Pharmaceutical Sciences
December 2016, 22, 234-243
doi: 10.15171/PS.2016.37
http://journals.tbzmed.ac.ir/PHARM

Research Article

Preparation and Physicochemical Characterization of Biodegradable mPEG-PCL Core-Shell Micelles for Delivery of Artemisinin

Hamid Reza Kheiri Manjili[1], Hojjat Malvandi[3], Mir-Sajjad Mousavi[1], Hossein Danafar[2,3*]
[1]*Pharmaceutical Nanotechnology Department, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*
[2]*Zanjan Pharmaceutical Biotechnology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.*
[3]*Department of Medicinal Chemistry, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*

Article Info

Article History:
Received: 9 June 2016
Accepted: 20 October 2016
ePublished: 30 December 2016

Keywords:
-Artemisinin
-Drug delivery
-Micelles
-mPEG-PCL

ABSTRACT

Background: Artemisinin is a sesquiterpene lactone chemical extract from Artemisia annua, is poorly resolvable in water and a fast-acting blood active in treating the acute attack of malaria.
Methods: Artemisinin was encapsulated within mPEG-PCL micelles with a single-step nano-precipitation method, leading to formation of ART/ mPEG-PCL micelles. mPEG-PCL copolymers was characterized *in vitro* by HNMR, FTIR and DSC techniques. Copolymers with artemisinin were self-assembled into micelles in aqueous solution. The consequential micelles were further characterized by various techniques such as DLS and AFM.
Results: The results exhibited the successful formation of spherical artemisinin-loaded micelles. The artemisinin-loaded micelles showed high loading efficiency. The encapsulation efficiency of artemisinin was 63±2.31%. *In vitro* release of artemisinin from artemisinin-entrapped micelles followed remarkably sustained release profile.
Conclusion: The results indicated that the successful formulation of artemisinin loaded mPEG-PCL micelles can improve the drug delivery of artemisinin. The results showed that nanomicelles can be promising drug delivery systems for sustaining release of artemisinin.

Introduction

Artemisinin (ART) and its derivatives are widely used throughout the world. ART is an end peroxide-containing sesquiterpene developed from an ancient Chinese herbal remedy, which was used by Chinese herbal medicine practitioners for at least 2000 years.[1] ART has been of special biological interest for many years. Because of its strong cytotoxic activity, it was reported that ART has special antitumor activity against melanoma, breast, ovarian, prostate, central nervous system, and renal cancer celllines.[2-4] However, ART presents some problems: ART has low bioavailability due to its low solubility; ART metabolizes quickly in vivo and has an initial burst effect and high peak plasma concentrations; ART is not very stable and easily decomposes, most probably by the opening of the lactone ring, due to its unusual peroxy group: it is difficult for drug crystals to disperse homogeneously in solution or blood because of their hydrophobicity; and a greater number of injections is necessary because of the short-duration effect. These problems can potentially be overcome by developing a controlled release scheme. Consequently, it is very important to prepare ART micro- or nanocapsules to increase their *in vitro* dissolution and to prolong their release time. However, there are a few literature reports on encapsulation of ART, particularly ART nanomicelles. Gharib, et al, Prepared magnetic nanoliposomes and characterized for delivery of artemisinin and transferring.[5] However, there are few literature reports on encapsulation of ART, especially nanoscale ART.[6-7] Li and Zhou prepared a solid dispersion of ART using solid- dispersion technique to improve bioavailability of its dosage form.[8] To achieve drug controlled release, biodegradable polymeric nanoparticles are often used in advanced anticancer drug delivery systems.[9-12] Some drug delivery systems via biodegradable polymers such as nanoparticles delivering anticancer agents are commercially

***Corresponding Author:** E-mail: danafar@zums.ac.ir

available.[13-14] Micelles as drug carriers welfare some benefits such as increased bioavability, reduced toxic side effects, possibility for attachment of targeting moieties, storage stability, long blood circulation time, and lower contacts with reticuloendothelial system (RES).[15-17] Moreover the most significant feature of micelles is that in spite of other drug carriers they can be used for both hydrophilic and hydrophobic drugs. This is because of amphiphilic nature of micelles which cause the drug molecule to be located in dissimilar micellar sections depending on hydrophobic–hydrophilic balance of drug molecule and, hence, associate with the micelle with varying affinities. Diblock copolymer of methoxypoly (ethylene glycol)- poly (caprolactone) (mPEG–PCL) is a good candidate to prepare micelle-like nanomicelles with the hydrophobic PCL block and hydrophilic mPEG block.[18] The hydrophobic core can serve as reservoir for hydrophobic drugs and mPEG shell play a dual role in stabilizing particles and associating with hydrophilic drugs. Poly (caprolactone) -poly (ethylene glycol) (PCL-PEG) copolymers are biodegradable, easy to produce, amphiphilic, and have a strong potential application in drug delivery systems.[19-22] The purpose of this research is to minimize the doses amount, toxicity and to improve the therapeutic efficacy by formulating ART nanoparticles. For this reason, it seems that encapsulating of artemisinin in mEEG-PCL micelles could be a novel delivery vehicle for artemisinin *in vitro* cancer therapy.

Materials and Methods

Materials

Artemisinin (98% purity), stannous 2-ethyl-hexanoate (Sn (Oct) $_2$), mPEG (Mn=5000 Da), ε-caprolactone (98% purity), Methanol, acetonitrile, acetone, ethanol, and the other chemicals and solvents were was shown in Table 1.

Synthesis of mPEG-PCL copolymer

The synthesis of mPEG–PCL copolymers was performed by a ring opening polymerization of ε-caprolactone with mPEG as initial molecule and Sn (Oct) $_2$ as catalyst.[23-25] mPEG was kept under a high vacuum at 80 °C for 2 hours(h) to remove all moisture. Briefly, ε-caprolactone (5 g), mPEG (2.5 g), and Sn (Oct) $_2$ (0.01 mmol) were heated to 120 °C under a nitrogen atmosphere. The polymerization reaction was performed under stirring situations. After 12 h, the resulting product was cooled to room temperature (23°C), dissolved in dichloromethane, and precipitated in cold diethyl ether. Then the mixture was filtered and the purification process was repeated twice more. The obtained di block copolymer was dried under vacuum at room temperature for 24 h.

Table 1. Source and purity of chemical used in this study

Component	Source
Acetone	Merck
Ethanol	Merck
Methanol	Merck
Artemisinin(98% purity)	Aldrich
Stannous 2-ethyl-hexanoate (Sn (Oct) $_2$)	Aldrich
mPEG (Mn=5000 Da)	Aldrich
Acetonitrile	Merck
ε-caprolactone (98% purity)	Aldrich
Tween 80	Merck
Dialysis bag	Amin saan
Choloroform	Merck
KH_2PO_4	Kimiagaran
KCl	Merck
HCl	Merck
H_3PO_4	Merck

Characterization of di block copolymer

Proton Nuclear Magnetic Resonance Spectroscopy (1H NMR) in $CDCl_3$ at 400 MHz (Bruker, Evans, 400) and Fourier Transform Infrared spectroscopy (FT-IR) (Bruker, Tensor 27) were used for characterization of the chemical structure of the mPEG-PCL copolymer. Molecular weight and distribution of the mPEG-PCL copolymers were calculated by HNMR. Thermal analysis of di block copolymers was determined by differential scanning calorimetry (DSC) (Mettler Toledo, model Star SW 9.30). Copolymers were heated at a rate of 10 °C min^{-1} and the data were recorded from 0 to 250 °C.

Preparation of micelles

Artemisinin loaded micelles were prepared by nanoprecipitation process and acetone was used as the solvent.[23-25] mPEG-PCL copolymer (20 mg) and artemisinin (6 mg) were dissolved in 2 ml of acetone. The solution was added drop-wise through a syringe (G=22) into 25 ml of distillated water under certain mixing rates (1500 rpm) and was continuously stirred magnetically at room temperature to evaporate all the organic solvent. Micelles were obtained by self-associating of amphiphilic mPEG-PCL copolymers. The micelles were separated by centrifuging at 20000 g for 20 min and freeze-dried under a pressure of 14(paskal) Pa at -78 °C to remove the residual solvent to obtain the final dried form.

Physicochemical Characterization of the micelles

Particle morphology

Atomic Force Microscopy (AFM) (JPK, Berlin, Germany, and model Nano Wizard 2) was used for evaluation of nanomicelles morphology. For AFM sample preparation, micelles were diluted with water and a droplet of 2 µL was placed onto a freshly cleaved mica substrate (1 cm^2) and air-

dried. AFM measurements were performed in intermittent contact mode.

Determination of particle size and zeta potential of artemisinin loaded micelles

Dynamic Light Scattering (DLS) using a nano/zetasizer (Malvern Instruments, Worcestershire, UK, model Nano ZS) was used for determination of the particle size distribution and zeta potential of the prepared micelles (1 mg/ml).

Stability study of micelles

Stability of micelles was evaluated by monitoring the size of the micelles in phosphate-buffer saline(PBS, pH=7.4) at ambient temperature at times 0, 15, and 30 days after preparation using the method described in section of preparation of micelles.

Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency of micelles were evaluated be equations 1 and 2. Drug loading (DL) efficiency was evaluated by equation 1.

$$\%DL = \left(W_{drug\ \ of\ \ micelles}\right) / W_{micelles} \qquad \text{Eq.(1)}$$

$W_{drug\ in\ micelles}$ and $W_{micelles}$ show weight of the encapsulated drug and the total weight of the corresponding drug-encapsulated micelles, respectively. DL% is the drug loading ratio (%). For assessment of the drug loading efficiency, 1 mg of the final freeze-dried nanomicelles was dissolved in 1mLof dichloromethane. Drug loading was determinated by high performance liquid chromatography (HPLC). A C_8 analytical column (150 mm ×4.6 mm, 5 micron) equipped with a guard column of the same packing was used. The mobile phase was consisted of acetonitrile and water in the volume ratio of 60:40, and was delivered at a flow rate of 1.0 ml/min using a double-reciprocating pump and the analysis wavelength was at 210 nm (Waters, MA, USA, model Breeze). The sample was injected through a 20 µL sample loop. Encapsulation efficiency was evaluated using the following equation 2:

$$\%DL = \frac{weight\ of\ \ drug\ in\ micelles}{weight\ of\ \ micelles} * 100 \qquad \text{Eq.(2)}$$

DSC analysis

Any possible drug-polymer interaction(s) as well as the physical changes occurred on the drug or polymer can be studied using the thermal analysis. DSC analysis was carried out on pure drug and drug-loaded micelles. Samples were heated at a rate of 10 ° C min^{-1} and the data were documented from 0 to 200 °C.

In vitro drug release study

This test was performed to measure the release behavior of artemisinin from nanomicelles. At first, 5 mg of artemisinin loaded micelles was dispersed in 2 mL PBS with pH= 7.4 containing 5% (v/v) Tween 80 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, 3 ml of the dialysate was taken out and replaced by 3 mL fresh PBS. The concentration of artemisinin in the dialysate was determined by HPLC method at the wavelength of 210 nm.[26] All the release studies were performed in triplicate. As the release control, the release of free artemisinin was measured in PBS at pH= 7.4. To determine the pH-dependency of the drug release, the study was also carried out, as specified earlier, using PBS with pH of 5.5. To evaluate the drug release behavior of carriers while incubated in real human plasma, the release experiments were repeated in plasma of a healthy male volunteer in the same condition except that the samples were incubated in plasma as a substitute of PBS.

Results

Synthesis and characterization of mPEG-PCL copolymer

Diblock copolymers were successfully synthesized using the ring-opening polymerization of caprolactone in presence of PEG, whose hydroxyl end group initiated the ring opening. Characterization of the mPEG-PCL di-block copolymer structure was evaluated by HNMR spectroscopy in $CDCl_3$ (Figure 1). The presence of methylenes (CH_2) in PCL was observed around 1.4 (i,j)ppm, 1.7(h) ppm, 2.4(g) ppm and 4.1(k) ppm, the methoxy and methylene protons in methoxy(OCH_3) (f)and methylene (CH_2) groups(a) of PEG were around 3.39 and 3.7 ppm.). NMR results showed that the average molecular weights of copolymer were 16.1. FT-IR spectrum of mPEG-PCL copolymer has been shown in Figure 2. The sharp and intense bands at 1725 cm^{-1} and 1109 cm^{-1}were attributed to the presence of carboxylic ester (C=O) and ether (C–O) groups, The signals at 2995 and 2891cm^{-1} were assigned to the absorption of the C-H stretching bonds of -CH_2CH_2, which are similar to those of ε-CL. The signals at 1430 and 1325 cm^{-1} were assigned to the bending bonds of C-H of CH_2 of caprolactone , the signal of 3500 cm^{-1} was assigned to the O-H band.

Preparation and characterization of copolymeric micelles

AFM was used for confirmation of nanomicelles. As expected for the micelles, the mPEG-PCL copolymeric micelles showed a homogeneous spherical morphology (Figure 3).The size of copolymric micelles measured by AFM was about 110.34 nm.

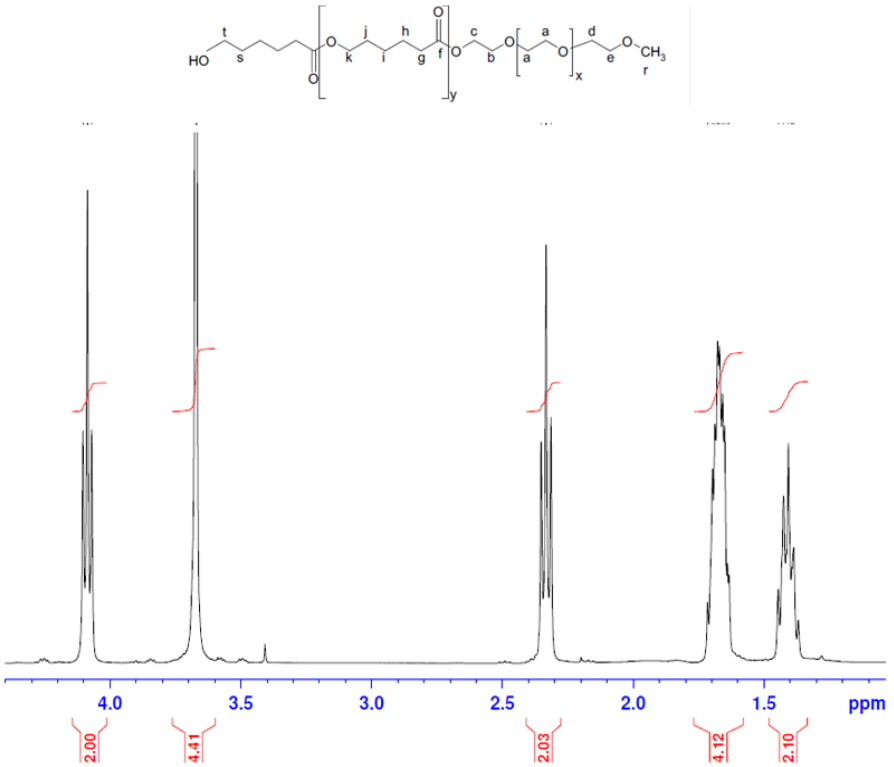

Figure 1. H NMR spectrum of mPEG-PCL di-block copolymer in CDCl3.

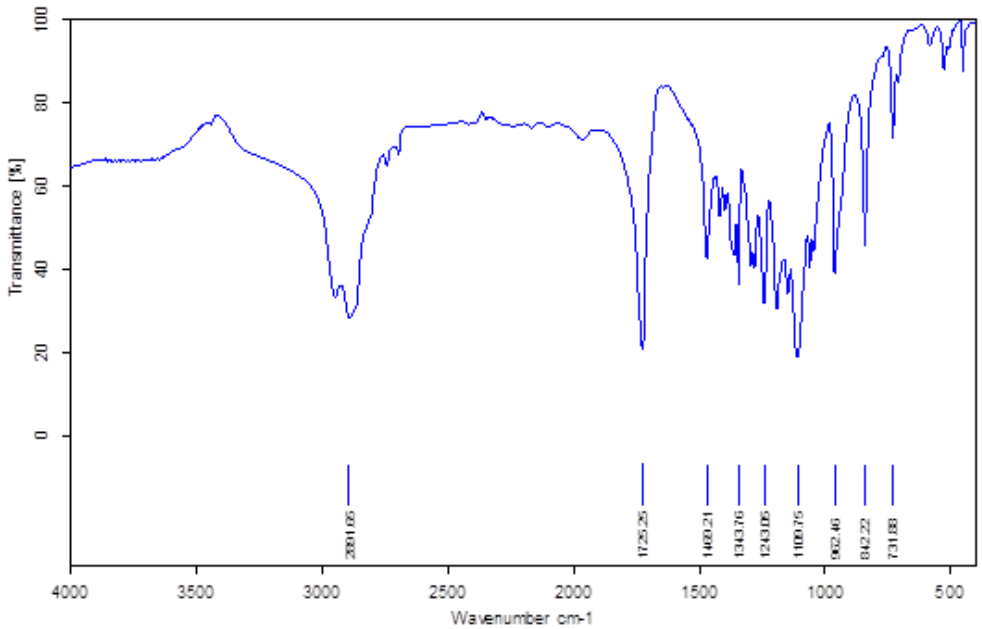

Figure 2. FT-IR spectrum of mPEG-PCL di-block copolymer.

The size of micelles measured by AFM was smaller comparing to measuring by DLS, because the size measured by AFM is related to the collapsed micelles after water evaporation while micelles diameter measured by DLS represents their hydrodynamic diameter. The size and zeta potential of prepared micelles was also measured by dynamic light scattering technique (Figure 4).The size and Zeta potential of artemisinin loaded micelles were about 142.9 nm and -4.93 mV, with their corresponding PDI being 0.142. The encapsulation efficiency and loading efficiency of artemisinin loaded copolymeric micelles were determined 63±2.31%, and 15% ± 1.43% respectively (Table2).

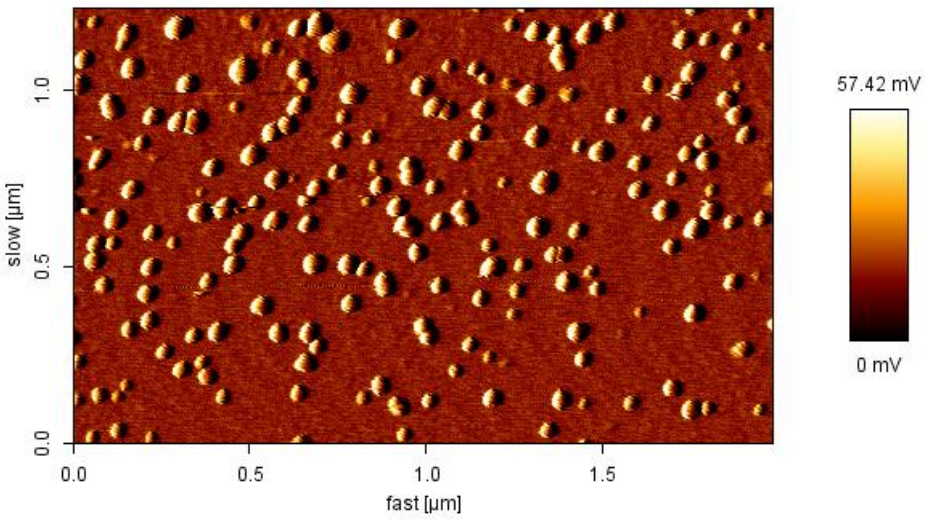

Figure 3. AFM image of micelles.

Table 2. Characterization of micelles (Size, drug loading, zeta potential and encapsulation efficiency).

Micelles	Mean size of micelles (nm) by AFM	Mean size of micelles (nm) by DLS	PdI	Zeta potential	Drug loading efficiency%	Encapsulation efficiency %
ART- m-PEG-PCL micelles	110.34	142.9	0.142	-4.93	15% ± 1.43%	63±2.31%

DSC analysis

Figure 5 shows the DSC thermograms corresponding to mPEG-PCL copolymer, artemisinin and micelles loaded by artemisinin. The thermogram of mPEG-PCL copolymer displayed an endothermic peak at 52.34 $^{\circ}$C which is indicative for the melting of the crystalline PCL segment of copolymer, the thermogram of artemisinin displayed an endothermic peak at 154.35 $^{\circ}$C and micelles displayed an endothermic peak at 45.08 ^{0}C which related to the melting of copolymers associated in the form of micelles. This endothermic peak of micelles presumably confirms a physical interaction between copolymer and artemisinin upon loading of the drug in micelles, since the melting point of PCL micelles was lower than melting point of copolymer (58.68 $^{\circ}$C). The characteristic peak of artemisinin was not observed in micelles. It could be concluded that the ART in the micelles was in an amorphous or disordered crystalline phase or in a solid solution state.

Artemisinin release from micelles impacted by different release media

Study of artemisinin release was determined on artemisinin-loaded nanomicelles in neutral and acidified PBS solution (pH=5.5). The release of free artemisinin was evaluated to verify that the diffusion of drug molecules across the dialysis membrane was not a rate-limiting step during the release process. The free artemisinin to released rapidly and reached to70.95% of the total amount in 12 h. The release profiles of artemisinin from the artemisinin-loaded micelles, at pH 7.4, 5.5, and plasma was showed at Figure 6. No considerable initial artemisinin burst release was observed from the micelles. As it can see in Figure 6, with pH decreasing from 7.4 to 5.5, the artemisinin release from micelles was increased. For example, percentage of artemisinin released from the micelles after 72 h incubation, in the media with pH values of 7.4, 5.5 and human plasma were about 46.23, 42.32, and 49.32%, respectively, Figure 6 shows that the maximum artemisinin releases form the nanomicelles is 60.99%, 65.56% and 66.54% for PBS pH=7.4, pH=5.5 and plasma after a period of 96 h respectively. The reason of artemisinin sustained release can be related to the entrapment of artemisinin in inner part of micelles or the core of micelles. Therefore, our nanomicelles could be a highly attractive nanomicelles for drug delivery of hydrophobic drugs for the achievement of different therapeutic objectives

Physical stability of micelles

In the clinical administration of nanoparticle dispersions, the stability of the sizes of the nanomicelles 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 a 30-days course. The variation of the sizes of nanomicelles as a function of incubation time is shown in Table 3. As it can be seen, the size of all micelles was increased slightly throughout the measurement period. This observation cannot be a sign of aggregation, 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 nanomicelles surfaces can be responsible for this event.

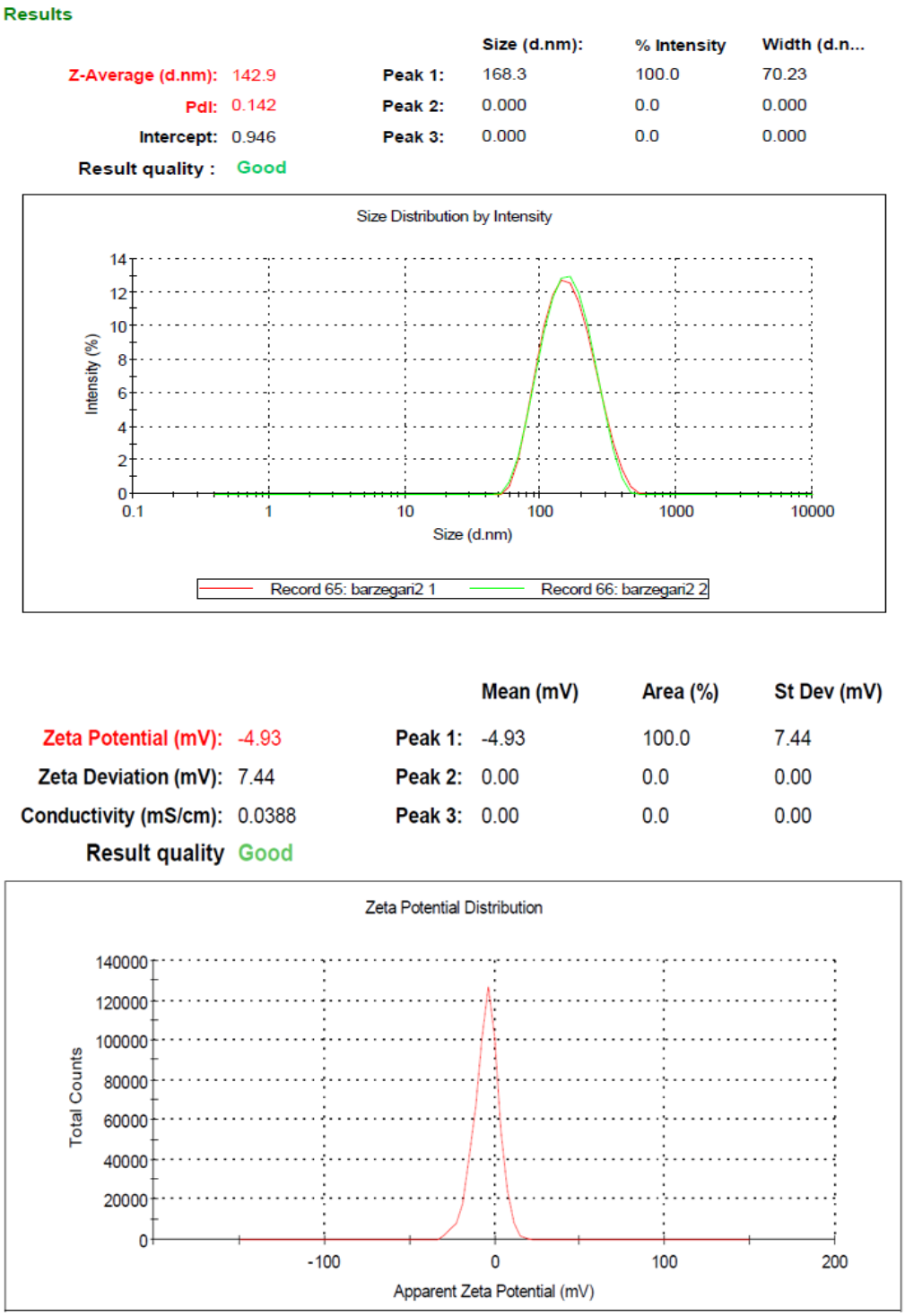

Figure 4. Particle size distribution and zeta potential of ART/mPEG-PCL micelles (a) particle size distribution (b) zeta potential.

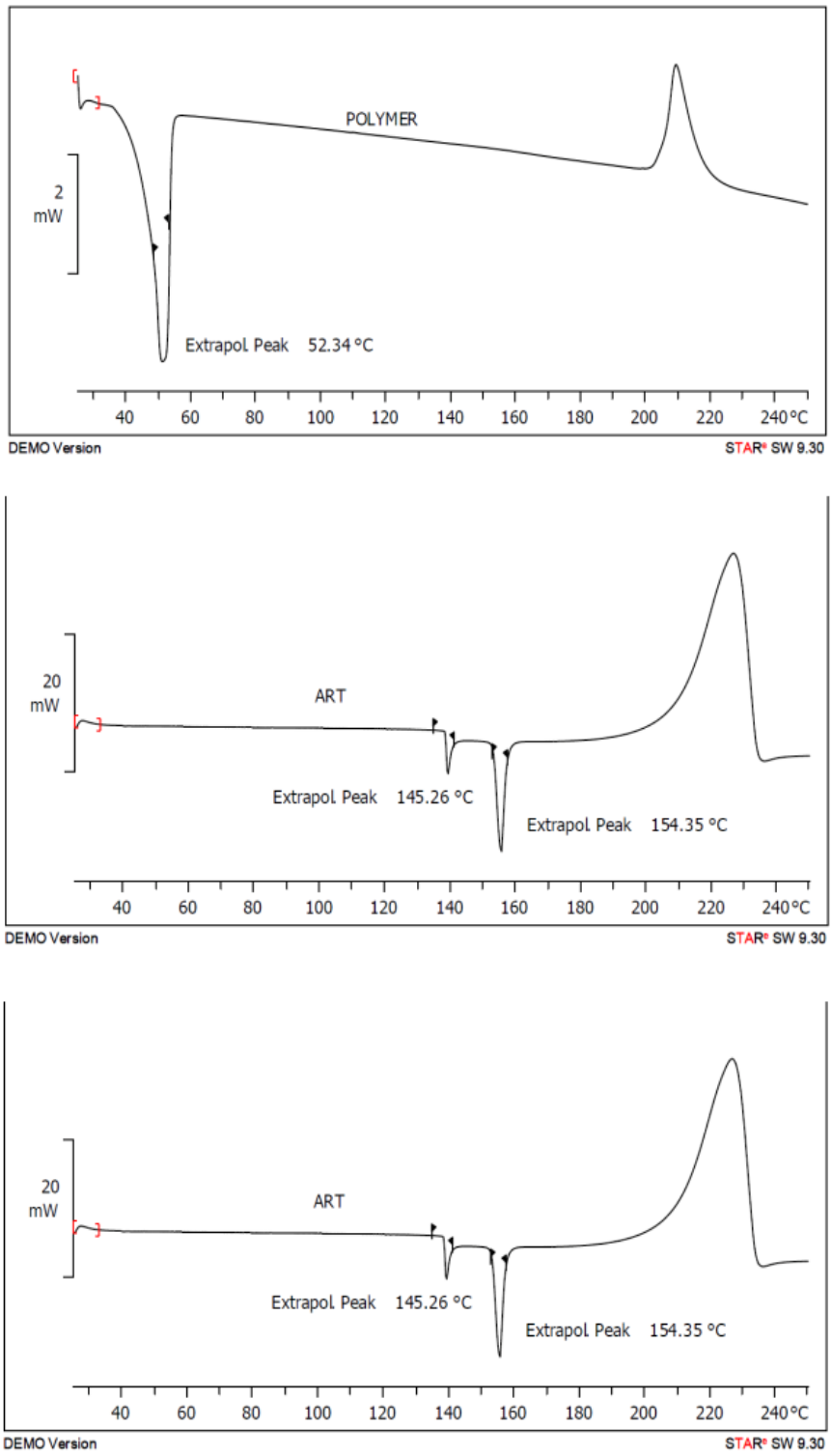

Figure 5. DSC spectra of (a) mPEG-PCL, (b) artemisinin and (c)ART - mPEG-PCL micelles.

Table 3. Stability of nanoparticles dispersions.

Micelles	Mean size of micelles immediately after preparation (nm)	Mean size of micelles after 15day (nm)	Mean size of micelles after 30 days (nm)
ART- m-PEG-PCL micelles	142.9	171.3	190.2

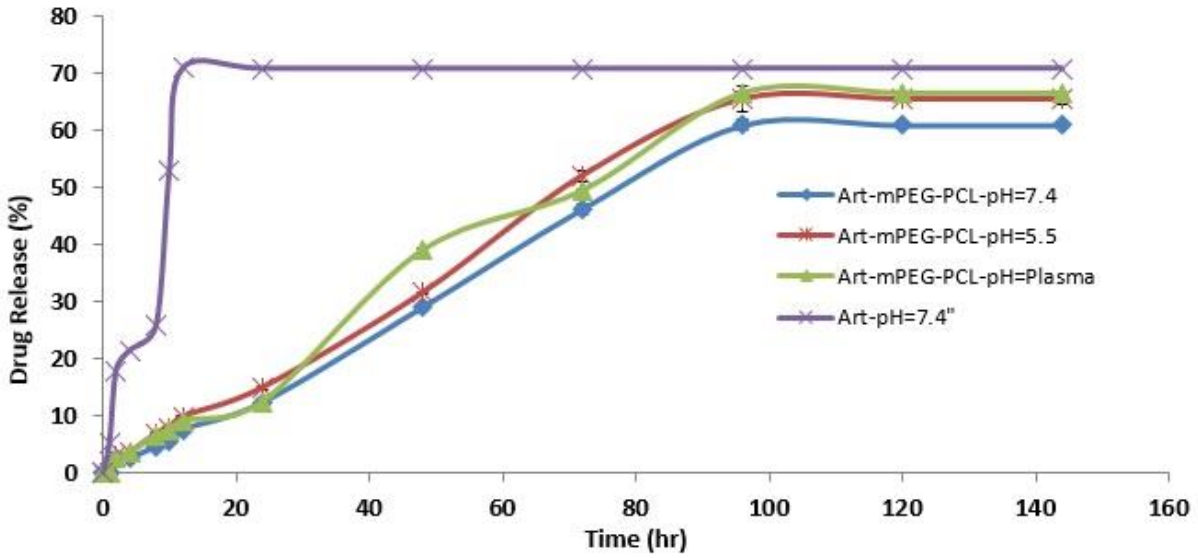

Figure 6 The release profiles of artemisinin fromART- mPEG-PCL micelles in different release media (a) PBS, pH= 7.4, (b) plasma, (c)PBS, pH=5.5, (d) free artemisinin release PBS, pH=7.4.

Discussion

Artemisinin is a sesquiterpene lactone and phytochemical found naturally in Artemisia annua L.[1] Data for artemisinin's advantage was attributed for anti-malaria, anti-oxidative, anti-inflammatory, and anti-cancer effects.[2,5] It is reported that the anti-cancer effect of artemisinin was increased several fold in the presence of iron sources such as transferring.[3] In this study, we evaluated the probable of loading artemisinin into nanomicelles. We found that the encapsulation efficiencies of artemisinin in the nanomicelles were suitable. Amphiphilic nature of mPEG-PCL with hydrophilic PEG and hydrophobic PCL blocks provides an opportunity to form micelles in water. This behavior can be explained as a consequence of copolymer self-assembling into micellar structure because of its amphiphilic nature which, subsequently forces the hydrophilic PEG segments to serve as hydrophilic shell and the hydrophobic PCL segments to become the micellar core. The ART/mPEG-PCL micelles having a minor negative surface charge of −4.93 mV can increase the circulation time of the drug. Surface charge is important in determining whether the nanomicelles will cluster in blood flow or will stick to or interact with oppositely charged cell membrane. The plasma and blood cells constantly have a negative charge; nanomicelles with slight negative surface charge can minimize nonspecific contact with these components through electrostatic interactions.[27-30] 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 micelles. Therefore, the obtained copolymeric micelles can be regarded as highly attractive nanomicelles for drug delivery of hydrophobic drugs to attainment of different therapeutic objectives.

Conclusion

With the potential managing as drug carriers for mPEG-PCL di block copolymer micelles in delivery systems, the efficacy of the micelles for the entrapment and controlled release of ART were investigated. The mPEG-PCL copolymers were self-assembled into micelles in aqueous solution in presence of artemisinin. The resulting micelles were characterized by various techniques such as DLS and AFM. The encapsulation efficiency and loading efficiency of artemisinin to these micelles is very good. In vitro release of artemisinin from artemisinin-entrapped micelles was clearly sustained in all the media tested for this purpose. The results of AFM revealed that the micelles formed had spherical structure. In summary the results suggested that the ART/mPEG-PCL micelles is suitable candidate for delivery of ART and may have potential application in cancer treatment.

Acknowledgment

This work has been supported financially by Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Global malaria control and elimination: report of a meeting on containment of artemisinin tolerance. Geneva: World Health Organization; 2008.
2. Efferth T, Kaina B. Toxicity of the antimalarial artemisinin and its dervatives. Crit Rev Toxicol. 2010;40(5):405–21. doi:10.3109/10408441003610571
3. Lai HC, Singh NP, Sasaki T. Development of artemisinin compounds for cancer treatment. Invest New Drugs. 2012;31(1):230-46. doi:10.1007/s10637-012-9873-z
4. Efferth T, Willmar SA. Antiplasmodial and antitumor activity of arternisinin—from bench to bedside. Planta Med. 2007;73(4):299-309.
5. Gharib A, Faezizadeh Z, Mesbah-Namin S, Saravani R. Preparation, characterization and in vitro efficacy of magnetic nanoliposomes containing the artemisinin and transferring. Daru. 2014;22(1):44-54. doi:10.1186/2008-2231-22-44
6. Nakase I, Gallis B, Takatani-Nakase T, Oh S, Lacoste E, Singh NP, et al. Transferrin receptor-dependent cytotoxicity of artemisinin-transferrin conjugates on prostate cancer cells and induction of apoptosis. Cancer Lett. 2009;274(2): 290-8. doi:10.1016/j.canlet.2008.09.023
7. Chen Y, Lin X, Park H, Greever R. Study of artemisinin nanocapsules as anticancer drug delivery systems. Nanomedicine. 2009;5(3):316-22. doi:10.1016/j.nano.2008.12.005
8. Li G, Zhou Q. Study of preparation and dissolution in vitro of qinghaosu solid dispersion. Pharma J Chin PLA. 2000;1:15-7.
9. Danafar H, Kheiri H, Sharafi A. Sulforaphane delivery using mPEG- PCL co-polymer nanomicelles to breast cancer cells. Pharm Dev Technol. 2016;1-10. doi:10.3109/10837450.2016.1146296
10. Shutava TG, Balkundi SS, Vangala P, Steffan JJ, Bigelow RL, Cardelli JA, et al. Layer-by-layer-coated gelatin nanomicelles as a vehicle for delivery of natural polyphenols. ACS Nano. 2009;3(7):1877-85. doi:10.1021/nn900451a
11. Shieh YA, Yang SJ, Wei MF, Shieh MJ. Aptamer-based tumor-targeted drug delivery for photodynamic therapy. ACS Nano. 2010;4(3):1433-42. doi:10.1021/nn901374b
12. Wang T, He N. Preparation, characterization and applications of low-molecular-weight alginate-oligochitosannanocapsules. Nanoscale. 2010;2(2):230-9. doi:10.1039/b9nr00125e
13. Safari J, Zarnegar Z. Advanced drug delivery systems: Nanotechnology of health design A review. J Saudi Chem Soc. 2014; 18(2): 85-99. doi:10.1016/j.jscs.2012.12.009
14. Danafar H, Rostamizadeh K, Davaran S, Hamidi M. Drug-conjugated PLA–PEG– PLA copolymers: a novel approach for controlled delivery of hydrophilic drugs by micelle formation. Pharm Dev Technol. 2016;1-11. doi:10.3109/10837450.2015.1125920
15. Chen W, Zhang JZ, Hu J, Guo Q, Yang D. Preparation of amphiphilic copolymers for covalent loading of paclitaxel for drug delivery system. J Polym Sci A Polym Chem. 2014;52(3):366-74. doi:10.1002/pola.27009
16. Danafar H, Hamidi M. Simple and sensitive high-performance liquid chromatography (HPLC) method with UV detection for mycophenolic acid assay in human plasma. Application to a bioequivalence study. Adv Pharm Bull. 2015;5(4):563-8. doi:10.15171/apb.2015.076
17. Cabral H, Kataoka K. Progress of drug-loaded polymeric micelles into clinical studies. J Control Release. 2014;190:465-76. doi:10.1016/j.jconrel.2014.06.042
18. Danafar H. MPEG–PCL copolymeric nanomicelles in drug delivery systems. Cogent Medicine. 2016;3(1):1142411. doi:10.1080/2331205x.2016.1142411
19. Wang X, Yang L, Chen ZG, Shin DM. Application of nanotechnology in cancer therapy and imaging. CA Cancer J Clin. 2008;58(2):97-110. doi:10.3322/ca.2007.0003
20. Wei X, Gong C, Gou M, Fu S, Guo Q, Shi S, et al. Biodegradable poly(epsilon-caprolactone)-poly(ethylene glycol) copolymers as drug delivery system. Int J Pharm. 2009;381(1):1-18. doi:10.1016/j.ijpharm.2009.07.033
21. Manjili HK, Sharafi A, Danafar H, Hosseini M, Ramazani A, Ghasemi MH. Poly (caprolactone)–poly (ethylene glycol)–poly (caprolactone)(PCL–PEG–PCL) nanoparticles: a valuable and efficient system for in vitro and in vivo delivery of curcumin. RSC Adv. 2016;6(17):14403-15.
22. Gou M, Wei X, Men K, Wang B, Luo F, Zhao X, et al. PCL/PEG copolymeric nanoparticles: potential nanoplatforms for anticancer agent delivery. Curr Drug Targets. 2011;12(8):1131-50. doi:10.2174/138945011795906642
23. Danafar H, Rostamizadeh K, Davaran S, Valizadeh H, Hamidi M. Biodegradable m-PEG/PCL Core-Shell Micelles: Preparation and characterization as a sustained release formulation for Curcumin. Adv Pharm Bull. 2014;4(Suppl2):501-10. doi:10.5681/apb.2014.074
24. Yodthong B. Surfactant-Free Nanospheres of m/PEG-PCL for controlled release of ibuprofen. J Appl Sci. 2009;9(12):2278-9.
25. Weihui X, Weipu Z, Zhiquan S. Synthesis, isothermal crystallization and micellization of

mPEG-PCL diblock copolymers catalyzed by yttrium complex. Poly. 2007;48(23):6791-8. doi:10.1016/j.polymer.2007.09.021
26. Danafar H, Hamidi M. Pharmacokinetics and bioequivalence study of amlodipine and atorvastatin in healthy male volunteers by LC-MS. Pharm Sci. 2015;21(3): 167-74. doi:10.15171/ps.2015.32
27. Danafar H. Applications of Copolymeric Nanoparticles in drug delivery systems. Drug Res. 2016;66(10):506-19. doi:10.1055/s-0042-109865
28. Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. Circ Res. 1996;79(3):581-9. doi:10.1161/01.res.79.3.581
29. Danafar H, Rostamizadeh K, Davaran S, Hamidi M. PLA-PEG-PLA copolymer-based polymersomes as nanocarriers for delivery of hydrophilic and hydrophobic drugs: preparation and evaluation with atorvastatin and lisinopril. Drug Dev Ind Pharm. 2014;40(10):1411-20. doi:10.3109/03639045.2013.828223
30. Dellian M, Yuan F, Trubetskoy V, Torchilin V, Jain R. Vascular permeability in a human tumour xenograft: molecular charge dependence. Br J Cancer. 2000;82(9):1513-8. doi:10.1054/bjoc.1999.1171