Iranian Journal of Pharmaceutical Sciences
2018: 14 (2): 25-38
www.ijps.ir

Original Article

Synthesis and Characterization of mpeg-PCL Copolymers as a Polymersomes for Delivery of Enalapril as a Model Hydrophilic Drug

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Abstract

Enalapril maleate (EPM) was used for hypertension and congestive heart failure. In this way, an innovative delivery system with mPEG–PCL was synthesized and the release profile of the EPM from the drug-loaded polymersomes was evaluated. Di-block methoxy-poly (ethylene glycol) - poly (caprolactone) (mPEG-PCL) copolymers were synthesized and used to prepare polymersomes for the controlled release of EPM as hydrophilic model drug. mPEG-PCL copolymer was characterized *in vitro* by HNMR, FTIR, DSC, and GPC techniques. The resulting polymersomes were characterized further by various techniques such as dynamic light scattering (DLS) and transmission electron microscopy (TEM). The results of TEM show the polymersomes formed had spherical structure and the size of polymersomes is 80 nm. The loading and encapsulation efficiency of EPM were determinate by HPLC at 215 nm with loading and encapsulation efficiency 19.8% ± 2.12% and 85.6% ± 1.26%, respectively. *In vitro* release of EPM from polymersomes was clearly sustained in all the time tested for this purpose. The sustained release of drug was hypothetically due to the entrapment of EPM in core of polymersomes. The results indicate the successful formulation of EPM loaded m-PEG/PCL polymersomes. Overall, the results demonstrated that m-PEG-PCL polymersomes can be considered as a promising carrier for hydrophilic drugs such as EPM.

Keywords: m-PEG-PCL, Polymersomes, EPM, Hydrophilic drugs, Drug delivery

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Cite this article as: Danafar, H., Synthesis and Characterization of Mpeg-PCL Copolymers as a Polymersomes for Delivery of Enalapril as a Model Hydrophilic Drug, 2018, 14 (2): 25-38.

1. Introduction

Enalapril maleate (EPM) is the maleate salt of EPM; the prodrug of enalaprilat. EPM was used for patients with hypertension and congestive heart failure [1-2], and is an angiotensin-converting enzyme (ACE) inhibitor.

EPM is soluble in water (1 g in 60 mL z at 25°C) and freely soluble in methanol, ethanol, and acetone. The daily dose of EPM is as low as 5 mg, and recommended for progress of a practical inject able dosage form for sustained release. However, the drug concentration rapidly decreases below therapeutic levels by excretion in urine. Delivery EPM is very vital at the ACE site to the hypertensive patients because of its sturdy dose dependency. Additionally, when oral medication of EPM cannot be useful an injectable dosage form with a sustained-release attribute has been preferred for patients in emergency situations. Polymersomes are hollow spheres, made of polymers that contain an aqueous solution in the core surrounded by a bi-layer membrane. The bi-layer membrane is composed of hydrated hydrophilic coronas both at the inside and outside of the hydrophobic middle part of the membrane separating and protecting the fluidic core from the outside medium. In drug delivery context, the aqueous core can be utilized for the encapsulation of hydrophilic therapeutic molecules such as small-molecule drugs, enzymes, proteins, peptides, DNA, and RNA fragments [3-5], while the membrane can incorporate hydrophobic drugs within its hydrophobic core [6-8]. Based on their multidrug loading capacity, stability, long blood circulation times, membrane robustness and stealth properties, polymersomes are highly attractive for drug delivery of highly toxic therapeutics with tuned pharmacokinetics in order to greatly increase therapeutic efficacy [15-17]. Polymersomes are used for drug delivery applications, usually degradable preferred. Feijen and coworkers synthesized biodegradable polymersomes from block copolymers based on poly (ethylene glycol) (PEG) and biodegradable polyesters or polycarbonates [13-15]. Polymersomes were assigned as a basis for simulated cells for drug entrapment on and release [16-18]. Block copolymers such as mPEG-PCL and tri-block poly(lactide)–poly(ethyleneglycol)–poly(lactide) (PLA–PEG–PLA) have been used widely for drug delivery applications because of their greater biocompatibility and biodegradability. Biodegradable polymeric nanoparticles are often used to reach controlled release of drugs in anticancer drug delivery systems [18-21]. Also, some biodegradable polymer-derived drug delivery systems, such as nanoparticles delivering anticancer agents, are commercially available. Poly (caprolactone)-poly (ethylene glycol) (PCL-PEG) copolymers are biodegradable, amphiphilic, easy to produce, and have potential application in drug delivery systems. We previously described the polymersome and micellar formation of the PLA–PEG–PLA and mPEG-PCL in an aqueous milieu. In this study we assigned successful method to synthesis of mPEG-PCL polymersomes with reduced initial burst. In this study, we aimed to prepare and mPEG-PCL polymersomes with respect to the loading and release properties toward hydrophilic drugs. Until recently there have been very few

published reports for comparison of polymersomes as a carrier for hydrophilic drugs. EPM selected as a model hydrophilic drugs according to their polarity difference, were selected as model drugs with extreme polarities to elucidate the potential of the mPEG-PCL polymersomes for being loaded efficiently with a wide spectrum of drug molecules.

2. Materials and Methods

2.1. Materials

Methoxypoly (ethylene glycol) (mPEG, Mn=5000Da) Aldrich, St. Louis, USA, CAS.81323), ε-caprolactone (98% purity) (Aldrich, St. Louis, USA, USA, CAS.502443), EPM maleate (gifts from Alborz Daroo Pharmaceutical Co, Iran) and stannous 2-ethyl-hexanoate (SnOct2), (CAS. 301100). Chloroform, methanol, and diethyl ether were provided all from Merck (Darmstadt, Germany), and used as received.

2.2. Synthesis and Characterization Of Mpeg-PCL Copolymer

The mPEG–PCL copolymer was synthesized by a ring opening polymerization of ε-caprolactone with mPEG as initial molecule and Sn $(Oct)_2$ as catalyst. In brief ε-caprolactone (6 g), mPEG (3 g), and Sn $(Oct)_2$ (0.01 mmol) were heated to 120°C to start polymerization. After 12 h, the consequential polymer was cooled to room temperature and dissolved in chloroform, in addition was precipitated in cold diethyl ether. The copolymer was dried under vacuum at room temperature for 24 h. The chemical structure of copolymer was known by proton nuclear magnetic resonance spectroscopy (1H NMR) in $CDCl_3$ at 400 MHz (Bruker, Avance 400) and Fourier transform infrared spectroscopy (FT-IR) (Bruker, Tensor 27).The average molecular weight and distribution of the mPEG–PCL copolymers were evaluated by gel permeation chromatography (GPC) (Knaure, Berlin, Germany) operational with differential refract metric detector and an ultraastyragel column (4.6*30 mm) (Waters, Milford, USA, model HR 4E). The mobile phase was tetrahydrofuran (THF) with a flow-rate of 1 ml/min and the injection volume was 100 µL of stock solutions (0.1-0.5 w/v %). Polymers were characterized by relative elution time to polystyrene monodisperse standards in the range of 4500-29500 Da (Varian Palo Alto, CA) using the calibration curve obtained before measurements. Thermal analysis property of the synthesized copolymers was determinate by differential scanning calorimetry (DSC) (Mettler Toledo, model Star SW 9.30) was used for. The samples were heated at a rate of 10 °C min^{-1} and the data were recorded from 0 to 200 °C.

2.3. Preparation of EPM-Loaded Polymersomes

A double emulsion (w:o:w) technique was used for preparation of polymersomes [33]. The aqueous solution of drug with determined

concentration was first poured into mPEG-PCL copolymer solution to form a w:o emulsion. The w:o emulsion was then injected drop-wise through a syringe(G=20) into 20 mL of distillated water containing 0.4 wt.% of poly(vinyl alcohol) under certain mixing rates and stirred magnetically at room temperature until complete evaporation of the organic solvent. The resulting polymersomes were separated by centrifuging at 20000 g for 20 min and freeze-dried under a pressure of 14 Pa at -78 °C in order to remove all the residual solvents and to produce the final dried form.

2.3.1. Characterization of the Polymersomes

2.3.2. Particles Morphology

To study the polymersomes sizes and morphology, the samples were observed by transmission electron microscopy (TEM) (Hitachi H-7650, Tokyo, Japan) at the voltage of 80 KV.

2.3.3. Determination of Particle Size and Zeta Potential

Photon Correlation Spectroscopy (PCS) using a Malvern Nano/zetasizer (Malvern Instruments, UK) was used for determination of the particle size distribution (mean diameters and polydispersity index) and zeta potential of polymersomes.

2.3.4. Stability of Polymersomes

The stability of polymersomes was evaluated by following size variation of suspension of polymersomes in aqueous medium. As detail, after synthesis of polymersomes suspension, the polymersomes were incubated over determined time until size measurement.

2.3.5. Drug Loading

To assign the quantity of the EPM loaded in the polymersomes, 1 mg of each sample was dissolved in 1mL of NaOH (1N), and the drug content was considered by high performance liquid chromatography (HPLC).[31] The mobile phase consisted of methanol and PBS in the volume ratio of 45:55(v/v) and was delivered at a flow rate of 1.0 mL/min using a double-reciprocating pump and the analysis wavelength was at 215 nm (Waters, MA, USA, model Breeze).4.6mm, particle size 5µm; Perfectsill, MZ-Analysen. The sample was injected through a 20 µL sample loop. A C_{18} analytical column (250 mm, Technik, Germany) equipped with a guard column of the same packing was used. The drug loading efficiency (DL) of the polymersomes was designed as follows.

$$\%DL = \frac{\text{weight of drug in polymersomes}}{\text{weight of polymersomes}} * 100$$

W_{drug} in polymersomes and $W_{polymersomes}$ show weight of the entrapment drug and the total weight of the corresponding drug-encapsulated

polymersomes, respectively. Where DL% is the drug loading ratio (percent).

Efficiency of entrapment was calculated using the following equation:

$$\%EE = \frac{\text{weight of drug in polymersomes}}{\text{weight of initial drug}} * 100$$

where EE% is the efficiency of entrapment (percent), W drug in polymersomes and Winitial drug show the total mass of powders obtained after freeze-drying and the drug fed initially in the polymersomes preparation step, respectively.

2.3.6. DSC Analysis

Thermal analysis was studied any possible drug-polymer contact(s) as well as the physical changes occurred on the drug or polymer. DSC analysis was carried out on pure drug and drug-loaded polymersomes. The samples were heated at a rate of 10 ° C min^{-1} and the data were recorded from 0 to 200 °C.

2.3.7. Drug Release of EPM

For evaluation of release of polymersomes two milliliters of PBS were added to the each vial containing 10 mg of freeze-dried polymersomes and placed within the dialysis bag and incubated at 37 °C while immersed in 15 mL of phosphate-buffered saline (PBS, pH 7.4 and pH 5.5).Then at suitable time intervals, 2 mL of the dialysate was taken out and replaced by 2 mL fresh PBS. The concentration of free EPM in the medium was monitored by HPLC at 215 nm. All the release studies were studied in triplicate.

3. Results and Discussion

3.1. Synthesis and Characterization of Mpeg–PCL Copolymer

mPEG– PCL di-block copolymer was synthesized using the ring-opening polymerization of caprolactone in presence of mPEG, whose hydroxyl end group initiated the ring opening. HNMR spectroscopy in CDCl3 was used for evaluating of the structure and composition of the synthesized mPEG–PCL di-block copolymer. As shown in figure 1, the presence of methylenes (CH2) in PCL was observed around 1.3 ppm, 1.6 ppm , 2.2 ppm and 4.06 ppm, the methoxy and methyl protons in methoxy(OCH3) and methylene (CH2) groups of PEG were around 3.38 and 3.64 ppm,

Table 1. Molecular characterics of the synthesized copolymer.

Copolymer	CL / EG feed	M_n(KDa) [a]	M_w (KDa)[a]	PdI[b]	T_m (°C) [c]	DP_{PEG}
mPEG–PCL	0.5	19.6	22.6	1.16	58.57	136.36

a: Determined by GPC analysis using narrow molecular weight polystyrene standards.
b: M_w/M_n = Polydispersity index of the polymers (PdI) determined by GPC analysis
c: Calculated from the first run of DSC as half of the extrapolated tangents
d: DP: degree of polymerization

respectively. The ratio of the peak area at 3.64 and 2.2 ppm was indicative of the number of

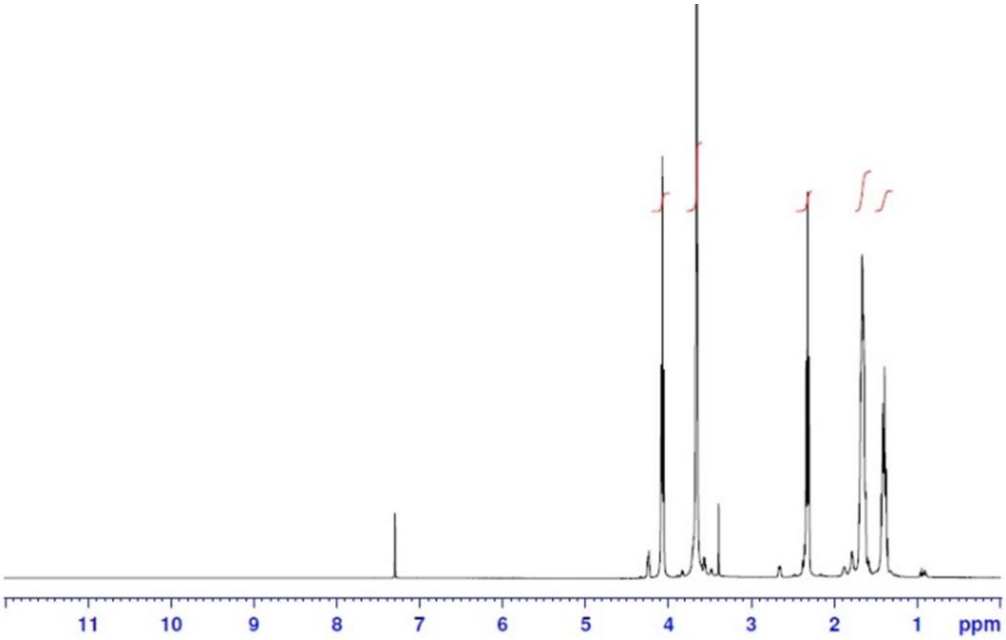

Figure 1. H NMR spectrum of mPEG–PCL di-block copolymer in $CDCl_3$.

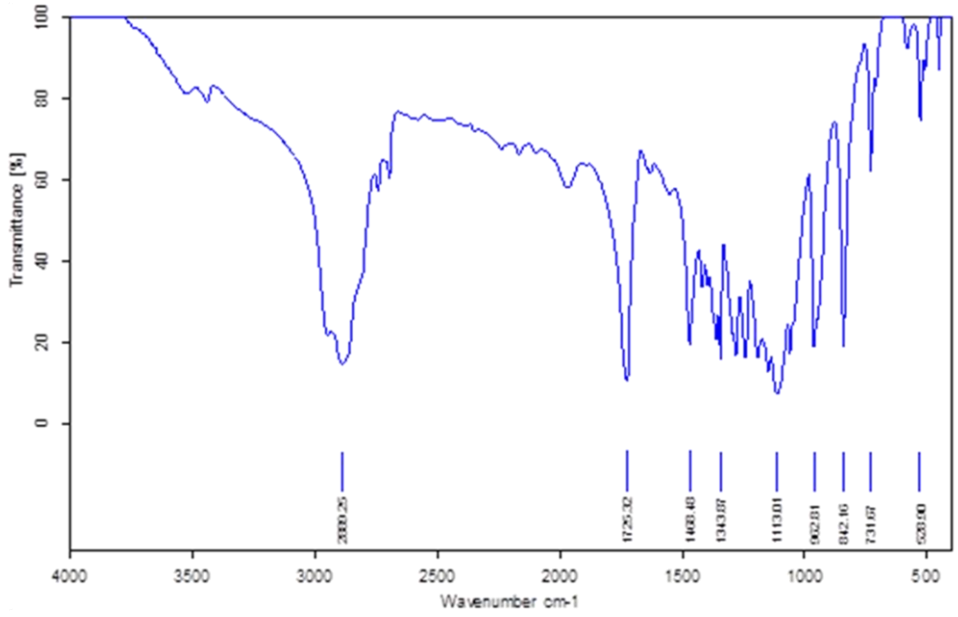

Figure 2. FT-IR spectrum of mPEG–PCL di-block copolymer in $CDCl_3$.

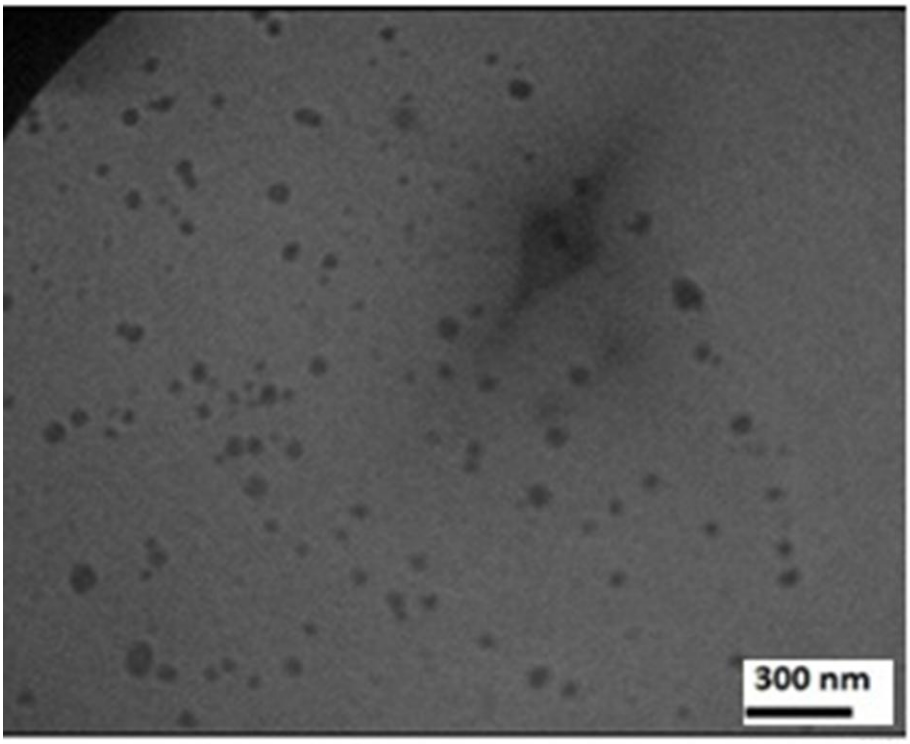

Figure 3.TEM image of polymersomes.

each repeating units, so it was considered in calculation of the number-average molecular weight of the synthesized copolymer. Table 1 shows the characteristics of synthesized copolymer. FT-IR spectrum of mPEG–PCL copolymer is shown in figure 2. In the spectrum shown, the sharp and intense bands at 1725 cm^{-1} and 1113 cm^{-1} were assigned to the presence of carboxylic ester (C=O) and ether (C–O) groups, thereby showing that the formation of mPEG–PCL copolymer has occurred successfully. The weight- and number-based average molecular weights of copolymer were 19.6 and 22.6 KDa, respectively that was evaluated by GPC.

3.2 Preparation and Characterization of Polymersomes

The formation of polymersomes was assigned by TEM. Apparently, polymersomes showed a homogeneous spherical morphology, as predictable (figure 3). Dynamic Light Scattering (DLS) technique was used for determination size of polymersomes. As shown in figure 4, the z-average and zeta potential of EPM loaded mPEG–PCL polymersomes were

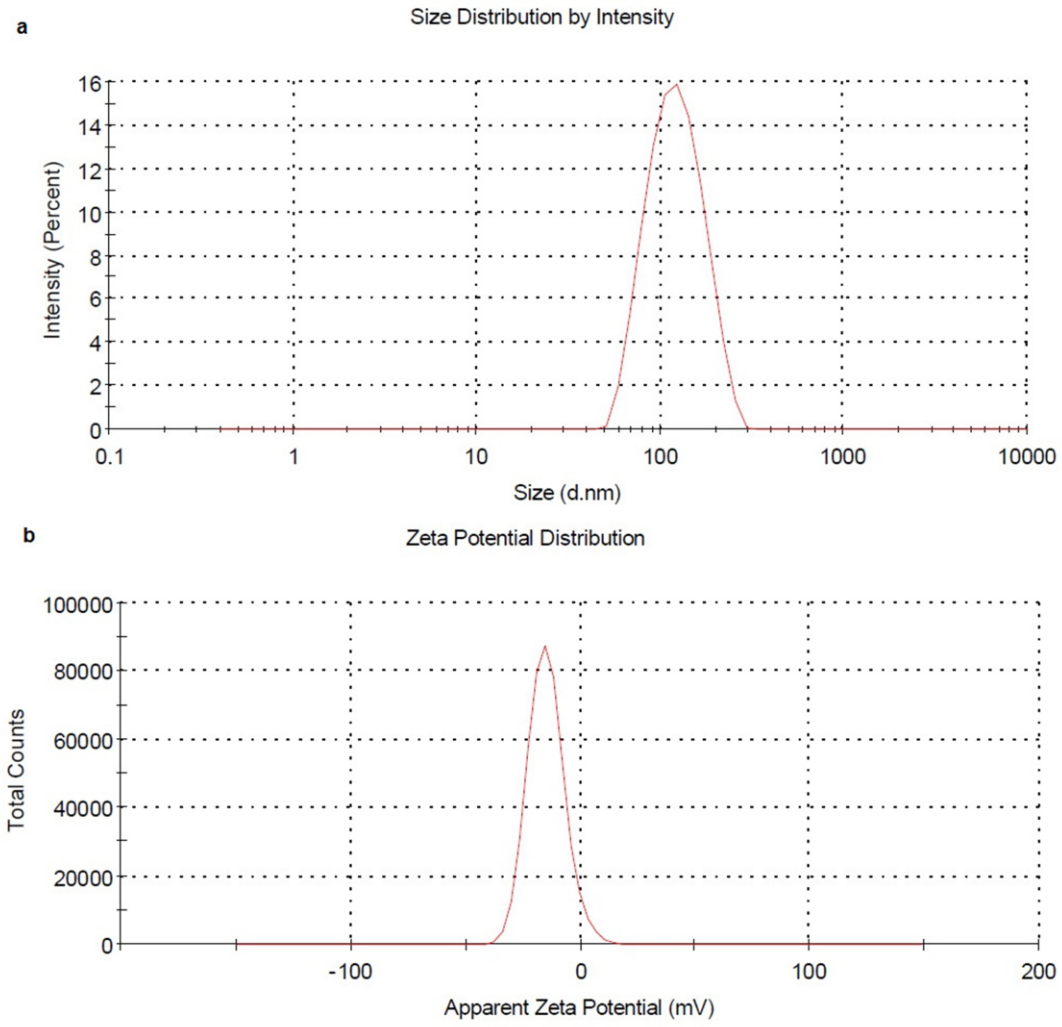

Figure 4. Particle size distribution and zeta potential of mPEG-PCL-DOX polymersomes (a) particle size distribution (b) zeta potential.

established to be about 116.1 nm and -15 mV, with their corresponding PDI being 0.229. The micelle size measured by TEM was about 80 nm, a little smaller than that determined by DLS. It can be related by the fact that the polymersome diameter measured by DLS shows the hydrodynamics diameter while that determinate by TEM is associated with the collapsed polymersomes after water evaporation. The loading ratio and encapsulation efficiencies of EPM loaded to mPEG–PCL polymersomes were determined to be 19.8% ± 2.12% and 85.6% ± 1.26%, respectively.

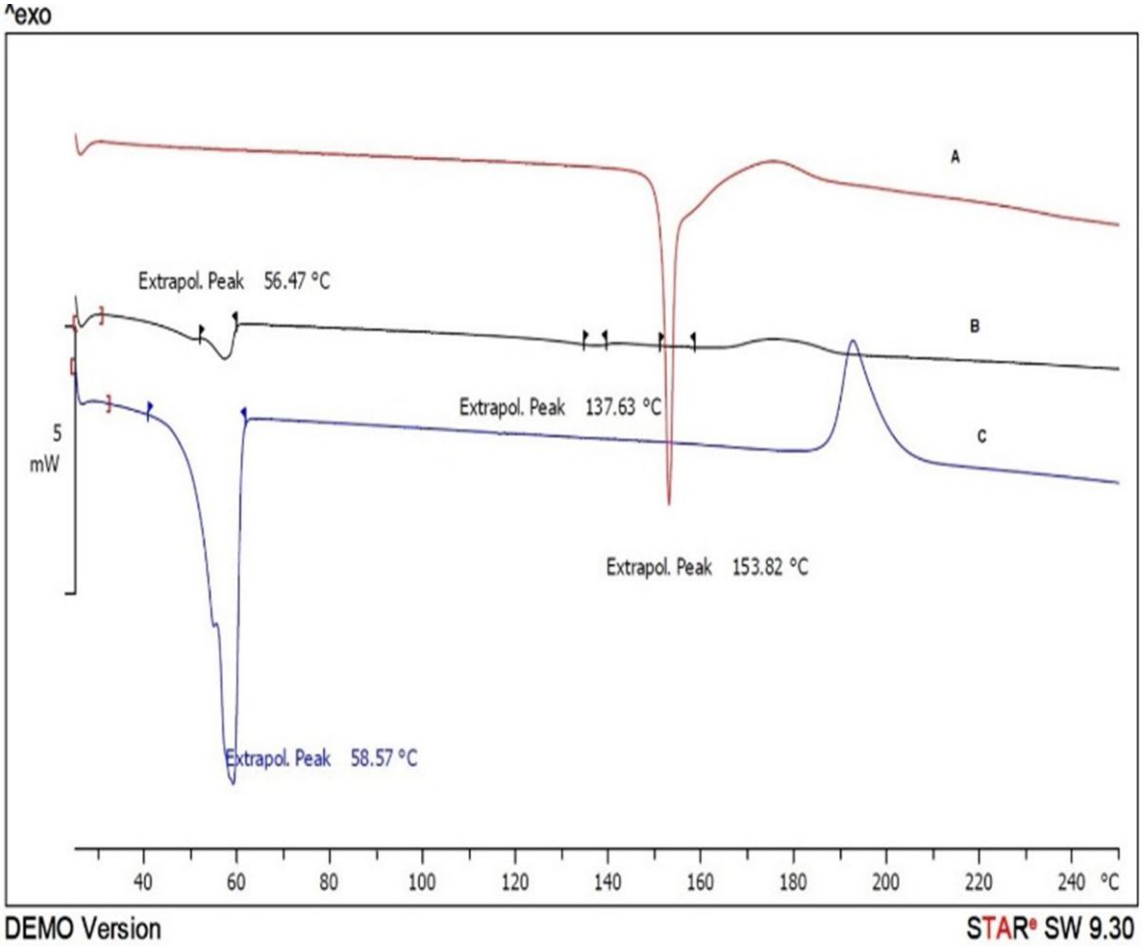

Figure 5. DSC spectra of (a) EPM,(b) EPM/mPEG-PCL polymersomes and (c)mPEG-PCL.

3.3. DSC Analysis

Figure 5 shows the DSC thermograms resultant to mPEG–PCL copolymer, EPM and polymersomes loaded by EPM. The thermogram of mPEG–PCL copolymer displayed an endothermic peak at 58.57 °C which is investigative for the melting of the crystalline PEG, PCL segment of copolymer, the thermogram of EPM showed an endothermic peak at 151.76 °C and polymersomes showed two endothermic peaks at 57.96 ^{0}C and 137.61 ^{0}C which set for the melting of copolymer and EPM connection in the form of polymersomes. This endothermic peak of polymersomes apparently confirms a physical interaction between copolymer and EPM upon loading of the drug in polymersomes, while the melting point of PCL polymersomes was lower than

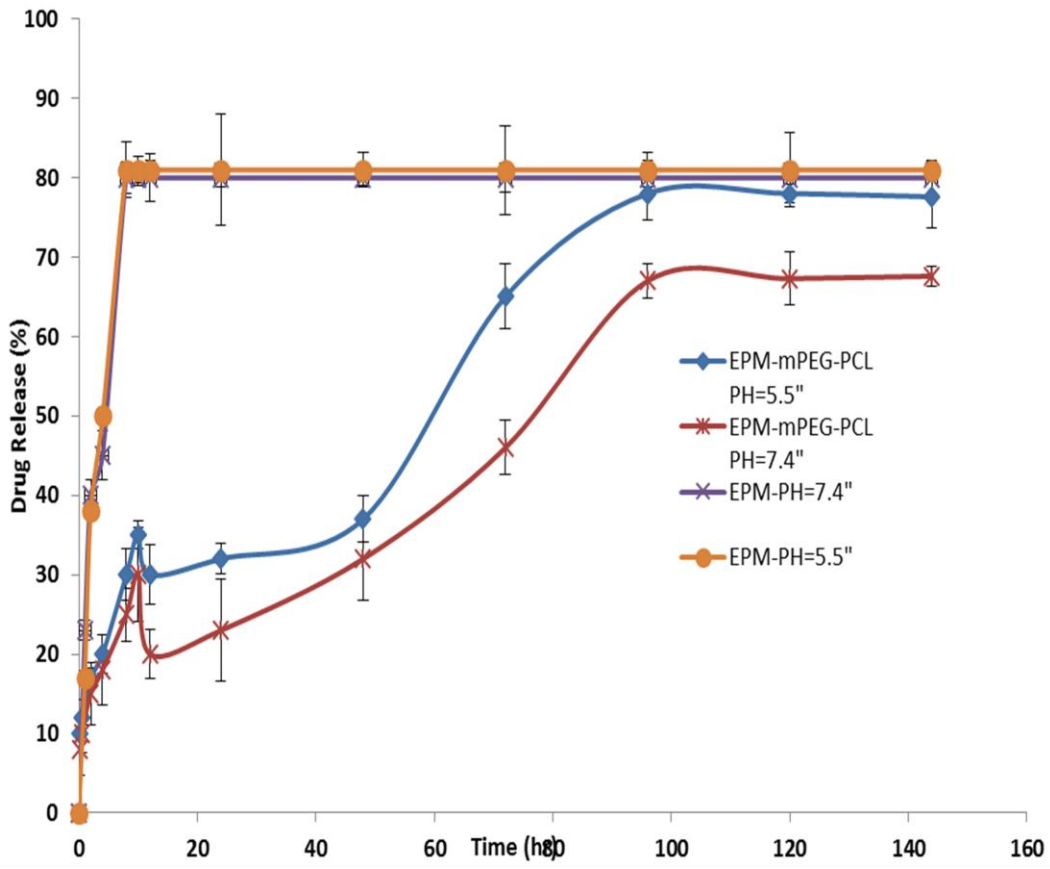

Figure 6.The release profiles of EPM from EPM/mPEG-PCL polymersomes in PBS.

melting point of both copolymer (58.97 °C) and pure EPM (151.76 ^{0}C).

3.4. In vitro Release of EPM

In order to examine the influence of the chemical and biochemical factors on the release of EPM from polymersomes, the release study was performed on drug-loaded polymersomes in neutral (pH=7.4) and acidified PBS solution (pH=5.5). As controls, the release of free EPM was studied to verify that the diffusion of drug molecules across the dialysis membrane was not a rate-limiting step during the release process. Free EPM was observed to be rapidly released and reached its peak of 80.10 % and 81.62 % of the total in the first 8 hours at pH 7.4 and 5.5 , respectively. Figure 6 shows the release profiles of EPM from the polymersomes, at pH 7.4 and 5.5. As expected, no considerable initial burst EPM release was observed from the polymersomes. As shown in figure 6, the percentage of EPM released from the polymersomes increased as the pH value decreased from 7.4 to 5.5. For example, after 72 h incubation, the amounts of EP, released in the media with pH values of pH 7.4 and 5.5 were

Table 2. Stability of nanoparticles suspension.

polymersomes	Mean size of polymersomes immediately after preparation (nm)	Mean size of polymersomes after 15 day (nm)	Mean size of polymersomes after 30 days (nm)
EPM/ mPEG-PCL polymersomes	116.1	145	180

about 47.2, and 68.15 %, respectively. The reason behind this phenomenon lies in the pH sensitivity of the release rate of EPM from the polymersomes because the copolymer is degradable in acidic condition by hydrolysis. Also, the release is faster in acidic pH than in neutral, as in acidic environment the polymer matrix swells due to protonation of polymer. This behavior is a highly desirable characteristic in many applications especially in anticancer drug delivery where the micro-environments of extracellular spaces of tumors, intracellular lysosomes and endosomes are acidic, which can potentially facilitate the drug release from obtained polymersomes. The results revealed that the maximum drug releases were 69.32, and 79.23%, respectively for PBS pH=7.4 and pH=5.5 after a period of 96 h. The most important feature of the release behavior of EPM-loaded polymersomes is that a triphasic release profile with an initial release of the surface-associated drug, an intermediate lag phase, and a secondary release with slower release rate was observed. A possible explanation for this observation could be an initial release due to the drug desorption from the particle shell, as described earlier, secondly, a plateau for a certain period, representing the drug travel from deeper parts to polymersomes surface, and thirdly, a constant sustained release of the drug from the surface simultaneously to transfer the drug from hydrophilic core to shell. This can be related to this fact that EPM, as hydrophilic protein, can be encapsulated either in the core of polymersomes or in the hydrophilic shell of nanoparticles which can lead to different drug release patterns. Therefore, the obtained copolymeric polymersomes can be regarded as highly attractive nano-carriers for time-controlled protein delivery for hydrophilic proteins to achievement of different therapeutic objectives.

3.5. Physical Stability of Polymersomes

In the clinical administration of nanoparticle dispersions, the stability of the sizes of the nanocarriers is of vast importance both as a determinate of the particle structure integrity and as a sign of the possible inter-particular associations (aggregation). For this study, the particle size stability was assigned in this study over a 30-days course. The variation of the sizes of polymersomes as a function of incubation time is shown in Table 2. As it can be seen, the

size of all polymersomes was increased slightly throughout the measurement period. This observation cannot be a sign of associations, which usually leads to several fold increases. Probably some kind of copolymer swelling and/or hydration as a result of presence of the hydrophilic PEG portions in polymersomes surfaces can be responsible for this event.

4. Conclusion

Methoxy poly (ethylene glycol)-poly caprolactone- (mPEG-PCL) copolymer was synthesized and characterized by HNMR, FTIR, DSC, and GPC techniques. Then,–PCL copolymer were self-assembled into polymersomes in aqueous solution in presence of EPM. The resulting polymersomes were characterized by various techniques such as DLS and TEM. The results revealed that the polymersomes formed had spherical structure. In summary, mPEG-PCL polymersomes were used to encapsulate EPM, creating EPM/mPEG-PCL polymersomes. The EPM/mPEG-PCL polymersomes improved release of EPM. As a model hydrophilic drug and may have probable appliance in hypertension treatment.

Acknowledgment

This work has been supported financially by Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran (Grant NO: A-12-430-6).

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