Atom transfer radical polymerization (ATRP), the one of most used of CRP methods, is extensively used in macromolecular through the combination of suitable catalyst-ligand systems with halogen-containing initiators [6]. This technique has also been proven for the controlled polymerization of various monomers, including those with a range of functional groups [1,7]. Reversible addition-fragmentation chain transfer (RAFT) polymerization replicates the characteristics of living polymerization and enables the polymerization of a wide variety of functional monomers that are generally not compatible with other CRP methods [8].

Poly(*N*-isopropylacrylamide) (PNIPAM) is a hydrophilic character polymer widely employed to form the temperature-responsive copolymers with other polymers [9]. Advancements in synthetic methods have made it possible to prepare various block copolymers consisting of a hydrophobic part containing PLA and a hydrophilic part containing PNIPAM. Most of the studies have focused on preparation of AB-type [9,10] diblock or ABA-type [11] or ABC-type [12] triblock copolymers consisting of PLA and PNIPAM through various polymerization methods have been studied extensively by numerous researchers. The amphiphilic block copolymers consisting of polyester and PNIPAM with advanced macromolecular architectures have also been investigated as carriers for drug delivery systems. The copolymerization of PNIPAM with PLA combines the thermosensitivity of PNIPAM and degradability of PLA, enabling its use as a carrier in drug delivery systems [11]. Poly(*N*-vinyl pyrrolidone) (PNVP) is a hydrophilic polymer that can be used in the pharmaceutical and biomedical fields due to its properties such as water solubility and low toxicity [13,14]. PNVP has been used extensively in pharmaceutical tablets and hydrogels [15,16]. The studies focusing on synthesis of block copolymers including PNVP and PLA or PCL using the combination ROP and RAFT polymerization have been investigated extensively by many researchers in recent years. Polymeric micelles formed from biocompatible PNVP-based block copolymers, such as PNVP-*b*-PCL [17,18] and PNVP-*b*-PDLLA [19] have been synthesized using via combination ROP and RAFT and their properties in terms of micelle formation have been evaluated for drug delivery applications. In addition to these literatures, Shin et al. prepared PVP-*b*-PLLA using a dual initiator via combination ROP and RAFT in one-step procedure [20]. In this work novel AB-type block copolymers were prepared in five stages via combination of ROP and ATRP or RAFT polymerizations of L-lactide, NIPAM or NVP, respectively. Firstly, PLLA **(2)** was prepared by ring opening polymerization (ROP) of (L-LA) at 120 °C using **(1)** as initiator. Secondly, bromoester end-functionalized PLLA macroinitiator **(3)** was synthesized by esterifying hydroxyl groups of **(2)**. Thirdly, PLLA macro chain transfer agent (CTA) **(4)** was then synthesized via substitution reaction of **(3)** with potassium ethyl xanthogenate (KEX). The first diblock copolymer, (PLLA-*b*-PNIPAM) **(4),** was prepared by ATRP of NIPAM as monomer using **(3)** in presence of copper(I) chloride/tris[2-(dimethylamino)ethyl]amine (CuCl/Me6TREN) as catalyst system in DMF/water at 25 °C. Finally, the second block copolymer, PLLA-*b*-PNVP **(6)**, was synthesized RAFT polymerization of NVP

using **(5)**. Characterization of the molecular structures for the novel polymers were elucidated by spectroscopic (FTIR and 1H NMR) methods. In the application phase of this study, the effectiveness of copolymers was examined on cervical cancer cells. The cytotoxicity of polymers was evaluated in vitro on HeLa cell lines.

Experimental Section

Materials

Reactions were carried out under an atmosphere of argon using standard Schlenk techniques. *N*-isopropylacrylamide (NIPAM, Sigma-Aldrich, 97%), was purified by re-crystallization from *n*-hexane/toluene mixture and dried in vacuum. *N*-Vinyl pyrrolidone (NVP) was dried over anhydrous magnesium sulfate and distilled under reduced pressure. L-lactide (L-LA, TCI, 98%) was purified by recrystallization from ethyl acetate/*n*-hexane twice and dried in vacuum at room temperature and kept in freezer. Copper(I) chloride (CuCl) (98%; Aldrich) was purified by stirring it overnight in glacial acetic acid to eliminate Cu^{2+} ions, followed by filtration, washing with ethanol, and drying under vacuum at 70 °C for two days. 2,2′-Azobis(isobutyronitrile) (AIBN) was received from TCI (>98%). After recrystallisation from methanol it was stored at 4 °C. Tris[2-(dimethylamino)ethyl] amine (Me_6TREN) was prepared according to published procedure [21]. Dichloromethane (DCM ≥99.5%) was dried over calcium hydride (CaH_2) and stored over molecular sieves (4 Å). 2-Bromopropionyl bromide (Sigma-Aldrich, 97%), 2,4-difluorobenzaldehyde (Sigma-Aldrich, 98%), potassium ethylxanthate (TCI, >95%), triethylamine (TEA, Sigma-Aldrich, ≥99%), sodium borohydride $NaBH_4$ (Sigma-Aldrich, 98%), pyridine (Sigma-Aldrich, ≥99%) and tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, Sigma-Aldrich, >92.5%) were used as received. Conventional methods were employed for purification of all solvents [22].

Measurement

Transmission IR spectra was recorded on a FTIR-ATR spectrophotometer (Perkin-Elmer 1600) in the spectral range 4000–400 cm^{-1} with samples. ^{1}H NMR spectra were recorded on the Bruker AVANCE III 400 MHz NMR instrument was used for the characterization Varian Mercury 400 MHz spectrometer with CDCl3 as solvent at ambient temperature. Tetramethylsilane (TMS) is used as the internal standard and the chemical shifts were given in parts per million (ppm) relative to this standard.

Synthesis of 2,4-difluorobenzyl alcohol (1)

2,4-Difluorobenzyl alcohol was synthesized the reduction of 2,4-difluoro benzaldehyde in presence of $NaBH_4$ in methanol according to published literature [23]. To a stirred solution of 2,4-difluorobenzaldehyde (1.67 mL, 2.18 g, 15 mmol) in methanol (10 mL) was added $NaBH_4$ (752 mg, 19.5 mmol) portion wise at 0 °C overnight. After completion of the reaction, methanol was removed under reduced pressure, diluted ice-cold water (50 mL) and extracted with ethyl acetate (2x25 mL). The combined organic layers were washed with H_2O (2x25 mL) and brine (2x25 mL) and dried over $MgSO_4$ and concentrated to obtain the light-yellow liquid. Yield was 90%.

^{1}H NMR (400 MHz, $CDCl_3$, **δ**): 7.46-7.34 (Ar**H**, 1H), 6.89-6.78 (Ar**H**, 2H), 4.72 (Ar–C**H**$_2$–,2H), 1.78 (CH_2–O**H**, O*H*).

Synthesis of (2) in the presence of initiator (1) via ROP

PLLA-OH **(2)** was prepared as follows: Into a Schlenk tube equipped with a magnetic stirrer, ROP initiator **(1)** (0.06 g, 0.4 mmol), L-LA (1.47 g, 10 mmol) and $Sn(Oct)_2$ ($3.41x10^{-3}$ mL, 0.01 mmol) in dry toluene (3 mL) were introduced. The flask with reaction mixture was degassed and then immersed into an oil bath at 120 °C for 24 h. The reaction mixture was poured into methanol with stirring and the polymer was precipitated. The white powder polymer **(2)** was re-precipitated using dissolve/precipitate process (DCM/methanol), collected, and then dried in the vacuum at 30 °C.

Yield: 1.29 g, Conversion: 84%. M_n (theo.); 3160 g/mol; M_n (NMR); 3320 g/mol; FTIR (ATR, cm^{-1}): 2994, 2946, 1756, 1453, 1358, 1185, 1083, 868, 753; ^{1}H NMR ($CDCl_3$, δ) = 7.34 (Ar***H***, H^b), 6.85 (Ar***H***, H^{a+c}), 5.16 (main chain, –C***H***(CH_3)OCO) H^e), 4.34 (terminal, –C***H***(CH_3)OH, H$^{e'}$), 1.57 (main chain, –CH(C***H***$_3$)OCO-, H^f), 1.49 (terminal, –CH(C***H***$_3$) OH, H$^{f'}$).

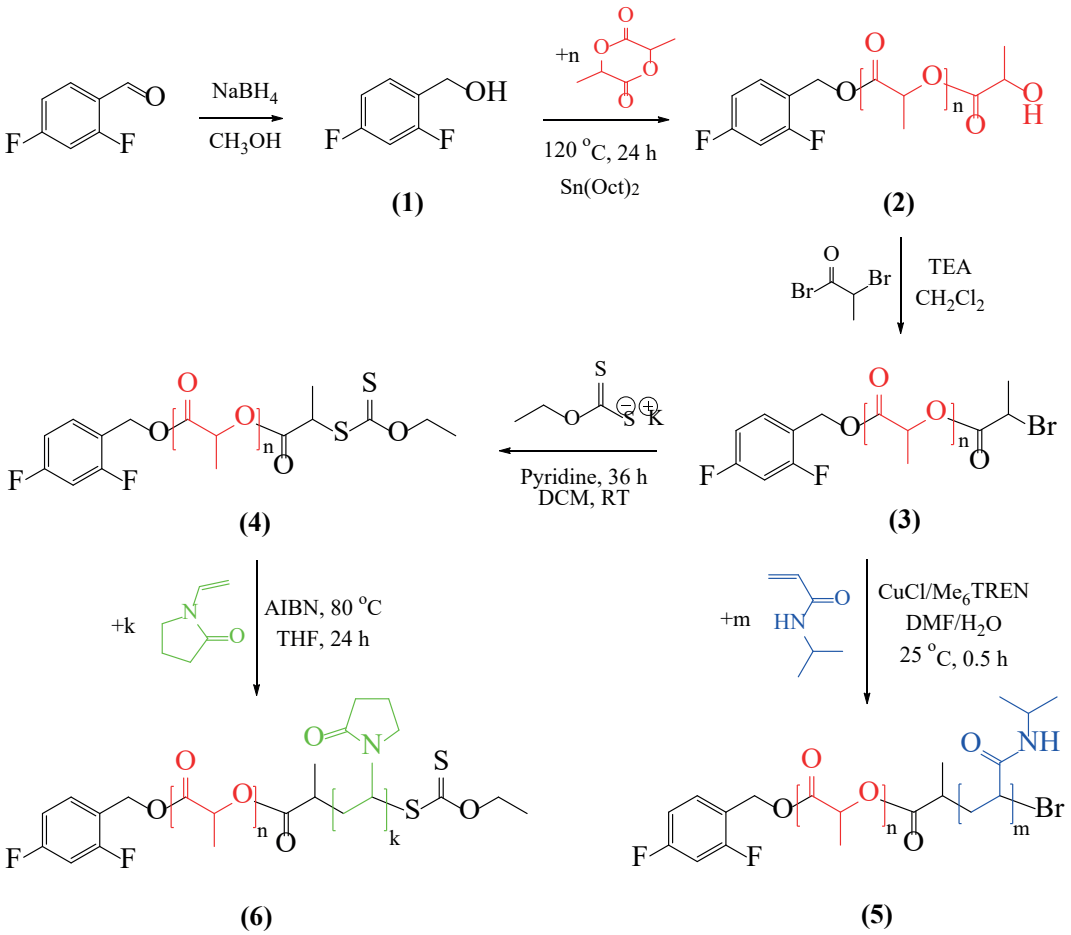
Synthesis of bromoester-ended PLLA (3)

PLLA-OH **(2)** was converted to bromoester functionalized (PLLA-Br) by using 2-bromopropionyl bromide. **(2)** (1.0 g, 0.301 mmol, M_n (NMR) =3320 g/mol) was charged a round-bottom two-necked flask and dissolved in dry DCM (15 mL). TEA (0.123 mL, 0.903 mmol) was added to the mixture. The reaction was cooled down to 0 °C and 2-bromopropionyl bromide (0.066 mL, 0.588 mmol) in dry DCM (5 mL) was added dropwise for 30 min. The reaction continued at room temperature for 36 h with stirring. After the removal of precipitated salt, the filtrate was diluted with 30 ml of DCM and washed with 5% aqueous $NaHCO_3$ (3x20 mL), then water (3x20 mL), dried over $MgSO_4$, and filtered. The concentrated solution was precipitated into cold methanol and the bromoester ended PLLA **(3)** was dried overnight in vacuum at 40 °C.

M_n (NMR): 3460 g/mol; ^{1}H NMR ($CDCl_3$, δ) = 7.34 (Ar***H***, H^b), 6.85 (Ar***H***, H^{a+c}), 5.16 (main chain, –C***H***(CH_3)OCO) H^e), 4.43 (–C***H***(CH_3)Br, H^g), 1.85 (–CH(C***H***$_3$)Br, H^h), 1.57 (main chain, –CH(C***H***$_3$)OCO-, H^f), 1.49 (–CH(C***H***$_3$)O, $H^{f'}$)

Synthesis of xanthate terminated PLLA (4)

The functional polyester **(3)** was converted into a PLLA-based RAFT-CTA **(4)** via substitution reaction of **(3)** with potassium ethyl xanthogenate (KEX) using molar ratio of reagents; [**(3)**: KEX: pyridine]: 1:3:55. In a typical synthesis process, **(3)** (0.15 g, 0.043 mmol, Mn, (NMR) =3460 g/mol) and potassium ethylxanthate (KEX) (0.021 g, 0.129 mmol) were taken in a dried and argon purged round-bottom flask and the flask was immersed in a cold ice bath. In another dried flask, pyridine (0.19 mL, 2.37 mmol) dissolved in 20 mL DCM and this solution was added dropwise to first reaction mixture during stirring for 30 min. The reaction mixture continued at room temperature for 48 h with stirring. The reaction mixture diluted with 60 mL of DCM was washed successively with saturated NH_4Cl solution (3x30 mL), saturated $NaHCO_3$, solution



Scheme 1. Synthesis of PLLA-based macroinitiators and block copolymers.

(3x30 mL), and water (3x 50 mL), dried over $MgSO_4$ and filtered. After the filtrate was brought to dryness, the residue was dissolved in THF and precipitated into hexane. The PLLA macro-CTA **(4)** was dried overnight under vacuum.

M_n (NMR); 3620 g/mol; ^{1}H NMR ($CDCl_3$, δ) = 7.33 (Ar***H***, H^b), 6.86 (Ar***H***, H^{a+c}), 5.17 (main chain, –C***H***(CH_3)OCO) H^e), 4.64 (O–C***H***2CH_3, H^i), 4.45 (–C***H***(CH_3)Br, H^g), 1.57 (–CH(C***H***$_3$)OCO-, H^f), 1.52 (–CH(C***H***$_3$)O, H^h), 1.41 (O–CH_2C***H***$_3$, H^j).

Synthesis of PLLA-*b*-PNIPAM (5)

PLLA-*b*-PNIPAM **(5)** was prepared using **(3)** as macroinitiator via ATRP. In a dried Schlenk flask, **(3)** 0.09 g (0.026 mmol, M_n (NMR)= 3460 g/mol), NIPAM 304 mg (2.6 mmol) and 2.6 mg (0.065 mmol) CuCl were dissolved in 1.5 mL DMF, then 0.3 mL of distilled water were added to the reaction mixture. After three freeze-pump-thaw cycles, 13.7 µL (0.065 mmol) Me_6TREN were added under argon atmosphere. The reaction was then allowed to proceed under stirring at 25 °C for 0.5 h. The viscous solution was precipitated by adding diethyl ether. The crude product dissolved in DCM was passed through a neutral alumina column. The resulting filtrate was concentrated, precipitated in cold diethyl ether and the obtained polymer **(5)** was dried under vacuum for 48 h.

Conversion: 19%. M_n (theo.):5610 g/mol; M_n (NMR): 5460 g/mol; FTIR (ATR, cm^{-1}): 3293, 3070, 2972, 2924, 1756, 1638, 1536, 1455, 1365, 1183, 1088, 1043, 872, 837; ^{1}H NMR ($CDCl_3$, δ): 6.61 (–N***H***CH(CH_3)$_2$, H^3**,** in PNIPAM), 5.13 (–C***H***(CH_3) OCO) H^e), in PLLA], 3.98 (–C***H***(CH_3)$_2$, H^4, in PNIPAM), 2.12 (–CH_2–C***H***– , H^2), 1.79 (–C***H***$_2$–CH– , H^1, in PNIPAM),1.55 (–CH(C***H***$_3$)OCO-, H^e, in PLLA), 1.12 (–CH(C***H***$_3$)$_2$, H^5, in PNIPAM).

Synthesis of PLLA-*b*-PNVP (6)

In a dried Schlenk flask, **(4)** (54 mg, 0.015 mmol, M_n (NMR)= 3620 g/mol) was dissolved in 1 mL THF and then NVP (0.16 mL, 1.50 mmol) and AIBN (1.23 mg, 0.0064 mmol) were added. The homogeneous solution was degassed with argon and continued for 0.5 h with stirring. The flask was immersed in an oil bath at 80 °C and the reaction was allowed to proceed for 24 h. The reaction mixture was diluted with THF (4 mL), precipitated with 250 mL of hexane and the precipitated polymer was separated by centrifugation. The obtained polymer **(6)** was purified by dissolution/precipitation procedure two more times and dried under vacuum at 30 °C.

Conversion: 23%. M_n (theo.): 6175 g/mol; M_n (NMR); 5890 g/mol; FTIR (ATR, cm^{-1}): 3435, 2974, 2946, 2925, 2886, 1755, 1650, 1421, 1289, 1182, 1084, 842, 735; ^{1}H NMR ($CDCl_3$, δ) = 5.14 (H^e, in PLLA), 4.62 (O–C***H***$_2$$CH_3$, H^i), 4,04–3.51 (CH_2C***H***, H^7 in PNVP backbone), 3.51–3.02 (–NC***H***$_2$$CH_2$$CH_2$, H^{10}, in PNVP ring), 2.55–1.81 (–NCH_2CH_2C***H***$_2$, H^8 and –NCH_2C***H***$_2$$CH_2$, H^9**,** in PNVP ring)), 1.67–1.12 (H^{f+6+i}).

Cell Culture

For this study, human cervical cancer (HeLa) cell lines obtained from Kırşehir Ahi Evran University, Department Medical Pharmacology, were used. The cells were cultured at 37 °C with 5% CO_2 in RPMI medium supported with 10% Fetal Bovine Serum and 1% penicillin-streptomycin.

Cytotoxicity Analyses

The cytotoxic impact of polymers HeLa cells was evaluated using the XTT assay kit (Biological Industries, USA). 800 cells were seeded per well in a 96-well plate. Following a 24-hour incubation period, the cells were exposed to copolymer. After 72 h of incubation, the solutions from the XTT kit were introduced to the cells. Cell viability was then measured using a microplate reader (BIOTEK ELX808, USA) at a wavelength of 450 nm. The IC50 value was determined. As a result of the readings the obtained values, the inhibition rates of cells were calculated using the following formula: % inhibition: (A450 nm test – A450 nm control/A450 nm control) ×100.

Results and Discussion

The synthetic route is depicted in Scheme 1. The main route used in the preparation of block copolymers, according to monomers or methods, is as follows:

The PLLA block was first synthesized by the ROP of L-LA using $Sn(Oct)_2$ as catalyst, followed by the ATRP of NIPAM or RAFT polymerization

of NVP. In this study the novel synthesized PLLA-based block copolymers were prepared in five stages; the synthesis of PLLA via ROP (i), esterification of PLLA hydroxyl group with 2-bromopropionyl bromide (ii), substitution reaction of bromine end group with potassium ethyl xanthogenate (KEX), synthesis of PLLA-*b*-PNIPAM **(5)** via ATRP of NIPAM initiated by **(3)** (iv), synthesis of PLLA-*b*-PNVP **(5)** via RAFT polymerization of NVP using macroCTA **(4)** (v). For this purpose, at first PLLA was synthesized ROP of L-LA with molar ratio of ([monomer]:[initiator]=25:1) using **(1)** as the initiator and $Sn(Oct)_2$ as catalyst. The structure of **(2)** was elucidated by FTIR and ^1H NMR.

FTIR spectra of PLLA **(2)**, PLLA-*b*-PNIPAM **(5)** and PLLA-*b*-PNVP **(6)** are depicted in Figure 1. In the spectrum of **(2)** (in Figure 1A), the absorption band of the carbonyl group of PLLA block was observed at 1756 cm^{-1}. The bands at 1185 and 1083 cm^{-1} were attributed to carbon-oxygen stretching.

Figure 2A showed ^1H NMR spectrum of PLLA **(2)**. In the ^1H NMR spectrum of **(2)** the signal ($H^{e'}$) of terminal methine group of PLLA was observed at 4.34 ppm. The peaks of methine (H^e) and methyl (H^f) protons, corresponding to PLLA repeating units, were detected at 5.15 and 1.57 ppm, respectively. The peaks at δ =7.34 (H**b)** and 6.85 (H^{a+c}**)** are also assigned to the aromatic protons of **(1)**. M_n (NMR) of polymer **(2)** was determined by using the integral ratio of the methyl proton peaks of PLLA (δ = 1.57 ppm) to the methine proton peak of terminal unit of PLLA (peak **e'** in Figure 2A). The calculated value of molecular weight by NMR spectra is close to M_n (theo.). M_n (NMR) of PLLA was calculated by integral area of related peaks displacements according to equation (1)

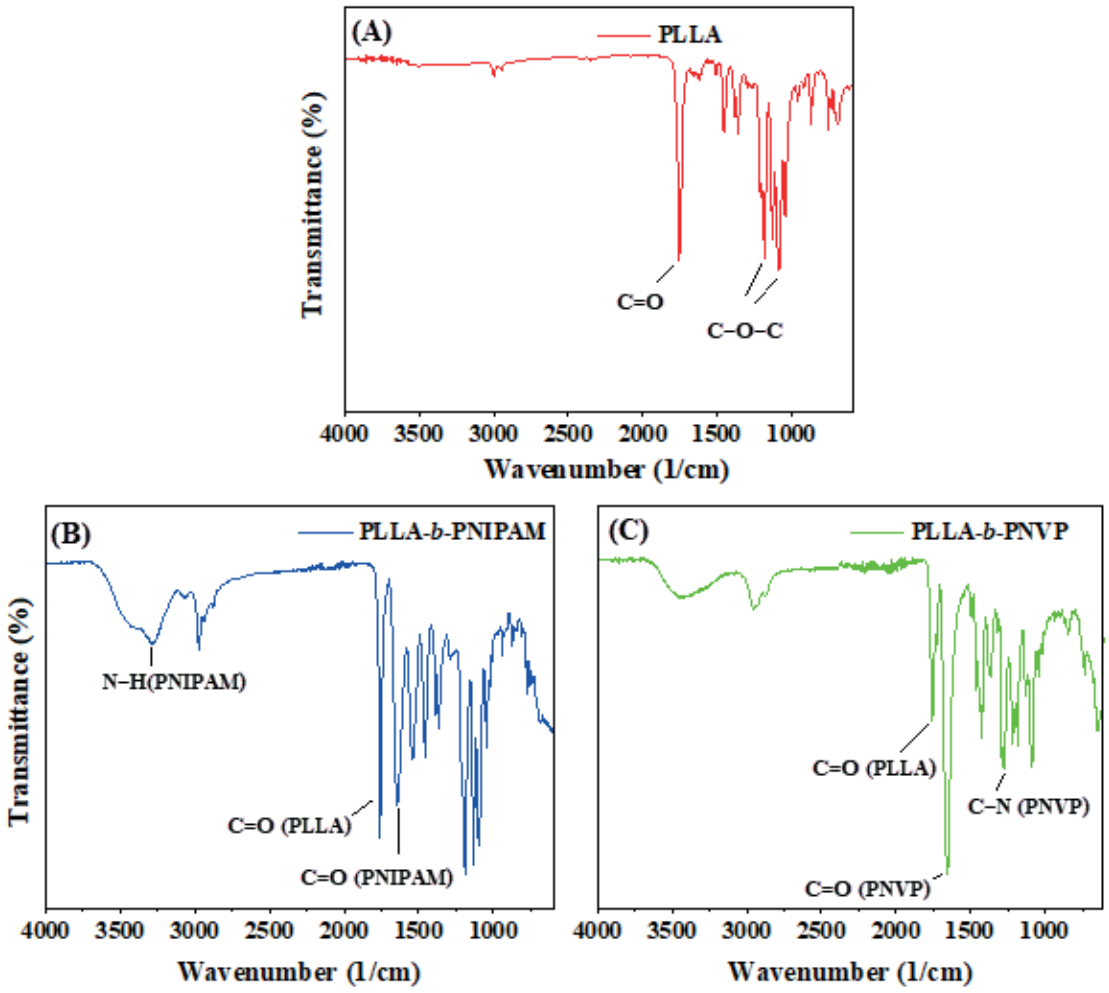


Figure 1. FTIR spectra of PLLA (A), PLLA-*b*-PNIPAM (B) and PLLA-*b*-PNVP (C).

$$M_n\,(NMR) = \left(\frac{I_f}{I_{e'}} \times M_{monomer}\right) + M_{initiator} \quad (1)$$

Here, $M_{monomer}$ and $M_{initiator}$ are molecular weights of the L-lactide and initiator, respectively.

M_n (theo.) was calculated according to equation

$$M_n(theo.) = \left(\frac{[M]}{[I]} \times M_{monomer} \times Conversion\%\right) + M_{ini} \quad (2)$$

In the second step, ATRP macroinitiator **(3)** was synthesized by esterifying of end group of **(2)** with 2-bromopropionyl bromide. ^{1}H NMR spectrum of **(3)** is displayed in Figure 2B. After esterification of hydroxyl end groups of PLLA, two novel signals appeared at 4.43 and 1.85 ppm. These signals were attributed to methine (H^g) and methyl (H^h) protons of bromo propionate end, respectively. The disappearance of peak at 4.34 ppm, which corresponds to the methine protons adjacent to terminal hydroxyl end groups of PLLA end, indicates that the esterification was successful. The peaks of methine and methyl

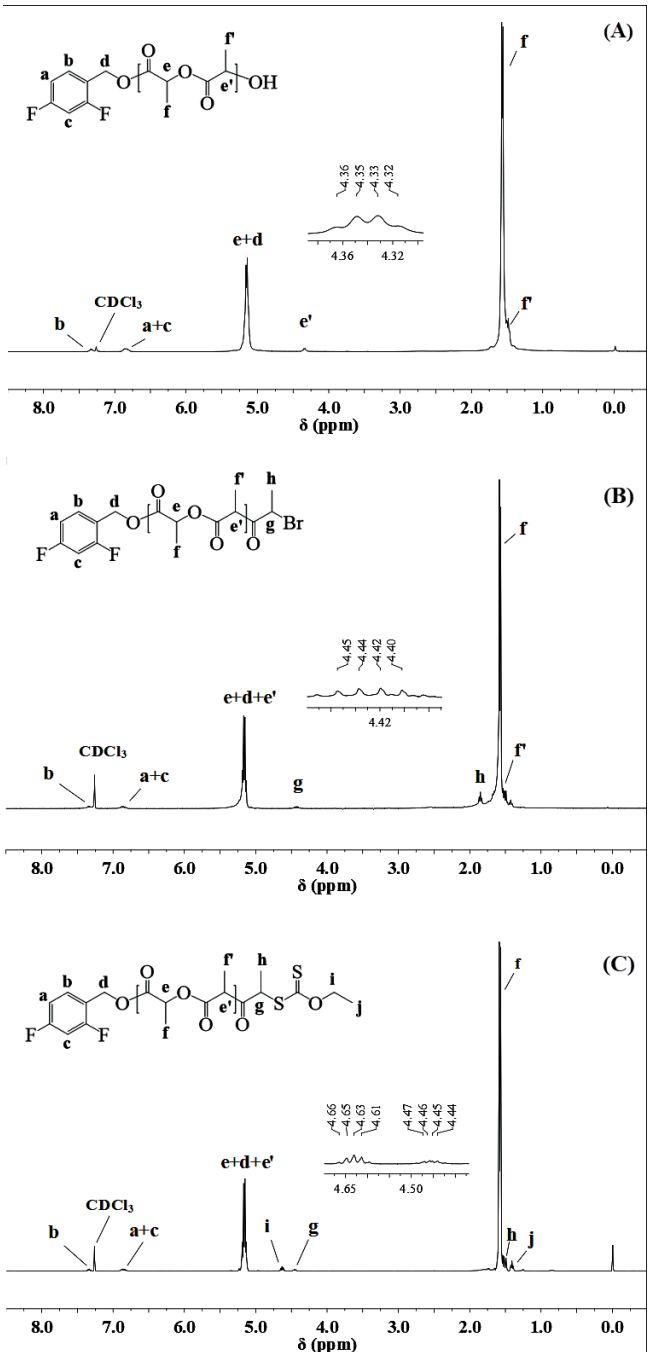


Figure 2. ^{1}H NMR spectra of (A) PLLAOH, (B) PLLA-Br macroinitiator, (C) PLLA macro CTA.

protons of the repeating units of PLLA main chain at 5.16 (H^e) and 1.57 (H^f) ppm were detected, respectively. The aromatic protons of **(1)** gave multiple signals at 7.35 and 6.86 ppm. M_n (NMR) of **(3)** calculated by comparing the peak integrals derived from the methyl protons' peaks of PLLA (δ = 1.57 ppm) and the methine proton peak of end group (peak g in Figure 2B).

In the third step, PLLA macro CTA **(4)** was synthesized via substitution reaction of **(3)** with KEX. ^{1}H NMR spectrum of **(4)** is displayed in Figure 2C. After the substitution reaction two novel signals appeared at 4.64 and 1.41 ppm, which correspond to methyl (H^i) and methyl (H^j) protons of xanthate end group, respectively. The peaks of methine and methyl protons of the PLLA at 5.17 (H^e) and 1.57 (H^f) ppm were detected, respectively. The signals for the aromatic protons of initiator fragment **(1)** in macro-CTA agree with the values recorded the previous spectrum (Figure 2B).

In the fourth step, PLLA-*b*-PNIPAM **(5)** was synthesized by ATRP of NIPAM as monomer using **(3)** as macroinitiator with molar ratio of monomer to initiator, i.e. [M]:[I] =100:1. FTIR spectrum of block copolymer is depicted in Figure 1B. As seen in FTIR spectrum of **(5)**, the appearance of bands at 3285 cm^{-1} (N-H stretching), 1640 cm^{-1} (C=O stretching) and 1538 cm^{-1} (N-H bending) belonging to PNIPAM indicated polymerization of NIPAM was successful [24]. In addition to these data, C=O

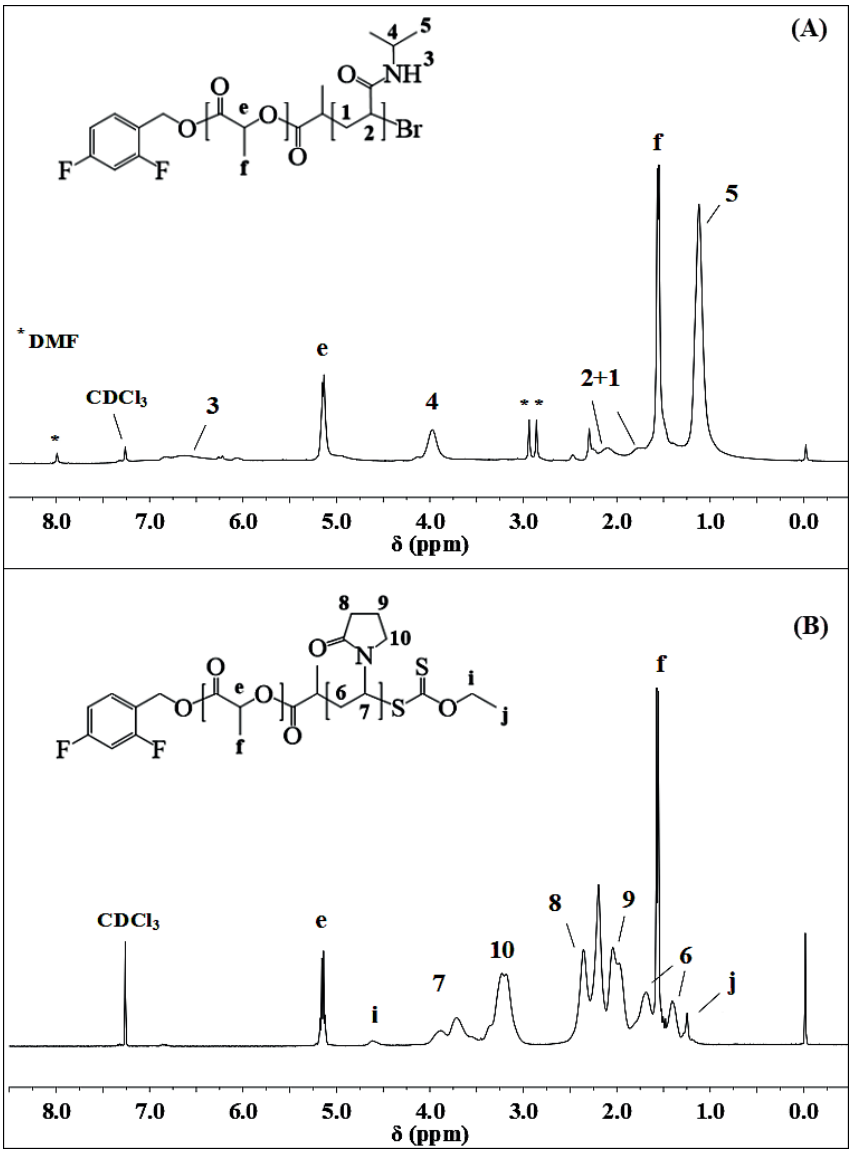


Figure 3. ^{1}H NMR spectra of (A) PLLA-*b*-PNIPAM, and (B) PLLA-*b*-PNVP.

band at 1755 cm^{-1}, attributable to the PLLA block, supports the formation of block copolymer.

^{1}H NMR spectrum of **(5)** (in Figure 3A) displayed characteristic signals at 3.98 and 1.12 ppm assigned to the methine (H^4) and methyl (H^5) protons of PNIPAM, respectively. The signals at 5.13 and 1.55 ppm were ascribed to methine and methyl protons of PLLA, respectively. A broad signal appeared at 6.61 ppm (H^3), which show the presence of proton of –NH group. The signals at 2.12 (H^2) and 1.79 (H^1) ppm are also attributed to the methine and methylene group of PNIPAM backbone, respectively. M_n (NMR) of **(5)** was determined by comparing the peak integrals derived from the methine proton peaks of PNIPAM (δ = 3.98 ppm, peak '**4**') and the methine proton signal of PLLA (δ = 5.13 ppm, peak '**e**' in Figure 3A) according to equation (3 and 4)

$$M_n\,(NMR) = (\,DP_{NIPAM} \times M_{monomer}\,) + M_{n,PLLA} \quad (3)$$

$$DP_{NIPAM} = \left(\frac{I_4}{I_e} \times DP_{PLLA}\right) \quad (4)$$

Here, DP_{PNIPAM} and DP_{PLLA} are degree of polymerization for PNIPAM and PLLA segments, respectively. $M_{monomer}$ is also molecular weight of the NIPAM.

Finally, PLLA-*b*-PNVP **(6)** was synthesized RAFT polymerization of NVP using **(4)**. FTIR spectrum of **(6)** is shown in Figure 1C. As seen in the FTIR spectrum of **(6)**, peaks corresponding to PVP can be detected by the appearance of C=O and C–N peaks at 1656 and 1289 cm^{-1}, respectively.

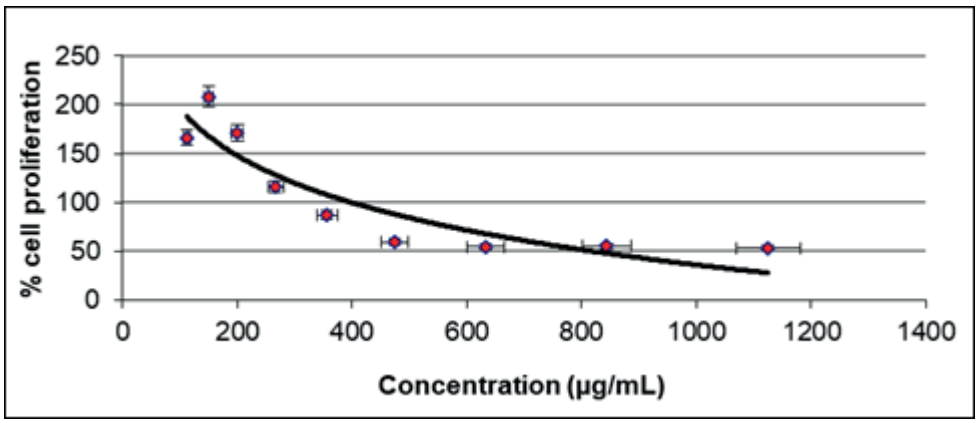


Figure 4. Cytotoxicity assay of PLLA-*b*-PNIPAM block copolymer.

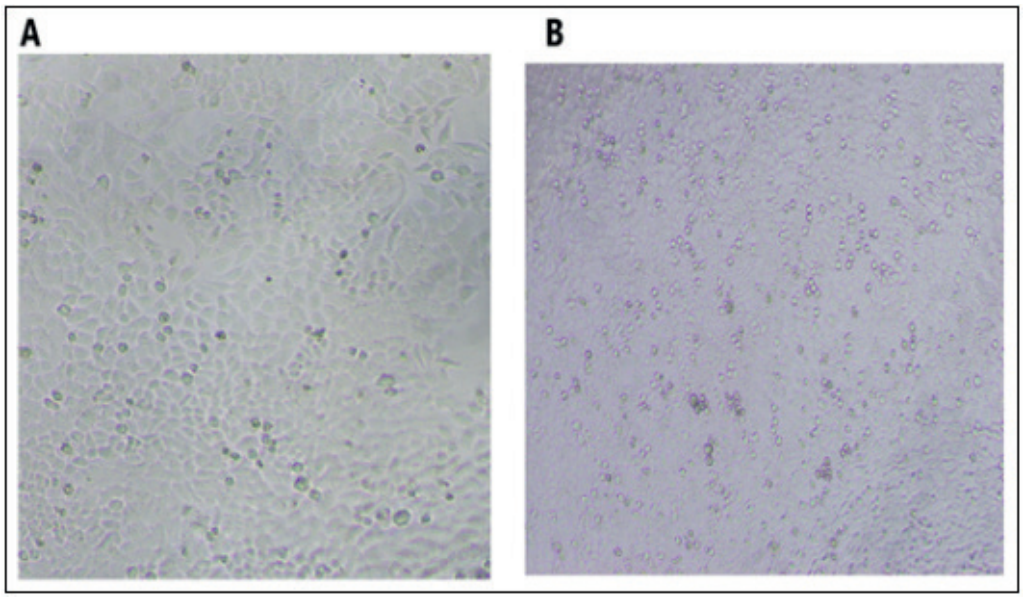


Figure 5. A; Control group (Non copolymer treated) Hela cell line B; PLLA-*b*-PNIPAM block copolymer treated cells (1000 ug/ml) (X40).

In addition to these values, the peak belongs to PLLA the appearance of C=O at 1755 cm^{-1} supports the formation of block copolymer.

^{1}H NMR spectrum of PLLA-*b*-PNVP **(6)** is displayed in Figure 3B. In the spectrum, methylene protons of pyrrolidone ring, corresponding to the characteristic peaks of PVP backbone, were detected at 3.51–3.02 (H^{10}) and 2.55–1.81 (H^{8+9}) ppm. The other peaks of methine proton (H^{7}) of PNVP chain appeared at 4,04–3.51 ppm. The observation of the peak at 5.14 of the methine (H^{e}) protons of PLLA block and the overlapped peaks at 1.67–1.12 ppm methylene (H^{6}) of PNVP block with methyl (H^{f}) protons of PLLA indicates the formation of block copolymer. M_n (NMR) of **(6)** was calculated by comparing the peak integrals derived from the methylene protons' peaks of PNVP (δ =3.24 ppm, peak '**10**') and the methine proton peaks of PLLA (δ = 5.19 ppm, peak '**e**' in Fig. 3B) according to equation (5 and 6)

$$M_n\,(NMR) = (\,DP_{NVP} \times M_{monomer}\,) + M_{n,PLLA} \quad (5)$$

$$DP_{PNVP} = \left(\frac{I_{10}}{I_e} \times DP_{PLLA}\right) \quad (6)$$

Here, DP_{PNVP} and DP_{PLLA} are degree of polymerization for PNVP and PLLA segments, respectively. $M_{monomer}$ is also molecular weight of the NVP.

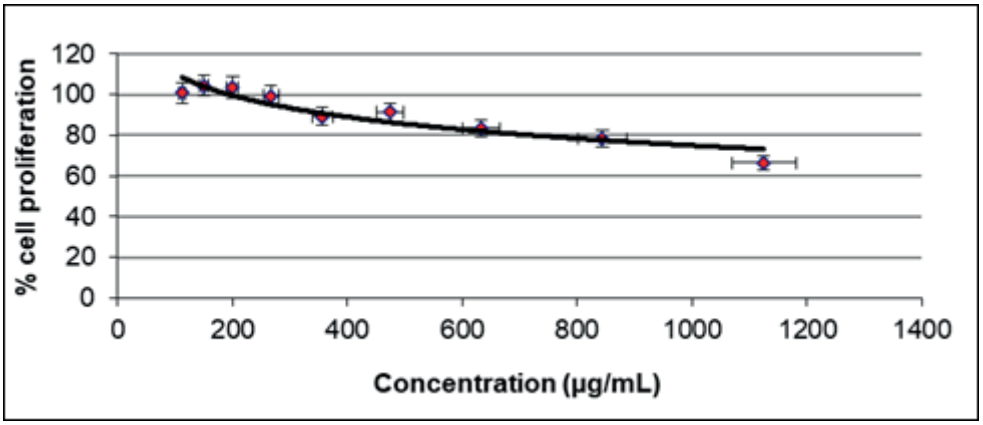


Figure 6. Cytotoxicity assay of PLLA-*b*-PNVP.

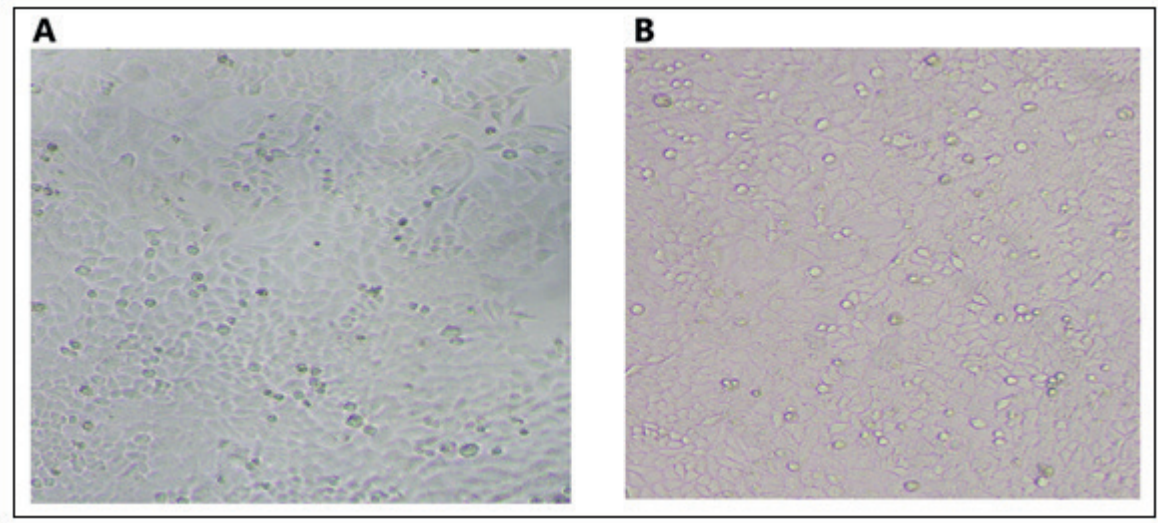


Figure 7. A; Control group (Non copolymer treated) Hela cell line B; (PLLA-*b*-PNVP) block copolymer treated cells (1000 ug/ml) (X40).

In Vitro Investigation

In vitro cytotoxicity studies in cancer cell lines are an important assay to evaluate the potential activity and toxicity of new drug carriers [25]. XTT analysis was performed to investigate the cytotoxic effect of synthesized block copolymers on cervical cancer (HeLa) cells. Considering vitro analyses, the cytotoxicity of synthesized block copolymers was investigated in cervical cancer cell lines at dose ranges of 100-1125 ug/mL. According to the obtained analyses results, it was observed that the first synthesized block copolymer PLLA-*b*-PNIPAM had no toxic properties on cells at doses below 350 ug/ml and the doses above 350 ug/mL killed approximately 50% of the cells. The results obtained are shown in Figure 4 and Figure 5. This result may be attributed to the trace amounts of copper that could remain in the block copolymer during the polymer purification process.

For PLLA-*b*-PNVP block copolymer, the cytotoxic effect of (6) on HeLa cell lines was also evaluated at dose ranges of 100-1125 ug/mL. According to the results obtained, (6) had no significant toxic effect on cells (Figure 6 and Figure 7).

Conclusion

In this work novel AB-type block copolymers, (PLLA-*b*-PNIPAM) and (PLLA-*b*-PNVP), were prepared by combining of ROP and controlled/living radical polymerization (CRP) techniques, ATRP or RAFT polymerization of NIPAM or NVP as monomers. The synthesis utilized a novel PLLA-based macroinitiators, created using a novel initiator, 2,4-difluorobenzyl alcohol, not previously used in polymerization of L-LA. The molecular structures of the compounds were confirmed using FTIR and ^{1}H NMR methods. The M_n of the obtained polymers was calculated by comparing the peak integrals of related NMR spectra and agreed with theoretical values. Considering vitro analyses, the cytotoxicity of synthesized block copolymers was investigated in cervical cancer cell lines at dose ranges of 100-1125 ug/ml. According to the obtained analyses results, it was observed that the first synthesized block copolymer PLLA-*b*-PNIPAM **(5)** had no toxic properties on cells at doses below 350 ug/ml and the doses above 350 ug/mL killed approximately 50% of the cells. This effect may be due to the fact that trace amounts of copper in the block copolymer are not completely removed during the purification process. The cytotoxic effect of (PLLA-*b*-PNVP) on HeLa cell lines was also evaluated at dose range of 100-1125 ug/ml. PLLA-*b*-PNVP **(6)** had no a significant toxic effects according to the results obtained. Furthermore, it was observed that if the (PLLA-*b*-PNIPAM) is further purified, both block copolymers could be used in future cancer treatments and drug delivery applications.

Acknowledgment

The author thanks Dr. Serap Yalçın Azarkan for to use of her laboratory.

Funding

This study was supported by Kırşehir Ahi Evran University Scientific Research Projects Unit Project number: MMF.A4.23.003.

Conflict of interest statement

The author declared no conflict of interest.

Ethics approval and consent to participate

Not applicable

Data availability statement

Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

References

1. Mısır M, Yılmaz SS, Bilgin A. Synthesis and characterization of ABA-Type Triblock Copolymers using novel Bifunctional PS, PMMA, and PCL Macroinitiators Bearing p-xylene-bis (2-mercaptoethyloxy) Core. Polymers. 2023;15(18):3813. doi: 10.3390/polym15183813.
2. Babikova D, Kalinova R, Zhelezova I, Momekova D, Konstantinov S, Momekov G, et al. Functional block copolymer nanocarriers for anticancer drug delivery. RSC Adv. 2016;6(88):84634-44. doi: 10.1039/C6RA19236J.

3. Dash TK, Konkimalla VB. Polymeric modification and its implication in drug delivery: Poly-ε-caprolactone (PCL) as a model polymer. Mol Pharm. 2012;9(9):2365-79. doi: 10.1021/mp3001952.

4. Yu C-F, Rwei S-P, Yang S-J, Tsen W-C, Lin L-H. Synthesis and characterization of Poly (DL-lactide) Containing Fluorene structures. Polymers. 2023;15(11):2555. doi: 10.3390/polym15112555.

5. Karanikolopoulos N, Pitsikalis M, Hadjichristidis N, Georgikopoulou K, Calogeropoulou T, Dunlap JR. pH-responsive aggregates from double hydrophilic block copolymers carrying zwitterionic groups. Encapsulation of antiparasitic compounds for the treatment of leishmaniasis. Langmuir. 2007;23(8):4214-24. doi: 10.1021/la0628827.

6. Tang W, Matyjaszewski K. Effects of initiator structure on activation rate constants in ATRP. Macromolecules. 2007;40(6):1858-63. doi: 10.1021/ma062897b.

7. Aliyev E, Shishatskiy S, Abetz C, Lee YJ, Neumann S, Emmler T, Filiz V. SI-ATRP Polymer-functionalized graphene oxide for water vapor separation. Adv Mater Interfaces. 2020;7(19):2000443. doi: 10.1002/admi.202000443.

8. Semsarilar M, Abetz V. Polymerizations by RAFT: Developments of the technique and its application in the synthesis of Tailored (Co) polymers. Macromol Chem Phys. 2021;222(1):2000311. doi: 10.1002/macp.202000311.

9. Lee RS, Lin CH, Aljuffali IA, Hu KY, Fang JY. Passive targeting of thermosensitive diblock copolymer micelles to the lungs: Synthesis and characterization of poly (N-isopropylacrylamide)-block-poly (ε-caprolactone). J Nanobiotechnology. 2015;13:1-12. doi: 10.1186/s12951-015-0103-7.

10. Ayano E, Karaki M, Ishihara T, Kanazawa H, Okano T. Poly (N-isopropylacrylamide)–PLA and PLA blend nanoparticles for temperature-controllable drug release and intracellular uptake. Colloids Surf B Biointerfaces. 2012;99:67-73. doi: 10.1016/j.colsurfb.2011.10.003.

11. Hu Y, Darcos V, Monge S, Li S. Synthesis and self-assembling of poly (N-isopropylacrylamide-block-poly (l-lactide)-block-poly (N-isopropylacrylamide) triblock copolymers prepared by combination of ring-opening polymerization and atom transfer radical polymerization. J Polym Sci A Polym Chem. 2013;51(15):3274-83. doi: 10.1002/pola.26721.

12. Zhang D, Xia Y, Gong H, Zhang D, Chen X, Shi S, Lei, L. Preparation and self-assembling of PLA-b-PNIPAM-b-PS Triblock Copolymer thin films. J Nanosci Nanotechnol. 2021;21(4):2174-84. doi: 10.1166/jnn.2021.19072.

13. Franco P, De Marco I. The use of Poly (N-vinyl pyrrolidone) in the delivery of drugs: A review. Polymers. 2020;12(5):1114. doi: 10.3390/polym12051114.

14. Gigmes D, Trimaille T. Advances in amphiphilic polylactide/vinyl polymer based nano-assemblies for drug delivery. Adv Colloid Interface Sci. 2021;294:102483. doi: 10.1016/j.cis.2021.102483.

15. Liu X, Xu Y, Wu Z, Chen H. Poly (N-vinylpyrrolidone)-modified surfaces for biomedical applications. Macromol Biosci. 2013;13(2):147-54. doi: 10.1002/mabi.201200269.

16. Mishra RK, Datt M, Banthia AK. Synthesis and characterization of pectin/PVP hydrogel membranes for drug delivery system. AAPS PharmSciTech. 2008;9:395-403. doi: 10.1208/s12249-008-9048-6.

17. Leiva A, Quina FH, Araneda E, Gargallo L, Radić D. New three-arm amphiphilic and biodegradable block copolymers composed of poly (ε-caprolactone) and poly (N-vinyl-2-pyrrolidone). Synthesis, characterization and self-assembly in aqueous solution. J Colloid Interface Sci. 2007;310(1):136-43. doi: 10.1016/j.jcis.2007.01.013.

18. Zhai C, Liu X, Yuan J, Gao Q. (2013). Synthesis, characterization, and drug delivery research of an amphiphilic biodegradable star-shaped block copolymer. Polym Bull. 2013;70:419-29. doi: 10.1007/s00289-012-0800-4.

19. Luo L, Ranger M, Lessard DG, Le Garrec D, Gori S, Leroux JC, et al. Novel amphiphilic diblock copolymer of low molecular weight poly (n-vinylpyrrolidone)-block-poly (d,l-lactide): Synthesis, characterization, and micellization. Macromolecules. 2004;37(11):4008-13. doi: 10.1021/ma035910q.

20. Shin SJ, Yu YC, Seo JD, Cho SJ, Youk JH. One-step synthesis of poly (N-vinylpyrrolidone)-b-poly (l-lactide) block copolymers using a dual initiator for RAFT polymerization and ROP. J Polym Sci A Poly Chem. 2014;52(11):1607-13. doi: 10.1002/pola.27160.

21. Fu Q, McKenzie TG, Tan S, Nam E, Qiao GG. Tertiary amine catalyzed photo-induced controlled radical polymerization of methacrylates. Polym Chem.

2015;6(30):5362-8. doi: 10.1039/C5PY00840A.

22. Perrin DD, Armarego WL, Perrin DR. Purification of laboratory chemicals. 2nd Edition. Pergamon Press: Oxford, UK. 1988.

23. Hoekstra, WJ., Schotzinger, RJ, Rafferty, SW. (2012). U.S. Patent No. 8,236,962. Washington, DC: U.S. Patent and Trademark Office.

24. Gong HL, Lei L, Shi SX, Xia YZ, Chen XN. Synthesis and self-assembly of block copolymers containing temperature sensitive and degradable chain segments. J Nanosci Nanotechnol. 2018;18(5):3266-73. doi: 10.1166/jnn.2018.14708.

25. Ali, A, Bhadane R, Asl AA, Wilén CE., Salo-Ahen O, Rosenholm JM, et al. Functional block copolymer micelles based on poly (jasmine lactone) for improving the loading efficiency of weakly basic drugs. RSC Adv. 2022;12(41):26763-75. doi: 10.1039/D2RA03962A.