International Journal of Medical, Pharmaceutical and Health Sciences (2024);1(4):153-162

International Journal of Medical, Pharmaceutical and Health Sciences

Journal home page: www.ijmphs.com

Research Article

Design, Formulation, and *In Vitro* Evaluation of Docetaxel-Loaded Nanostructured Lipid Carriers

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Article Info

Article history:
Received: 25/10/2024
Received in revised format: 20/12/2024
Accepted: 23/12/2024
Available online: 30/12/2024

Keywords: Nanostructured lipid carriers; Docetaxel; Cytotoxicity; Skin cancer; Box-Behnken design

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DOI:10.62946/IJMPHS/1.4.153-162

ABSTRACT

Nanostructured lipid carriers were prepared using the solvent diffusion melt dispersion method. A 3-factor, 3-level Box-Behnken design was employed to optimize the formulation, considering lipid concentration, surfactant concentration, and stirring time as independent variables. The optimized NLCs were characterized for their size, polydispersity index, zeta potential, entrapment efficiency, and in vitro drug release profile using a Franz diffusion cell. Furthermore, *in vitro* cytotoxicity and cellular uptake studies were conducted to evaluate the effectiveness of docetaxel-loaded NLCs against skin cancer cells. The optimized nanostructured lipid carriers exhibited desirable characteristics, including an average size of 329.1±5.07 nm, a narrow PDI of 0.102, a high entrapment efficiency of 71.52±2.49%, and a negative zeta potential of -19.4±2.4 mV, indicating good stability. *In vitro* drug release studies demonstrated a sustained release profile, with 79.34±2.1% of docetaxel released over 72 hours. Importantly, the docetaxel-loaded nanostructured lipid carriers exhibited significantly higher cytotoxicity against skin cancer cells compared to free docetaxel. The MTT assay revealed a higher growth inhibition rate of 74.62±1.3% for docetaxel-loaded nanostructured lipid carriers compared to 38.3±0.3% for plain docetaxel after 48 hours of incubation at a concentration of 10 µg/mL. The optimized formulation effectively encapsulates docetaxel, provides sustained drug release, enhances cellular uptake, and improves cytotoxicity against skin cancer cells.

INTRODUCTION

The challenges of chemotherapy and systemic drug delivery in the treatment of malignancies includes limitations of achieving sufficient drug concentration in the skin for the treatment of skin cancer. The response rates to chemotherapy vary, and in many cases, they are below 20%. Even in patients who are more responsive to chemotherapy, such as those with breast cancer, the observed clinical outcomes often do not meet expectations. Common toxic effects associated with chemotherapy include

nausea, vomiting, fatigue, and hair loss, which can significantly impact patients' quality of life [1].

In addition to these general challenges, the treatment of skin diseases, including skin cancer, faces specific hurdles due to the difficulty of achieving effective drug concentrations in the skin following systemic drug delivery. The avascular nature of the epidermis makes it relatively inaccessible to drugs administered through systemic routes. As a result, drug concentration tends to be higher in highly perfused organs than in the skin, leading to suboptimal therapeutic outcomes [2]. To overcome these limitations, topical administration of anticancer drugs has emerged as an interesting alternative. By delivering drugs directly to the skin's outer surface, this approach allows for selective targeting and increased accessibility to the viable epidermal and dermal tissues. This localized drug delivery strategy holds the potential to enhance drug targeting and improve therapeutic benefits in the treatment of skin diseases [3]. The investigation of various nano delivery systems, such as liposomes, dendrimers, polymersomes, carbon-based nanoparticles, nanotubes, and nanowires, offers promising opportunities for the development of effective and targeted therapies for skin cancer [4]. These systems have the potential to improve drug solubility, stability, skin penetration, and controlled release, ultimately enhancing the efficacy of skin cancer treatment. In addition to their drug delivery capabilities, nanoparticles can control the release of drugs from formulations, providing sustained and controlled drug delivery. This feature is particularly important in skin cancer treatment, where continuous drug exposure is often desired. By manipulating the properties of nanoparticles, such as size, surface charge, and composition, we can optimize their drug release kinetics [5]. Nanostructured lipid carriers (NLCs) are the attractive colloidal lipid nanocarriers for topical application in the treatment of skin cancer. They offer several desirable effects on the skin and possess characteristics that make them effective as a colloidal carrier system. One of the advantages of NLCs is their solid-state particle-matrix, which provides stability to the formulation and protects chemically labile ingredients from decomposition. This is particularly important for delivering drugs or therapeutic agents that may be sensitive to degradation. NLCs can help maintain the integrity and potency of these ingredients during storage and upon application to the skin [6].

NLCs also have film-forming properties, which enable them to create a protective layer on the skin surface. This controlled occlusion can help enhance the penetration of active ingredients into the skin, increasing their bioavailability and therapeutic efficacy [7]. Additionally, the film formed by NLCs can contribute to skin hydration by reducing water loss from the skin surface, leading to improved skin barrier function. The use of NLCs as a carrier system allows for the modulation of drug release kinetics. This means that the release of the active ingredient can be controlled and sustained over time, providing a prolonged therapeutic effect. This is particularly beneficial in the treatment of skin cancer, where continuous exposure to the drug is often desirable.

NLCs are composed of non-irritant and non-toxic lipids, making them well-suited for use on damaged or inflamed skin. This reduces the risk of adverse reactions or irritation associated with topical application. Furthermore, the formulation of NLCs using physiological, biodegradable, and biocompatible lipid materials and surfactants eliminates concerns regarding the presence of solvent residues or cytotoxicity associated with other nanocarriers. This enhances the safety profile and regulatory acceptance of NLCs as a pharmaceutical carrier. Another advantage of NLCs is their ability to offer improved drug loading capacity and long-term stability [8]. They can accommodate high concentrations of drugs, allowing for efficient delivery of diverse anticancer cytotoxic agents. The stability of NLC formulations over extended periods ensures that the therapeutic agents remain active and effective during storage and application. Hence the present study focused on optimizing and preparing NLCs that are loaded with the drug docetaxel. The objective was to enhance the delivery of docetaxel to skin tumor cells, thereby improving the effectiveness of the treatment.

MATERIALS AND METHODS

Materials

Docetaxel was obtained as a gift sample from Sun Pharma Pvt Ltd. Ahmadabad, India. Stearic acid was purchased from Himedia, Mumbai, India. Oleic acid was purchased from Loba Chemical, Mumbai, India. Tristearin, cholesterol, and soya PC were purchased from Sigma Aldrich, USA. Other chemicals and solvents were used of analytical reagent grade.

Methods

Preparation Of Docetaxel Loaded NLCs

The solvent diffusion melt dispersion technique was employed to prepare drug-loaded NLCs. In a 50 mL beaker, stearic acid (3.0% w/v), oleic acid (0.5% w/v), and soya phosphatidylcholine (1.5% w/v) were dissolved in a solvent system consisting of 10 mL of methanol and acetone in a 1:1 ratio. The mixture was stirred until the lipids were completely dissolved. The lipid mixture obtained was heated to 70°C until it melted and formed a clear lipid phase. This heating process helped ensure the homogeneity of the lipid phase. The drug, at a concentration of 10% w/w of the lipid phase, was added to the melted lipid mixture. The drug was carefully dispersed in the lipid solution by stirring the mixture at 10,000 rpm. The lipid solution containing the dispersed drug was sonicated using a probe sonicator (Lark, BTI-48) for a duration of 10 minutes. In a separate beaker, 60 mL of double distilled water containing tween 80 (a surfactant) was stirred using a magnetic stirrer. The aqueous phase was heated to 70°C using a heating mantle [9].

The aqueous phase, maintained at 70°C, was placed under a high-speed homogenizer (IKA T 25 digital Ultra-Turrax®, Germany) set at 10,000 rpm was mixed with the oil phase containing the drug. After the complete addition of the oil phase, the mixture was stirred for 30 minutes. After this stirring period, the heating was stopped. Then, 20 mL of cold water was added dropwise to the dispersion. The addition of cold water helps in reducing the temperature of the dispersion, facilitating the removal of ethanol and acetone present in the NLCs. The dispersion was continuously stirred for 2 hours to allow for the removal of residual ethanol and acetone from the NLCs. This stirring process aids in the evaporation of these solvents, leaving behind the desired NLC formulation.

After the stirring period, the prepared formulation was separated, likely through a centrifugation or filtration process. The separated NLC formulation was then lyophilized, a process of freeze-drying, using mannitol as a cryoprotectant. The lyophilization was performed using a freeze dryer (Labconco, Cascade Freezone Plus 4.5 L Benchtop Freeze dryer, USA). The lyophilized NLC formulation was stored at 2-8°C until further use for characterization [9,10].

Experiment Design (Response Surface Methodology/Box-Behnken Design)

Optimization of drug-loaded nanostructured lipid carrier formulation was done using design expert software 13.0 (trial version Stat- Ease Inc., Minneapolis USA). Based on pre-optimization studies, it was inferred that lipid concentration, surfactant concentration and stirring time affect the particle size, % entrapment efficiency and % drug release. So the concentration of lipid, surfactant concentration, and stirring time, were taken as the independent variable. Various parameters affecting the effectiveness of NLCs were taken as response variables. Particle size, % entrapment efficiency and % drug release impact the performance of NLCs, so selected as response variables. Box-Behnken Design (BBD) was used for the optimization of process parameters. Box-Behnken design found a most suited design for analyzing quadratic response surface and second-order, polynomial model, allowing the process optimization with the minimum possible number of runs (17 runs) including 5 replicated center points. A computer-generated non-linear, polynominal model quadratic equation explaining three-factor three-level design is given below.

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

Where Y stands for dependant variable, b0 for the intercept, b1 to b33 denotes the regression coefficient calculated from the observations of individual responses and A to C shows the coded levels of prefixed independent variables (A stands for lipid concentration, B for surfactant concentration and C for

stirring time). The encoded values of various independent and dependent variables, along with their respective levels, are defined in Table 1.

Table 1: Defining different variables and their respective levels of Box-Behnken design for optimization of NLCs.

Variables	**Levels**		
Independent variables	-1	0	+1
A = Lipid concentration (mg)	100	200	300
B = Surfactant concentration (%)	0.5	1	1.5
C = Stirring time (min)	5	7.5	10
Dependent variables	Constraints		
Y1 = Particle size (nm)	Minimum		
Y2 = Entrapment efficiency (%)	Maximize		
Y3 = Drug release (%)	Maximize		

Average Particle Size, Zeta Potential and Polydispersity Index

The particle size and polydispersity index (PDI) of the NLC formulations were determined using a dynamic light scattering technique with a particle size analyzer (Malvern ZEN3600 instrument from Malvern Panalytical Ltd.) was used for this purpose. To measure the particle size and PDI, the drug-loaded nanocarriers were first diluted with double distilled water. The analysis was conducted at room temperature, specifically at 25 ℃. The diluted samples were then subjected to dynamic light scattering analysis in triplicate, meaning each sample was measured three times to obtain reliable and statistically significant data [11-12].

Drug Entrapment Efficiency

The entrapment efficiency of the prepared DTX-NLCs (Docetaxel-loaded Nanostructured Lipid Carriers) was determined following a method as reported by Fan and colleagues [13]. Lyophilized NLCs equivalent to 10 mg of the drug were taken and dissolved in 10 mL of ethanol. The solutions were individually centrifuged at 10,000 rpm for a duration of 10 minutes. After centrifugation, 1.0 mL of the supernatant from each solution was carefully withdrawn using a micropipette and transferred into two separate 10 mL volumetric flasks. The samples in the volumetric flasks were subsequently analyzed spectroscopically for drug content using a UV-spectrophotometer (Shimadzu 1800 UV-spectrophotometer). The absorbance of the samples was measured at a wavelength of 232 nm. % drug entrapment efficiency was calculated according to the formula given below-

$$\% \text{ Drug entrapment efficiency} = \frac{W\alpha}{Wi} X\ 100$$

Where Wα is the Amount of drug released from the NLCs and Wi is the amount of drug initially taken to prepare NLCs.

Shape And Surface Morphology

To examine the shape and surface morphology of the prepared NLCs, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were conducted, as reported by Varela-Fernández and colleagues [14]. The analysis was performed at the Indian Institute of Science Education and Research, Bhopal, India. The NLCs were subjected to TEM imaging to visualize the internal structure and morphology of the nanoparticles at a nanoscale level. SEM analysis, on the other hand, utilizes a scanning electron microscope to examine the surface morphology of the samples.

***In-vitro* Release Studies From DTX Loaded NLCs**

The dialysis tube-based diffusion technique was employed to evaluate the in vitro drug release behavior of the formulations. Lyophilized drug-loaded NLCs weighing 100 mg were accurately weighed and dispersed in 1 mL of phosphate buffer solution (PBS) with a pH of 7.4. The dispersion was then placed inside a sealed dialysis membrane. The dialysis membrane used had a molecular weight cut-off of 3500 kDa and was obtained from Himedia. The sealed dialysis membrane containing the drug-loaded NLC dispersion was immediately suspended in 50

mL of PBS (pH 7.4), which served as the receptor medium. To maintain the sink condition in the receptor compartment, constant stirring was carried out using a magnetic stirrer. Specifically, a Remi 2-MLH magnetic stirrer was used for this purpose. The temperature was maintained at 37±0.5°C throughout the experiment to mimic physiological conditions. At predetermined time intervals, 1 mL samples were collected from the receptor compartment, and an equal volume of fresh buffer solution was added to replace the withdrawn sample. The collected samples were appropriately diluted, and the drug release was determined by measuring the absorbance using a UV spectrophotometer (UV-1800, Shimadzu) [15].

Cytotoxicity Studies

The *in vitro* cytotoxicity of optimized drug-loaded NLC formulation was assessed on B16F10 melanoma cells using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. B16F10 melanoma cells were cultured and maintained in DMEM medium supplemented with 10% fetal calf serum (FCS) under suitable conditions. This involved maintaining the cells in a humidified incubator at 37°C with 5% CO_2. For the cytotoxicity assay, 2×10^4 B16F10 cells were plated in each well of a 96-well plate and allowed to attach for 24 hours. The cells were then exposed to serial concentrations of the test samples, which contained equivalent concentrations of Docetaxel. The concentrations used ranged from 0.1 µM to 10 µM. The samples were incubated with the cells at 37°C for 24 hours. After the incubation period, the cells were washed thrice with PBS to remove any residual test samples. Fresh media was added to the wells, and the cells were further incubated at 37°C until the control wells (without any treatment) reached confluency. To assess cell viability, 20 µl of MTT solution (5 mg/ml) was added to each well and incubated for another 4 hours at 37°C. During this time, viable cells reduced the MTT reagent into purple formazan crystals. After the incubation with MTT, the media in each well were replaced with 200 µl of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. The absorbance of the dissolved formazan was measured at 550 nm using an automated microplate reader (Bio-Rad Hercules, CA, USA). The percentage inhibition of cell viability was calculated based on the absorbance readings, and the data were plotted against the concentrations of the test samples. The concentration required to inhibit 50% of cell growth (IC50) was determined from the plotted data. The percentage cell viability was calculated as:

$$\%\ cell\ viability = \frac{Abs\ sample}{Abs\ control} x\ 100$$

where, Abs_{sample} is the absorbance of cells tested with free drugs and NLC formulations and $Abs_{control}$ is the absorbance of control cells [16].

RESULTS AND DISCUSSION

Optimization Of Drug Loaded NLC Formulation

In the experimental design and process optimization of DTX-NLCs, a three-factor and three-level Box-Behnken design was utilized. The analysis aimed to identify the best experimental parameters and assess the influence of the factors on the selected responses. The design resulted in 17 experimental runs, which included different combinations of the chosen factors and their levels. These runs were randomly assigned to ensure statistical validity and reduce bias. Among the 17 runs, 5 runs were designated as central points, where the responses were recorded and analyzed.

The best-fitting model terms for all three variables are quadratic polynomial models. The model terms indicated a direct relationship between independent variables (predictors) and dependent variables (responses). All observed responses were compared in order to determine the optimal experimental parameter. As desired, model terms were found to be significant, and lack of fit for each observed response was not significant. Table 2 displays the 17 iterations of the experimental design and the respective responses of each run. Figure 4 depicts the 3D response surface plots of all responses illustrating the effects of various factors. Below is the final regression equation of the applied quadratic model for individual responses, such as particle size (Y1), drug entrapment

efficiency (Y2), and drug release (Y3), derived by response surface methodology utilizing DesignExpert® software:

$Y1 = 121.08-12.92A-8.23B+0.0450C-15.78AB+9.03AC-1.08BC+126.48A^2+123.10B^2+64.30C^2$

$Y2 = 90.13+1.89A+4.65B-1.60C+0.8625AB-5.74AC+0.1900BC-5.85A^2-11.34B^2-8.58C^2$

$Y3 = 91.59+3.05A-0.6150B-3.99C+4.52AB-3.54AC+0.6850BC-4.11A^2-7.94B^2-11.88C^2$

Table 2: 17 runs of the experimental design, including the factor combinations and the respective responses obtained from the runs.

Std	Run	Factor A: Lipid Conc. A (mg)	Factor B: Surfactant conc. B (%)	Factor C: Stirring time C (min)	Response 1 Particle size Y1 (nm)	Response 2 Drug Release Y2 (%)	Response 3 Entrapment Efficiency Y3 (%)
8	1	1	0	1	295.26	70.44	72.64
7	2	-1	0	1	297.15	77.22	73.68
6	3	1	0	-1	308.52	85.65	84.58
2	4	1	-1	0	377.68	67.52	78.76
16	5	0	0	0	70.95	92.58	91.58
3	6	-1	1	0	395.18	76.64	71.26
15	7	0	0	0	177.7	90.53	93.18
9	8	0	-1	-1	300.98	68.42	78.56
17	9	0	0	0	86.13	92.74	88.64
1	10	-1	-1	0	377.87	66.39	81.64
5	11	-1	0	-1	346.51	69.49	71.47
14	12	0	0	0	136.44	87.4	91.36
11	13	0	-1	1	334.64	65.38	66.1
10	14	0	1	-1	284.48	74.66	76.06
13	15	0	0	0	134.16	87.38	93.18
12	16	0	1	1	313.8	72.38	66.34
4	17	1	1	0	331.89	81.22	86.48

ANOVA (analysis of variance) was used to analyze the responses in order to determine the optimal mathematical model and parameters. Individual and combined factor coefficients and p-values indicated the influence of respective factors on selected responses. P-value also indicated the significance of the employed model and other ANOVA-evaluated parameters (Table 3).

Table 3: Summary of ANOVA and statistical parameters.

Model fit parameters	Y1: Particle size		Y2: Drug release		Y3: Entrapment Efficiency	
	p-value	Interpretation	p-value	Interpretatio	p-value	Interpretatio
Model	0.0011	Significant	0.0002	Significant	0.0001	Significant
R^2	0.9469		0.9703		0.9761	
R^2 adjusted	0.8786		0.9322		0.9454	
R^2 predicted	0.7496		0.7941		0.7613	
Lack of fit	0.7785	Not significant	0.5589	Not significant	0.2750	Not significant

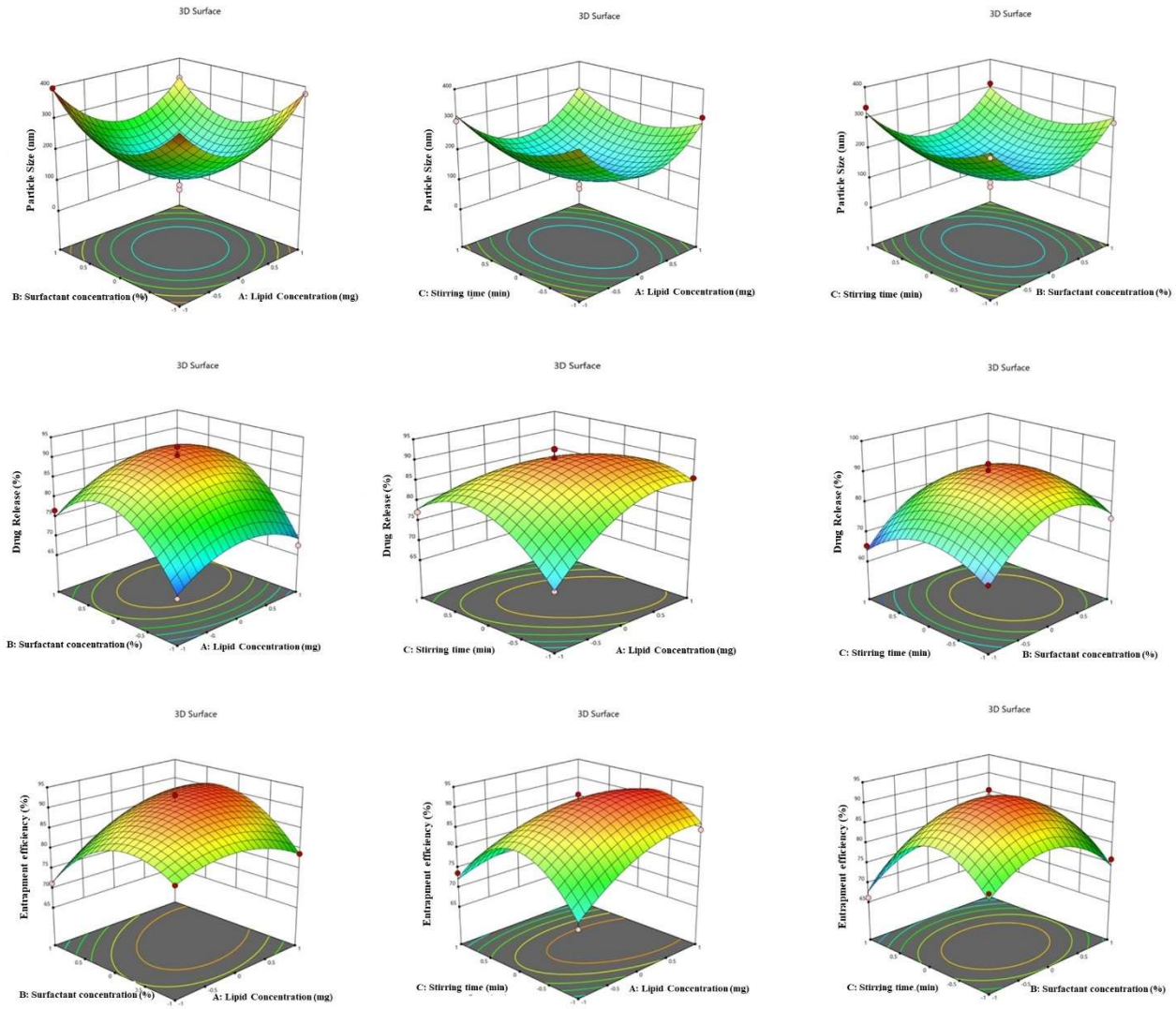

Fig. 1. Presents the 3D response surface plots, illustrating the effects of different factors on the responses.

The response surface diagram and coefficient of the quadratic equation revealed that the selected variables (Y1, Y2, and Y3) are significantly influenced by the selected factors (Figure 1). The positive and negative indications preceding individual terms of the regression equation indicated, respectively, an increase and a decrease in the response as a result of the factors.

According to the results, the particle size of all the prepared formulations ranges between 70.95 and 395.18 nm. According to the regression equation, it was evident that particle size (Y1) was directly influenced by the lipid concentration (A), inversely related to the surfactant concentration (B), and the stirring time (C) had no significant effect. Figure 1 depicts a 3D response surface plot that provides more specific information on the influence of various independent factors on particle size. The graph displayed an initial decrease in particle size with a transition from 1 to 0 for the solid/liquid lipid ratio (X1), followed by a significant increase from 0 to + 1. This response may be the result of an initial increase in solid lipid, which will result in a perfect combination of solid and liquid lipid and the smallest possible NLC structure. In contrast, as the concentration of solid lipids increases, so does the accumulation of solid content and particle size. In addition, an increase in surfactant concentration (X2) initially caused a

significant decrease in particle size, followed by a minor increase as the surfactant concentration rose further. Initial increase in surfactant concentration is intended to decrease interfacial tension and generate steric hindrance on the NLC surface in order to prevent the accumulation of individual particles and enhance stability. Once the surfactant concentration reaches the point of saturation, further increases result in the deposition of excess surfactant molecules on the NLC surface and a modest increase in size. The particle size was not significantly affected by ultrasonication time. With increasing ultrasonication time, minute particle size was determined to be at its smallest at the central value. It was evident from the 3D plot that the interaction between factors A and B and factors A and C affects particle size in a synergistic manner. Maximum particle size was observed when X1 and X2 were at their highest levels, while minimal particle size was observed when all the independent factors were at their midpoint.

Table 4: Predicted optimized variables for drug loaded NLCs

S. No.	Independent Variable	Quantity
1.	Lipid	300.00 mg
2.	Drug	26.2 mg
3.	Tween 80	0.50%
4.	Stirring speed	9999.97

Average Particle Size, Zeta Potential And PDI

The size and size distribution of formulation are key parameters that affect drug release kinetics, cellular uptake efficiency, and stability. The average particle size was found to be 329.1nm for DTX bearing NLCs. Polydispersity index of DTX loaded NLCs were found to be 0.102, 0.150, and 0.131, respectively. A low PDI value (<0.2) indicated the narrow distribution of size (monodispersity).

Drug-loaded NLCs were also characterized for zeta potential (ZP). ZP is essential to determine the stability of the nanosuspensions. The high ZP values play an important role in stabilizing the formulations due to high repulsive forces, hindering aggregation NLCs. A minimum zeta potential of ±20mV is desirable for the stability and redispersion of NLCs. The stability of DTX loaded NLC formulations were indicated by the zeta potential value of -19.4. The zeta potential value was negative in charge because of the presence of stearic acid. As the obtained zeta potential values for drug-loaded NLCs were near -20mV, the NLCs were found to be physically stable.

% Drug Entrapment Efficiency

Entrapment efficiency expresses the fraction of the total drug incorporated into the elastic liposomal formulation. Percent drug entrapment efficiency for DTX loaded NLC formulation was found 71.52%. Percent drug entrapment was found to be very near to the predicted values. Higher drug entrapment was observed in DTX-NLCs. This might occur due to possible hydrophilic interaction between the OH group of lipid and the NH_2 group of DTX.

Shape and Surface Morphology

Scanning electron microscopy and Transmission electron microscopy was used to determine the shape and surface morphology of various NLC formulations. Various characteristics such as particle size, shape, and morphology of NLCs can be efficiently evaluated using SEM and TEM studies. TEM images revealed the spherical shape of drug-loaded NLCs and confirmed particle diameter in nanometer range similar to that obtained from particle size measurement in Litesizer 500. Detailed SEM images confirmed that all of the particles were spherical with a smooth outer surface. SEM images also confirmed the morphological uniformity of NLC formulations (Figure 2).

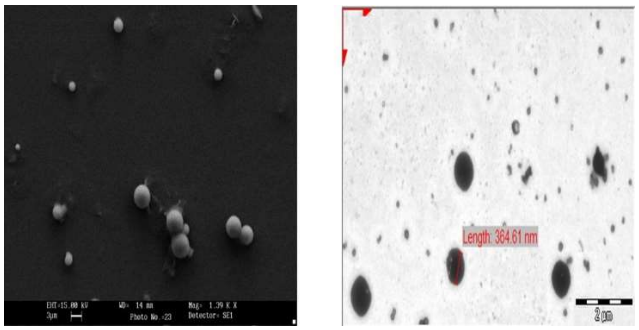

Fig. 2. SEM and TEM photographs of DTX loaded NLCs

***In-vitro* Drug Release Studies**

Drug release from NLC formulations may occur by desorption of surface-bound drug and diffusion through the NLCs matrix. The release profiles of DTX-NLCs formulations were performed at physiological pH 7.4 (37±0.5°C) using dialysis methods. The obtained in vitro release pattern of all the formulations is shown in Fig. 10.15. The initial release is reflecting a burst release of the drugs from NLCs. This may be due to some surface adsorbed drug or may be due to entrapment of the drug in the outer stratum of the NLCs. After 8 hrs, the drug release rate was observed comparatively slower and sustained for 72 hrs. The approximate drug release from DTX-NLCs formulations after 72 hrs was 79.34±2.1% (Figure 3). The existing biphasic drug release behavior may be due to the lipophilic nature of the drugs, which might result in the barrier effect for drug diffusion.

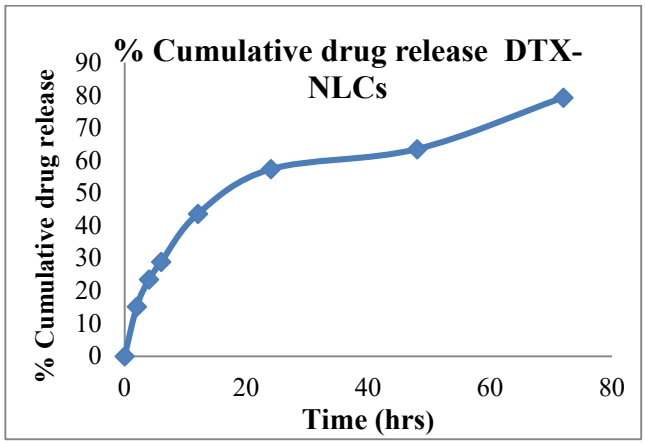

Fig. 3. *In vitro* Drug Release Profile of Dtx-NLCs Formulation (Mean ± Sd, N = 3)

***In vitro* Cytotoxicity Study**

B16F0 cells were employed as normal cell control. Measuring the DNA content of cells is a well-established method to monitor the cell cycle. In inflow cytometry, plasma membrane permeability is assessed using propidium iodide, a dye that is not permeant to live cells. PI fluorescence of 10,000 events was analyzed using a flow cytometer. Percentages of cells in each phase were calculated using FlowJo. Flow cytometry analysis showed that drug-loaded NLC treatment affects the cell cycle. As represented in Figure 4, drug-loaded NLCs induced a decrease in the number of cells in the G0/G1 phase, S, and G2/M phases. These data are in agreement with the above-described cell cytotoxicity data.

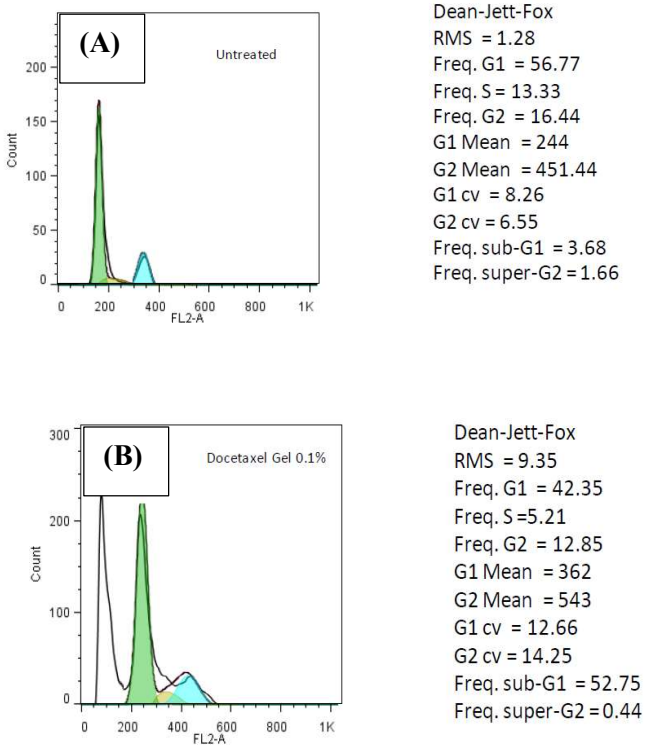

Fig. 4. Dean-Jett-Fox Univariate cell cycle analysis by flow cytometry: Representative DNA content frequency histograms (A-untreated, B-DTX-NLCs)

CONCLUSIONS

Lipid-based drug delivery systems have been proved promising carriers for cytotoxic drugs because of their potential to increase the solubility and bioavailability of poorly water-soluble and/or lipophilic drugs. In the present investigations we have successfully prepared DTX loaded NLC formulation and optimized using the Box-Behnken Design approach of design of experiments (DoE). The DTX loaded NLC formulation was evaluated for size, shape, drug encapsulation, invitro drug release, ex vivo and in vivo animal studies. The DTX loaded NLC formulation showed a stronger antitumor activity in a mouse model with pre-established tumors, likely because the DTX-NLCs significantly increased the accumulation of the

DTX in tumor tissues. Our results confirmed that the effects of DTX loaded NLC on activation of apoptosis are accompanied with changes in the cell cycle progression. The present study established that NLC formulations can be used to develop Docetaxel nanoparticulate delivery systems with increased anticancer efficacy.

Ethics approval: The animal study protocol was approved by the Institutional Animal Ethical Committee of Adina Institute of Pharmaceutical Sciences, Sagar (Registration No. 1546/PO/Re/S/11/CPCSEA).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors appreciate Dr. Vandana Arora for her valuable input and guidance in the preparation of this research article. We would also like to acknowledge the support provided by Lloyd Institute of Management and Technology, Greater Noida.

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