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Study of the Copolymer Structure Effect on Physicochemical Characteristics and In Vitro Stability of PLGA–PEG Nanoparticles Loaded 9-Nitrocamptothecin

Katayoun Derakhashandeh[a,b*], Maryam Haghkhah[b,d], Mahmood Amiri[c]

[a]Novel Drug Delivery Research Center, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Iran
[b]Department of pharmaceutics, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Iran
[c]Medical Biology Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran
[d]Student research committee, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

9-nitrocamptothecin (9-NC) is a semisynthetic and a low soluble analogue of camptothecin alkaloids that target nuclear enzyme topoisomerase I. The unstable lactone form of 9-NC in biological fluids is required for its cytotoxic activity. To improve aqueous solubility and stability in biological media, 9-NC was loaded in polymeric nanoparticles. In this paper, we studied the effect of PEG percent (0, 5, 10, 15) in PLGA-PEG copolymer on physicochemical properties of nanocarriers. To acquire an optimum formulation, a Generalized Regression Neural Networks (GRNN) and a Multi-Layer Perceptron (MLP) as potent statistical methods were employed. The drug loaded parameters were the input vectors of the GRNN and included the amount of polymer and emulsifier, volume of external and internal phases. The nanoparticles drug loading constitutes the output vector of each network. In this way, GRNN and MLP are trained to investigate the functional influence of input variables on the output response. PLGA-PEG nanoparticles were prepared by nanopercipitation method. Zeta Sizer, DSC, SEM and Franz diffusion cell were used to measure physicochemical properties of optimized formulations. The PEG percent in PLGA-PEG copolymer has an effective role in drug loading which can be attributed to the hydrophobic nature of drug and amphiphilic nature of copolymer. The size of nanoparticle decreased by increasing the PEG percent in copolymer which can be attributed to emulsifying nature of PEG. Release rate decreased by increasing the percent of PEG in PLGA-PEG nanoparticles but in vitro stability increased. DSC thermograms and FTIR results showed that 9-NC was encapsulated in PLGA-PEG nanoparticles in its amorphous form. According to artificial neural network (ANN) data, we found that PLGA-PEG (15%) had best physicochemical characteristic compare to the other copolymers. The optimum formulation showed that nanoparticles could be potential carriers for delivery of unstable and low soluble drugs especially for anticancer agents.

[*]**Corresponding author: Katayoun Derakhashandeh**, E-mail: kderakhshandeh@kums.ac.ir

Introduction

9-Nitrocamptothecin (9-NC) is a potent semisynthetic analogous of camptothecin alkaloids [1,2]. Like camptothecin family, 9-NC inhibits the nuclear enzyme DNA topoI, by stabilization of the DNA- topoI complex through a nuclophilic attack [3,4]. This interaction enables 9-NC to inhibit the growth of a wide range of tumors such as breast, ovarian and prostate cancer [5].

However the lactone ring in the structure of 9-NC absolutely required for cytotoxic activity, is insoluble and unstable in physiologic pH and convert to an inactive carboxylate form that cause adverse effects such as neutropenea and diarrhea (Fig.1.) [6,7].

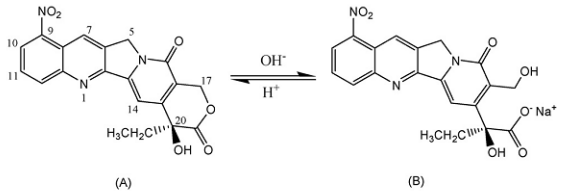

Fig. 1. The pH dependence equilibrium between lacton and carboxylate forms

To overcome these problems, several pharmaceutical formulations were used. First, the liposomal drug delivery system was used to stabilize the lactone form of CPT in the blood and increase tissue exposure [8,9]. Second, polymeric micelles were used and lactone stability of CPT was improved greatly with nearly zero-order invitro release profile [10-12]. In these polymeric micelles, biocompatible and biodegradable PLGA nanoparticles have more stability and lower toxicity but these nanoparticles were uptaken by phagocytic systems easily and have a rapid initial release [13-15]. However, opsonin proteins present in the blood serum, quickly bind to PLGA nanoparticles, allowing macrophages of the mononuclear phagocytic system (MPS) to easily recognize and remove these drug delivery devices before they can perform their designed therapeutic function [16-18]. To address these limitations, several methods have been developed to mask nanoparticles from the MPS. Of these methods, the most preferred is the adsorption or grafting of poly (ethylene glycol) (PEG) to the surface of nanoparticles. Addition of PEG and PEG-containing copolymers to the surface of nanoparticles results in an increase in the blood circulation half-life of the particles [19-21]. This method creates a hydrophilic protective layer around the nanoparticles that is able to repel the absorption of opsonin proteins via steric repulsion forces, thereby blocking and delaying the first step in the opsonization process. These novel micelles would be potential carriers for poorly soluble anticancer drugs [22-24]. However, the best choice for lipophilic drugs such as 9-NC are amphiphilic polymers, hydrophilic outer shell and hydrophobic inner core, because of self assembly in aqueous medium and no need for stabilizer [25-27].

Recently, there has been increased interest in applications of artificial neural networks (ANNs) in biomedical researches [28]. ANNs are used in pharmaceutical and pharmacokinetic areas to model complex relationships and to predict the nonlinear relationship between causal factors and response variables [29]. The distinct features of the ANN make this approach very useful in situations where the functional dependence between the inputs and outputs is not clear.

The applicability of the ANN in modeling and predicting drug release profiles was investigated to evaluate an experimental study in transdermal iontophoresis [30]. Rafienia and colleagues compare the potential of neural network models in order to estimate the release profiles of betamethasone and betamethasone acetate from in situ forming implants [29]. The MLP has played a central role in the research of neural networks. Their study began with the nonlinear and adaptive response characteristics of neurons and it was discovered that the MLP is a universal approximate of relations between inputs and output variables [31]. The GRNN was not commonly used but has the advantage of being easily trained and required only one free parameter.

In this article, we encapsulated 9-NC in amphiphilic PLGA-PEG nanoparticles in different PEG percents and evaluated the physicochemical properties of drug such as size and morphology, zeta potential, encapsulation efficiency, drug release profile and invitro stability. To obtain an optimum formulation, the GRNN and MLP neural networks are employed. Then, the GRNN and MLP are trained using the experimental data sample collected from drug loading. Moreover, it is investigated that the obtained optimum formulation could be effectively used as a new drug delivery systems.

Material and methods

Materials

9-Nitrocamptothecin (9-NC), 99.8% pure, was purchased from Yuanjian Pharmaceutical Technology Develope Co., (China). Poly (DL-lactide-co-glycolid) (PLGA 50:50, inherent viscosity of 0.16–0.24 dl/g) and PLGA-PEG 5, 10 and 15% were obtained from the Boehringer Ingelheim Co. (Ingelheim, Germany). Polyvinyl alcohol (PVA, MW 30000 Da, 87% hydrolyzed) was a gift from Mowiol (Germany). Analytical grade acetone, pure potassium dihydrogen phosphate, dichlromethane was purchased from Merck (Darmstadt, Germany). All other chemical reagents used, were of pharmaceutical grade.

Methods

Neural network experiments

PLGA-PEG nanoparticles were prepared based on artificial neural network. The independent variables were the amount of polymer (X_1), amount of emulsifier (X_2) and volume of internal and external phase (X_3, X_4). On the other hand, the dependent variable of drug loading (Y_1) was the response. Table 1 shows the independent variables and their lower and upper limits. To achieve an optimum formulation, MLP and GRNN are employed. In this way, ANN was trained to investigate the functional dependence of input variables on the output response.

Table 1. Independent variables of formulation parameters

Independent variables	*Low level*	*High level*
X_1 amount of polymer (mg)	50	200
X_2 amount of emulsifier (mg)	50	200
X_3 volume t of internal phase (ml)	4	20
X_4 volume of external phase (ml)	6	24

Preparation of nanoparticles

Nanoparticles were prepared by the nanopercipitation method as reported previously [36]. In brief, PLGA-PEG copolymer and drug were dissolved in acetone and added drop wise in aqueous solution containing PVA (pH was adjusted to 3 by 0.1 N HCl) under magnetic stirring. The acetone was evaporated at room temperature and suspension of nanoparticle was separated by ultracentrifuge (Beckman XL 90) at 40,000 rpm for 30 min and washed. This stage repeated three times to remove impurities of nanoparticles. The precipitated nanoparticles were freeze-dried and were used to characterize.

Characterization of 9-NC loaded PLGA-PEG nanoparticles

Characterization of size, zeta potential, and morphology

The mean size and zeta potential of nanoparticles were measured by dynamic light scattering (DLS) (Zeta nanosizer Malvern) at 25^{o}C at a scattering angle of 90^{o}.

Morphology of nanoparticles was performed by SEM (XL30) and samples were coated with a thin layer of colloidal gold.

Encapsulation Efficiency (EE)

For calculating the encapsulation efficiency, 20mg of nanoparticles were dissolved in 4 ml acetone by ultrasonicator (Tecna 3). The amount of 9-NC was determined by UV-Vis (UVmini-1240) absorption at 370 nm for three replicated samples. The encapsulation efficiency was calculated from the following equation:

Encapsulation efficiency (%) = Amount of 9-NC in NPs / Amount of 9-NC used in the formulation×100

Structural characterization of nanoparticles loaded 9-NC

For the characterization of the physical state of 9-NC in PLGA and PLGA-PEG nanoparticles, 8 mg of sample was sealed into aluminum pans and temperature ramp speed was set at 10 ^{o}C/min. The DSC (DSC-60, Shimadzu Co., Kyoto, Japan) standard reference material to calibrate the temperature was indium.

The transformation infrared spectroscopy investigations of nanoparticles obtained were carried out. 2 mg of the sample was mixed with 10 mg KBr and pressed as a pellet. Infrared spectra were recorded over a range of 400-4000 cm^{-1}, using a Shimadzu IR-prestige 21.

In vitro stability of 9-NC

To evaluate the lactone ring stability of 9-NC, the freeze-dried NPs were stored at 4 and 25°C for 1, 2, 7 and 14 days. After each time, samples were dissolved in acetone and sonicated 5 min in PBS (0.1M, pH 7.4) and the amount of lactone ring of 9-NC was determined by UV-Vis absorption at 370 nm. For evaluation of release kinetics, the obtained release data were fitted into first order, zero order and Higuchi equations. Selection of the best model was based on the comparisons of the relevant correlation coefficients.

Optimization of nanoparticle formulation

Neural Network Models

ANNs are characterized in principle by a network topology, a connection pattern, neural activation properties and train strategy. The rapid development of ANN technology in recent years has led to an entirely new approach for biomedical applications. Two types of neural networks that are MLP and GRNN will be used in the next section to obtain the optimum formulation [33,35].An MLP, illustrated in Fig. 2A, is used as a first structure for simulations. In the conventional structure of an MLP, a neuron receives its input either from other neurons or from external inputs (input vector). In Fig.2, the output *Y* of the MLP is a vector with *n* components determined in the terms of
m components of an input vector *X* and *l* components of the hidden layer [36,37].

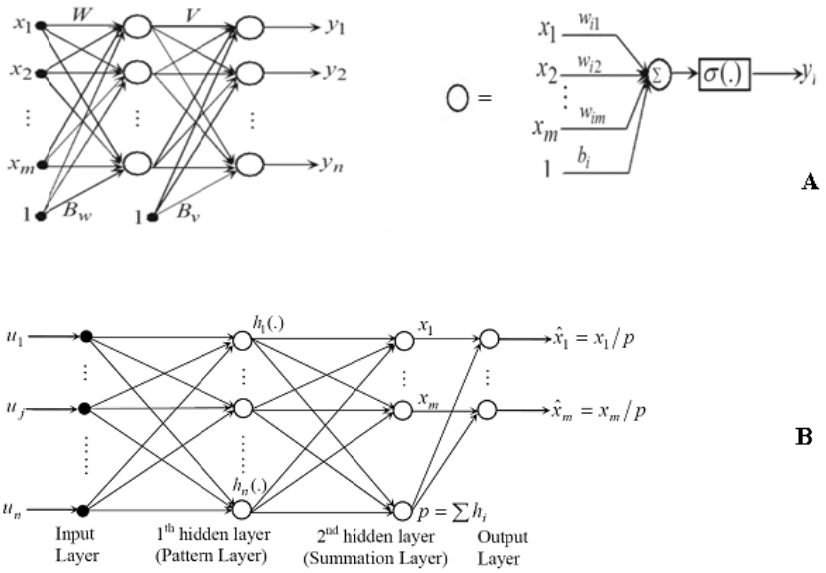

Fig. 2. The MLP and 1GRNN Structure

GRNN belongs to the class of neural networks widely used for the continuous function mapping. The main function of GRNN is to estimate a linear or nonlinear regression surface on independent variables [32].An important advantage of the GRNN is its simplicity and fast approximation procedure [38,39]. In addition, the training process with a GRNN-type algorithm is much more efficient than with the BP-NN algorithm. The topology of a GRNN is described in Fig. 2B, and it consists of four parts.
To find the optimum formulations, at first, GRNN and MLP are trained. In so doing, we divide the data samples into two groups, the training and validation data sets [40,41]. The training group contains 15 randomly selected samples and the validation group

includes the other three remaining samples. As can be seen in Table 2, the performance of the GRNN network in the estimation of output variables is better than the MLP performance.

Table 2. Mean prediction errors of the individual output variables for each neural network

Network	AMPE	MPEs
MLP	0.21	5%
GRNN	0.25	7%

Therefore, in this application, GRNN can effectively approximate the function between input and output vectors to find the optimum formulation and is more reliable than MLP[42].

Results

Nanoparticle characterization

Optimum formulations (Table 3) were characterized according to morphology, particle size, size distribution, surface charge and physical state of encapsulated drug (Table 4).

Table 3. Optimum formulation of PLGA-PEG nanoparticles loaded 9-NC

Composition	External phase	Polymer	Emulsifier	Internal phase	
	PLGA	21.33	165	28	12
	PLGA-PEG(5%)	18	125	50	4
	PLGA-PEG(10%)	18	200	50	4
	PLGA-PEG(15%)	22	200	50	4

Table 4. Physicochemical characteristics and encapsulation Efficiency of the nanoparticle compositions involved in the present study

Composition	Size (nm)	Size polydispersity	ζ-potential (mV)	%EE
PLGA	212	0.143	-12.3	32
PLGA–PEG(5%)	156	0.095	-14.0	45.7
PLGA–PEG(10%)	98	0.126	-18.0	55.8
PLGA–PEG(15%)	84	0.098	-22.6	57.4

Fig. 3. Was investigated by SEM showed the spherical and smooth surface of nanoparticles. The average size of nanoparticles was reduced related to the emulsifying nature of PEG in the structure of copolymer and size distribution was narrow. PLGA-PEG nanoparticles were negatively charged with approximated zeta potential of -12.3 to -22.6 due to change of a PEG percent of copolymers (0-15%).

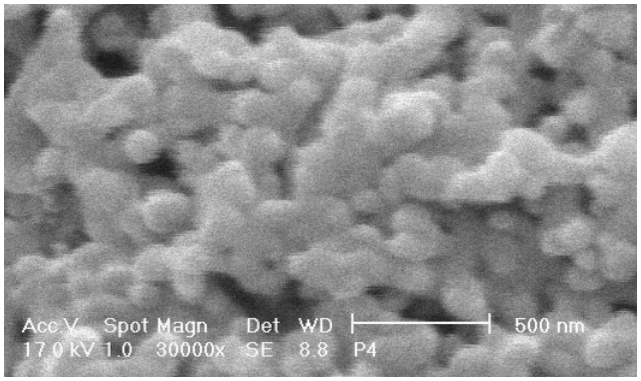

Fig. 3. Scanning electron microscopy image of 9-NC nanoparticles

The FTIR spectra of 9-NC and physical mixtures and loaded nanoparticles were shown in figure 5. The characteristic peaks of 9-NC at 1357 and 1500 were due to nitro and the presence of peaks in 1658 and 3520 was related to hydrogen of hydroxyl and carbonyl in the structure of the drug.

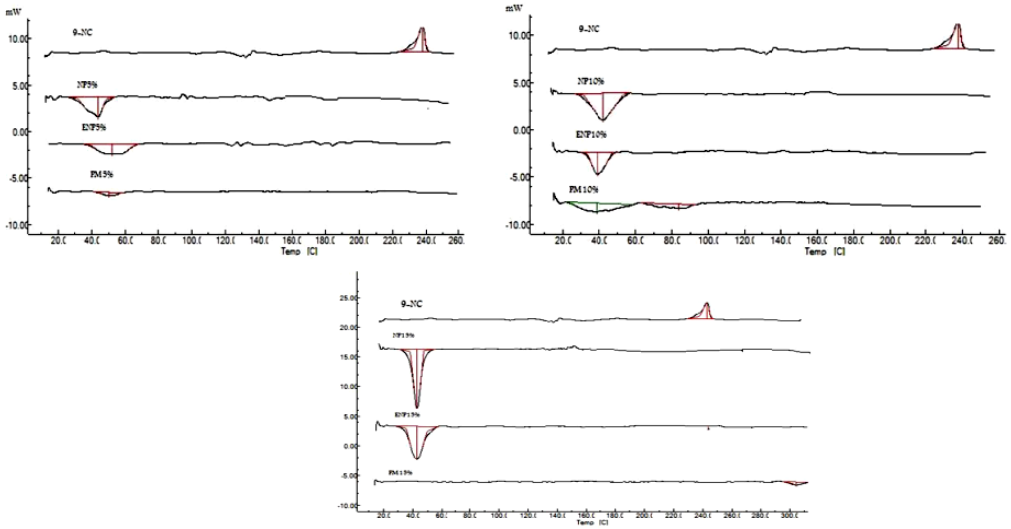

Fig. 4. DSC thermograms of drug, physical mixture of polymer and drug, polymer and nanoparticles

As shown in Fig.5., the C=O stretching vibration at 1750 were related to the PLGA and PLGA-PEG copolymer. In nanoparticles loaded 9-NC the peak of carbonyl group of polymers shift to 1730 that confirmed drug was loaded in hydrophobic inner core of amphiphilic polymers.

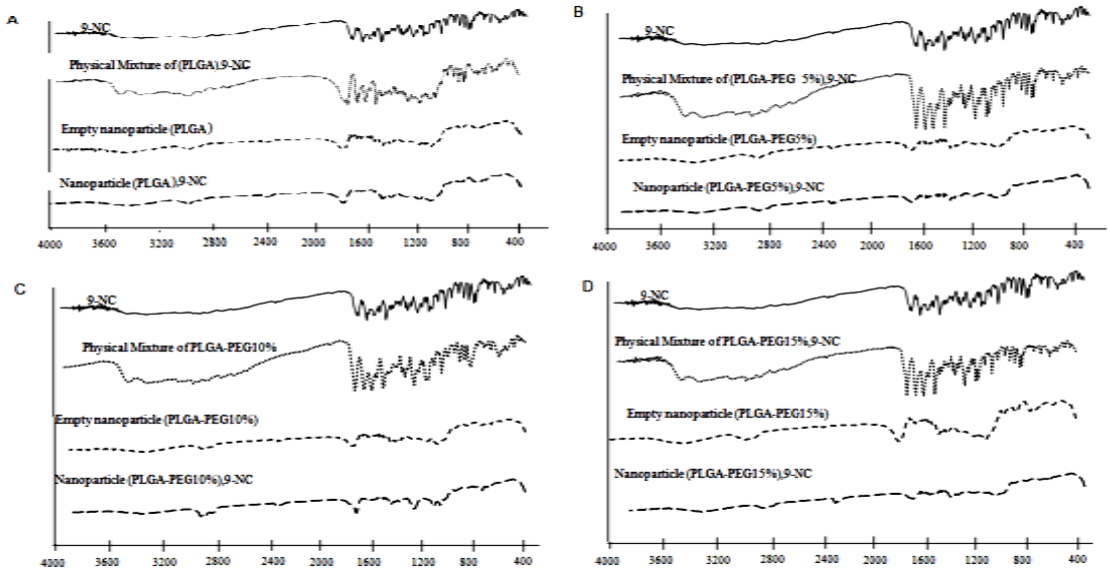

Fig. 5. FTIR of polymers, physical mixtures of drug and polymers and nanoparticles loaded 9-nitrocamptothecin

Encapsulation efficiency

As shown in Fig.6., with the increasing of polymer amount and aqueous phase volume, drug loading increased whether with the increasing of emulsifier amount and organic phase volume, drug loading decreased.

Table 4 was shown the effect of a PEG percent in PLGA-PEG nanoparticles on encapsulation efficiency.

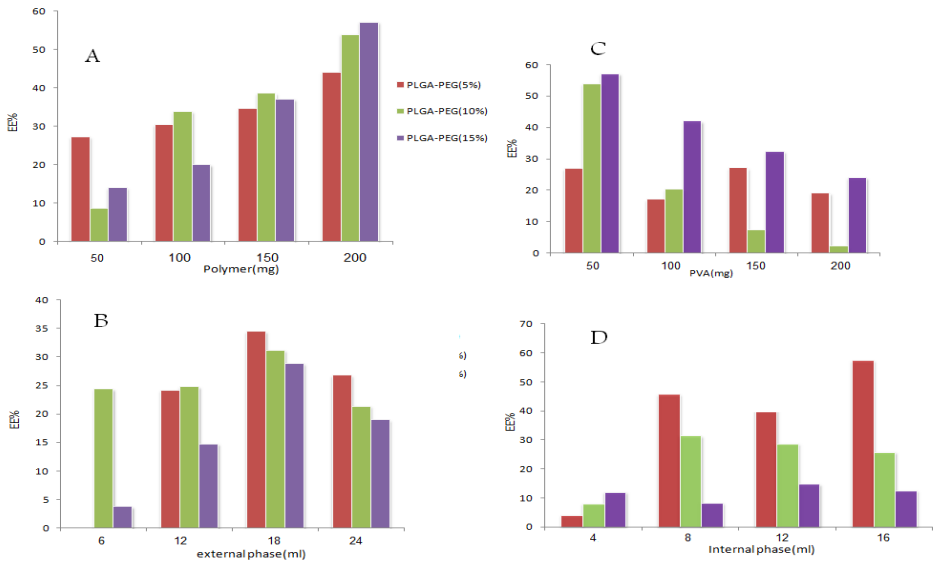

Fig. 6. The effect of polymer amount, emulsifier amount, internal phase volume and external phase volume on Encapsulation efficiency of nanoparticles

The hydrophilic nature of PEG shell caused hydrophobic drug encapsulated in the hydrophobic PLGA core. Because of the hydrophobic nature of 9-NC with increasing the PEG percent in PLGA-PEG nanoparticle drug loading increase (Fig. 7.).

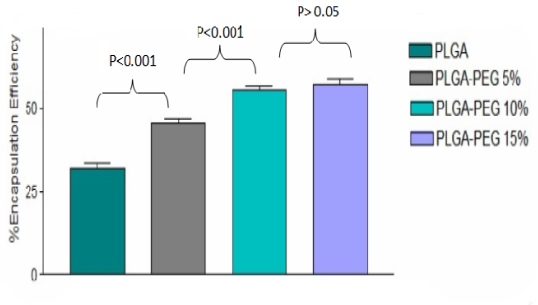

Figure 7. Effect of PEG percent of copolymer structure on drug loading

In vitro drug release profile

In vitro release of 9-NC loaded PLGA-PEG nanoparticles with different percent of PEG (0-15%) were performed in PBS (0.1M, pH 7.4) at the 37 ^{o}C and sink condition.The release profiles of 9-NC from nanoparticles with different percent of PEG are shown in Fig. 8. The release profiles showed sustained release up to more than 190 h. By increasing of PEG percent in PLGA-PEG nanoparticles, the release rate reduced but the time of release become longer.

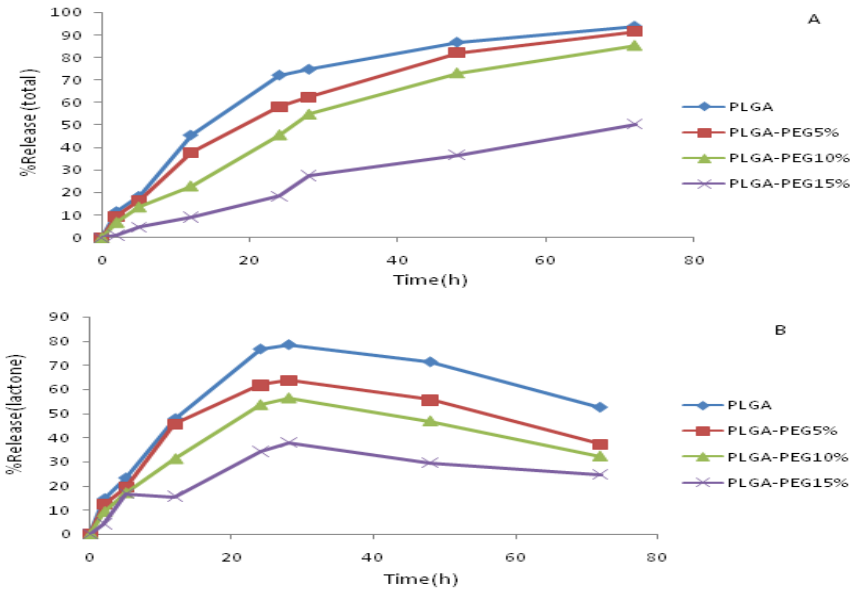

Fig. 8. Effect of PEG percent in PLGA-PEG nanoparticles on the release profile of 9-NC (A: total and B: lactone form)

The best release was observed in PLGA-PEG nanoparticles contian15% of PEG.

The release profiles were fitted in to release kinetic models and Higuchi model was best fitted to them (R^2= 0.913, %error =14.09).

In vitro stability of 9-NC

The lactone (active) form of 9-NC is unstable at 37^{o}C and rapidly converts to the inactive carboxylate form. The stability of lactone form of encapsulated drug was evaluated at 1, 2, 7 and 14 days in different conditions. As shown in Fig. 9., the increasing of a PEG percent in PLGA-PEG nanoparticles, the stability of lactone form increased and the best stability was observed in PLGA-PEG (15%) nanoparticles.

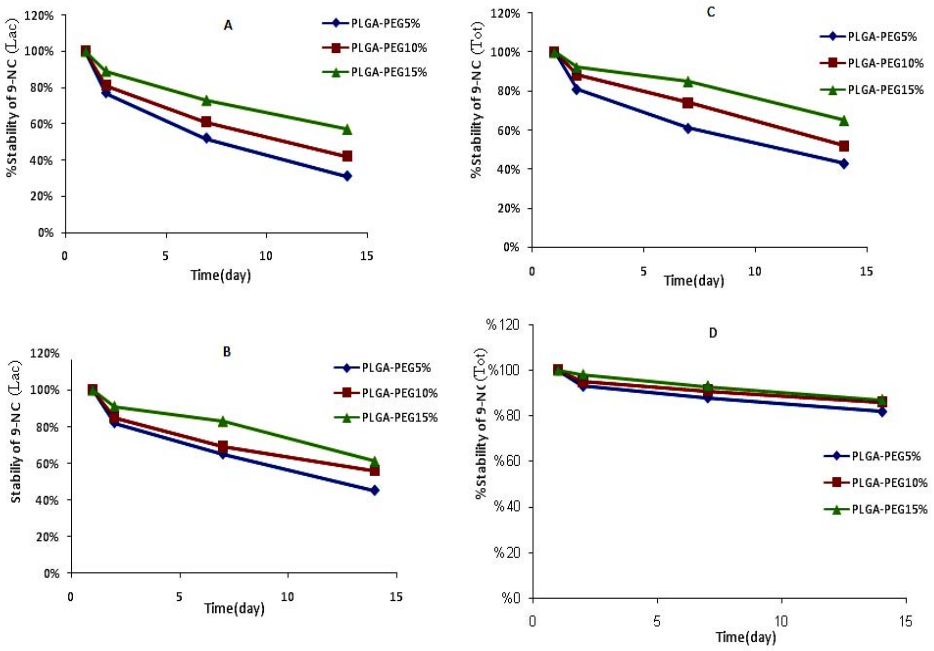

Fig. 9. In vitro stability of 9-NC(A: nanoparticles dried with desiccator at room temperature, B: nanoparticles dried with desicatore at 4 ^{o}C, C: nanoparticles dried with freeze dryer at room temperature, D: nanoparticles dried with freeze drier in 4 ^{o}C)

Discussion

Carrier-mediated drug delivery offers a number of design opportunities to tailor a delivery route around the details of particular drugs, including their modes of action and potential side effects.

Such carrier functions can be quite diverse, including transport to the targeted tissue, control of intracellular distribution, and protection against degradation or elimination of the mononuclear phagocytic systems (MPS).All these features prompted us to investigate the possibility of developing these nanoparticles as a controlled release drug formulation. In the develop-pment of new colloidal structures as circulating carriers, the assessment of their performance as drug carriers necessarily comprise the knowledge of the loaded amount of drug, its physical state and distribution inside the carrier, as well as its release kinetics. Similar to our investigation, Gu et al used polymeric micelle loaded with 9-NC to improved stability and proved that over 90% of 9-NC keep its lactone form in micelle solution after incubating in phosphate-buffered saline for loomining, while the corresponding proportion for free drug solution was 25% [43]. Derakhshandeh et al. used PLGA nanoparticle loaded 9-NC to improve its physic-cochemical characteristics and concluded that drug loading was more than 30% and nanoparticle size was 207 nm. They found that this carrier increased the cytotoxicity effect of 9-NC about 10 times higher than free drug [7].

In another study on encapsulated 9-NC in PLGA-PEG copolymer (15%), they showed that optimized formulation had narrow size distribution and drug loading of more than 45%.[11]

Lu et al indicated that 9-NC loaded folate-conjugated of polymers were stable during storage at 4^{o}C for 4 weeks [1].Li et al used nanocrystal formulation for Camptothecin (CPT) anticancer agent as a way to circumvent poor stability challenges and storage stability study. It is indicated that the nanocrystal were stable for at least six months and exhibited a significant suppression of tumor growth [43].

Lee et al chose MPEG-PLGA diblock copolymer with different hydrophilic and hydrophobic balances by changing PLGA segments under constant MPEG segments and the droplet size nano-emulsified paclitaxel were found in the range of 200-300 nm by dynamic light scattering. It is concluded that PLGA-

PEG nanoparticle could stabilize paclitaxol during storage and increased its cytotoxicity efficacy [44].
In this paper we evaluated the effect of PEG percent in PLGA-PEG copolymer on physicochemical characterization of nanoparticles loaded 9-NC and concluded that by increasing the PEG percent in PLGA-PEG copolymer the zeta potential, drug loading and stability increased while the size and release rate decreased. The best formulation according to physicochemical properties and stability data was PLGA-PEG nanoparticles with 15% of PEG.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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References

[1] Lu W, Feng L, Zhan Ch. 9-NC-Loaded folate-conjugated polymer micelles as tumor targeted drug delivery system. Int J of Pharm. 2009;372:125–131.
[2] Fang X, Sha X, Xin H, Jiang Y. Enhanced anti-tumor effect of 9-NC complexes by Hydroxy propyl-b-cyclodextrin. Int J Pharm. 2011;415:252–258.
[3] Jiang X, Liu Ba, Zhang L, Wang Q, Yang M. 10-Hydroxy camptothecin loaded nanoparticles: preparation and antitumor activity in mice. J Controlled Release. 2007;119:153–162.
[4] Verma RP. Understanding of topoisomerse I and II in terms of QSAR. Bioorganic and Medicinal chemistry. 2005;13:1059–1067.
[5] Ping Q, Chen J, Guo J. Effect of injection routs on pharmacokinetics and lactone/carboxylate equilibrium of 9-NC in rats. Int J Pharm. 2007;340:29–33.
[6] Yadav SC, Kumari A. Biodegradable polymeric nanoparticles based drug delivery systems. Colloid and surface. 2010;75:1–18.
[7] Derakhshandeh K, Erfan M, Dadashzadeh S. Encapsulation of 9-nitro camptothecin, a novel anticancer drug, in biodegradable nanoparticles: factorial design, characterization and release kinetics. Eur J Pharm Biopharm. 2007;66:34–41.
[8] Fang X, Xu B, Wu J, Xing B. Biodistribution in mice and severity of damage in rat lungs following pulmonary delivery of 9-NC liposomes. Pulmonary Pharmacology & Therapeutics. 2008;21:239–246.
[9] Gref R, Minamitake Y, Peracchia MT, Trochilin V. Biodegradable long-circulating polymeric nanospheres. Science. 1994;263:1600–1603.
[10] Kommareddy S, Tiwari SB, Amiji M. Long-circulating polymeric nanovectors for tumor selective gene delivery. Technol. Cancer Res. Treat. 2005;4:615–625.
[11] Derakhshandeh K, Soheili M, Dadashzadeh S, Saghiri R. Preparation and in vitro characterization of 9-nitrocamptothecin-loaded long circulating nanoparticles for delivery in cancer patients. Int J Nanomedicine. 2010;5:463–471.
[12] Luo Y, Prestwich GD. Cancer-targeted polymeric drugs. Curr. Cancer Drug Targets. 2002;2:209–226.
[13] Red head HM, Davis SS, Illum L. Drug Delivery in PLGA nanoparticles surface modified with poloxamer 407 and poloxamine 908. J. Controlled Release. 2001;70:353–363.
[14] Dunne M, Corrigan I. Influence of particle size and dissolution condition on the degradation properties of PLGA particles. Biomaterials. 2000;21:1659–1668.
[15] Panyam J, Dali M, Sahoo S. Polymer degradation and in vitro release of a model protein from PLGA nano and micro particles. J. Controlled release. 2003;92:173–187.
[16] Peracchia MT. Stealth nanoparticles for intravenous administration. S.T.P. Pharma Sci. 2003;13:155–161.
[17] Johnson RJ. The complement system. In: Ratner, B.D., Hoffman, A.S., Schoen, F.J., Lemons, J.E. (Eds.), Biomaterials Science: An Introduction to Materials in Medicine. Elsevier Academic Press, Amsterdam, 2004, P: 318–328.
[18] Mitchell RN. Innate and adaptive immunity: the immune response to foreign materials. In: Ratner, B.D., Hoffman AS, Schoen FJ, Lemons JE. (Eds.), Biomaterials Science: An Introduction to Materials in Medicine. Elsevier Academic Press, Amsterdam, 2004; 304–318.
[19] Bhadra D, Bhadra S. Pegnology: a review of PEG-ylated systems. Pharmazie. 2002;57:5–29.
[20] Gref R, Domb A, Quellac P, Blunk T, Muller RH. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanosphere. Adv Drug Del Rev. 1995;16:215–233.
[21] Small E, McMillan A, Meyer M, Chen L, Slichenmyer W, Lenehan P. Serum prostate-specific antigen decline as a marker of clinical outcome in hormone-refractory prostate cancer patients: associated with progression-free survival, pain end points, and survival. J Clin Oncol. 2001;19:1304–1304.
[22] Gu Zh, Gao J, Ming J, He B, Fan Y, Zhang X. Preparation and characterization of novel polymeric

micelles for 9-nitro-20(S)-camptothecin delivery. Eurpean J pharm sc. 2008;34:85–93.
[23] Kim K, Jeong S, Jo H, Bae S, Lee S. Hydrophobically modified glycol chitosan nanoparticles –encapsulated camptothcin enhance the drug stability and tumor targeting in cancer. J controlled release. 2008;127:208–218.
[24] Alvarez-Lorenzo C, Barreiro-Iglesias R, Bromberg L, Temchenko M, Hatton T.A, Cocheiro A. Solubilization and stabilization of camptothecin in micellar solutions of pluronic-g-poly(acrylic acid) copolymers. J control release. 2004;97:537–549.
[25] Peracchial MT. PEG-Coated nanopheres from amphiphilic diblock and multiblock copolymers. J. Controlled Release. 1997;46:223–231.
[26] Hinz H, Harris N, Natelson E, Giovanella B. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res. 1994;54:3096–100.
[27] Govender T, Riley T, Stohik S, Davis S. Definding the drug incorporation properties of PLA-PEG nanoparticles. Int. J. Pharm. 2000;199:95–110.
[28] Hosseini SM, Amiri M, Najarian S, Dargahi J. Application of artificial neural networks for the estimation of tumour characteristics in biological tissues. International Journal of Medical Robotics and Computer Assisted Surgery. 2007;3:235–244.
[29] Rafienia M, Amiri M, Janmaleki M, Sadeghian A. Application of Artificial Neural Networks in controlled drug delivery Systems. Applied Artificial Intelligence. 2010;24:807–820.
[30] Lim C P, Quek SS, Peh KK. Prediction of drug release profiles using an intelligent learning system: an experimental study in transdermal iontophoresis. J. Pharma. Biomed. Anal. 2003;31:159–168.
[31] Amiri M, Rafienia M, Sadeghian A. Estimation of betamethasone release profiles from an in situ forming system based on the biodegradable polymer using artificial neural networks. IFMBE Proceedings. 2009:2016–2019.
[32] Wachowiak MP, Elmaghraby AS, Smolikova R, Zurada JM. Generalized regression neural networks for biomedical image interpolation. Int. Joint Conf. on Neural Networks (IJCNN) 2001:2133–2138.
[33] Amiri M, Davandeh H, Sadeghian A, Seyyedsalehi S.A. In Proceedings of the International Joint Conference on Neural Networks. (IJCNN) 2007;18:2664–2664.
[34] Amiri M, Davandeh H, Sadeghian A, Seyyedsalehi S.A. Auto-associative neural network based on new hybrid model of SFNN and GRNN. In Proceedings of the International Joint Conference on Neural Networks (IJCNN) 2007:2664–2670.
[35] Amiri M, Davandeh H, Sadeghian A, Chartier S. Feedback associative memory based on a new hybrid model of generalized regression and self-feedback neural networks. Neural Network. 2010;23:892–904.
[36] Quintanar D, Fessi H, Doelker E. Preparation techniques and mechanism of formation of biodegradable nanoparticles from performed polymers. Drug-Dev Ind. Pharam. 1998;24:1113–1128.
[37] Chen T, Chen H. Universal approximation to nonlinear operators by neural networks with arbitrary activation functions and its application to dynamical system. IEEE Trans. Neural Networks. 1995;6:911–917.
[38] Davande H, Amiri M, Sadeghian A, Chartier S. Associative memory based on a new hybrid model of SFNN and GRNN: performance comparison with NDRAM, ART2 and MLP. IEEE World Congress on Computational Intelligence (WCCI) 2008:1699–1704.
[39] Specht DE. A general regression neural network. IEEE Transactions on Neural Networks. 1991;2:568–576.
[40] Amrouche A, Rouvaen JM. Efficient system for speech recognition using general regression neural network. International Journal of Computer Systems Science and Engineering. 2006;1:183–189.
[41] Simon L, Frenandes M. Neural network-based prediction and optimization of estradiol release from ethylene-vinyl acetate membranes. Comp. Chem. Eng. 2004;28:2407–2419.
[42] Riedmiller M, Braun H. A direct adaptive method for faster backpropagation learning: the RPROP algorithm. IEEE Int. Conf. on Neural Networks. 1993;1:586–591.
[43] Li T, Zhang H. Preparation and antitumor study of camptothecin nanocrystals. International Journal of Pharmaceutics. 2011;415:293–293.
[44] Lee SY, Hyun H, Youn JY, Kim BS, Song IB, Kim MS, Lee B, Khang G, Lee HB. Preoparation of nano-emulsified paclitaxel using MPEG-PLGA diblock copolymers. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2008;313:126–130.

