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Research paper

Encapsulation of 9-nitrocamptothecin, a novel anticancer drug, in biodegradable nanoparticles: Factorial design, characterization and release kinetics

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Abstract

This study was aimed at developing a polymeric drug delivery system for a new and potent antitumor drug, 9-nitrocamptothecin (9-NC), intended for both intravenous administration and improving the therapeutic index of the drug. To achieve these goals, 9-NC loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles were prepared by nanoprecipitation method and characterized. The full factorial experimental design was used to study the influence of four different independent variables on response of nanoparticle drug loading. Analysis of variance (ANOVA) was used to evaluate optimized conditions for the preparation of nanoparticles. The physical characteristics of PLGA nanospheres were evaluated using particle size analyzer, scanning electron microscopy, differential scanning calorimetry and X-ray diffractometry. The results of optimized formulations showed a narrow size distribution with a polydispersity index of 0.01%, an average diameter of 207 ± 26 nm, and a drug loading of more than 30%. The *in vitro* drug release profile showed a sustained 9-NC release up to 160 h indicating the suitability of PLGA nanoparticles in controlled 9-NC release. Thus prepared nanoparticles described here may be of clinical importance in both stabilizing and delivering camptothecins for cancer treatment.

Keywords: 9-Nitrocamptothecin; PLGA; Nanoparticles; Full factorial experimental design; Release kinetics

1. Introduction

Camptothecins and related analogues are a promising family of anticancer agents with a unique mechanism of action, targeting the nuclear enzyme of Topoisomerase I. Chemically, the analogues share the common features of a planner aromatic five-ring system with a lactone moiety. Structure–activity investigations elucidated that the preservation of the lactone ring of camptothecins is crucial for their antitumor activity. 9-Nitrocamptothecin (9-NC, RFS2000, Fig. 1) is a novel, lipophilic analogue of camptothecin with a high antitumor activity against advanced pancreatic carcinoma, ovarian epithelial cancer and leukemia [1–5]. Unfortunately, all camptothecin derivatives undergo a pH-dependent, rapid and reversible hydrolysis from closed lactone ring to the inactive hydroxy carboxylated form with loss of antitumor activity. In acidic medium, the lactone structure predominates, while at alkaline pHs, including physiological pH, the formation of the carboxylate is favoured [6].

Because of instability at biological pH and low water solubility, the delivery of lipophilic derivatives like 9-NC is quite challenging.

To address these problems, some drug delivery systems have been offered. For example camptothecin was prepared as nanocrystalline suspensions [7], incorporated into

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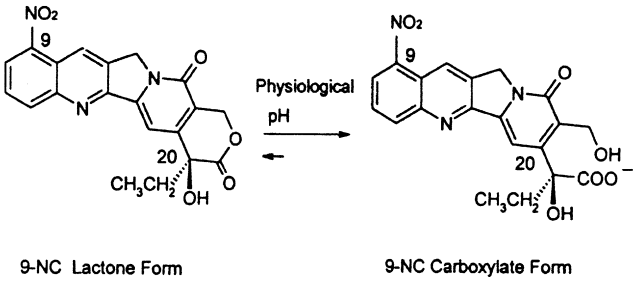

Fig. 1. Chemical structures and equilibrium reaction between the lactone and carboxylate forms of 9-nitrocamptothecin (9-NC).

PLGA microspheres [8] and solid lipid nanoparticles (SLNs) [9,10]. However, with the exception of aerosolized liposome preparation, no suitable delivery system especially for IV administration of 9-NC has been offered. Aerosolized liposome preparation of 9-NC has been remarkably effective in the treatment of human cancer subcutaneous xenografts and pulmonary cancer metastasis in nude mice [11] as well as in patients with advanced pulmonary malignancies [12].

Recently, increasing attention has focused on formulating therapeutic agents in biocompatible nanocomposites such as liposomes, nanocapsules, micellar systems and conjugates [13–18]. Since these systems are often polymeric and submicron in size, they can in general be used to provide targeted delivery (cellular/tissue) of drugs, to improve bioavailability, to sustain drug effect in target tissue, to solubilize drug for intravascular delivery and to improve the stability of therapeutic agents from enzymatic degradation [19–21].

Polymeric nanospheres composed of PLGA as a biocompatible and biodegradable compound have lower toxicity compared to other polymers and have more stability than liposomes or SLNs and other drug delivery systems in biological environments. PLGA is composed of lactic and glycolic acids linked together by ester bonds. The polymer degradation proceeds with formation of free carboxylic end groups. Several current reports have indicated the presence of low micro environmental pH in large specimens of PLGA [23–26].

The commonly reported methods of preparing nanoparticles from biodegradable polymers include solvent evaporation, monomer polymerization, nanoprecipitation and salting out procedure [27]. The nanoprecipitation method developed by Fessi et al. [28] represents an easy and reproducible technique and very often used to prepare colloidal carriers both matricidal (nanosphere) and vesicular type (nanocapsules). This method were based on the interfacial deposition of a polymer following diffusion of a semi-polar and miscible solvent in the aqueous medium in the presence of a surfactant [29].

Several important factors contribute to the effectiveness of this method in preparing particles with acceptable size range, shape and the percentage of the drug load, namely the amount of polymer, percentage of surfactant and volume of organic and aqueous phases. It is difficult to assess the effect of the variables individually or in combination, therefore deriving a mathematical model suitable for establishing a quantitative relationship between the formulation variables is necessary [9,30,31].

The aim of present study was to prepare 9-NC loaded nanoparticles by nanoprecipitation method. A two-level factorial design experiment was used for obtaining a mathematical model and prediction of optimized formulations. The resulted 9-NC nanoparticles were characterized with regard to morphology, size, drug loading and in vitro drug release.

2. Experimental

2.1. Materials

9-Nitrocamptothecin (9-NC), 99.8% pure, was purchased from Yuanjian Pharmaceutical Technology Develop Co., (China). Poly(DL-lactide-co-glycolid) (PLGA, 50:50 M_W 12,000, inherent viscosity of 0.16–0.24 dl/g) was obtained from Boehringer Ingelheim Co. (Ingelheim, Germany) in the form of Resomer(R) 502H. Polyvinyl alcohol (PVA, M_W 30,000 Da, 87% hydrolyzed) was a gift from Mowiol (Germany). Analytical grade acetone, pure potassium dihydrogen phosphate, dichlromethane were purchased from Merck (Darmstadt, Germany). All other chemical reagents used were of pharmaceutical grade.

2.2. Preparation of 9-NC- loaded nanoparticles

Nanoparticles were prepared by nanoprecipitation method as described by Fessi et al. [25]. The preparation procedure was as follows: exact quantity of PLGA polymer and 9-NC (1 mg) was accurately weighted and dissolved in acetone. The organic phase was added drop-wise (0.5 ml/min) into PVA aqueous solution (pH was adjusted to 3 by 0.1 N HCL) and stirred magnetically at room temperature until complete evaporation of the organic solvent. Subsequently, nanoparticles were separated by ultracentrifugation (Beckman, XL-90) at 40,000 rpm and 4 °C for 1 h. The separated nanoparticles were redispersed and centrifuged three times in distilled water (pH 3) to completely remove free drug and excess surfactants. The acidic condition in this procedure was used to stabilize the lactone form of 9-NC.

2.3. Experimental design

Based on preliminary study of the effect of parameters on the drug loading of nanosphere, the experiments were performed by nanoprecipitation method using a two-level full factorial design. Design-expert version 6 software was applied for designing the experiment. Four variables were taken at its two levels: low and high, which were represented by transform values of −1 and +1, respectively. Values of these selected variables are shown in Table 1. Sixteen batches of nanoparticles were prepared according to

Table 1
Values and coded units of 2^4 factorial design for preparation of 9-NC nanoparticles by nanoprecipitation method

Variables	Coded units	Levels	
		1	2
PLGA (mg)	*A*	50	100
Organic phase (ml)	*B*	10	15
PVA (%)	*C*	1	2
Aqueous phase (ml)	*D*	20	40

experimental design shown in Table 2 (four variables and one response of drug loading). Each batch was prepared three times and mean drug loading values were determined (Table 2).

Based on the results and mathematical models the software offered optimum setting of formulation as shown in Table 4.

2.4. Characterization of 9-NC nanoparticles

2.4.1. Size measurement

Nanoparticle mean diameter and polydispersity index were determined using photon correlation spectroscopy (PCS) (SEMATech). The analysis was performed at a scattering angle of 90° and at a temperature of 25 °C using samples appropriately diluted with ultrapurified water.

Table 2
Experimental design and percentage of drug loading ($n = 3$)

Batch No.	*A*	*B*	*C*	*D*	Drug loading % (mean ± SD)
1	100	15	1	40	15.61 ± 0.62
2	50	10	1	25	19.67 ± 0.80
3	50	15	2	25	20.41 ± 0.74
4	100	10	2	40	16.53 ± 0.66
5	50	15	1	40	13.52 ± 0.55
6	100	10	2	25	27.37 ± 1.08
7	100	15	1	25	26.58 ± 0.93
8	50	15	2	40	13.66 ± 0.86
9	50	10	2	25	21.54 ± 0.81
10	100	10	1	40	17.33 ± 0.75
11	100	10	1	25	28.61 ± 1.17
12	50	10	2	40	14.79 ± 0.66
13	100	15	2	25	24.68 ± 0.85
14	50	10	1	40	14.51 ± 0.61
15	50	15	1	25	18.50 ± 1.01
16	100	15	2	40	15.61 ± 0.89

Table 3
Analysis of variance for a 2^4 Factorial design experiment for evaluation of some variables' effect on the 9-NC loading in PLGA nanoparticles

Variables	Sum of squares	df*	*F* value
A	79.52	1	404.55*
B	8.45	1	43.01*
C	1.806e−003	1	9.189e−003
D	270.52	1	1376.20*
AC	4.01	1	20.40*
AD	21.09	1	107.31*
CD	0.07	1	0.34
ACD	2.04	1	10.37

df, degrees of freedom.
* Significance level based on 1 df; $p < 0.01$.

Table 4
Factorial design optimized formulations

Batch No.	*A*	*B*	*C*	*D*	Drug loading %	
					Theoretical	Experimental[a] (mean ± SD)
17	165.73	14.05	1.88	25.62	25.63	29.41 ± 1.56
18	152.89	10.07	1.27	20.71	28.12	30.72 ± 1.64
19	165.27	12.00	1.31	21.33	29.65	33.50 ± 1.31
20	141.45	13.91	1.09	23.38	26.14	21.59 ± 0.93
21	146.03	10.53	1.22	20.15	27.43	17.82 ± 0.86
22	148.95	13.92	1.48	20.82	27.55	16.84 ± 0.84
23	182.94	11.62	1.59	22.08	32.36	30.36 ± 1.18
24	144.96	14.78	1.98	22.96	25.38	21.43 ± 0.95
25	192.65	14.66	1.47	25.67	27.67	30.22 ± 1.47
26	150.01	14.71	1.19	20.20	28.12	25.11 ± 0.98

[a] Each batch was prepared three times.

2.4.2. Particle morphology

The morphology of nanospheres was determined using scanning electron microscopy (SEM) (Phillips, the Netherlands), the sample were prepared on aluminum stabs and coated with gold prior to examination by SEM.

2.4.3. Differential scanning calorimetry (DSC)

DSC experiments were carried out to characterize the physical state of 9-NC in PLGA nanospheres. Five to ten milligrams of the 9-NC, PLGA, nanospheres and physical mixture of drug and polymer was put in aluminum pans and hermetically sealed. The heating rate was 10 °C/min, nitrogen served as purge gas and the system was cooled down by liquid nitrogen. The DSC (DSC-60, Shimadzu Co., Kyoto, Japan) instrument was calibrated for temperature using octadecane and indium. Furthermore, for enthalpy calibration, indium was sealed in aluminum pans with a sealed empty pan as a reference. Triple runs were carried out on each sample to check reproducibility.

2.4.4. X-ray diffractometry (XRD)

X-ray diffractometer, Philips APD, was used for diffraction studies. XRD studies were performed by exposing samples to CuK_α radiation (40 kV, 20 mA) and scanned from 2° to 70°, 2θ at a step size of 0.02° and step time of 5 S. Samples used for XRD analysis were the same as those used for DSC analysis.

2.4.5. Determination of drug loading and nanoparticle recovery

Nanoparticles were dissolved in dichloromethane (2 ml) a common solvent for PLGA and the drug. The organic solvent was evaporated under a gentle stream of nitrogen gas then it was dissolved in 1 ml mobile phase of HPLC assay. The concentration of 9-NC was analyzed by our previously reported HPLC method [32]. In brief, the HPLC

system consisted of a model 1001 solvent delivery equipped with a model 2700 ultraviolet detector at 370 nm (all from Knauer, Germany). The analytical column was Nucleosil-100 C8 and the mobile phase was a mixture of acetonitrile-water (43:57, v/v, pH 5.5). The retention time of 9-NC was about 4 min. The intra-day and inter-day variability of assay were less than 5%.

The amount of drug loading in nanoparticles and the nanoparticle recovery, also known as nanoparticle yield, was calculated using the following equations:

Drug loading (%) = (amount of 9-NC in nanoparticles/amount of 9-NC used in formulation) × 100.

Nanoparticle recovery (%) = (mass of nanoparticles recovered/mass of polymeric material and drug) × 100.

2.4.6. In vitro drug release

The in vitro drug release of the nanoparticles was determined by dialysis method using Franz diffusion cell. As regards to sink condition, 2 ml of nanoparticle suspension in 0.1 M, pH 7.4, phosphate buffer saline (PBS) was placed in donor site and 50 ml PBS in receptor chamber incubated at 37 °C under magnetic stirring (200 rpm). At specific time intervals, 1 ml of medium was taken and replaced with the same volume of fresh PBS. For assay of total form of 9-NC, the taken samples were acidified with 5% perchloric acid before HPLC analysis. The concentration of released 9-NC was determined by HPLC method as described in Section 2.4.5. Each dissolution study was carried out in triplicate.

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.

3. Results and discussion

In recent years significant efforts have been devoted to develop nanotechnology for suitable means of delivering small molecules like anticancer drug and antimicrobials as well as macromolecules such as protein, peptides and genes by either localized or targeted delivery to the tissue of interest [22,23]. Numerous investigations have shown that both tissue and cell distribution of anticancer drugs can be controlled by entrapment in submicron colloidal systems such as liposomes and nonoparticles. The rational behind this approach is to increase antitumor efficacy, while reducing systemic side effects [17]. For instance, it has been demonstrated that doxorubicin nanospheres as compared to free drug have lower cardiotoxicity and renal damages [33]. Likewise, administration of paclitaxel (PTX) as one of the most effective anticancer agents exhibited high incidences of adverse reactions like neurotoxicity and myelosuppression. NK105, a micellar nanoparticle formulation, was developed to overcome these problems and to enhance the antitumor activity of PTX [34].

9-NC is a novel and potent anticancer drug which has demonstrated high anti-tumor activity against various tumors [1–5]. In order to increase antitumor efficacy, while reducing systemic side effects, 9-NC loaded PLGA nanoparticles were prepared and characterized in the present study. Furthermore, it was anticipated that loading 9-NC in nanoparticles would protect the active lactone form of the drug from rapid hydrolysis under physiological pH [24,25]. As mentioned before camptothecins clinical applications are limited by drug inactivation at physiological pH. These potent chemotherapeutic agents undergo rapid hydrolysis ($t_{1/2}$ ~20 min at 37 °C, pH 7.4). This reaction is a reversible pH sensitive interconversion from the potent lactone form (stable below pH 5) to the poorly active carboxylate form (stable above pH 8) (6, 25). It is well documented that PLGA microspheres can develop acidic microclimate [25,38] and there are several reports regarding the central role of microclimate pH in PLGA for controlling the stability of encapsulated substances for numerous molecules [24,25,36,37]. 10-Hydroxy camptothecin (10-HCPT), an analogue of camptothecin with a hydrolysis half-life of 21 min, was stabilized in PLGA microspheres for more than 10 weeks (>95% lactone) under a simulated physiological environment [24]. Further mechanistic investigation revealed that PLGA microparticles develop an acidic microclimate that stabilizes the lactone form of 10-HCPT [25]. Vincristin degradation in PLGA through acid-catalyzed loss of the *N*-formyl group was completely inhibited by neutralization of acidic PLGA microclimate [36].

Although there is no published report on the microclimate pH in the bulk of PLGA nanoparticles, one according to abovementioned reports can speculate that PLGA nanoparticles are expected to stabilize camptopthecins due to their acidic microclimate. However, this hypothesis should be confirmed by further studies.

3.1. Preparation of nanoparticles

A technique of two-level factorial design offers the possibility of investigating four independent variables at two levels after performing only sixteen experiments. The selection of factors and levels in the design, which most affects drug loading, would be based on the results of preliminary investigations.

The nanoparticle preparation process by nanoprecipitation method, apparently simple, may involve complex interfacial hydrodynamic phenomena. Addition of acetonic-oily solution resulted in spontaneous emulsification of the oily solution in the form of nanodroplets, due to some kind of interface instability arising from rapid diffusion of the acetone across the interface and marked decrease in the interfacial tension. The origin of the mechanism of nanosphere formation could be explained in terms of interfacial turbulence or spontaneous agitation of the interface between two unequilibrated liquid phases, involving flow, diffusion and surface processes (Marangoni effect) [28]. Davis and Rideal suggested that the interfacial turbulance is caused by localized lowering of the interfacial tension

where the oil phase undergoes rapid and erratic pulsations or "kicks" each of which is quickly damped out by viscous drag. The molecular mechanism of interfacial turbulence could be explained by the continuous formation of eddies of solvent (e.g. acetone) at the interface. Such eddies originate either during drop formation or in thermal inequality in the system. Thus, once the process has started, movements associated with previous kicks change the pressure inside the solvent by increasing the surface pressure inside the solvent or decreasing the interfacial tension. Thus, if the solvent droplets formed contain polymer, these will tend to aggregate and form nanoparticles because of continuous diffusion of solvents and because of the presence of a nonsolvent medium [27].

Among various factors, solute transfer out of the phase of higher viscosity, concentration gradients near the interface and interfacial tension sensitive to solute concentration are the most important factors. The presence of surfactant may markedly complicate the situation since they act to suppress interfacial flow and the rapid diffusion of acetone to the aqueous phase. The main advantage of surfactants in process is the instantaneous and reproducible formation of nanometric, monodispersed nanospheres exhibiting a high drug loading capacity [28].

Primary independent factors that might affect drug loading in nanoprecipitation method are: amount of polymer (A), amount of surfactant (B), volume of organic phase (C) and volume of aqueous phase (D). We evaluated the influence of these parameters on drug loading by a full factorial experimental design. Sixteen batches of different combinations were prepared by taking values of selected variables: A, B, C, D at two levels as shown in Table 1.

The mathematical modeling of preparation of 9-NC nanoparticles was carried by the following equation:

$$Y = 19.28 + 2.23 \times A - 0.73 \times B + 0.011 \times C - 4.11 \times D - 0.5 \times AC - 1.15 \times AD - 0.064 \times CD + 0.36 \times ACD$$

where Y is dependent variable of drug loading. The result of analysis of variance (ANOVA) of the factorial design is presented in Table 3. Based on the results of ANOVA, for stability of the resulting suspension and the formation of high drug loaded nanoparticles the optimum setting was significantly ($p < 0.001$) affected by amount of polymer, external and internal phase volumes and interaction of these parameters. The optimum formulations offered by software are shown in Table 4. Each formulation was performed three times and mean drug loading values were recorded (Table 4). The calculated desirability factor for offered formulations was 1.00 indicating suitability of the designed factorial model. Nevertheless distinct deviations between theoretical and experimental values of response (drug loading) were found for batches 21 and 22. This may be due to the presence of some minor contributing variables for drug loading of prepared nanoparticles which were not considered in our study to reduce the number of experiments.

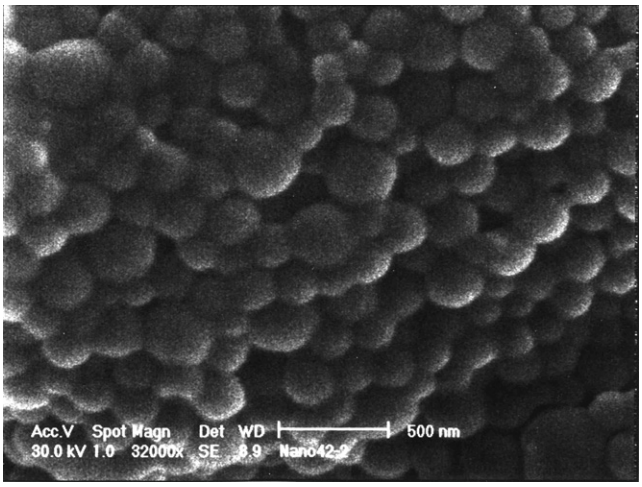

Fig. 2. Scanning electron microscopy image of 9-NC nanoparticles (32,000×).

As shown in Table 4 the optimum formulation with highest drug loading was achieved by 165.27 mg of polymer, 1.31% of emulsifier, 21.33 ml external phase and 12 ml internal phase volumes. The resulting 9-NC nanoparticles showed the best response to size with average of 207 nm, best recovery of 95% and optimum loading of about 33%.

3.2. Nanoparticle characterization

Freshly prepared 9-NC nanoparticles according to the optimized formulation and preparation conditions were rigid and spherical as shown in Fig. 2 by SEM. The number average diameter was 207 ± 26 nm and showed polydispersity of 0.01%. The particles were capable of redispersing homogeneously making them applicable for IV administration.

The DSC thermograms corresponding to 9-NC,PLGA, physical mixture and 9-NC nanoparticles are shown in Fig. 3. The PLGA thermogram displayed an endothermic peak at 45 °C, corresponding to the polymer transition temperature (T_g). The DSC curve of 9-NC showed a single melting peak at 217 °C and started to degrade as it melted. No 9-NC melting peak was visible in the case of drug loaded nanoparticles. This might be due amorphous state of the drug dispersed in the nanoparticles. Since there was no shift in the T_g of the polymer, it can be concluded that there is no significant interaction occurring between the drug and the polymer. For physical mixture no peak was detectable at the melting point of 9-NC. Since glass–liquid transition of PLGA occurs at relatively lower temperature (45 °C), it is possible that at higher temperatures molecular dispersion of drug in polymer occurs during DSC process. To confirm these results XRD was performed and results are presented in Fig. 4. As shown XRD patterns of 9-NC and physical mixture exhibit a sharp peak at about 2θ scattered angle 27 indicating crystalline nature of 9-NC. This characteristic peak for drug was absent in the nanoparticle XRD pattern

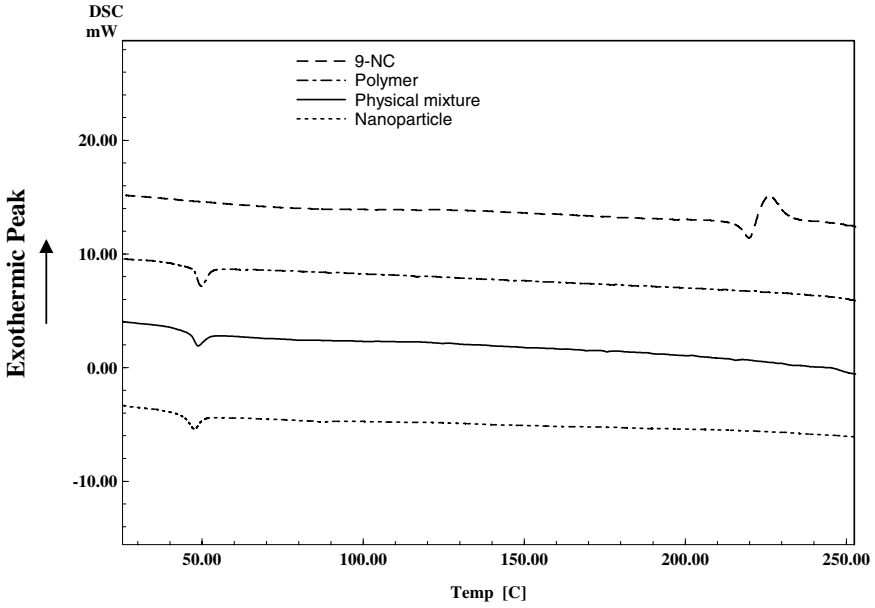

Fig. 3. Differential scanning calorimetry (DSC) thermograms obtained for 9-NC, polymer, drug–polymer physical mixture and nanoparticles.

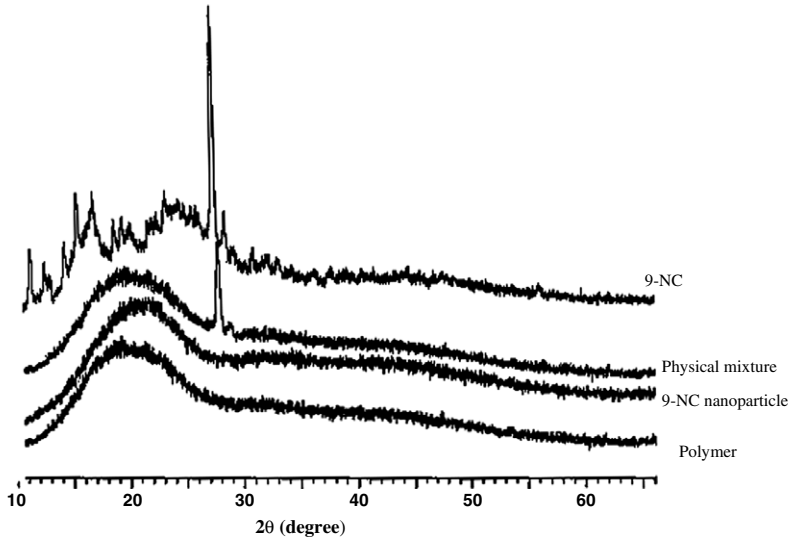

Fig. 4. XRD patterns of 9-NC, polymer, physical mixture of drug-polymer and nanoparticles.

suggesting that 9-NC was not in crystalline form in nanoparticles.

3.3. In vitro release of 9-NC nanoparticles

The amount of 9-NC released from nanoparticles during 1 week was evaluated using a dialysis technique. Dialysis membrane with a molecular weight cut-off of 12,000 Da (Sigma) was fixed on Franz diffusion cell and donor and acceptor medium were PBS (pH 7.4). The release profiles of total form of 9-NC from nanoparticles prepared with different amounts of polymer are shown in Fig. 5. About 20% of drug was released over a period of 20 h, followed by a distinct prolonged release up to more than 160 h. The followed delayed release may be attributed to diffusion of the dissolved drug within the PLGA core of the nanoparticle into the dissolution medium. As shown in Fig. 5, the amount of PLGA affected drug release rate and as the polymer content increased drug release rate was decreased distinctly.

Release kinetics was evaluated by fitting obtained data into first order, zero order and Higuchi equations. Based on the results, 9-NC release from nanoparticles followed Higuchi equation and related correlation coefficients were better than both zero order and first order kinetics (Table 5)

Two possible mechanisms may be involved in the release of 9-NC from nanoparticles.

The dissolution diffusion of the drug from the matrices and matrix erosion resulting from degradation of polymer. According to the model developed by Baker and Lonsdale,

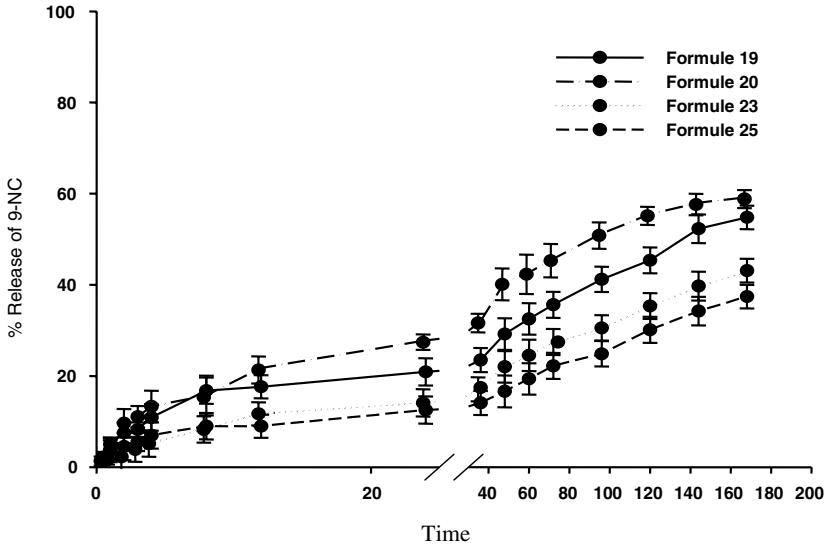

Fig. 5. In vitro release profile of 9-NC from PLGA nanoparticles in PBS (pH 7.4), data represent means (n=3).

Table 5
Kinetic fitting results of 9-NC released from nanoparticles in PBS

Batch No.	R^2			K^a (μg/h$^{0.5}$)
	Zero order	First order	Higuchi	
19	0.959	0.987	0.995	4.044
20	0.898	0.914	0.989	4.771
23	0.948	0.960	0.996	3.350
25	0.959	0.964	0.989	2.665

[a] Calculated based on the Higuchi model.

for a drug incorporated in a spherical matrix, a straight line is expected for the $3/2[1-(1-Q)^{2/3}]-Q$ versus time plot if drug release from the spherical matrix is based on a diffusion mechanism [35]. By fitting the observed data, shown in Fig. 5, to the spherical matrix model, correlation coefficients of 0.944–0.978 were obtained. The Hixson–Crowell cube root model $(100-Q_t)^{1/3}=100^{1/3}-K_{HC}.t$, where K_{HC} is the Hixson–Crowell rate constant describes the release from the systems, where it depends on the change in surface area and diameter of the particles with time and mainly applies in case of systems, which dissolve or erode over time [39]. By fitting the release data to this model, correlation coefficients of 0.901–0.941 were obtained. These results indicate that the release of 9-NC from nanoparticles may be more consistent with a diffusion mechanism than with a matrix erosion mechanism.

Overall *in vitro* release data indicate that PLGA based nanoparticles are capable to sustain 9-NC release rate successfully.

4. Conclusion

In the present study, the preparation and characterization of nanoparticles of 9-NC as a novel potent anticancer drug was carried out. For simultaneous analysis of the influence of different factors on the properties of the nanoparticles and to find optimum formulations, the formulation was optimized using a 2^4 factorial experimental design. The polymer amount and both external and internal phase volumes had a statically significant influence on the drug loading. The optimum formulation with highest drug loading was achieved by using 165.27 mg of polymer, 1.31% of emulsifier, 21.33 ml external phase and 12 ml internal phase volumes. In vitro release studies showed that PLGA nanoparticles could successfully control the release of 9-NC up to more than 160 h.

In general the results show that the PLGA nanoparticles may be considered as a promising carrier system for controlled release and targeted delivery of lipophilic drugs like 9-nitrocamptothecin with possible clinical application.

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