



Preparation and Characterization of PCL-PEG-PCL Copolymeric Nanoparticles as Polymersomes for Delivery Hydrophilic Drugs

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Abstract

A novel drug delivery system using poly (ϵ -caprolactone) - poly (ethylene glycol) -poly (ϵ -caprolactone) (PCL-PEG-PCL) was established in this study. Ceftriaxone (CTX) was encapsulated within PCL-PEG-PCL nanoparticles by a double emulsion technique (w/o/w), leading to creation of ceftriaxone-loaded PCL-PEG-PCL (CTX/PCL-PEG-PCL) polymersomes. The resulting polymersomes were characterized by various techniques such as dynamic light scattering (DLS). The release profile of the CTX from the polymersomes was evaluated. The findings showed the successful formation of spherical CTX/PCL-PEG-PCL polymersomes. The loading efficiency of CTX was 17.50 ± 1.17 %. The results of DLS showed that the polymersomes have size of 115.7 ± 0.48 nm. *In vitro* release of CTX from polymersomes was remarkably sustained. The sustained release of drug was hypothetically due to the encapsulation of CTX in core of polymersomes. The results indicate the successful formulation of CTX loaded PCL-PEG-PCL polymersomes. It can be concluded that polymersomes may be considered as an effective treatment strategy to improve the therapeutic effect of CTX in the future.

Keywords: Ceftriaxone, Drug delivery, Nanoparticles, PCL-PEG-PCL, Polymersomes.

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Cite this article as: Danafar H, Preparation and Characterization of PCL-PEG-PCL Copolymeric Nanoparticles as Polymersomes for Delivery Hydrophilic Drugs, 2018, 14 (2): 21-32.

1. Introduction

Ceftriaxone (CTX) has proved activity against most *Salmonella* spp. To *in vitro* [1–2] it was verified to be ineffective in killing intracellular pathogens [3-6]. CTX, a third-generation cephalosporin having a broad spectrum of activity against Gram-positive and Gram-negative aerobic and some anaerobic bacteria, has been indicated for the treatment

of a range of infections caused by susceptible microorganisms, including infections of the lower respiratory tract and central nervous system [7]. Although CTX has the advantage of superior efficacy and a long elimination half-life that permits once daily administration, its outpatient use was limited due to the lack of an oral formulation. The availability of new oral forms of therapy may provide an impetus for expanding the usage of CTX through community-based prescribing, as well as for following up on parenteral antibiotics using oral therapy. However, switching from intravenous or intramuscular to oral formulation is confronted with a difficulty because CTX is hydrophilic and has a low octanol/water partition coefficient ($\log K = -2.10 \pm 0.19$). Due to the poor passage of CTX through epithelial membranes, many efforts have been made to find improved methods and carriers for enhancing the absorption from the small intestine. The poor cellular penetration of the antibiotic was recognized to its high molecular weight as well as its hydrophilicity ($\log P -0.6$) [8-12]. Some efforts have been made to increase its oral absorption, promotion of intracellular delivery [13-14], and improvement of the antibacterial effect [15-16]. Nanoparticulate systems which increase the selectivity of antibiotics in phagocytic cells have been reviewed elsewhere [17], and most of them were based on biodegradable poly (lactide-co-glycolide) polymer [18-20]. Chitosan nanoparticles were recently used as a carrier for aminoglycosides [21]. Polymersomes, hollow spheres, contained an aqueous solution

in the core surrounded by a bi-layer membrane. The bi-layer membrane is composed of hydrated hydrophilic coronas at the inside and outside of the hydrophobic middle part of the membrane which can separate and protect 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 [22-23] while the membrane can incorporate hydrophobic drugs within its hydrophobic core [24-26]. 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. Poly (caprolactone)-poly (ethylene glycol) (PCL-PEG) copolymers are co-friendly, easy to produce, amphiphilic, and have a sturdy probable function in drug delivery system [27-30]. It seems that encapsulating CTX in PCL-PEG-PCL polymersomes may be a capable method for developing an improved CTX formulation. In this study, we are aimed to encapsulate CTX in PCL-PEG-PCL polymersomes as a promising carrier with sustained release characteristics. So, a novel drug delivery system with PCL-PEG-PCL was synthesized and the release profile of the CTX from the polymersomes was evaluated.

2. Materials and Methods

2.1. Materials

All used chemicals were purchased from commercial sources with analytical grade. Poly (ethylene glycol)PEG(Mn=6000 Da) (Aldrich, St. Louis, USA, CAS.81323), ϵ -caprolactone (98% purity) (Acros, New Jersey, USA, CAS.502443), ceftriaxone (CTX) (Zahravi Company, Iran), stannous 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) (Aldrich, St. Louis, USA, CAS. 301100), PBS (20 mM, pH=7.8), poly(vinyl alcohol), $M_w=145000$ (Aldrich, St. Louis, USA) and salts which were used throughout this research were obtained from Sigma-Aldrich or Merck.

2.2 Synthesis of PCL-PEG-PCL Copolymer

The PCL-PEG-PCL copolymers were synthesized according to our previous report [28]. It was synthesized by a ring opening polymerization of ϵ -caprolactone with PEG as initial molecule and $\text{Sn}(\text{Oct})_2$ as catalyst. In brief, PEG (2 g) was added to a dry three-necked flask and heated at 120 °C under vacuum for 3 hours (h) to remove moisture. The ϵ -caprolactone (ϵ -CL)(4 g) and $\text{Sn}(\text{Oct})_2$ (0.01 mmol) were introduced into the flask under a nitrogen atmosphere and then the polymerization reaction was performed at 120 °C with vigorous stirring for 12 h. After 12 h, the resulting copolymer was cooled at room temperature, dissolved in chloroform and precipitated in cold diethyl ether. The copolymer was dried under vacuum at room temperature for 24 h. The obtained copolymers were characterized by Fourier Transform Infrared spectroscopy (FT-IR) (Bruker, Tensor

27) for characterization of the chemical structure of the PCL-PEG-PCL copolymer and Proton Nuclear Magnetic Resonance Spectroscopy (^1H NMR) in CDCl_3 at 400 MHz (Bruker, Evans, 400). Thermal analysis of triblock copolymers were determined by differential scanning calorimetry (DSC) (Mettler Toledo, model Star SW 9.30) and the data were recorded from 0 to 250 °C. Molecular weight and distribution of the PCL-PEG-PCL copolymers were calculated by gel permeation chromatography (GPC) (Knaure, Berlin, Germany) composed of an ultrastayragel column and differential refractometric detector (4.6*30 mm) (Waters, Milford, USA, model HR 4E). Tetrahydrofuran (THF) as a mobile phase with a flow-rate of 1 mL/min and the injection volume was 100 μL of stock solutions (0.1-0.5 w/v %). The standards of polystyrene mono disperse in the range of 4500- 29500 DA (Varian Palo Alto, CA) were obtained before measurements for use as the calibration curve for determination of average molecular weight of the PCL-PEG-PCL copolymers[28].

2.3. Preparation of CTX-loaded Polymersomes

CTX loaded polymersomes were prepared by a double emulsion technique (w/o/w). 1 mL aqueous solution of CTX with known concentration 6 mg/mL was first poured drop-wise through a syringe (G=22) into the PCL-PEG-PCL copolymer solution in 2 mL chloroform (25 mg/mL) under certain mixing rates (1500 rpm) to form a w/o emulsion. The resulting emulsion was then injected drop-wise through a syringe (G=22) into 20 mL of

distilled water containing 0.4 wt % of poly (vinyl alcohol) under certain mixing rates (1500 rpm). Then, the mixture stirring was continued magnetically at room temperature until complete evaporation of the organic solvent. Subsequently, evaporation of the organic solvent in aqueous environment leads the amphiphilic copolymers to self-associate and form the nanoparticles. Finally, all of the samples were centrifuged at 20000 g, and freeze-dried (at pressure of 14 Pa and -78 °C) to remove all of the residual solvents and to produce the final nanoparticle form.

2.4. Characterization of the Polymersomes

2.4.1. Determination of Particle Size

The particle size distribution of the prepared polymersomes was determined by dynamic light scattering (DLS) using a nano/zetasizer (Malvern Instruments, Worcestershire, UK, model Nano ZS).

2.4.2. Stability of Polymersomes

The physical stability of polymersomes was evaluated by monitoring the particle size distribution of the polymersomes while suspended in phosphate-buffer saline (PBS, pH=7.4) and kept in room temperature at times 0, 15, and 30 days after preparation using the method was described in 2.3.

2.4.3. Determination of Loading Efficiency

To determine the loading efficiency of the drugs in the polymersomes, two parameters including the drug loading ratio and encapsulation efficiency were evaluated. Drug loading (DL) ratio was determined as:

$$\%DL = \frac{\text{weight of drug in polymersomes}}{\text{weight of polymersomes}} \times 100 \quad (1)$$

Weight of drug in polymersomes and weight of polymersomes show weight of the encapsulated drug and the total weight polymersomes, respectively. For determination of the drug loading ratio, 1 mg of the final freeze-dried nanodispersion polymersomes was dissolved in 1 mL of NaOH 1N, and the drug content was measured by spectrophotometry(UV-Vis) (Thermo Fisher Scientific, USA, Madison, model GENESYS™ 10S) at wavelength of 272 nm. Encapsulation efficiency was obtained using the following equation:

$$EE\% = \frac{(\text{Weight of the drug in the polymersomes})}{\text{Weight of the initial drug}} \times 100$$

2.5. Drug Release Study

This test was performed to evaluate the release behavior of CTX from polymersomes. Briefly, 5 mg of freeze-dried drug-loaded carriers were dispersed in 2 mL phosphate-buffered saline (PBS) and the resulting suspension was placed within a dialysis sac (Mw 12 kDa) and incubated at 37 °C while immersed in 15 mL of PBS. Then, at predetermined time intervals, 2 mL of the dialysate was taken out and replaced by 2 mL fresh PBS. The concentration of CTX in the dialysate was measured at 272 nm using UV-Vis. All the release studies were carried out in triplicate. In order to study the pH-dependency of the drug release, the experiments were performed using PBS at pH of 5.5. As control,

the release of free CTX was studied in PBS with pH= 7.4 and pH= 5.5.

3. Results and Discussion

3.1 Synthesis and Characterization Of PCL-PEG-PCL Copolymers

PCL-PEG-PCL tri-block copolymers were synthesized using the ring-opening

polymerization of caprolactone in presence of PEG which was explained in our previous work[28]. The structure and composition of the synthesized PCL-PEG-PCL tri-block copolymers were determined by ¹H NMR spectroscopy in CDCl₃ (Figure 1). The presence of methylene's (CH₂) in PCL was observed around 1.41 ppm, 1.75 ppm, 2.48

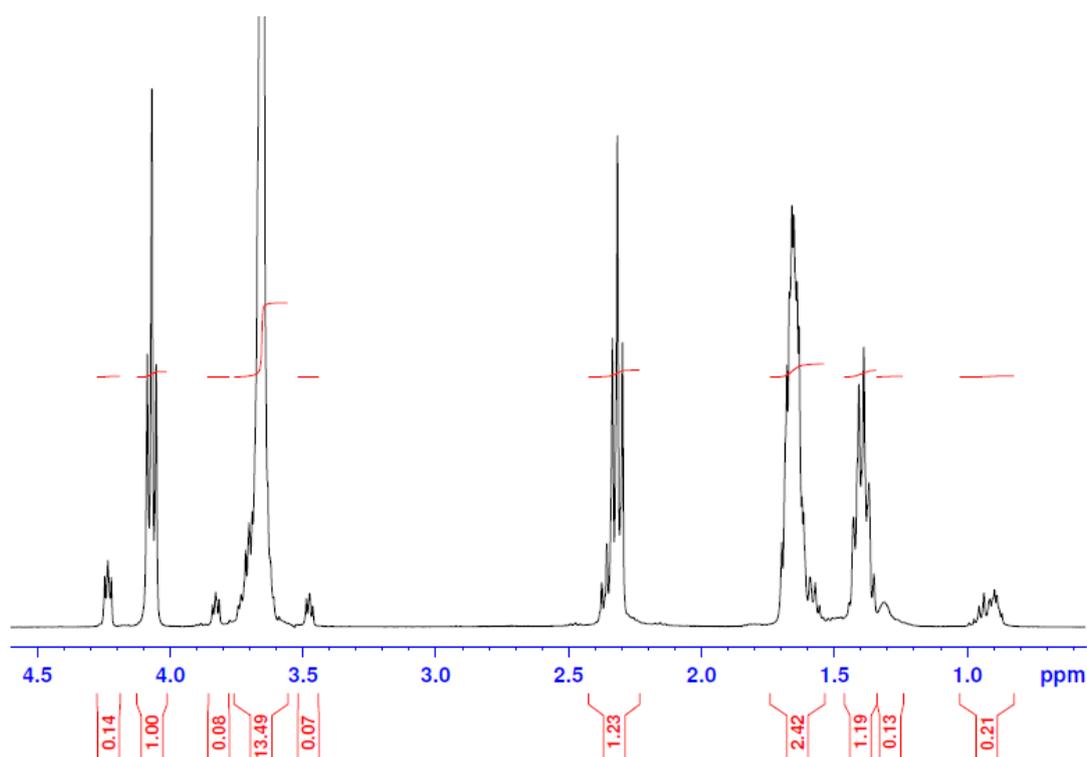


Figure 1. H NMR spectrum of PCL-PEG-PCL tri-block copolymer in CDCl₃.

Table 1. Molecular characteristics of the synthesized copolymers.

Copolymer	EG	CL / feed	M _n (Da) ^a	M _w (Da) ^a	M _w I ^b	Pd (°C) ^c	T _m EG	DP _{PCL} ^d	DP _P ^d
PCL-PEG-PCL	2		14235	5	987	1.4	53.	136.	72.1

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

ppm and 4.11 ppm, the methylene (CH_2) groups of PEG were around 3.75 ppm. Table 1 shows the characteristics of synthesized

To DSC thermograms, the endothermic peak (53.21 °C) including two merged peaks of PEG and PCL was presented in figure 3.

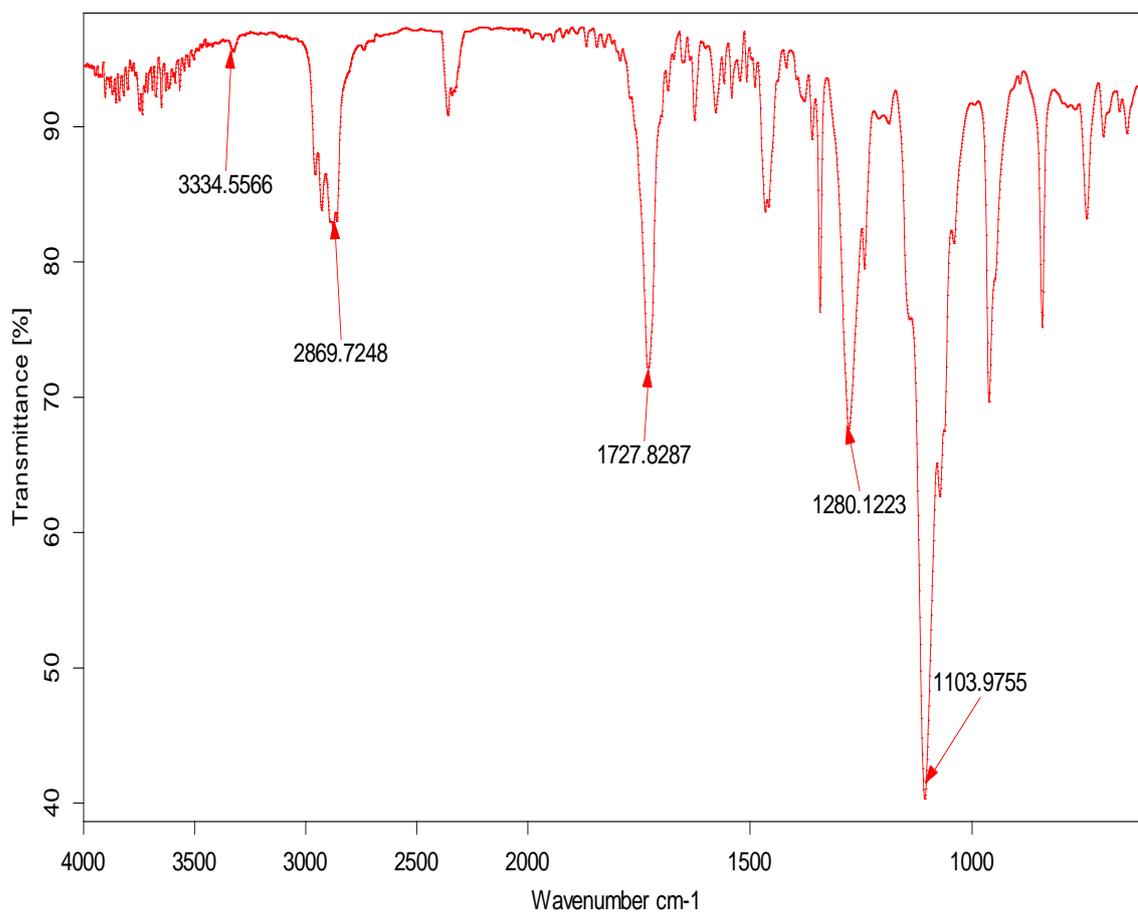


Figure 2. FTIR spectrum of PCL-PEG-PCL tri-block copolymer.

copolymers. FT-IR spectrum of PCL-PEG-PCL copolymer show the sharp and intense bands at 1727 cm^{-1} and 1103 cm^{-1} were awardable to the presence of carboxylic ester ($\text{C}=\text{O}$) and ether ($\text{C}-\text{O}$) groups, thereby indicating that the formation of PCL-PEG-PCL copolymer has occurred successfully (Figure 2).

GPC results showed that the weight- and number-based average molecular weights of copolymer were 14.23 and 9.87 KDa, respectively.

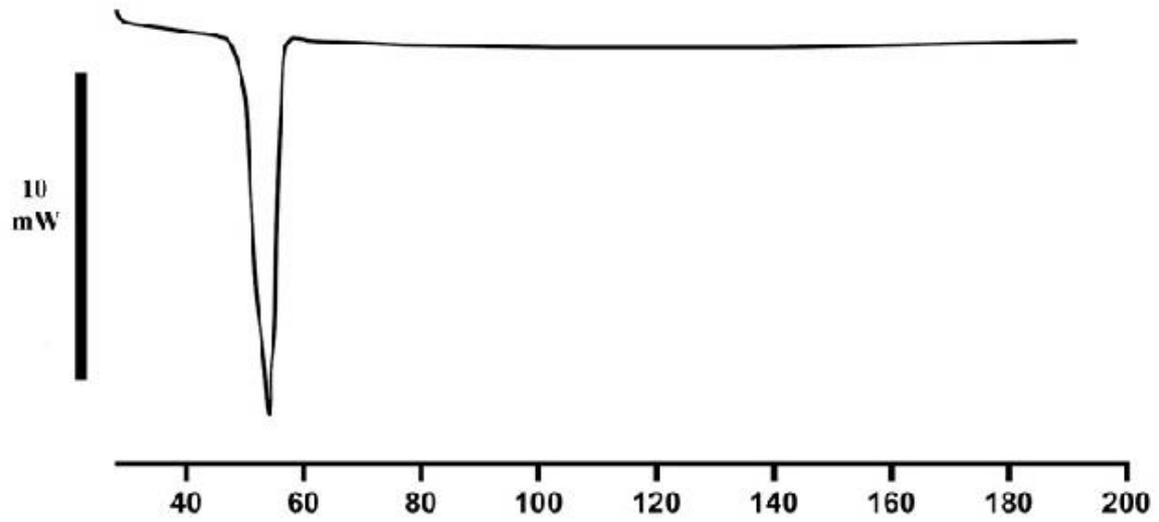


Figure 3. DSC thermogram of PCL-PEG-PCL copolymer.

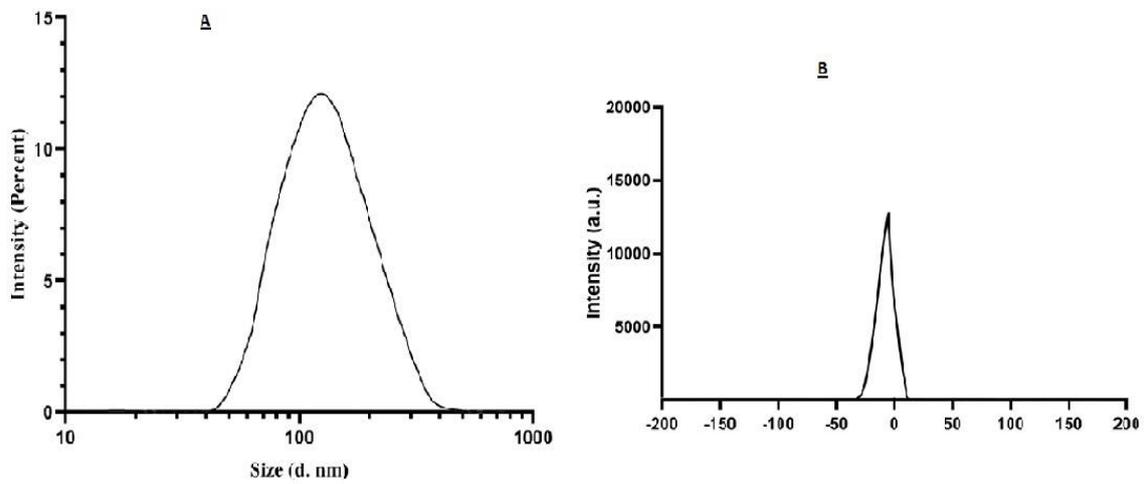


Figure 4. Particle size distribution and zeta potential of CTX/PCL-PEG-PCL nanoparticles(a) particle size distribution (b) zeta potential.

3.2. Preparation and Characterization of Copolymeric Polymersomes

The size of polymersomes was measured by dynamic light scattering technique (DLS). The Z-average and Zeta potential of CTX loaded PCL-PEG-PCL polymersomes were found to be about 115.7 ± 0.48 nm and -7.57 ± 1.12 mV, with their corresponding PDI being 0.096 (Figure 4). The loading ratio and encapsulation efficiency of CTX loaded PCL-PEG-PCL polymersomes were determined by UV-Vis to be 17.50 ± 1.17 % and 75.83 ± 1.41 %, respectively.

3.3. In Vitro Release of CTX

In order to examine the influence of the chemical and biochemical factors on the release of CTX 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 CTX 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 CTX was observed to be rapidly released and reached its peak of 85.95% and 88.13 % of the total in the first 12 h at pH 7.4 and 5.5 respectively. Figure 5 shows the release profiles of CTX from the drug-loaded polymersomes, at pH 7.4 and 5.5. As expected, no considerable initial burst CTX release was observed from the polymersomes. As shown in Fig. 5, the percentage of CTX released from the polymersomes increased as the pH value decreased from 7.4 to 5.5. For example, after 96 h incubation, the amounts of CTX released

in the media with pH values of 7.4 and 5.5 were about 45.36 and 76.21 %, respectively. The reason behind this phenomenon lies in the pH sensitivity of the release rate of CTX 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 the acidic environment, the polymer matrix swells due to protonation of a polymer. The results showed that the maximum drug releases were 60.87, and 80.21 %, respectively for PBS with pH=7.4 and pH=5.5 after a period of 120 h. The sustained release of CTX can be attributed to the entrapment of CTX in core of polymersomes. So, the obtained polymersomes can be considered as highly attractive nanocarriers for time-controlled drug delivery hydrophobic drugs to achievement of different therapeutic objectives.

3.4. Physical Stability of Polymersomes

In the clinical administration of nanoparticle dispersions, the stability of the size and volume of the nanocarriers is of great importance both as a measure of the particle structure integrity and as an indicator of the possible inter-particular associations (aggregation). For this purpose, the particle size stability was monitored in this study over 30-days course. The size of all polymersomes was increased slightly throughout the measurement period. This observation cannot be a sign of aggregation which usually leads to several fold increases. Possibly, some kind of copolymer swelling and/or hydration (as a result of presence of the hydrophilic PEG

portions in polymersome surfaces) can be responsible for this event [31].

suitable system for delivery of CTX. This developed nano drug delivery system can also

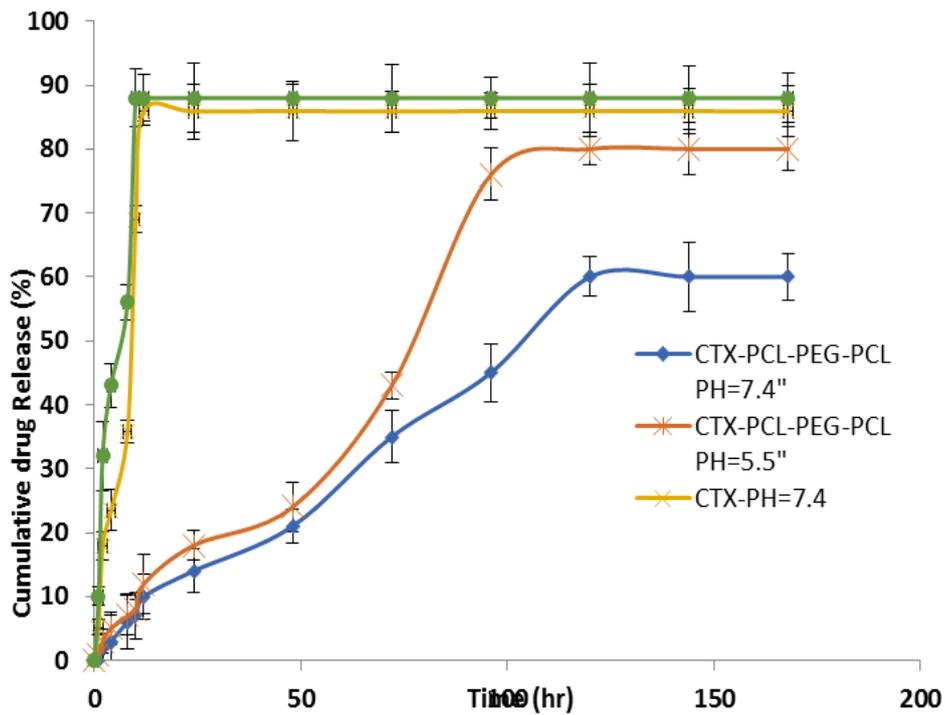


Figure 5. The release profiles of CTX from CTX-PCL-PEG-PCL polymersomes in different release media (a) pH= 7.4, (b) pH=5.5.

4. Conclusion

PCL-PEG-PCL copolymer was synthesized and characterized by ^1H NMR, FTIR, DSC, and GPC techniques. These copolymers were self-assembled into polymersomes in aqueous solution in presence of CTX. The polymersomes were characterized by DLS. The encapsulation efficiency of CTX was $75.83 \pm 1.41\%$. The results revealed that the polymersomes had size of 115.7 ± 0.48 nm. *In vitro* release of CTX from polymersomes was clearly sustained in all the media tested for this purpose, with the apparent release plateau reached late at about 120 h. Our study demonstrated that this nanocarrier provided a

be used as a promising base to design further nanoparticle systems to improve the therapeutic effect of CTX in the future.

Acknowledgment

This work has been supported financially by research deputy of Zanjan University of Medical Sciences, Zanjan, Iran (grant No. A-12-430-6).

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