RESEARCH ARTICLE

Optimized Nanostructured Lipid Carriers for Dutasteride Delivery: Improving Therapeutic Efficiency and Minimizing Adverse Effects

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ABSTRACT

Dutasteride is a BCS Class II medication that is frequently employed in the treatment of benign prostatic hyperplasia. It has limited solubility and strong penetration. Nevertheless, using it frequently results in side effects like sexual dysfunction and urination problems. This work aims to decrease these side effects by developing a novel delivery system entitled Nano-Structured Lipid Carriers (NLCs) that will maintain the expulsion of dutasteride, potentially boosting their therapeutic efficiency while minimizing unwanted effects. This study details the construction of a unique NLC composition with two different variables at three levels using a design of experiments (3^2 factorial layouts) and pseudo-ternary phase diagrams. Following the design phase, the right proportions of water, Omix, and surfactant were chosen to create several DUT-NLC formulations using the Hot Melt-Emulsification Ultrasonication Technique. Among the techniques used to evaluate the formulations were analyses of particle sizes, zeta potency measurement, polydispersity index (PDI) assessment, the use of transmission electron microscopy (TEM), differentiation scanning calorimetry (DSC), X-ray diffraction (XRD), release kinetics, as stability testing, and in vitro cytotoxicity analysis. The design, advancement, & characterization of a novel NLC composition are described in this paper. 7% (Omix) and 20% (Surfactant concentration) were the optimized batch parameters, resulting in an 81% mean Percentage EE, a zeta potential value of -37.5 MV, and an average particle size of 109.3 nanometers. Further in vitro cytotoxicity tests employing PC3 cell lines showed that the created system was more efficient because IC50 values in DUT-NLCS treated cells were lower than in pure drug-treated cells. The experiment revealed that surfactant (Omix) and water phases had a significant effect on the physicochemical features of NLCs. Based on the results of an in vitro cytotoxicity research (MTT Assay), it appears that the suggested formulation has a higher cytotoxic effect on cancer cells. With notable anticancer benefits and long-term release, DUT-NLCs show great promise for a drug delivery technology that may lessen systemic adverse effects in clinical circumstances when DUT dosages are lower.

Keywords: Dutasteride, Prostate Cancer, Nanostructured Lipid Carriers, DUT-NLCs, Hot Emulsion Ultrasonication Technique.

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Conflict of interest: None

INTRODUCTION

The most common hyperplasia in males worldwide is prostate cancer.[1] Approximately thirty percent of all malignancies in men are diagnosed as prostate cancer. There is evidence as of 2019 that 9.8% of male cancer-related deaths are attributable to prostate cancer. Active monitoring is becoming a potential treatment for localized PC; however, the two major treatments are radiation therapy and radical prostatectomy.[2,3] In the US, it was projected that 29,720 prostate cancer patients would pass away from their illness in 2013 and that roughly 238,590 males would receive a prostate cancer diagnosis.[4] Numerous individuals who pass away from prostate cancer initially have tumors that appear to be restricted to the gland; this may indicate that the patient has a truly "high-risk" condition, necessitating the development of new treatment strategies. According to current estimates, 15% of all prostate cancer diagnoses are related to "high-risk" disease.[5] Prostate cancer is treated with dutasteride, a class II BCS drug having restricted porosity as well as solubility. Through the promotion of sustained release and the reduction of systemic toxicity, nanotechnological techniques can enhance the effectiveness of chemotherapy. By blocking 5-alpha reductase, the antiandrogenic medication dutasteride cures adult males with symptoms of benign prostatic hyperplasia (BPH). Type 1 along with type 2 are the two variants of 5α-reductase. When prostate cancer develops, type 1 expression in the prostate rises while type 2 expression either stays the same or decreases.[6] Dutasteride reduces both isoforms of 5α-reductase, in contrast to finasteride. In this study, we examined the impact of dutasteride on the number of cases of prostate cancer discovered after a prostate biopsy in males who were previously predisposed to the disease. Dutasteride has been

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Table 1: Preliminary Observation of Solid and Liquid Lipid in Various Ratio

Lipid Ratio (Stearic Acid: Oleic Acid)	Observation
1:1	Miscible, but the mixture gets solidified at room temperature
1:2	Miscible, but the mixture gets solidified at room temperature
1:3	Miscible and stable at room temperature
1:4	Miscible and stable at room temperature
1:5	Miscible and stable at room temperature
2:1	Miscible, but the mixture gets solidified at room temperature
3:1	Miscible, but the mixture gets solidified at room temperature
4:1	Miscible, but the mixture gets solidified at room temperature
5:1	Miscible, but the mixture gets solidified at room temperature

studied as a treatment for androgenic alopecia. It is authorized for use as an oral medication to treat benign prostatic hypoplasia. This study investigated the possibility of topically administering dutasteride to lessen systemic exposure, skin irritation, and cytotoxicity. This medication is used to treat a condition called benign prostatic hyperplasia, usually a prostate cyst, as well as male-related baldness.

Inhibiting the conversion of testosterone to dihydrotestosterone, dutasteride helps in the therapy of various ailments. In most cases, dutasteride was well tolerated. Although the incidence was generally modest, dutasteride recipients experienced impotence, diminished libido, gynaecomastia, and ejaculation dysfunction much more frequently than placebo receivers. Over time, incidence steadily declined except for gynecomastia.[7,8] Through the promotion of sustained release and reduction of systemic toxicity, nanotechnological techniques can enhance the effectiveness of chemotherapy. Pharmaceuticals use colloidal drug delivery devices called nanostructured lipid carriers (NLCs) to encapsulate and administer active components. It is used to provide medications with improved clinical efficacy. They are made up of a blend of solid as well as liquid lipids that combine to produce a nanoscale structure that can hold both hydrophilic and hydrophobic medications.[9] NLCs have benefits such as better drug solubility, increased bioavailability, controlled release, and the capacity to target particular tissues. NLCs, or nanostructured lipid carriers, were created to increase SLNs' ability to be encapsulated and their stability during storage. Solid plus liquid lipids combine to form NLCs, with the liquid lipid component typically ranging from 10% to 30%.[9] A larger drug load can be accommodated by a nanostructure matrix created by a liquid lipid's presence in a lipid mixture. Furthermore, a

Table 2: Independent and Dependent Variables Along with their Levels

Independent Variables	Levels		
	Low (-1)	Low (-1)	Low (-1)
% Omix	3	5	7
% Surfactant (Poloxamer 407)	10	15	20
Dependant Variables	Criteria		
Particle size	Minimum		
Zeta potential	Optimum		
% Entrapment efficiency	Maximum		

Table 3: Composition of NLC's Formulation According 3^2 Factorial Design

Trial No	O mix (%)	Surfactant (%)
E1	3	20
E2	7	15
E3	5	15
E4	7	20
E5	5	10
E6	7	10
E7	3	15
E8	3	10
E9	5	20

significant drug concentration gradient will be made possible by a large loading capacity of NLCs, improving drug penetration.[10,11] NLCs have better drug penetration along with stability, controlled release of drugs, and use of biocompatible and biodegradable lipids. The resulting NLC lipid blend maximizes drug loading and reduces drug ejection during storage since it is less crystalline than SLN. A mixture of solid as well as liquid lipids forms a nanostructured lipid composite (NLC), and its surface is stabilized by a surfactant or a combination of surfactants.[12] The study aims to treat prostate cancer by developing and describing a new dutasteride-loaded formulation of nanostructured lipid carriers (NLCs). Powder size, Zeta potential, PDI, TEM, DSC, XRD, in vitro release of drug profile, release kinetics, along in vivo studies were among the techniques used to evaluate the NLCs. The objective of the present investigation was to design, build, and analyze dutasteride-loaded nanostructured lipid carriers, or DUT-NLCs, to treat prostate cancer.

MATERIALS AND METHODS

Materials

Mac-Chem Labs Pvt. Ltd., Bombay kindly supplied a gift sample of the main component, dutasteride. The solid lipid stearic acid was acquired from Sigma-Aldrich Private Limited (India), whereas the liquid lipid oleic acid was obtained through Sun Pharmaceuticals Ltd., Mumbai. We acquired glyceryl monostearate from Croda Pharmaceuticals Chemicals (India) Ltd. Tween 20 was confirmed to have been purchased from National Drug

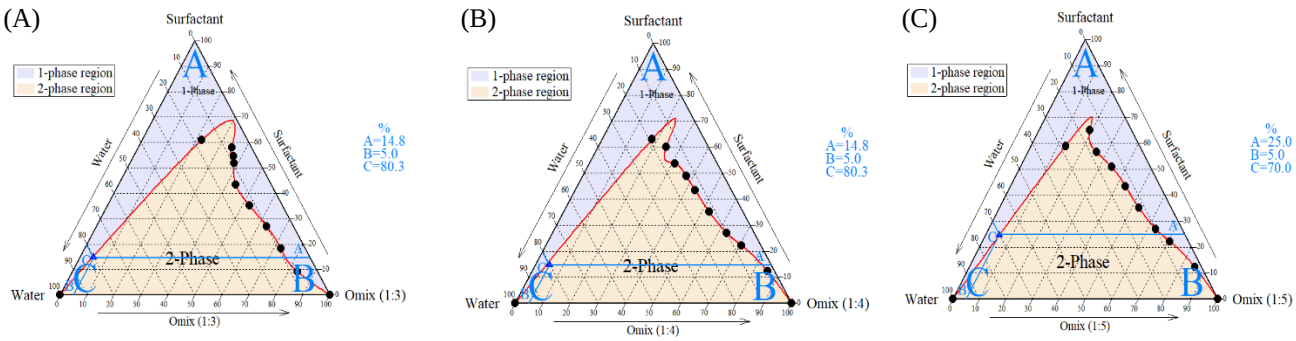

Figure 1: Psuedo-ternary phase diagram for all formulations.
Note: (A)Water + Surfactant + Omix (1:3) ; (B) Water + Surfactant + Omix (1:4); (C)Water + Surfactant + Omix (1:5)

Table 4: Results of formulation from the points selected from the pseudo ternary phase diagram

Trial	O_{mix} (%)	Surfactant (%)	Water (%)	Outcome
A	5.0 (1:3)	14.8	80.3	Stable
B	5.0 (1:4)	14.8	80.3	Stable
C	5.0 (1:5)	25.0	70.0	Stable

Table 5: Particle size, Zeta potential, and Entrapment Efficiency of DUT- NLC

Trial No	Particle Size (nm)	Zeta Potential (mV)	Entrapment (%)	Efficiency	Polydispersity Index
E1	110.2	-23.3	72		0.324
E2	187.9	-25.8	71		0.542
E3	149.3	-34.2	74		0.486
E4	109.3	-37.5	81		0.364
E5	188.4	-38.1	71		0.347
E6	221	-33.7	68		0.428
E7	117.2	-33.3	69		0.468
E8	170	-37.1	65		0.346
E9	113.1	-37.8	79		0.458

Table 6 :In vitro Release Kinetics of Dutasteride Loaded NLCs

Formulation code	Zero Order R^2	First Order R^2	Higuchi Model R^2	Hixon Crowell Model R^2	Koresmeyer Peppas	
					R^2	n (slope)
DT1	0.9508	0.8083	0.8492	0.8228	0.8902	0.2455
DT2	0.9733	0.7618	0.8723	0.8424	0.9102	0.3195
DT3	0.9752	0.7314	0.8755	0.8527	0.859	0.3922
DT4	0.9204	0.9358	0.9312	0.9414	0.915	0.281
DT5	0.9666	0.8874	0.9126	0.9363	0.9257	0.2905
DT6	0.9796	0.8511	0.9164	0.9333	0.9244	0.3137
DT7	0.9583	0.8788	0.894	0.9209	0.9199	0.2841
DT8	0.9866	0.8366	0.9325	0.9355	0.9008	0.2881
DT9	0.9658	0.863	0.9004	0.9232	0.9038	0.302

House (Pvt) Inc., in New Delhi, while it was determined that Poloxamer 407 was obtained as gifting samples through BASF Indian Ltd., Mumbai, India. Laboratory-grade reagents and chemicals were utilized in all other instances.

Methods

For solid lipid

To visually determine the drug's solubility within these solid fats, a specified amount of the drug (1 mg) was placed in an Eppendorf tube, and various solid lipids that had been previously liquefied at temperatures 5–10 °C above their respective melting points were added in 50 mg increments. After each addition, eppendrof tube was checked visually to ensure the drug was thoroughly dissolved.[13]

For liquid lipid

In an Eppendorf tube, one milligram of medication was dissolved with 0.5 ml containing various liquid fats to assess liquid lipids. Eppendrof tubes were sonicated at 37°C ± 0.5°C for 10 min. After each addition, eppendrof tube was checked visually to ensure drug was thoroughly dissolved.

For surfactant

Surfactant and co-surfactant screening was performed by administering 1 mg of drug to 1% w/v (1mg surfactant/co-surfactant in 1 ml water) surfactant mixtures in an

Table 7. Accelerated Stability Testing (75 ± 5% Relative The level of humidity, 40 ± 2°C)

Time Interval	Particle size	Zeta Potential	Polydispersity Index
0 Days	109.3	-37.5	0.306
7 Days	109.3	-37.5	0.306
15 Days	109.8	-37.2	0.308
30 Days	110.2	-36.9	0.309
45 Days	110.5	-36.5	0.310
60 Days	110.7	-36.1	0.311
90 Days	111.2	-35.6	0.318

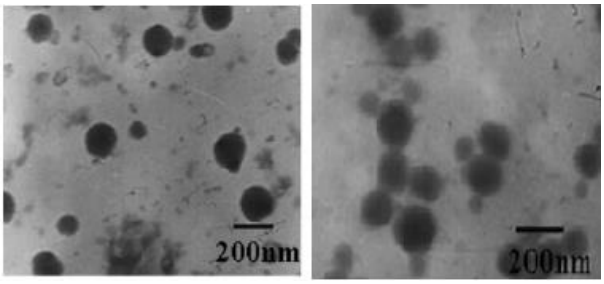

Figure 2: TEM of optimized dutasteride loaded NLC suspension. A) Image at 200nm (freshly prepared) B) Image at 200nm (Freeze-dried)

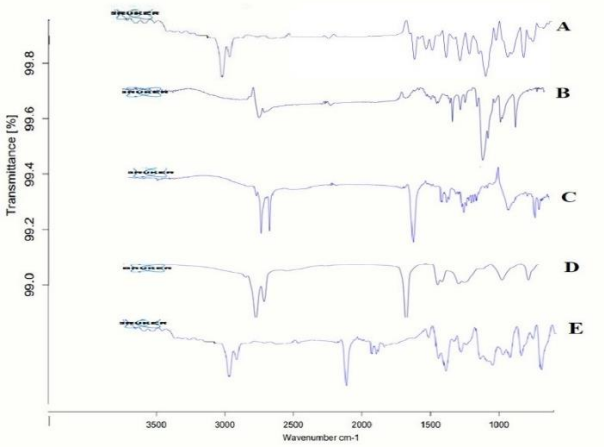

Figure 3: Overlay of FTIR study: A=Dutasteride, B=Poloxamer, C=Stearic Acid, D=Oleic Acid, E=Optimized Formulation

eppendrof tube and tubes were sonicated at 37°C ± 0.5°C for 10 min. After each addition, eppendrof tube was checked visually to ensure drug was thoroughly dissolved.

Miscibility study of lipids

To determine the capability of lipids (solid and liquid) to mix in any ratio without separation of two phases was carried out by miscibility study. Solid and liquid lipid were weighed (w/w) and incorporated in Eppendorf tubes according to 1:1, 1:2, 1:3, 1:4, 1:5, 2:1, 3:1, 4:1and 5:1 ratio respectively and can be observed in Table 1. The average temperature at which these mixtures were cooked was approximately 5$^{°C}$over the melting point of the solidified lipid. They were then stored at room temperature for a full day. We looked at the compatibility and clarity of these combinations.[14]

Fabrication of pseudo-ternary phase diagram

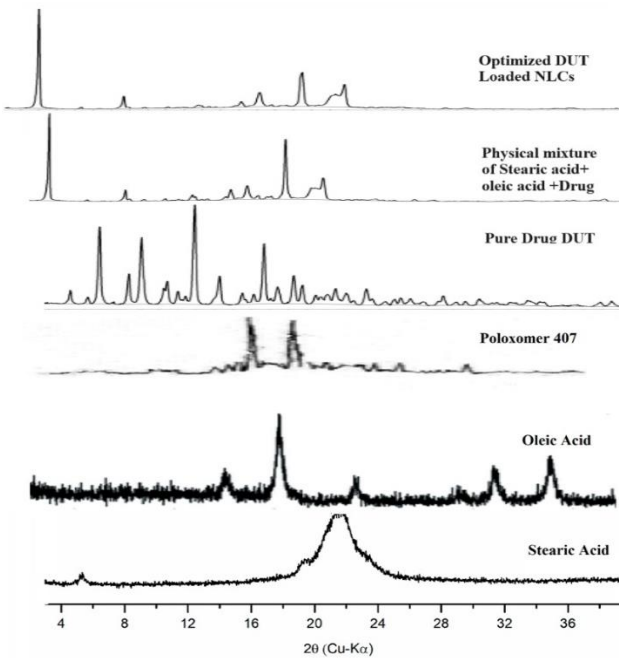

Figure 4: XRD spectra of Optimized Formulation, Physical Mixture, Pure Drug, Poloxamer 407, Oleic Acid, and Stearic Acid.

The creation of a pseudo-ternary phase figure is the first leap in the fabrication of any microemulsion. The following elements were chosen for the development of a pseudo-ternary phase figure relying on the results of solubility and miscibility investigation of inspected solid lipid, liquid lipid, and surfactants. O_{mix} consists of a mixture of solid and liquid lipids. Distilled water is considered an aqueous phase, where surfactants (Poloxamer 407) are used for miscibility. By applying the water-based titration approach, pseudo-ternary phases were constructed. The preliminary mixture Omix consisted of various weight proportions of 1:3, 1:4, and 1:5, and surfactant (Poloxamer 407). After that, to create each phase diagram, the chosen lipid phase (Omix) and particular Surfactant (Poloxamer 407) were combined in weight proportions varying from 1:9 to 9:1, yielding 9 possible mixtures, including 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Needed amounts of Omix and Surfactant were combined, and resulting combination was warmed above lipid's melting point and shake to create a monophasic mixture. Then, at 75 °C, distilled water was progressively titrated into this monophasic combination to reach equilibrium. Distilled water was sequentially added dropwise until the appearance of the mixture changed from transparent to turbid and the volume require for distilled water was noted down and its weight was calculated. Weight of Omix, surfactant, and distilled water (1:9 to 9:1 ratios) were fed in Chemix School 12.0 software to construct a pseudo-ternary phase diagram as shown in Figure 1.

Formulation of dutasteride NLC's by using 3^2 factorial design

NLC formulation was significantly improved using the 3^2 Factorial Design experimentation strategy. Encapsulation effectiveness and particle size were dependent variables,

while cycles of, Omix ratio, and % Surfactant (Poloxamer 407) were independent variables. Based on the results of early tests, three variables were independently created: low (1), moderate (0), and highest (+1) values. Response criteria (i.e., the dependent variables) for NLC formulation's refinement are also shown in Table. Applying the program Design of Experiment version 13, a factorial plan with 9 trial runs was created s illustrated in Table 2.

Hot melt-emulsification ultrasonication technology for the fabrication of NLCs

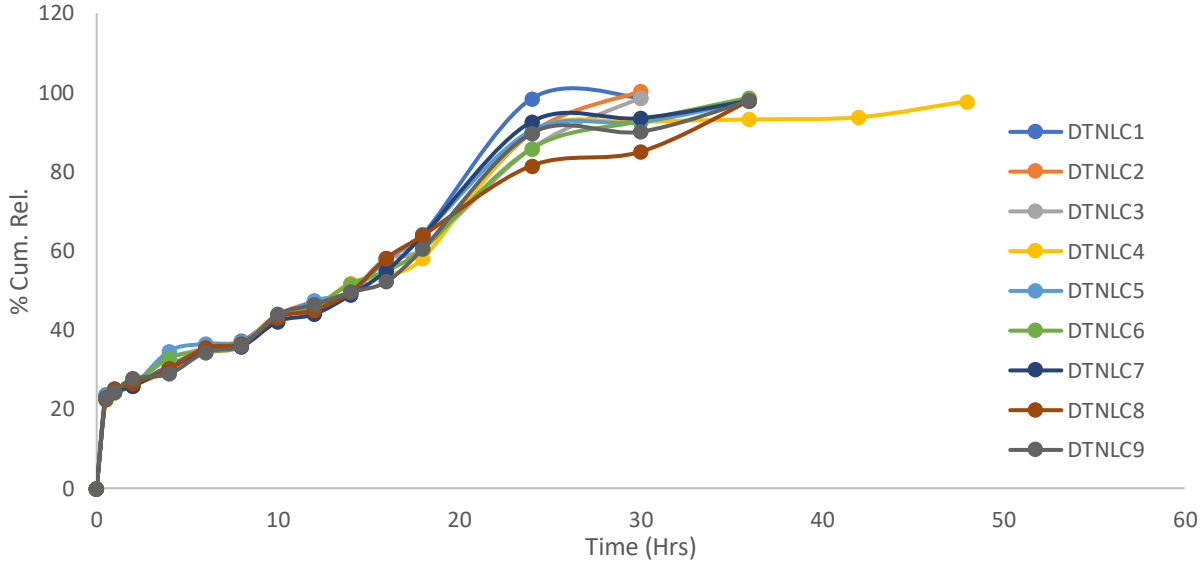

Figure 5: In Vitro Release of NLC Coupled with Dutasteride

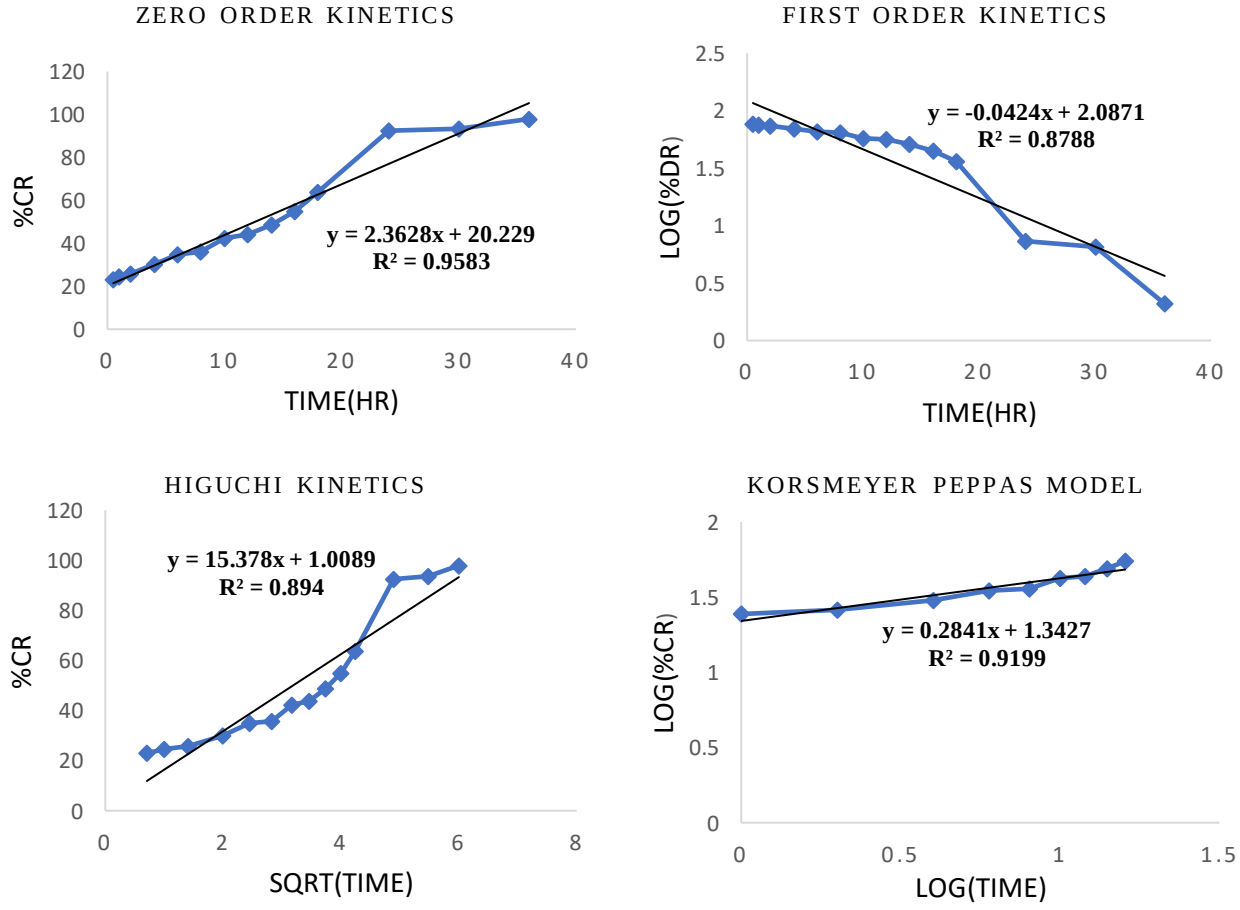

Figure 6: Graphs of Release Kinetics Study

Nine trial runs were produced using a 3^2-factor design and the hot melt-emulsification procedure with only minimal modifications. the one Stearic acid plus oleic acid was combined in a standard beaker at a lipid ratio of 1:4. The drug (0.5 mg) was incorporated into the above mixture. Using a water-based bath, the combination (Omix + drug) was brought up to 80 °C, a temperature roughly 10 °C above the point of melting of stearic acid. This was done to melt & dissolve the medicine. A magnetic stirring device (Remi Equipment Pvt. Ltd., Mumbai) was used to continuously stir the combination (Omix + drug) at 80 °C while the water phase (distilled water + surfactants) was heated to an identical temperature (80 °C). This mixture was kept for continuous stirring for 1 h at 500 rpm. After completion of 1 h, this emulsion was sonicated by using an ultra-probe Sonicator (Dakshin Pvt. Ltd., Mumbai) at a power of 170 volts (0.430 AMP) for 1 h. To avoid excess heating, specific time interval breaks were taken in between probe cycles. The subsequent mixture is then instantly cooled. The detailed composition of NLC's Formulation According 3^2 Factorial Design is given in Table 3

Evaluation dutasteride loaded NLC

Particle size, zeta potential, and poly dispersibility index

The Horiba SZ-100, Japan device was used to measure the dimension and distribution of the size of the dutasteride-optimized nanostructured lipid carrier. Using dynamic light scattering (DLS) on the SZ 100 V2 series. Using distilled water, 0.1 ml of the NLC mixture was diluted to make 10 ml.. The sample was filled in four opening quartz cuvettes and particle size was analysed. The sample obtained from the optimized batch has been placed in a carbon 6mm electrode cell to determine the Zeta potential. The Horiba SZ-100, made in Japan, was used to analyze the surface charge of NLCs at 25 °C.[15]

Percentage entrapment efficiency (% encapsulation efficiency)

NLCs were diluted with 0.5 M methanolic HCl solution and sonicated for 5 min to ascertain total drug concentration (A). Following that, a double-beam Shimadzu (UV-1800) spectrophotometer was used to assess total drug concentration. By centrifuging 1 mL of NLCs at 12,000 rpm for 45 minutes, we were able to assess how much drug was left unentrapped. 0.1 mL of supernatant was diluted with 10 ml 0.5 M methanolic hydrochloride solutions and unentrapped drug concentration (B) was determined by UV-spectrophotometer. Values of A and B were placed in the following equation to determine the entrapped drug.[16]

$$\text{Percentage entrapment efficiency (PDE)} = \frac{A-B}{A} \times 100$$

where "B" is the percentage of drug that is free & "A" is the overall quantity of drug.

Fourier transform infrared spectroscopy (FTIR)

Dutasteride, Poloxamer, Stearic Acid, Oleic Acid, and Optimized Formulation Fourier-transform infrared (FTIR) spectrum were examined. Solid samples were distributed in KBr, and a Bruker spectrometer fitted with an ATR diamond was used to scan the sample between 400 and 4000 cm−1.[17]

Transmission electron microscopy (TEM)

The morphology of carriers of nanostructured lipids has been studied using the transmission microscopy of electrons (TEM) method. For staining that was negative, a second droplet of 1% uranyl acetate was placed on the copper grid for 2 minutes after the first drop of each sample was applied. Grid was subjected to TEM examination after being dried at room temperature. (Holland, Philips, Technoi-20).[18]

Differential scanning calorimetry (DSC)

Utilizing Digital Signal Processing Q200 (TA instrument, USA), the DSC performance of a select few elements was investigated. The components were heated to a temperature of 10° C over a 20–200 C range, and a nitrogen purging of fifty milliliters per minute was implemented to carry out the research, multiple specimens of 2–8 mg of the individual medication, purified lipid, actual drug blending, & the final product was gathered and kept in a standard metal pan. As a comparison, an empty aluminum pan had been employed.[19]

Powder X-ray diffraction (pxrd) analysis

(XRD) had been carried out by D8 Discover, Bruker, Germany on DUT-NLC, DUT. XRD was used to analyze the formulations' crystalline or amorphous states. Between polyester films, powdered product additives and DUT were subjected to CuK" irradiation (45 kV, 40 mA, λ = 1.5418 Å). At 2□, steps of 0.026◦ and 200 s were used for the step size and time interval, respectively, during measurements from 2◦ to 60◦.[20]

***In-vitro* drug release study**

A bag for dialysis technique has been employed in the investigation on medication release in vitro. The bag needs to be activated by submerging it in filtered water for a full day before using it. Its atomic weight threshold ranges from 12,000 - 14,000 (150 represents the filtering layer price.), and its pores have a size of 2.4 nanometers. The drug-laden NLC was put inside bags with tight closures on both ends. The USP Dissolution apparatus Class Lab India, located in Mumbai, was used to hold the ambient temperature at 37 ± 0.5 ºC while stirring the bags at 100 rpm. The containers were set up in baskets. Up to 48 hours, 5 mL of the sample were taken out of the dissolution tube at 0.5,1,2,4.[21]

***In vitro* drug release kinetics study**

The usefulness of releasing data in numerous kinetics scenarios, such as the Zero order, First order, and Higuchi, Hixon Crowell, and Korsmeyer-Peppas releasing theories, was further studied using releasing data gathered from varied compositions. We were able to assess correlation values (R2) quality of fit and identify which model provided a better explanation of release pattern by using this compressive technique.

***In vitro* cytotoxicity studies**

Cell Culture

Ten percent fetal bovine serum is added to Dulbecco's modified Eagle's (DMEM) media. (Invitrogen, Carlsbad, CA), 4.5 g/l sugar, PC 3 cells, which are derived from prostate cancer, were cultured using 4 mM glutamine, 100 IU/ml of the antibiotic penicillin, with 100 milligrams per milliliter of streptomycin. Five days before the experiment began, 10,000 cells or so were planted into each well of 96-thoroughly cultured tissue plates. Following that, the plates were allowed to develop around 37 ℃ in an atmosphere with five percent carbon dioxide (CO2).

***In vitro* cell toxicity analysis (MTT assay)**

The harmful effect of dutasteride has been evaluated using the MTT test, and different composition doses were added to each well. Subsequently, the ceramic plates were placed back into the incubators. After 24 hours, aliquots of the 20 ml solution containing MTT were applied to each well. Then, the plates were put back in the incubators. Following three hours of incubation, the growth media in each well was taken out, and encapsulated purple formazan crystals were introduced to each well containing 150 ml of dimethyl sulfoxide (DMSO). Each well had a 100 ml aliquot that was taken out and put onto a fresh 96-well plate. After that, plates were analyzed with a microplate reader at 550 and 690 nm. The formazan crystals' absorbance readings were calculated by subtracting the reading at 690 nm from the reading at 550 nm. The negative control was an equivalent volume of sodium acetate buffer. Proportion of absorbance of negative control was employed to express the results. The

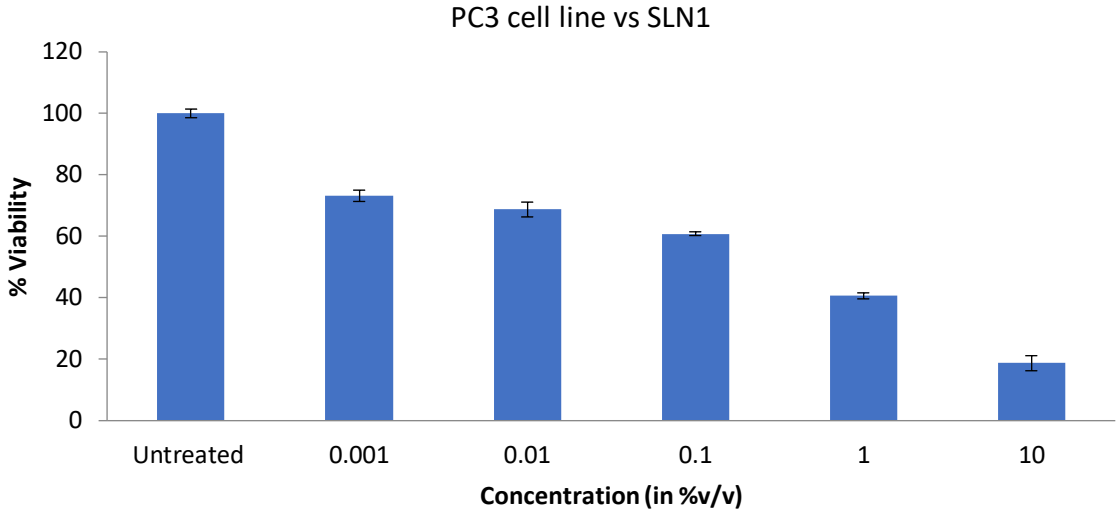

Figure 7: % Cell viability vs concentration (v/v)

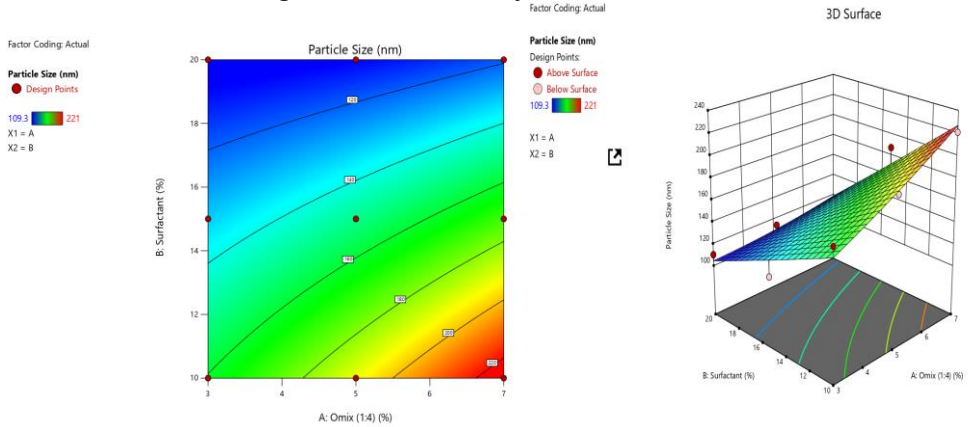

Figure 8: The impact on the factorial design batch's particle size is depicted in contour plot (a) along with the 3D response graph (b).

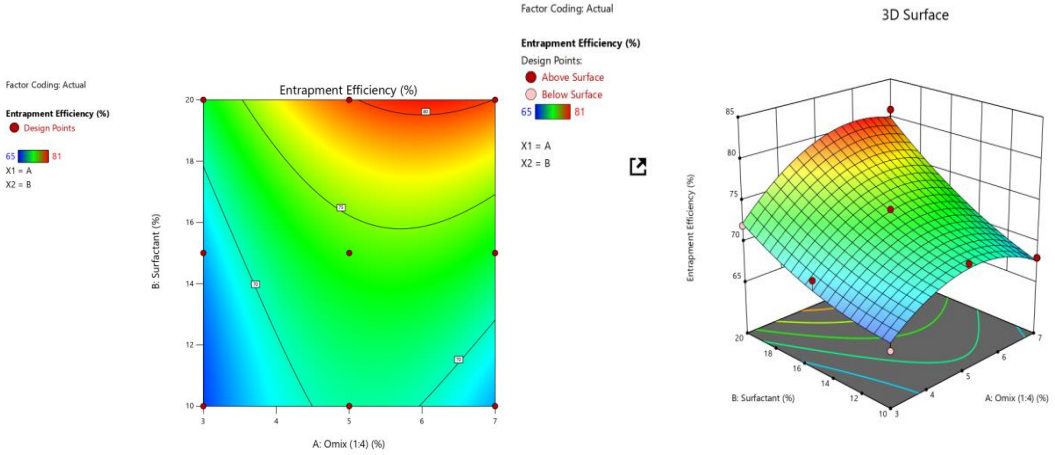

Figure 9: The impact on the factorial design batch's % entrapment efficiency is depicted in contour plot (a) along with the 3D response graph (b).

sample's half maximum concentration of inhibition (IC50) values was computed.[22]

Stability study

The optimal formulation was stored for 90 days at 4 ± 1ºC and 25 ± 1ºC in a dark environment to test the storage stability of NLC. At the 30th, 60th, and 90th day, 1 mL samples were taken out and analyzed for PS, ZP, PDI, and EE.[23-26]

Statistical analysis

For the statistical evaluation, an ANOVA was used; a statistically significant distinction is indicated by a p-value of below 0.05. The average ± the standard deviation was used for presenting all the data.

RESULT AND DISCUSSION

Choice of solid and liquid lipid

Dutasteride has a limited solubility in water of 0.038 ng/mL and a Log P score of 5.09. Stearic acid and other solid lipids had the highest drug solubility, followed by chitosan, lecithin, and GMS. Maximum solubility while dealing with liquid fat. Oleic acid was the most soluble of the liquid lipids, next to olive and soy oil. One stearic acid, as well as oleic acid, were chosen as both liquid and solid lipids, accordingly, during the production of DUT-NLC.

Selection of surfactants

Based on their capacity to generate a small size of particles (PS) and polydispersibility index (PDI), surfactants were chosen. Effects of surfactants like Poloxamer 407, Tween 20, and PEG 200 on particle size and PDI were studied.

Pseudo ternary phase diagram

From the results shown in Table 4, it is observed that all trials are stable but trials A & B have less percentage of surfactant as compared to C with the same percentage of O mix. In a comparison of trials A & B again there is a difference between O_{mix} ratio in which the same surfactant concentration, but trial B was found to be stable even though possessing a higher ratio of liquid lipids. Which is further helpful in improving the entrapment efficiency of the drug.

Particle size, zeta potential, and polydispersibility index

Dutasteride-loaded NLCs had particle sizes that ranged between 100 and 230 nm, it is advantageous. The size and size distribution of the enhanced composition (E4) are 109.3 nm, having a PDI near 0.364 with an apparent zeta potential near -37.5 The mv. These results are shown in Table 5. As for the zeta potential, values between −23 and −38mV are reasonable for particle repulsion and prevention of aggregation.

Percentage entrapment efficiency

Percentage Entrapment Efficiency of Dutasteride loaded NLC of optimized batch E4 was achieved high entrapment efficiency which is 81% respectively.

Transmission electron microscopy (TEM)

Photomicrograph of dutasteride loaded NLCs suspension revealed near spherical to elliptical morphology as depicted in Figure 2.

Fourier transform infrared spectroscopy (FTIR)

FTIR examination was determine the potential chemical bond interactions between Dutasteride and the excipients within the formulation. Figure 3 illustrates stacked IR spectra of the dutasteride, poloxamer 407, stearic acid, oleic acid, and physical, and formulation. Pure dutasteride's infrared spectra showed several peaks, such as one for N-H stretching (Amide) at 3320.05 cm-1, another for C-H stretching at 2987 cm-1, a peak with C=O extending (Ketone) at 1615 cm-1, and a peak for C-N stretch (Amine group) about 1278 cm-1. The existence of C-F stretches, which are connected to halogens, was suggested by the highest value at 700 cm-1, while C-H extending was linked to a peak at 3100 cm-1. A C-H stretching peak at 2834 cm-1, an O- H stretching spike at 3470 cm-1, and a C-O-C stretching signal (ether) around 1160 cm-1 were among the unique features of the poloxamer 407 spectra. The stearic acid spectra, on the other hand, showed two distinct peaks: one at 3322 cm-1, which was linked to O-H stretching, while the other at 1634 cm-1, which was linked to C=O stretch. In addition, peaks were seen that correlated to the stretching of C-O at 1225 cm-1 while C-H extended at about 2860 cm-1. The O-H stretching point of oleic acid is 3320 cm-1, the C-H stretching spike is 2855 cm-1, and the C=O stretching peak is 1634 cm-1. At conclusion, the FTIR spectrum as shown in Figure 3 has verified that there are no problems with dutasteride compatibility. poloxamer 407, stearic acid, oleic acid and DUT-NLC formulation (E4) (Fig. 3) There were no notable changes from the typical peaks of the pure medication in the spectral data from this DUT-NLC preparation (E4). The stretching peaks for O-H, C-H, C=O, C-N extending spike (Amine), C-O, & C=O were measured to be 3400 cm-1, 2900 cm-1, 1678 cm-1, and 1120 cm-1, respectively.

Differential scanning calorimetry (DSC)

Any changes in thermal behavior during DUT-NLC formulation that are connected to interactions between drug and excipient can be found via DSC analysis. Distinctive endothermic peaks at 251.69°C were seen in the DSC thermogram of pure DUT. DSC thermogram for Stearic acid was recorded between 60 and 57 °C.DSC thermogram for oleic acid revealed an endothermic peak at 242°C. DSC investigation for poloxamer 407 revealed an endothermic peak at 155.53°C. A large peak was visible in the physical combination of lipids around 57.49°C.

X-ray diffraction

XRD has been employed for inquiry into differences among the microstructural state of NLCs.

Figure 4 illustrates the study of scattering trends for purified medication, stearic acid, oleic acid, Poloxamer 407, physical mixture, and optimum formulation using Source Pro BVR 2018 plotting and evaluation program (Origin Lab Corporation, USA). A prominent peak was seen in the pure DUT diffraction spectra, with values ranging from 0° to 30°.

***In-vitro* drug release study**

A customized USP dissolution equipment has been used to examine the pharmaceutical release profile via NLC. A 48-hour continuous release was demonstrated by formulations, with E4 exhibiting the longest sustained release at 97.75 hours. Based on more sustained release and adequate trapping of 81 E4, the optimal formulation was chosen as illustrated in Figure 5.

***In vitro* drug release kinetics study**

The drug's release kinetics analysis as showed in Table 6 and Figure 6 that the most suitable model for all compositions had been determined to have a zero-order kinetics model with "n" = 0.281 with an R2 value of 0.915, indicating a non-Fickian diffusion release process for the Dutasteride laden NLC used in the E4 preparation.

***In vitro* cytotoxicity studies**

It was observed that DUT and DUT-NLCs exhibited dose-dependent cytotoxicity in cells. As compared to blank, untreated, 0.001% (v/v), 0.01% (v/v), 0.1% (v/v), and 1% (v/v) DUT-treated cells, there was a substantial reduction in percentage of cell viability of 10% (v/v) test concentration in DUT-NLCs treated cells (Figure 7), demonstrating the safety of developed formulation. Higher cytotoxicity was shown by lower IC50 in DUT-NLC-treated cells compared to IC50 of pure DUT. Higher cytotoxicity of DUT-NLCs is caused by improved intracellular penetration of DUT nanoparticles compared to their aqueous counterpart. This implies that suggested formulation has a higher cytotoxic result on cancer cells.

ANOVA for quadratic model

Response: impact on the size of particles

The study examined the impact of Omix quantity and concentration of surfactant on particle size. The corresponding contour plot as well as three-dimensional surface responsiveness are displayed in Figure 8. The size of particles expanded with an increase in Omix phase proportion while it decreased with a rise in surfactant content. The following polynomial equation (Equation (1)) illustrates the primary, interactive, and polynomial impacts of Omix quantity and surfactant concentration on particle size.

Final equation in coded factors

$$\text{Particle Size} = 149.91+20.13A-41.13B-12.97AB+2.33A^2+0.533B^2 \quad \text{(1)}$$

Response: entrapment efficiency

The impact of Omix percentage with surfactants concentration on the percentage efficiency of entrapment is depicted in a contour plot & 3D surface graph in Figure 9. The EE rose due to the Omix stage & surfactant concentrations raised. Due to the rise in the concentration of Omix phase increases in EE but after some extent of Omix there is no change in EE may be due to space occupied by the lipidic phase escape drug. In addition, polynomial equation (2) confirmed that the effect of the amount of Omix and surfactant concentration was more significant (p <0.05) on percent entrapment efficiency.

$$\text{Entrapment Efficiency} = +73.78+2.33A+4.67B+1.50AB-3.67A^2+1.33B^2 \quad (2)$$

Stability study

The formulation under investigation for physical stability investigations shows (Table 7) minor changes in zeta potential value, PDI, and particle size over 90 days. The findings showed that after 7 days, the optimized formulation generated 109.3nm, -37.5mV, and 0.306. With the optimized formulation, there were no appreciable changes; at 0 days, the zeta potential was 37.5, and at the conclusion, it was 35.6.

CONCLUSION

The optimized formulation of Dutasteride-loaded NLCs achieved desirable properties, including small particle size (109.3 nm), high entrapment efficiency (81%), and prolonged drug release. The formulation demonstrated compatibility between the drug and excipients, improved cytotoxicity in cancer cells, and maintained stability over time. These findings highlight the potential of DUT-NLCs for enhanced delivery and therapeutic efficacy in treating prostate cancer, with sustained release and minimal side effects.

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