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Research Article

Nanostructured Lipid Carriers of Eperisone Hydrochloride using Quality By Design Approach

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Abstract

Objective: The current work involves formulation development of nanostructured lipid carrier (NLC) of eperisone hydrochloride (EPE) using quality by design (QbD) approach.

Materials and Methods: Initial screening of solid and liquid lipids was performed based on the Hansen solubility parameters of EPE and the lipids and later confirmed using saturation solubility studies. The optimum formulation was identified using three-squared randomized full factorial design. High shear homogenization coupled with ultrasonication was used to prepare the NLCs. The effect of the independent variables on the dependent variables was analyzed using response surface graphs and ANOVA. The optimized formulation is characterized for mean particle size, zeta potential, entrapment efficiency, *in vitro* drug release and subjected to stability testing.

Results and Discussion: Precirol® ATO 5, Capryol™ 90, and Pluronic® F127 are found to be ideal solid lipid, liquid lipid and surfactant respectively based on the initial screening. Amount of lipid mixture (X1) and the amount of surfactant (X2) were selected as independent variables and the entrapment efficiency (Y1), mean particle size (Y2) and zeta potential (Y3) were selected as dependent variables. The optimized NLC formulation has mean particle size of 175 nm, zeta potential -24.4 mV and entrapment efficiency of 78.64%. The *in vitro* drug release data suggested that the drug release follows Quasi-Fickian diffusion. The stability data showed that the NLCs are stable up to 3 months at refrigerated conditions.

Conclusions: The Hansen solubility parameter approach coupled with QbD is a powerful tool for formulation of sustained release NLCs of highly soluble poorly soluble drugs.

Keywords: Nanostructured lipid carrier, Solubility Parameters, Nanoparticles, Lipid based drug delivery, Quality by Design

INTRODUCTION

Oral route is the most convenient and preferred route of administration. Popularity of oral route is attributed to its greater convenience, non- invasive nature, high patient compliance, ease of administration, reduced risk of cross-infection and an advantage in drug absorption due to large surface area of gastrointestinal tract (GIT). [1] However, some drugs are reported to have poor oral bioavailability as they get metabolized either during first pass metabolism or by gut cytochrome and efflux of P-glycoprotein. This makes oral route of administration a challenging route for certain drugs. [2, 3] To overcome these limitations, various drug delivery systems like liposomes, microemulsions, nanoemulsions, nanocapsule, polymeric nanoparticles, lipid nanoparticles etc. are being explored. [4, 5] Lipid-based drug delivery systems like solid lipid nanoparticles (SLN) and nanostructured lipid particles (NLC) have gained tremendous attention during the last few decades due to their ability to encapsulate the drug and prevent it from undergoing metabolism. [6]

NLCs represent a new and improved generation of SLNs and are made of a solid lipid matrix entrapping liquid lipid nano compartments, the blend being solid at body temperature. NLCs have good physical stability, high drug payload, controlled drug release, specific targeting, scalability, and feasibility of delivering both lipophilic and hydrophilic drugs. Their protective effect, coupled with their sustained/controlled release properties, prevents drugs/macromolecules from premature degradation and improves their stability in the gastrointestinal tract (GIT). [7, 8]

Eperisone (EPE) is an antispasmodic drug which relaxes both skeletal muscles and vascular smooth muscle and demonstrates a variety of effects such as reduction of myotonia, improvement of circulation, and suppression of the pain reflex. EPE is reported to have short biological half-life (1.6 to 1.8 hr) due to high first pass metabolism in the liver. Therefore, it is administered in 3 doses of 50 mg per day. [9] The pharmacokinetic study of immediate release EPE formulation observed rapid elimination from the body with inter-personal variation in the plasma concentration (C_{max}=0.80–44.8ng/ml, $AUC_{0-\infty}$ =1.16–76.1 ng/ml.h).

Due to short half-life of EPE and its poor bioavailability because of first-pass metabolism, it is a potential candidate for the development of sustained release (SR) NLCs. Sustained release formulations are needed for EPE to prolong its

duration of action and to improve patient compliance. If EPE can be incorporated into NLCs, direct lymphatic absorption of the NLCs avoids first pass metabolism and thus improves oral bioavailability because intestinal lymph vessels drain into the venous blood and thus bypass the portal circulation. [10] The function of lymphatic system is to facilitate the absorption of lipids especially long chain fatty acids. This specialized physiological transport mechanism is thus being widely explored for oral drug delivery through colloidal carriers, like nanoparticles, which by passes the first-pass metabolism pathway. [11] Thus, NLCs, a lipid-based carrier system in nanosized range may enhance the oral bioavailability of EPE.

In the current study, NLCs of EPE were prepared using a solid lipid, a liquid lipid, and a surfactant employing high shear homogenization coupled with ultrasonication method. Initial screening of solid lipid and liquid lipid was carried out using theoretical Hansen solubility parameters of the drug and the lipids and later confirmed using high throughput solubility testing. Quality by Design (QbD) approach was followed to obtain a robust formulation with the required target product attributes. A three squared factorial design was used to study the effect of amount of lipid mixture and the amount of surfactant on entrapment efficiency (%EE), mean particle size (MPS) and zeta potential (ZP) of the NLCs. The design space obtained using the experimental design was validated and the optimized formulation was determined.

MATERIALS AND METHODS

Eperisone (EPE) is kindly gifted by Sharon bio-medicine Ltd. (Mumbai, India). Capryol™ 90, Precirol® ATO 5, Compritol® 888 ATO, Labrafil® M 2125 CS and Transcutol® P were gifted by Gattefosse Ltd. (New Jersey, USA). BASF Corporation (New Jersey, USA) gifted Pluronic® F-127, Pluronic® F-68 and Cremophor® RH 40. Imwitro® 491 and Imwitro® 900 K were gifted from IOI Oleo GmbH (Hamburg, Germany). Ethyl oleate and Isopropyl mysristate were purchased by S.D. Fine chem Ltd. (Mumbai, India). Oleic acid, Tween® 20 and Tween® 80 were gifted by Loba Chemie Pvt. Ltd. (Mumbai, India). Capmul® MCM was gifted by Abitec corporation (India).

Screening of solid and liquid lipids:

Based on the results of the Hansen solubility parameters of lipids and EPE derived using the following equation. [12]

$$\delta_t = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \quad (1)$$

Where δ_d, δ_p and δ_h are calculated using the following equation:

$$\delta_d = \frac{\sum F_{di}}{V}; \quad \delta_p = \frac{\sqrt{\sum F_{pi}^2}}{V}; \quad \delta_h = \sqrt{\frac{\sum E_{hi}}{V}} \quad (2)$$

The values of F_{di}, F_{pi} and E_{hi} are calculated according to the group contribution method by Van Krevelen and the value of molar volume, V is calculated according to Fedors. [13]

The practical solubility of EPE in the lipids was determined by accurately weighing 10 mg of EPE in a test-tube to which solid lipid was added in increments. The test-tube was kept in water bath and the temperature was adjusted above the melting point of respective solid lipid. The test-tube was intermittently vortexed on cyclone mixer. This was continued until there were no EPE residues seen. The endpoint being clear solution. [14] The amount of solid lipid required to solubilize 10 mg of EPE was determined.

To determine the solubility of EPE in liquid lipids, 2 ml of liquid lipid was taken in glass vial and excess quantity of EPE was added to each vial. The mixture was vortexed on cyclone mixer for 10 min and then kept on mechanical shaker at 150 rpm for 24 h at room temperature. The content of each vial was centrifuged at 9000 rpm for 30 min to separate the undissolved EPE. The supernatant was then collected and analyzed by UV-visible spectroscopy at 255 nm by suitably diluting each sample with methanol.

Screening of surfactants:

The screening of surfactant was performed by determining the solubility of EPE in various surfactants and identify the surfactants in which EPE has least solubility. Based on the initial screening of EPE in various surfactants, it was found that the EPE exhibited least solubility in Tween 20, Poloxamer 407 and Tween 80. Preoptimized batches were prepared using these surfactants at three different concentrations i.e., 0.25%, 0.5%, 1% v/v. NLCs thus prepared were evaluated for the %entrapment efficiency, mean particle size and immediate stability at room conditions.

Quality by Design (QbD):

According to QbD approach, the quality target product profile (QTPP) was set for the development of NLCs of EPE, to ensure the desired quality, safety, and efficacy of the drug product. QTPP elements for developing EPE NLCs include key quality characteristics such as dosage form, dosage strength, route of administration, and stability requirements. [15] The QTPP was defined based on the required biopharmaceutical properties and the existing marketed formulations. The dosage form is to prepare an NLC formulation due to its ability to increase the oral bioavailability. A sustained drug release is desired to provide enough time for the permeation of NLCs. A dosage strength is assigned as 150 mg based on the marketed formulations and the dose required to show antispasmodic activity. The desired route is oral route due to the patience compliance and the biopharmaceutical properties of the drug. The target stability of the NLCs is taken as 3 months due to the time frame of the study. This should be at least 24 months for NLCs. Once the QTPP was set, the critical quality attributes (CQAs) and critical material attributes (CMAs) were identified. The CQA of NLCs that prominently influencing the performance of drug product include mean particle size, zeta potential and entrapment efficiency. The CMAs that influence the CQAs are predominantly, the lipid concentration, the amount of surfactant, the type of surfactant. To reduce the number of experiments, the dependent factors (CMAs) were further reduced by keeping the type of surfactant constant. The two remaining CMAs, amount of surfactant and lipid concentration were further analyzed using an experimental design to identify the optimal formulation and design space.

Experimental design:

A three-squared 3^2 factorial design was used to determine the effect of two factors varied at three different levels to optimize the NLC of EPE.[16] Based on preliminary runs lipids and surfactant concentration was known to have a great impact on entrapment efficiency and particle size of NLC. Thus, lipid concentration (X1) and surfactant concentration (X2) were considered as independent variables. Entrapment efficiency (EE), mean particle size (PS) and zeta potential (mV) was considered as dependent variables. The operating conditions i.e., the rpm and time of Ultra Turrax as well as the time and power for ultrasonication during preparation of NLCs dispersion were adjusted based on the results obtained in preliminary study and were later kept constant during the experimental runs. A 3^2 factorial design was employed to study the effect of two independent variables at three levels on dependent variables. This design allows estimating all main factor and interaction effects between the considerable factors. Multiple regression analysis (MLRA) was used to analyze the optimization data by mathematical modeling and

fitting the experimental data to a second-order quadratic polynomial model with added interaction factors. The model was analyzed based on the values of correlation coefficient (R) and lack of fit. Response surface plots and contour plots were constructed to understand the relationship among the studied independent parameters on the dependent parameters. Design Expert Version 11 (Stat-Ease, Inc. MN, USA) was used for the experimental design and data analysis.

Preparation of NLC:

Accurately weighed quantity of lipophilic excipients like solid lipid and liquid lipid were put in glass vial and maintained at 80°C with continuous stirring to mix the lipophilic excipients. Weighed quantity of drug was then added to the previous mixture and allowed to dissolve. In another beaker, surfactant was dissolved in double distilled water and maintained at 80°C. The surfactant solution (aqueous phase) was mixed with the lipophilic phase containing drug and homogenized at 8000 rpm using homogenizer (IKA T-10 Ultra-Turrax, Germany) at 80°C for 15 minutes. The coarser o/w emulsion was then sonicated using probe sonicator (Oscar Ultrasonics, Mumbai) at 80°C at 50 watts for 10 minutes. [17, 18] This resulted o/w nanoemulsion was rapidly cooled down to 4-8°C on magnetic stirrer. This step converts the lipidic nanodroplets to get converted into nanoparticles to give NLC dispersion.

Characterization of NLC:

Particle size and zeta potential

Particle size (z- average), polydispersity index and zeta potential were measured using photon cross correlation spectroscopy employing NANOPHOX particle size analyzer (Sympatec GmbH, Germany) using WINDOX 5 software. Before measuring the particle size NLC dispersion was suitably diluted to obtain suitable scattering intensity (count rate of 100- 1000 kbps). The diluted NLC dispersion was then poured in the cuvette which was then placed in the cuvette holder and then particle size was measured using Nanophox control software. All the experiments were performed in triplicate and the data is expressed as mean ± SD (n=3).

Drug entrapment efficiency

Drug entrapment efficiency (%EE) of NLC dispersion was determined by measuring the unentrapped drug (coagulation centrifugation method). Appropriate amount of NLC dispersion was diluted using saturated aqueous sodium chloride solution. This diluted solution was centrifuged using at 9800 rpm for 30 minutes. After centrifugation, the supernatant was withdrawn and analyzed by suitably diluting with methanol AR using UV-3000 Plus UV-visible spectrophotometer (Lab India Analytical Instruments, India) at 255 nm. [19] The % entrapment efficiency was determined by difference between initial drug content and unentrapped drug as shown below in equation 3. All the experiments are performed in triplicate and the data is expressed as mean ± SD (n=3).

$$\%\text{Entrapmet efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100 \qquad (3)$$

Characterization of optimized formulation:

Particle size, zeta potential and entrapment efficiency:

Mean particle size (MPS) and zeta potential (ZP) of the optimized formulation was determined using Nanophox particle size analyzer (Sympatec GmbH, Germany) and the data was analyzed using WINDOX 5 software (Sympatec GmbH, Germany) as discussed earlier. The entrapment efficiency (%EE) was determined using UV-3000 Plus UV-visible spectrophotometer (Lab India Analytical Instruments, India) as discussed earlier.

***In vitro* drug release:**

Drug release of EPE from the NLCs was determined by dialysis membrane technique in simulated gastric fluid for 2 hr, and then for 24 hr in simulated intestinal fluid using USP XXIII dissolution testing apparatus type II with rotating paddle at 50 rpm and temperature of 37 ± 0.5°C. 5 ml of optimized NLCs dispersion equivalent to 75 mg of EPE was used in dissolution study. The NLCs dispersion was filled in dialysis membrane with a molecular weight cutoff of 12kDa, which was soaked previously in double distilled water for 24 h and it was tied to the paddle of dissolution apparatus. Samples were withdrawn at various time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h from dissolution medium and replaced with fresh media to maintain sink condition. The aliquots withdrawn were filtered through syringe filters and analyzed using HPLC.

Stability studies:

The optimized formulation was subjected to stability testing for a period of 3 months at the refrigeration temperature (5 ± 1°C) and room temperature (25 ± 2°C / 60±5% RH). The samples were taken after 1 month and at the end of 3 months and the stability of the optimized formulation was evaluated based on the appearance, % entrapment efficiency, mean particle size, zeta potential and polydispersity index.

RESULTS

Selection of solid and liquid lipid:

The screening of solid lipids and liquid lipids was performed by initially comparing the Hansen solubility parameters of the lipids and EPE. The solubility parameters of the solid and liquid lipids, along with their solubility parameter difference (Δδ) with EPE are shown in **Table 1**. The results show that the miscibility of EPE in the solid lipids is in the order of Percirol® ATO 5 > Geleol™ > Imwistrol® 900K > Imwitrol® 491 > Stearic acid > Compritol® 888 ATO. Also, according to the solubility parameter difference, EPE is miscible in all the solid lipids studied. On the other hand, the miscibility of EPE in the liquid lipids was found to be in the order of Capryol® 90 > Ethyl oleate > Oleic acid > Isopropyl myristate > Capmul® MCM EP. These results are compared with the experimentally obtained solubility values of EPE in solid and liquid lipids. Amount of various solid lipids required to solubilized EPE per gm of solid lipids is shown in **Figure 1a**. Among various solid lipids screened, Precirol® ATO 5 was able to solubilize EPE to maximum extent as compared to other solid lipids i.e., 34.6 mg of EPE was soluble per gm of Precirol® ATO 5. These results are in line with the results obtained from the solubility parameter approach. Moreover, Jannin *et al* reported that sustained release properties of Precirol® ATO 5. [20, 21] Hence, it was selected as the solid lipid for further studies.

Table 1: Hansen Solubility Parameters of Solid and Liquid Lipids along with their solubility parameter difference with EPE.

Solid Lipid	δ_d	δ_p	δ_h	δ_t	$\Delta\delta$
Compritol® 888 ATO	16.5	1.0	1.2	16.57	3.27
Imwitrol® 491	16.2	2.4	7.6	18.05	1.79
Geleol™	16.3	4.2	10.3	19.65	0.19
Stearic acid	16.2	2.8	5.2	17.24	2.60
Percirol® ATO 5	16.4	2.3	11.1	19.93	0.09
Imwitrol® 900K	16.7	1.3	7.15	18.17	1.67
Liquid Lipid					
Capryol® 90	16.4	5.1	8.7	19.25	0.59
Campul® MCM EP	10.3	5.5	9.8	15.24	4.60
Ethyl oleate	16.2	3.2	7.9	18.30	1.54
Oleic acid	16.6	2.8	6.2	17.38	2.46
Isopropyl myristate	16.2	2.1	4.6	16.80	3.04

The selection criterion for liquid lipids was like that of solid lipids. The solubility of EPE in various liquid lipids is shown in **Figure 1b**. Among the various liquid lipids screened, Capryol™ 90 showed highest solubility of EPE (15.2 ± 1.2 mg/mL). This is in line with the results obtained using the solubility parameter approach. Hence, Capryol™ 90 was considered as the best choice for the preparation of NLCs to maximize the entrapment of drug.

Screening of surfactant:

The equilibrium solubility data of EPE in various surfactants is shown in **Figure 1c**. It was found that EPE has least solubility in Pluronic® F-127 (0.32 ± mg/mL) followed by Tween® 20 (0.42 ± mg/mL) and Tween® 80 (0.57 ± mg/mL). As the values of solubility in these three surfactants is close to each other, there three surfactants were screened for pre-optimization batches in the concentration range of 0.25 -1 % w/w and evaluated for stability, % EE and MPS. The formulations containing Tween® 80 failed to form nano emulsion. The formulations containing Tween® 20 formed nano emulsions but were unstable upon storage, resulting in phase separation. The failure of tween 80 and tween 20 to form a stable emulsion due to their poor emulsification power. Due to the poor emulsifying power of the surfactant it led to the absence of adsorption at the interface and therefore increase in the interfacial tension and formation of unstable emulsion [22]. The formulations containing 0.25% and 0.5% w/w of Pluronic® F-127 formed stable nano emulsions with %EE of > 70%. However, formulations containing 0.5% w/w Pluronic® F-127 resulted in larger particle size of 321 nm. The larger particle size is observed may be due to the accumulation of surfactant molecule on NLC surface due to hydrophobic interaction causing increased particle size. The formulations containing 0.25% w/w of Pluronic® F-127 resulted in % EE of 79.8 ± 4.2% and MPS of 167 ± 23 nm. Hence, Pluronic® F-127 was chosen as the surfactant at a concentration of 0.25% w/w for further optimization studies.

Formulation optimization using experimental design:

Based on the initial screening and risk assessment studies, the highly influential factors that affect the CQAs of NCLs were identified and systematic optimization was done using a three-squared factorial design. The nine experimental runs along with the observed responses are given in **Table 2**. To find the best fit, multiple models are fitted with the observed data. It was discovered that a second-order quadratic polynomial model could accommodate all the responses. The non-significant model coefficients are removed, and the significant coefficients—along with the interaction terms—are then identified. The summary of ANOVA results shown in **Table 3**, suggest that the model is significant for all the studied responses. The lack of fit was found to be not significant, which show that the experimental results have excellent goodness of fit.

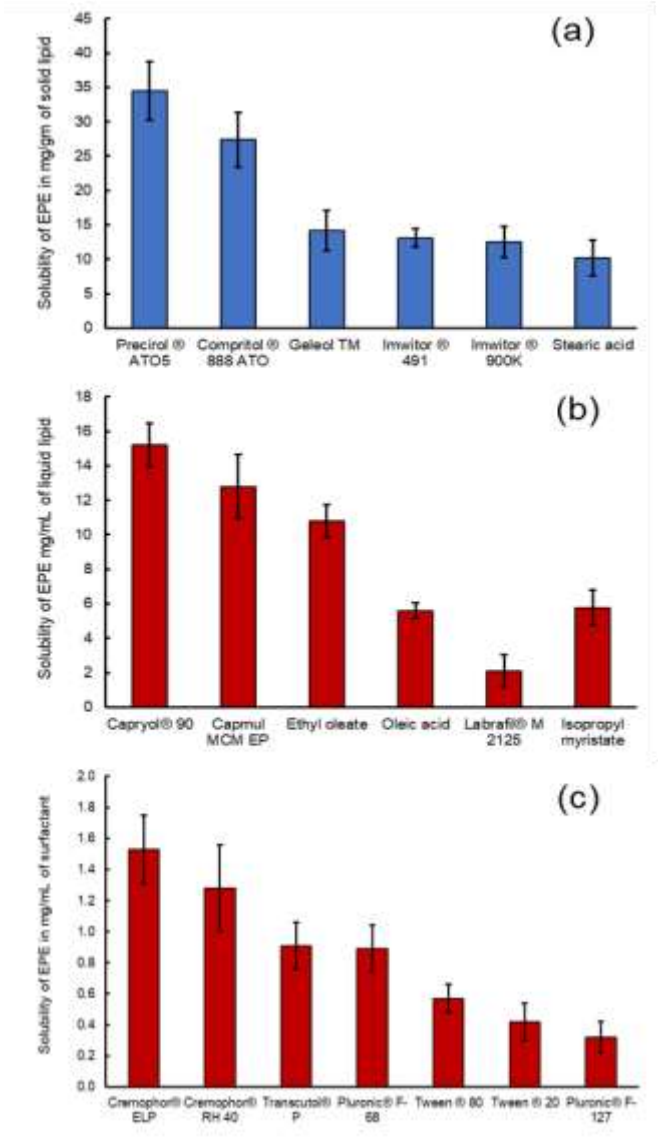

Figure 1: Equilibrium solubility data of EPE in various (a) solid lipids, (b) liquid lipids and (c) surfactants. The data is expressed as mean ± SD (n=3)

Table 2: The experimental design and the responses for experimental design

Formulation	CMAs		CQAs		
	Conc. of Lipid Mixture (mg)	Conc. of surfactant (mg)	Entrapment Efficiency (%)	Mean Particle Size (nm)	Zeta Potential (mV)
F1	700	175	88.59 ± 2.36	267.58 ± 12.36	-28.15 ± 2.41
F2	750	125	86.59 ± 5.85	225.02 ± 20.23	-36.63 ± 3.35
F3	650	175	74.02 ± 3.66	111.25 ± 06.34	-30.45 ± 5.22
F4	750	225	79.98 ± 3.58	153.44 ± 07.34	-37.17 ± 6.28
F5	650	125	79.89 ± 6.34	131.53 ± 14.57	-46.47 ± 5.45
F6	700	125	90.11 ± 5.46	289.44 ± 14.22	-39.22 ± 7.84
F7	700	225	85.73 ± 3.19	209.02 ± 11.23	-27.64 ± 3.39
F8	750	175	82.35 ± 4.22	160.87 ± 10.98	-24.32 ± 3.42
F9	650	225	71.42 ± 2.08	105.79 ± 08.65	-21.22 ± 2.88

Table 3: Summary of results from ANOVA results

Parameters	SS	DF	MS	*F* value	*p* Value	Significance
% Entrapment Efficiency						
Model	323.21	5	64.64	37.36	0.0067	significant
Residual	5.19	3	1.73			
Total	328.40	8				
Mean Particle Size						
Model	3476.68	5	6995.34	19.69	0.0168	significant
Residual	1066.08	3	355.36			
Total	36042.76	8				
Zeta Potential						
Model	484.50	5	96.90	9.07	0.0496	significant
Residual	32.04	3	10.68			
Total	516.54	8				

Response surface mapping:

Response surface plots give the enhanced product and process understanding by showing the cause-and-effect relationship among the factors which are studied. **Figure 2a and 2b** are 3D- response surface plot and counter plot for entrapment efficiency which illustrate that at lower levels of lipids, increasing the concentration of surfactant did not affect the entrapment efficiency significantly. Whereas, at increased levels of lipids, nearly asymptotic values of % entrapment efficiency was observed with increase in the concentration of surfactant. In contrast, at lower level of surfactant, increasing the concentration of lipid mixture increased the entrapment efficiency until certain lipid concentration, then decreased.

Entrapment Efficiency (Y_1) =

$$87.72 + 3.93X_1 - 3.24X_2 + 0.4650X_1X_2 - 9.10X_1^2 + 0.6333X_2^2$$

Figure 2c illustrate the response surface plot for particle size which shows that at low lipid level, the particle size decreases with increase in concentration of surfactant. At, low level of surfactant the particle size decrease with increase in lipid concentration. Lower particle size was observed at medium lipid concentration and high surfactant level. At lower lipid concentration, the mean particle size decreased with increase in the concentration of surfactant. Same phenomena were observed at high lipid concentration the mean particle size changed asymptomatically with increase in the concentration of surfactant. The lower particle size was observed at medium lipid concentration and lipid concentration. Contour plot depicted in **Figure 2d** indicate that the lower particle size was observed at medium lipid level and high surfactant. Increase in the concentration of lipid did not show any influence on the mean particle size.

Mean particle size (Y_2) =

$$251.48 + 31.79X_1 - 29.62X_2 - 11.46X_1X_2 - 107.36X_1^2 + 5.81X_2^2$$

Figures 2e and **Figure 2f** depict the response surface plot and contour plot for zeta potential, where at lower levels of lipid an increase in surfactant concentration from low to high values results in an increase in zeta potential. Also, increase in zeta potential was observed at lower of concentration of surfactant and increasing the concentration of lipid from low concentration to high concentration. Maximal vale of zeta potential was observed at higher level of surfactant concentration and low lipid concentration. Higher value of zeta potential indicates the stability of the prepared nanostructured lipid carrier.

Zeta potential (Y_3) =

$$-26.73 - 0.0283X_1 + 5.97X_2 - 6.54X_1X_2 - 0.9783X_1^2 - 6.98X_2^2$$

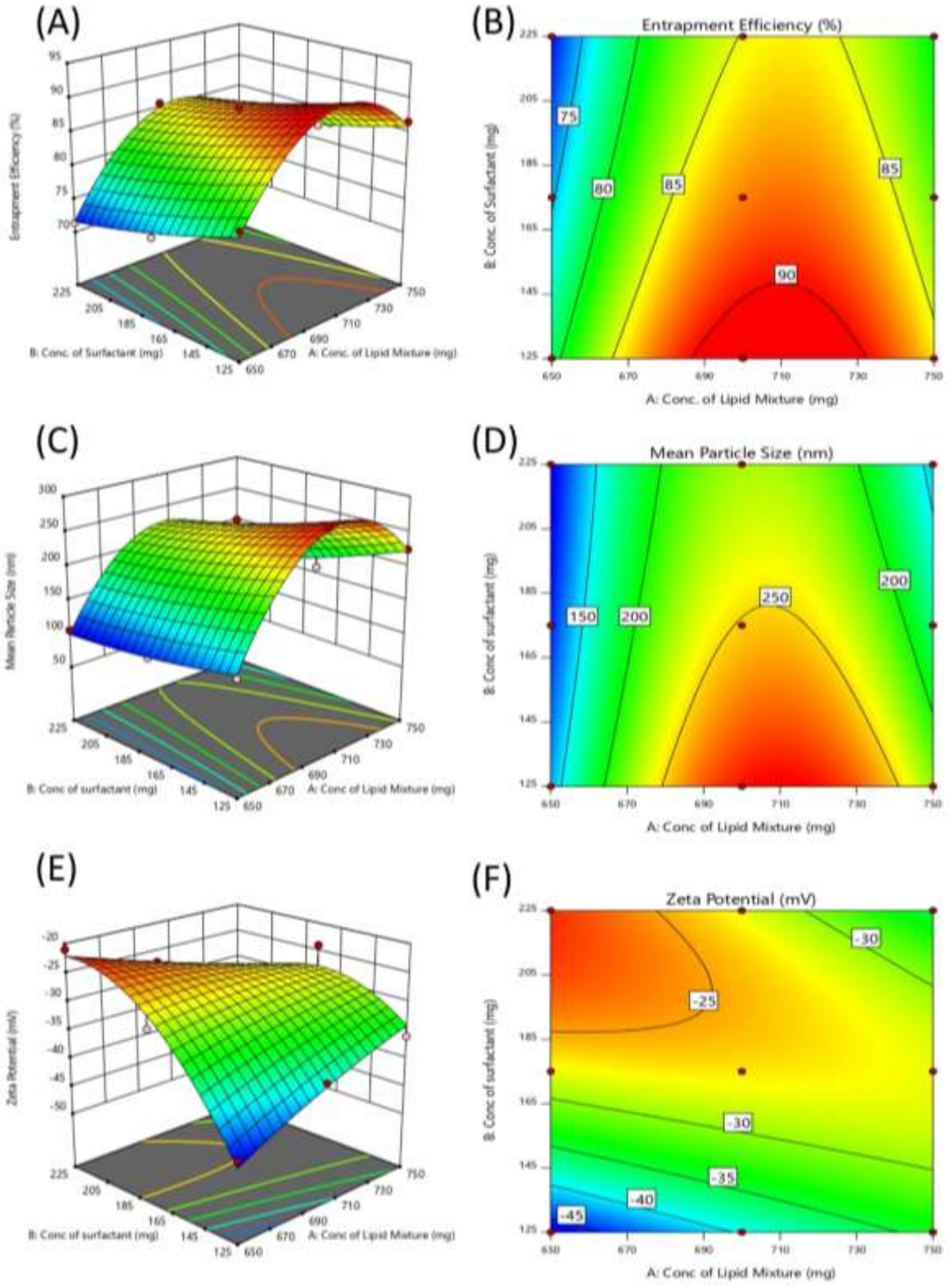

Figure 2: 3D response surface plot and 2D contour plots showing the effect of lipid and surfactant concentration on the dependent variables

Selection of optimum formulation:

The optimal formulation was chosen using numerical optimization and graphical optimization. Initially, each CQA's target values were assigned, and numerical optimization was used to determine possible solutions and desirability values. The optimized batch was selected from among the solutions obtained for the criteria (all factors are kept in range, % EE: maximum, MPS: minimum and ZP: in range). The optimal formulation was determined to be the solutions with desirability near to one. **Table 4** displays the outcomes of the numerical optimization of the NLCs of the EPE. Also, the differentiation of the optimum formulation was made using overlay plots within the design space region.

Table 4: Criterion for numerical optimization of NLCs of EPE

	Constraints			
	Goal	Priority	Lower limit	Upper limit
CMAs				
(X1): Concentration of lipid mixture	In range	-	-1	1
(X2): Concentration of lipid mixture	In range	-	-1	1
CQAs				
(Y1): Entrapment efficiency (%)	Maximize	****	70	90
(Y2): Mean particle size (nm)	Minimize	****	150	250
(Y3): Zeta potential (mV)	In range	****	-25	-20
Solution from numerical optimization				

X1	X2	EE	MPS	ZP	Desirability
661.2	212.2	76.86	150	-22.94	0.521

The optimum formulation was also chosen using an overlay plot with the same constraints as numerical optimization. **Figure 3** depicts the overlay plot of the CMAs, with the design space highlighted in yellow. The contour lines represent the boundaries established for each of the responses.[23] It was evident that the optimum formulation determined using the numerical and graphical methods is identical, which shows the accuracy of the applied QbD methodology.

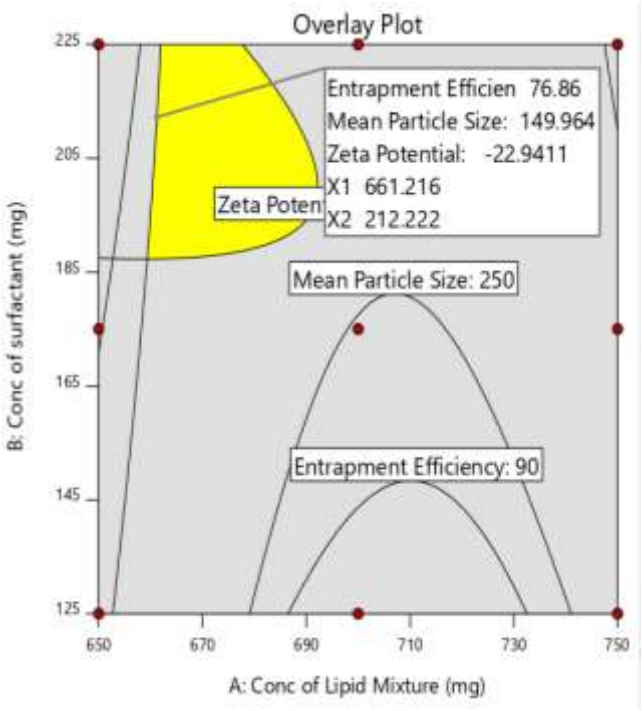

Figure 3: Design space overlay plot for optimized NLCs of EPE in the two-dimensional experimental domain

Characterization of the optimized formulation:

Particle size and zeta potential

The particle size and zeta potential value of the optimized formulation with L_{mix} concentration 661 mg and surfactant concentration 212 mg was found to be 174.9 nm and -24.4 mV respectively. The value of the particle size indicates the nanostructured nature of the formulation and a negative value of zeta potential indicates the stable nature of the preparation. The obtained values are within the predetermined CQAs that further effect the QTPP.

Entrapment efficiency

The entrapment efficiency of the optimized formulation was found to be 78.3% which is close to the predicted value and within the targeted CQA value. A high value of entrapment efficiency ensures that the required dose of the drug can be delivered using a single dose for sustained release.

In vitro drug release study

The *in vitro* drug release profile of optimized batch of EPE loaded NLCs dispersion is shown in **Figure 4**. The % EPE released at the end of 24 h from the optimized NLC formulation was found to be 79.5 ± 2.4 % in controlled manner. A biphasic release pattern was observed, i.e., a burst release in initial stage followed by a sustained release. The occurrence of burst release clearly indicates the location of certain amount of EPE onto the surface of NLCs, whereas the sustain release profile is due to the structure of NLCs where the drug is present as a reservoir which is surrounded by a lipid layer. The drug release from the lipids follows diffusion mechanism, resulting in the desired sustained release profile.[24] The drug release data was fitting in various mathematical models and the best-fit model was determined based on the value of regression coefficient (R^2). It was observed that the data fitted well with Korsmeyer-Peppas model with a R^2 value of 0.9872. The value of the diffusion exponent 'n' was found to be 0.23 indicating Quasi- Fickian diffusion. [25]

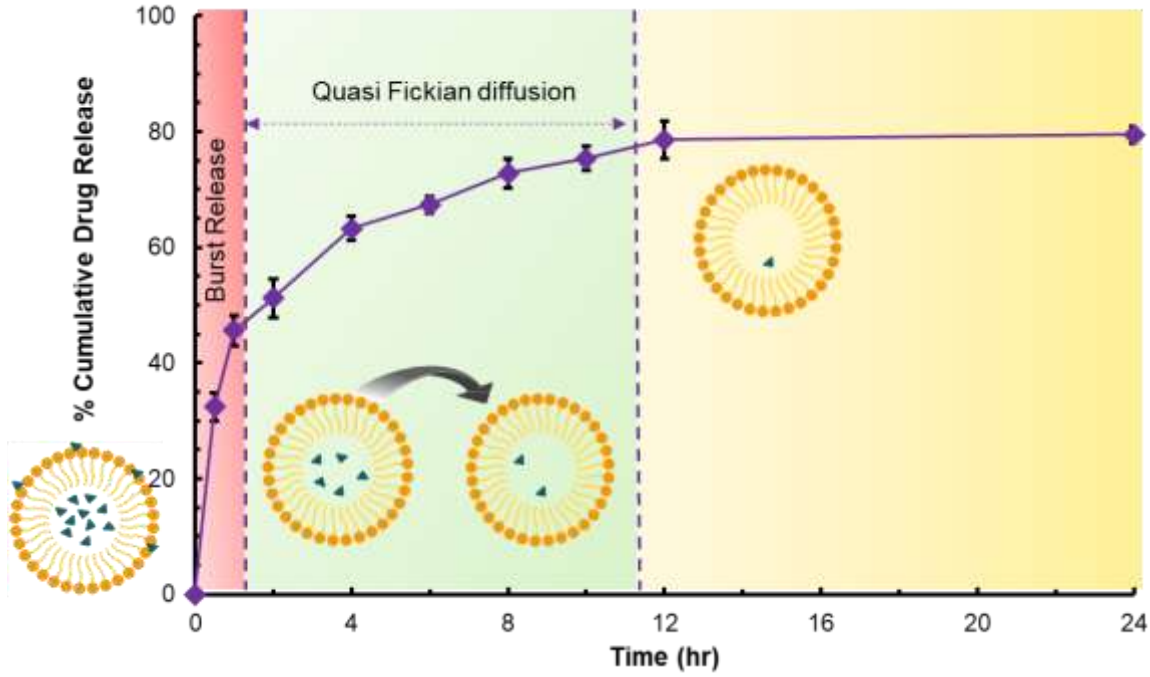

Figure 4: *in vitro* drug release of EPE from the optimized formulation. The data is represented as mean ± SD (n=3)

Stability studies:

The results of stability studies are summarized in **Figure 5**. Stability data of optimized batch of NLCs of EPE revealed that there were no substantial changes observed in the appearance, % entrapment efficiency, mean particle size and zeta potential on storage which showed formulation is stable at refrigeration temperature (5 ± 1°C) and room temperature (25 ± 2°C / 60 ± 5% RH). Initially, the particle size and zeta potential of optimized freeze dried NLC was found to be 164.2 ± 3.8 nm and -20.6 ± 2.9 mV respectively. The characteristics of the NCL formulation did not change significantly at the end of 3 months after storing at refrigerated conditions. This could be due to solid nature of the lipids that prevent any phase separation. At room temperature, the mean particle size changed significantly after 3 months, however, the characteristics of the optimized NLCs is still within the target CQA values.

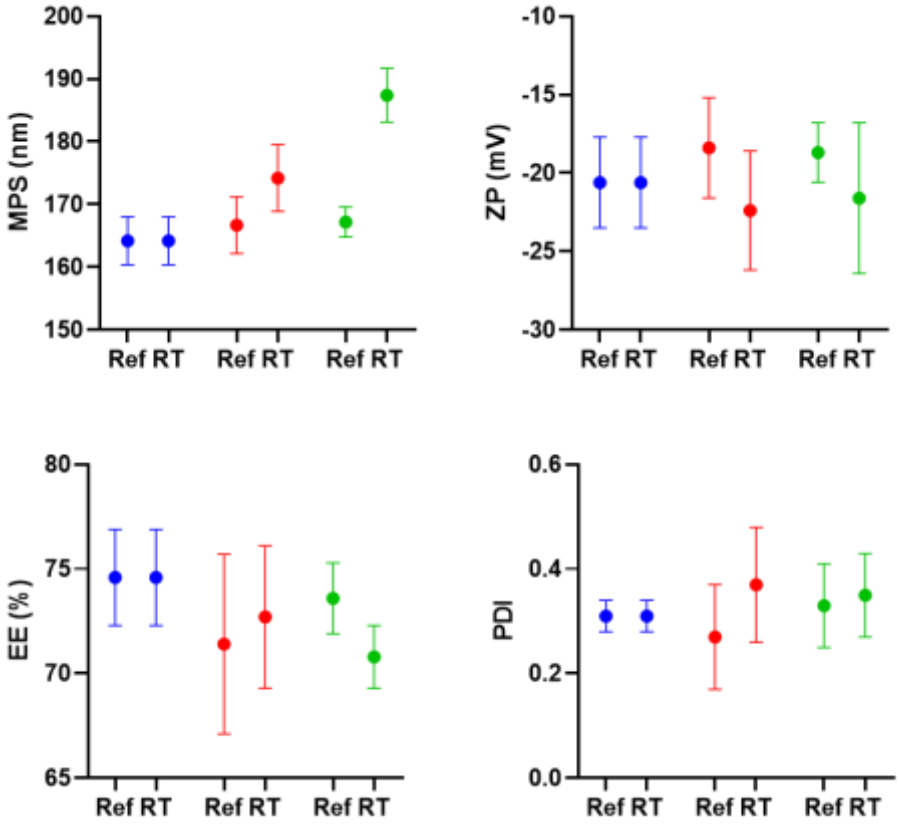

Figure 5: Stability data of optimized NLCs at refrigerated and room temperatures. The data is represented as mean ± SD (n=3).

CONCLUSION

NLCs of EPE was successfully developed using the QbD approach. Based on solubility studies of EPE in lipids and surfactants, suitable lipids and surfactant were selected for formulation of EPE NLCs. Employing QbD in formulation of NLCs helped in meeting the quality attributes by development of NLCs taking into consideration all the factors critically affecting the formulation. Risk assessment showed that the concentration of lipid mixture and the concentration of the surfactant are the two factors that majorly influence the CQAs. The experimental design showed that interaction effect was observed between the studied independent parameters and the responses. The numerical and graphical optimization both suggested an optimized formulation containing 661 mg of lipid mixture and 212 mg of surfactant to prepare NLCs with a mean particle size in nanometers, highly negative zeta potential and an entrapment efficiency of >70%. The in vitro release data showed the release of EPE from NLCs follows Quasi- Fickian diffusion from 2 hrs to 12 hrs, which is desirable for sustained release. The study reiterates the robustness of QbD approach in the formulation of nanoparticulate formulations which can also be employed for the delivery of large molecules.

Conflict of Interest

The authors report no conflict of interest in conducting this research. The authors alone are responsible for the content and writing of this article.

Author contributions

S.B. Conceptualization, Methodology, Investigation, Formal Analysis, Writing-original draft, Data Curation, Visualization; H.K.M. Methodology, Investigation, Data Curation, Formal Analysis, Writing-original Draft, Supervising.

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