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ORIGINAL RESEARCH PAPER

Encapsulation of irinotecan in polymeric nanoparticles: Characterization, release kinetic and cytotoxicity evaluation

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ABSTRACT

Objective(s): Irinotecan is a potent anti-cancer drug from camptothecin group which inhibits topoisomerase I. Recently, biodegradable and biocompatible polymers such as poly lactide-co-glycolides (PLGA) have been considered for the preparation of nanoparticles (NPs).

Materials and Methods: In this study, irinotecan loaded PLGA NPs were fabricated by an emulsification/solvent diffusion method to improve the efficacy of irinotecan. The effect of several parameters on the NPs' characteristics was assessed, including the amount of drug and polymer, the amount and volume of the poly vinyl alcohol as a surfactant, and also the internal-phase volume/composition. The irinotecan entrapment efficiency and the particle size distribution were optimized by changing these variables. The cytotoxicity of the particles was evaluated by cell viability assay.

Results: NPs were spherical with a comparatively mono-dispersed size distribution and negative zeta potential. Selected formulation (S9) showed suitable size distribution about120 nm with relative high drug entrapment. MTT assay showed a stronger cytotoxicity of S9 against HT-29 cancer cells than control NPs and irinotecan free drug. The release kinetic indicated Log-Probability model in S9.

Conclusion: Our results demonstrated that the designed NPs show suitable characteristic and also great potential for further *in vivo* cancer evaluation.

Keywords: *Cell culture, Formulation, Irinotecan, Nanotechnology*

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INTRODUCTION

Irinotecan,7-ethyl-10-[4-(l-piperidino)-l-piperidino carbonyloxycamptothecin (CPT-11) is a water-soluble derivative and semi synthetic analogue of the natural alkaloid camptothecin [1-4]. Currently, it is used for the treatment of small cell lung cancer [2] and advanced colorectal cancer [3]. Irinotecan itself is a prodrug and is converted to 7-ethyl-10-hydroxy-CPT (SN-38), a biologically active metabolite of irinotecan, by carboxylesterases [4]. The schematic structure of irinotecan is shown in Fig. 1. Although SN-38 is highly potent *in vitro*, it doesn't regularly present potent antitumor activity *in vivo* due to its pharmacokinetic attribute [3-5]. Irinotecan, exhibits potent efficacy against various tumors *in vivo*, and displays more efficacy *in vivo* than SN-38 [2]. Nevertheless, irinotecan was found to have serious side effects such as myelosuppression and gastrointestinal disorders (mainly diarrhea), which are identified as constituting dose-limiting toxicity for this drug [2-3].

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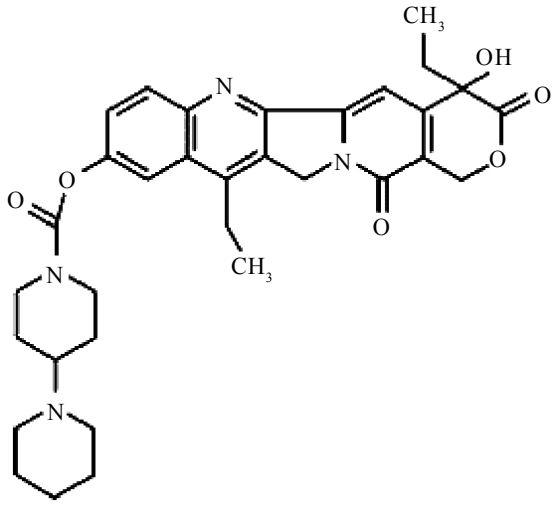

Fig. 1. The schematic structure of Irinotecan

A significant characteristic for camptothecins and related analogues is a rapid, reversible and pH-dependent hydrolysis from closed lactone ring to the carboxylate form [4-8]. More investigations have revealed that only lactone species is an inhibitor of topoisomerase I and exhibit low toxic effects [4-7]. Therefore it can be selected an attractive target for anticancer drug development. Consequently, finding an effective drug delivery system, to reduce toxicity and preserve the active form of the drug is very important. One of the essential challenges in cancer therapy is efficient systemic delivery of drugs so that they can reach disease parts throughout the body to keep the effective concentration in healthy cells and tissues [4-6].

Accordingly, dosage forms have been investigated to improve the antitumor effects of irinotecan. Since the antitumor characteristics of camptothecin is time dependent, novel formolations which exhibits long circulation residence in the body may enhance the efficacy of irinotecan [3-5].

Biodegradable polymers have been studied extensively over the past few decades for the fabrication of drug delivery systems [9-11]. Considerable research is being directed towards developing biodegradable polymeric NPs for drug delivery and tissue engineering, in view of their applications in controlling the release of drugs, stabilizing labile molecules (e.g., proteins, peptides, or DNA) from degradation, and site-specific drug targeting [12].

NPs with a diameter up to 400 nm can accumulate in diseased parts [13]. A possible explanation for the high level of selectivity observed with this system would be the enhanced permeability and retention (EPR) effect. This effect develops as follows: tumors induce angiogenesis by secreting various growth factors which in turn induce the development of a vascular system in tumors which is irregular in shape, dilated, and contains endothelial cells with multiple defects which create 'leaks' [13-14].

In this study PLGA formulation of irinotecan was developed and then the obtained NPs were fully characterized. The effect of several variables on the NPs' characteristics was also evaluated, including the amount of irinotecan, amount of poly vinyl alcohol (PVA) as surfactant, and internal-phase volume/composition. In addition the kinetic model of *in vitro* release study determined. Finally the cytotoxicity effects of prepared NPs and free drug determined against human tumor cell lines respectively.

MATERIALS AND METHODS

Irinotecan hydrochloride trihydrate was purchased from Aurisco Pharmaceutical (Shanghai, China). PLGA (50:50, Resomer 504) was purchased from BoehringerIngelheim, Germany. PVA (MW 22,000) was purchased from sigma-aldrich (St. Louis, MO, USA). Dichloromethane (DCM) and acetone (both analytical grade) were purchased from Merck (Darmstadt, Germany). HPLC-grade acetonitrile (ACN) was purchased from Merck (Germany). Water was purified in a Milli-QUV Plus System (Millipore, Bedford, MA, USA). Deionized water was used throughout the experiment. All other chemicals used were of reagent grade.

Nanoparticles preparation

The irinotecan loaded PLGA NPs were fabricated by a modified emulsification/solvent diffusion method [6, 9]. Concisely, as shown in Table 1, known amounts of polymer and irinotecan were added into DCM or mixture of DCM and acetone, which was stirred to ensure that all materials were dissolved. Then the dispersed phase was slowly dropped into the stirred PVA aqueous solution (pH was adjusted to 3 by 0.1N HCl) using a high speed homogenizer (ultra-turrax IKA, Wilmington, MA, USA) at 24,000 rpm. The formed o/w emulsion was gently stirred at room

Table 1. Various formulations of irinotecan loaded PLGA nanoparticles

Sample	Irinotecan (mg)	PLGA (mg)	Acetone (ml)	DCM (ml)	PVA (g)	Aqueous phase (ml)
S1	3	10	20	5	1	100
S2	3	10	20	5	0.5	100
S3	3	10	20	5	0.5	50
S4	3	10	20	5	0.3	100
S5	5	10	20	5	0.3	100
S6	5	10	20	5	1	100
S7	5	10	3	5	1	25
S8	5	10	10	5	0.5	100
S9	3	10	10	5	0.5	100
S10	3	5	5	5	0.5	50
S11	2	10	5	5	0.5	100
S12	2	10	5	5	0.5	30
S13	2	20	5	5	0.5	100
S14	3	30	5	5	0.5	100
S15	30	150	5	5	0.5	100
S16	20	200	5	5	0.5	100
S17	40	200	5	5	0.5	100
S18	20	200	20	10	0.5	100
S19	20	100	20	10	0.5	100

temperature by a magnetic stirrer to allow for the evaporation of the organic solvent. The NPs were collected by centrifuging (21,000 g, 30 min, sigma 3k30, Germany), followed by washing thrice with deionised water. The produced suspension was freeze dried for 48 hours (Lyotrap plus, LYE scientific Ltd, Oldham, UK) to obtain a fine powder of NPs, which was placed and kept in a freezer at -20 °C to protect the NPs from heat and degradation.

Particle size and size distribution

The Z-averageof particle size, polydispersity index and the distribution size of the prepared NPs were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern Instruments Limited, Worcestershire, UK). The dried powder samples were suspended in deionised water before measurement. The obtained homogenous suspension was examined to determine the volume mean diameter, size distribution and polydispersity.

Morphology

Scanning electron microscopy (SEM, Philips XL 30 scanning microscope, Philips, the Netherlands) was employed to determine the shape and surface morphology of the produced NPs. Particles were coated with gold under vacuum before SEM.

Surface charge

Zeta potential, an indicator of surface charge, was determined by a zeta potential analyzer (Zetasizer Nano ZS, Malvern Instruments Limited, Worcestershire, UK). The dried powder samples were suspended in deionised water and sonicated before measurement. The obtained homogenous suspension was examined to determine the zeta potential of the samples.

High performance liquid chromatography (HPLC) analysis

The drug entrapped in the NPs was determined in triplicate by HPLC. The release rate of irinotecan from the NPs was measured in phosphate buffered solution (pH 7.4) at 37 °C by HPLC in triplicate.

A simple HPLC method was developed for determination of irinotecanin NPs.

The HPLC equipment consisted of an integrated system with a Smartline pump (Knauer, Berlin, Germany), equipped with an ultraviolet detector (Knauer, Berlin, Germany).

A C18 column (Nucleosil H.P. 25 cm × 0.46 cm internal diameter, pore size 5 mm, Knauer, Germany) was used.

The mobile phase was comprised of tri ethyl ammonium acetate buffer (3%, pH=5) and acetonitrile, (70:30 v/v), and was delivered at a flow rate of 1.00 ml/min. The column effluent was detected at 254 nm [15].

Entrapment efficiency and drug loading

The drug entrapped in the NPs was determined in triplicate by HPLC analysis.

A 5-mg sample of freeze dried NPs was dissolved in 10 ml of acetonitrile and sonicated for 5 min.

The solution was filtered using RC-45/25 CHROMAFIL with pore size of 0.45 µm, Ø: 25 mm (MACHERY- NAGEL, Germany) into a vial and an aliquot of 20 µl was analyzed by the HPLC system to detect the irinotecan concentration.

The percentage of entrapment efficiency and drug loading of irinotecan in the NPs were calculated as follows:

$$\textit{Entrapment efficiency (\%)} = \frac{Weight\ of\ the\ actual\ drug\ in\ nanoparticles}{Weight\ of\ the\ feeding\ drug\ in\ nanoparticles} \times 100$$

$$\textit{Drug loading (\%)} = \frac{Weight\ of\ the\ actual\ drug\ in\ nanoparticles}{Weight\ of\ nanoparticles} \times 100$$

In vitro drug release

Drug release from irinotecan-loaded NPs was carried out using a modified dissolution method. A known mass of NPs was suspended in tubes of buffer solution at pH 7.4 (to maintain sink conditions) to simulate physiologic pH. The tubes were placed in a shaker bath (Memmert WB, Germany) at 37 °C and shaken horizontally at 90 cycles/min. At selected time intervals, the tubes were centrifuged and an aliquot of 900 ìl was taken from the supernatant. A volume of 100 ìl of ethanol was added and the concentration of released irinotecan was determined by HPLC method as described in previous section. Each dissolution study was carried out intriplicate. A calibration curve was prepared prior to the start of dissolution using a phosphate buffer : ethanol (9:1) medium.

After the aliquots were removed, the entire supernatant was replenished in order to maintain sink conditions. Drug release data were normalized by converting drug concentration in solution as percentage of the cumulative drug release.

Kinetic models

Various mathematical equations have been proposed for kinetic analysis of drug release from evaluated formulations. The kinetic models were those which have been used in interpretation of drug release data [8-11]. The equations of the kinetic models are presented in Table 2. In order to define a model, which will represent a better fit for the formulations, dissolution data can be further analyzed by equation in Table 2. Selection of the best model was based on the comparisons of the relevant correlation coefficients [16-17].

Cell culture study

Human colorectal adenocarcinoma cell lines (HT-29) (American Type Culture Collection) were cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a humidified incubator with 5% CO_2. Cells were maintained in an exponential growth phase by periodic subcultivation.

Table 2. Kinetic models used for analysis of drug nanoparticle release

No.	Model name	Model
1	Zero order	$F=k_0t$
2	First order	$\ln (1-F) = - k_ft$
3	Higuchi	$F = k_H\sqrt{t}$
4	Peppas (Power Law)	$\ln F = \ln k_p + p\ln t$
5	Hixon-Crowell	$1 - \sqrt[3]{1-F} = k_{1/3}t$
6	Square root of mass	$1 - \sqrt{1-F} = k_{1/2}t$
7	Three seconds root of mass	$1 - \sqrt[3]{(1-F)2} = k_{1/3}t$
8	Weibull	$\ln[-\ln(1-F)] = - \beta\ln t_d + \beta\ln t$
9	Linear Probability	$Z = Z_0 + qt$
10	Log-Probability	$Z = Z_0' + q'\ln t$
11	Reciprocal powered time	$(\frac{1}{F} - 1) = \frac{m}{t^b}$
12	Non-conventional order 1	$1 - (1-F)^{1-n} = (1-n)\ k_{1-n}t$
13	Non-conventional order 2	$\frac{1}{(1-F)^{n-1}} - 1 = (n-1)\ k_{n-1}t$

In vitro cell viability

HT-29 cells were seeded in 96-well plates (Costar, IL, USA) at the density of 1×10^4 viable cells/well and incubated 24 h to allow cell attachment. The medium was replenished every other day. The cells were incubated with the irinotecan or irinotecan NP suspension at concentrations of 0.1 to 100µg/ml for 48 h. The diluent for preparing the working solution was RPMI-1640 culture medium.

At designated time intervals 20 µl MTT (5 mg/ml in PBS) was added to the wells. After incubation for 3–4 h, the culture medium and MTT solution were removed and 100 µl di-methyl sulfoxide (DMSO) was then added to the wells before the plate was analyzed by the microplate reader. Cell viability was calculated by the following equation:

$$\text{Cell viability (\%)} = (Int_s/Int_{control}) \times 100,$$

Where Int_s is the fluorescence intensity of the cells incubated with the samples and $Int_{control}$ is the fluorescence intensity of the cells incubated with the PBS only (positive control). IC50, the drug concentration at which inhibition of 50% cell growth was observed in comparison with that of the control sample, was calculated by the curve fitting of the cell viability data.

Statistical analysis

One-way analyses of variance (ANOVA) test was performed on the data to assess the impact of the formulation variables on the in vitro results. P values of <0.05 were considered significant. All calculations were performed using a statistical software program (SPSS® 11.5, Microsoft).

RESULTS AND DISCUSSION

In order to increase antitumor efficacy, while reducing systemic side effects, irinotecan loaded PLGA nanoparticles were prepared and characterized in the present study. Furthermore, it was anticipated that loading irinotecan in nanoparticles would protect the active lactone form of the drug from rapid hydrolysis under physiological pH [21-30]. 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 versible pH sensitive inter conversion from the potent lactone form (stable below pH 5) to the poorly active carboxylate form (stable above pH 8) [6, 22]. It is well documented that PLGA microspheres can develop acidic microclimate [22, 26] and there are several reports regarding the central role of microclimate pH in PLGA for controlling the stability of encapsulated substances for numerous molecules [21-25]. 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 [21]. Further mechanistic investigation revealed that PLGA microparticles develop an acidic microclimate that stabilizes the lactone form of 10-HCPT [22]. Vincristin degradation in PLGA through acid-catalyzed loss of the N-formyl group was completely inhibited by neutralization of acidic PLGA microclimate [25]. According to above mentioned 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.Irinotecan loaded PLGA NPs were fabricated using various concentrations of PVA (0.3 % w/v, 0.5 % w/v, and 1 % w/v) and different ratio of drug: polymer. The mean particle size and the polydispersity of all samples were determined (Fig. 2). As shown in Table 3, the mean size of the NPs was between 124 to 365 nm as confirmed by the light scattering measurement.

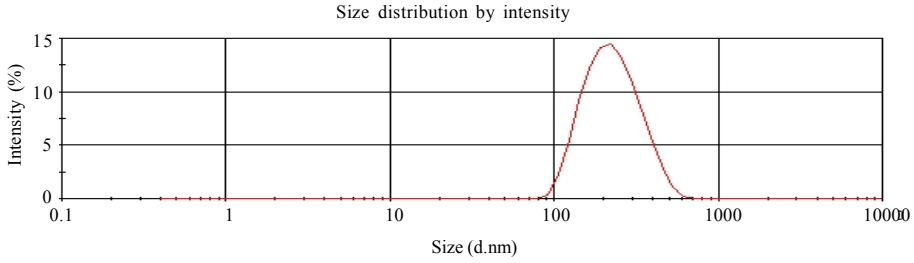

Fig. 2. The size distribution of Irinotecan loaded PLGA nanoparticles (S9) measured by laser light scattering method

Table 3. The physicochemical characteristics of irinotecan loaded PLGA nanoparticles

Sample	Mean Diameter (nm) ± SD	PdI*	Zeta Potential (mV)
S1	234 ± 3	0.19	-8.6
S2	210 ± 7	0.18	-11.9
S3	246 ± 10	0.11	-7.97
S4	266 ± 7	0.19	-7.1
S5	253 ± 3	0.20	-11.8
S6	264 ± 12	0.17	-2.99
S7	342 ± 6	0.24	-2.84
S8	298 ± 7	0.27	-12.2
S9	124 ± 12	0.28	-20.3
S10	278 ± 7	0.36	-6.45
S11	168 ± 3	0.40	-5.75
S12	180 ± 2	0.24	-9.28
S13	186 ± 4	0.21	-8.86
S14	233 ± 5	0.4	-3.79
S15	251 ± 3	0.46	-2.43
S16	219 ± 5	0.56	-1.07
S17	244 ± 3	0.34	-5.32
S18	365 ± 2	0.4	-4.06
S19	323 ± 5	0.35	-6.49

* PdI = polydispersity index

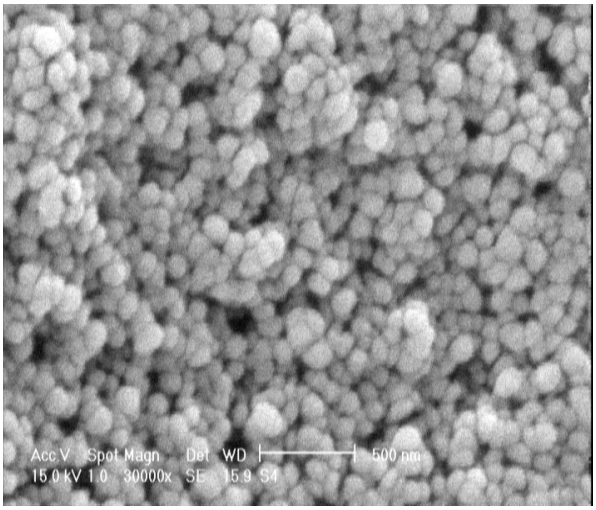

Fig. 3. SEM micrograph of irinotecan loaded PLGA nanoparticles (S9)

The NPs prepared in this study were spherical with smooth surface. Fig. 3 shows SEM image of NPs. The samples in the present work exhibited a zeta potential between -1.07 and -20.3 mV. The entrapment efficiency for S9 was 55 ± 2.7%, while drug loading was 11 ± 3%. As shown in Table 3, in different formulation the different ratio of drug-polymer was used. Obtained data shows that the more the amount of drug used the higher the size achieved. On the contrary by decreasing the amount of the drug used, the size of NPs decreased. This is correlated with the previous studies [18-22].

The effects of some solvent for dissolving both the drug and polymer were studied.

When DCM is used as the internal-phase solvent, the emulsification-evaporation technique produces larger particles [20]. Increasing the amount of solvent decreased the size of NPs. Based on the previous studies adding acetone to the internal phase reduced the surface tension and caused smaller particle size [4,7]. It was found that the best result was achieved using acetone/DCM with the ratio of 1:1. In addition, DCM alone may not be regarded as an ideal solvent for both the drug and the polymer. Therefore acetone was selected as a co-solvent and added into DCM to decrease the interfacial tension and hence the size of NPs. Emulsifier makes better mixing and suitable dispersing of internal and external phase. When no PVA was used in the external phase, no NP was formed. The size of the NPs was smaller when prepared with 0.5% (w/v) PVA as an emulsifier (n=3). The size distribution of NPs at this concentration of emulsifier was narrower than that of NPs prepared with higher concentration of PVA (1% w/v) or lower concentration of PVA (0.3% w/v). This reduction of particle size was significant ($P < 0.05$) in accordance with those found in the literature [6, 9]. Increasing the PVA makes larger particles.

This phenomenon is due to increasing the amount of emulsifier in external phase which makes higher viscosity in this phase. Without emulsifier no NPs

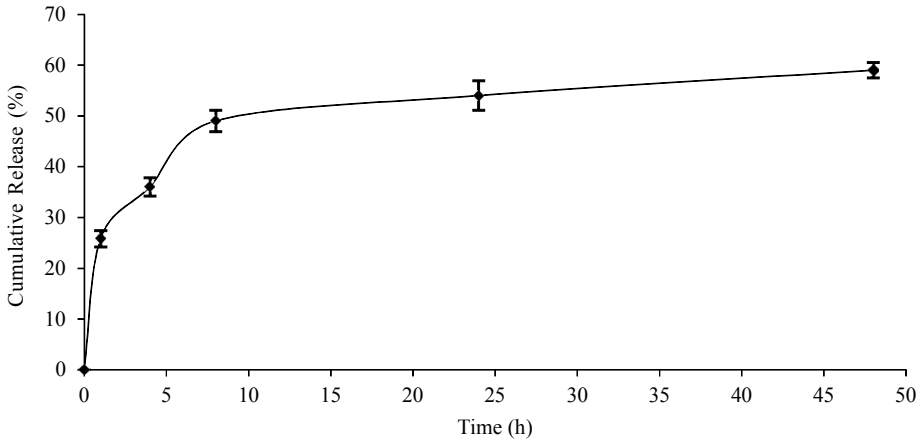

Fig. 4. *In vitro* drug release of irinotecan loaded PLGA nanoparticles (S9)

Table 4. Kinetic models fitting results of drug nanoparticle release (S9)

Model	MPE %	R^2	K	n	Slope	Intercept
Zero order	15.20	0.696	0.0058		0.006	0.348
First order	14.60	0.760	0.0108		-0.011	-0.433
Higuchi	9.97	0.848	0.0527		0.053	0.261
Peppas (Power Law)	5.43	0.943	0.2716	0.217	0.217	-1.304
Hixon-Crowell	14.78	0.739			0.003	0.134
Square root of mass	14.87	0.728			0.004	0.194
Three seconds root of mass	14.97	0.717			0.005	0.249
Weibull	4.76	0.954	0.0177	0.288	0.288	-1.162
Linear Probability	14.96	0.682	0.0152		0.015	-0.399
Log-Probability	4.24	0.962			0.231	-0.628
Reciprocal powered time	4.28	0.961			-0.374	1.017
Non-conventional order 1	15.09	0.706	0.0054	0.150	0.005	0.306
Non-conventional order 2	14.53	0.769	0.002	1.143	0.002	0.064
Maximum RSQ:	0.962	Log-Probability				
Minimum MPE:	4.240	Log-Probability				

mean percent error (MPE)

can be obtained. Decreasing the emulsifier below a certain concentration reduces the size again. It may be due to decreasing the viscosity of external phase that facilitate the coalescence of droplet to produce larger ones. Also small particles have larger surface so need more emulsifier, but too much emulsifier may cause aggregation as it remains on the particles' surface. Our data showed the best result, S9, was obtained by using PVA at 0.5 % concentration. The NPs in the present study were found to be stable in dispersion state, possessing high absolute values of zeta potential and having negative surface charges.

Zeta potential of NPs was negative due to the presence of terminal carboxylic groups of the polymers. As it is seen in the results S9 has more negative surface charges than other formulations

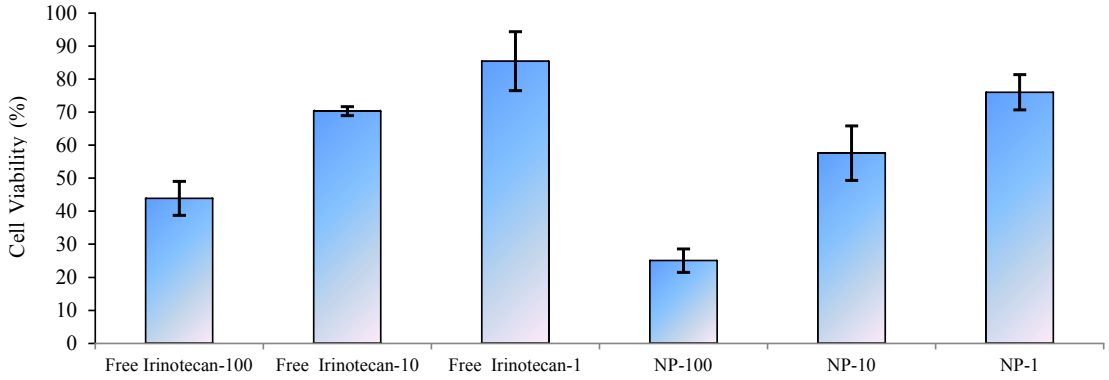

Fig. 5. Percentage survival of HT-29 cells after exposure to free irinotecan, and irinotecan NPs (S9) at different concentrations (n=6). Percentage survival was assessed by the MTT assay

which indicate stability in colloidal systems. Therefore S9 was selected as ideal among the formulations because of both suitable size and zeta potential.

The release rate of irinotecan from the NPs was measured in phosphate buffered solution (pH 7.4) at 37 °C. Although the release study seems would rather analyzed in plasma or in culture media, many references indicates that PBS is suitable for drug release of NPs [4, 7].

The *in vitro* release behavior of the irinotecan-loaded NPs (S9) is summarized in the cumulative percentage release shown in Fig. 4. Release during the first day was measured. The initial burst release was prominent during the first hour of release, being less than 10%. The release gradually decreased and remained constant. The constant slow irinotecan release was observed until 59% of the loaded drug released from S9, respectively during 50 hours. The initial *in vitro* burst release of drugs from PLGA NPs has been observed before by other investigators [8, 11]. The initial burst could be due to the diffusion release of drug distributed at or just beneath the surface of the NPs. A constant slow release of drug in NPs is thought to be due to the involvement of drug molecule entrapped in the polymer matrix which prevents its fast release [8, 11]. The followed delayed release may be attributed to diffusion of the dissolved drug within the PLGA core of the nanoparticle into the dissolution medium [8,11]. Overall *in vitro* release data indicate that PLGA based nanoparticles are capable to sustain irinotecan release rate successfully.

The release data of the ideal formulation in this report (S9) was fitted to 13 models mentioned in Table 2. Squared correlation coefficient (r^2) and the parameters of reciprocal powered time model for the selected nanoparticle formulation are given in Table 4. The release kinetic was Log-Probability model in S9. This is correlated with the previous study for drug release from NPs [23].These values indicated that release mechanism were similar and based on anomalous transport of drug from matrix [23].

HPLC analysis confirmed that the retention time of irinotecan was the same as the irinotecan lactone form, indicating that the chemical structure of irinotecan was not damaged during NP preparation.

in vitro cytotoxic effect of irinotecan–free drug and irinotecan–NPs (S9) for HT-29 cells (n =6) is presented in Fig. 5.

The result signifies that the drugformulated in the NPs has shown benefits inattaining a lower cell viability or equivalently, highercytotoxicity versus the irinotecan–free drug, for example, the cellviability measured at the 100μM drug concentration wasdecreased from 43.91% for irinotecan–free drug to 25.04%, respectively, for the NPformulation after 48 hours incubation with HT-29 cells whencompared with the control. The difference between free drug andNPs after 48 hours in concentrations of 1 and 100 μM was significant ($P<0.05$).

NPs demonstrate advantages in achieving a lower cell viability, or equivalently, higher cytotoxicity versus free drug.

Fig. 5. Percentage survival of HT-29 cells after exposure to free irinotecan, and irinotecan NPs (S9)

at different concentrations (n = 6).Percentage survival was assessed by the MTT assay.

The drug concentration at which 50% of growth is inhibited (IC_{50}) was calculated. The data show that the NP formulation decreases the IC_{50} value of irinotecan in 48 hoursincubation. The IC_{50} value of irinotecan (μM) in NPs is 36.2± 1.2%lower than that of free drug, 79.1±2.1%, in 48 hours. In MTT assay, the results of empty nanoparticles toxicity for the cells as control considered (data not shown).

The higher cytotoxic outcomes of irinotecan–loaded PLGA NPs were in agreement with other reports [8, 21]. The drug-loaded NPs might be internalized by endocytosis because of their mesoscopic size, which essentially increases the uptake of drug [8, 21].

Previous studies showed free drug molecules transmitted into the cytoplasm of the cell, in a passive diffusion way, were effluxed out by Pglycoprotein (P-gp) pumps, while some of them could enter the nucleus and bind to DNA, and NPs are taken up by the cells by endocytosis, resulting in a higher cellular uptake of the drug, thereby making possible them to find protection of P-gp pumps effect and indicate a high cytotoxic effect in comparison with free drug, which is in agreement with this study [8,24].

All the mentioned factors may denote to the considerable cytotoxicity of irinotecan–loaded NPs. These results suggest that the NP matrix impressively declined the *in vitro* cancer cell viability of the drug formulated in the NPs, which could hint the therapeutic activity of the NP formulation *in vivo*.

These *in vitro* cytotoxicity results were similar the data of liposomal and miscillar nanoparticles of irinotecan that were before reported, indicating drug was released from the NPs during incubation of irinotecan NPs in cell cultures and was valid for inhibiting cell growth [8,21].

CONCLUSION

Irinotecan-containing PLGA Nanoparticles were prepared by a modified emulsification/solvent diffusion method. Our results demonstrated that this method is simple and efficient for preparing spherical NPs with smooth surfaces and desired size and size distribution, morphological and physiochemical properties. Formulations showed a mean diameter in the range of 120-300 nm with higher cytotoxicity *in vitro* that is suitable to obtain an effective intracellular uptake of NPs, and can be selected for the future *in vivo* study.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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