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Formulation and *In Vitro* Characterization of Raloxifene Nanostructured Lipid Carriers for Oral Delivery With Full Factorial Design-Based Studies Using Quality by Design (QbD) Approach

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ABSTRACT

The main aim of the present study is to improve the dissolution rate of Raloxifene Hydrochloride by formulating nanostructured lipid carriers (NLC) using Quality by Design (QbD) approach. The formulations of NLC-RH were prepared by the ultrasonication method using stearic acid as solid lipid, medium-chain triglyceride as the liquid lipid and polysorbate 80 as the surface-active agent. Two most critical quality attributes (CQAs) for NLC-RH were particle size and entrapment efficiency. The other attributes of medium influence identified includes dissolution rate, zeta potential and particle size didtribution. The Critical Material Attributes (CMAs) identified were solid lipid/liquid lipid ratio and surfactant concentration. The time required for ultrasonication was selected as a Critical Process Parameter (CPP). The 2^3 full factorial design was used to evaluate the relationship between the CMAs and CPPs variable. Based on the experiments, the composition of the optimal formulation is achieved with solid lipid/liquid lipid ratio of 7:3 and 7 % of surfactant concentration with 15 min of ultrasonication time. The optimized formulation of NLC-RH was found to be with a mean particle size of 146 nm with narrow particle size distributions. From the above results, it is concluded that a promising Raloxifene HCl loaded NLC could give a novel and potential therapy for osteoporosis.

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INTRODUCTION

Osteoporosis(OP) is a disorder of bone elements and their quality (Lorentzon and Cummings, 2015). Out of many causes for OP, the most important is post-menopausal osteoporosis and is considered as high risk for women (Sozen *et al.*, 2017). About 30-40 % of women are estimated to suffer from this disorder (Curtis and Safford, 2012).

Pharmacological measures for treating post-menopausal osteoporosis include use of drugs like activated vitamin D3, Calcitonin, Biphosphonates, Hormone Replacement Therapy (HRT) with Estrogen, Anabolic steroids, Calcium supplements and use of selective estrogen receptor modulators (Brar, 2010). Though HRT is an effective treatment of

postmenopausal osteoporosis, complications like deep vein thrombosis and occurrence of endometrial and breast cancer make it less preferred as the first line of therapy (Gambacciani and Levancini, 2014). Raloxifene, a second-generation Benzothiopene derivative that is prescribed for treatment and prevention of osteoporosis (Maximov *et al.*, 2013).

Raloxifene, marketed as a Raloxifene Hydrochloride compound approved for the prevention and treatment of PMO fractures. It has significantly less solubility and very low bioavailability (of less than 2 %) with inter-subject variability. Raloxifene undergoes external hepatocellular metabolism in the liver.

The NLCs are formulated with combination of solid lipid with liquid lipid, which improved the encapsulation of drug and prevents drug expulsion during storage. Moreover, NLC formulations have the advantages of prolonged drug release, biocompatibility and easy of scaling-up its production (Mukherjee *et al.*, 2009; Mohammadi-Samani and Ghasemiyeh, 2018). The *in vivo* digestion (by lipases and co-lipases) of NLCs, once reaching the small intestine, converts them into mixed micelles that are ready for drug absorption (Elmowafy *et al.*, 2017). The objective of the present study was to explore the feasibility to encapsulate Raloxifene Hydrochloride into NLCs to improve the dissolution rate and eventually oral bioavailability. The NLC formulation has the advantages for prolonged drug release with improved bioavailability and easy for production. The improved bioavailability is due to its encapsulation of drug with lipid carriers.

MATERIALS AND METHODS

Materials

Raloxifene Hydrochloride was used as the drug candidate. Stearic acid (Stelliesters Glycerol) and MCT (Stelliesters MCT 65/35) were evaluated as the solid lipid and liquid lipid, respectively. Polysorbate 80 (Tween 80) and Lecithin were explored as the hydrophilic and lipophilic surfactants. Silicon Dioxide (Syloid XDP 3150) was used as the adsorbent. The compounds mentioned above were received as gift samples from Cadila Pharmaceuticals Ltd., Ahmedabad.

Methods

Defining QTPP

QbD based approach is to define the quality target product profile (QTPP) based on product requirement and intended performance. Target product profile (TPP) includes route of administration, maximum and minimum doses, and appearance is better appropriate quality, safety and efficiency features of drug product such as stability, pharmacokinetic properties, bioavailability, purity, drug release profile etc. depending on the specific dosage form and therapeutic properties.

Determination of CQAs

The CQAs are the critical attributes of the products which ensure the consistency in the product performance. The CQAs are derived from QTPP and are based on prior product knowledge and process understanding. CQAs include the physical, chemical, biological and microbiological attributes that are within an appropriate limit, range or distribution to ensure the desired product quality. The CQAs may include particle/droplet size, entrapment efficiency, zeta potential, poly dispersibility index (PDI), drug release as shown in Table 1.

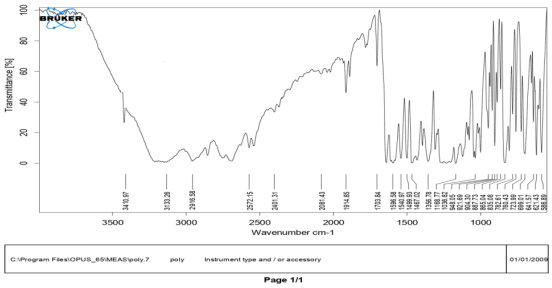


Figure 1: FTIR spectrum of Pure Drug RH

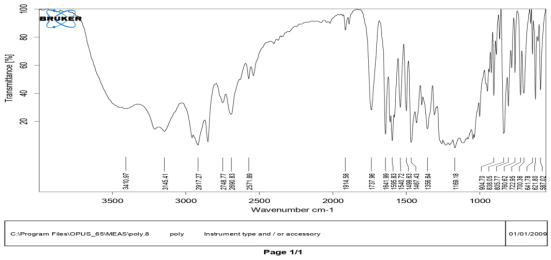


Figure 2: FTIR spectrum of Final Formulation

Determination of the CMAs and CPPs

The QbD determine the material attributes and process parameters based on experimental conditions and also relationships between these material attributes and process parameters to the drug product CQAs. Factors are considered based on properties of API and excipients used in the preparation of NLCs and from process parameters (ultrasonication time and temperatures) as shown in Table 2.

Pre Formulation Study

Drug polymer interaction study

To verify the purity of the obtained samples like drug and excipients, the obtained FTIR spectra were

Table 1: CQAs of NLC-RH

Profile Component	Target	Is it CQA	Justification
Dosage Form	Nanoparticle	Yes*	Novel dosage form for better product performance / targeted drug delivery.
Particle Size (nm)	NMT 150	Yes	Smaller particle size is required.
Entrapment Efficiency (%)	> 50	Yes	Higher entrapment is better for the nanoparticulate dosage form.
Drug Release (Hrs)	12		To achieve sustained drug release for a more extended time.
Zeta Potential (mV)	More than +30 or less than – 30.	Yes	For better stabilization of the nanoparticulate dosage form.
Poly Dispersibility Index (PDI)	< 0.3	Yes	Less the PDI (<0.3), more the homogeneity of the composition

Table 2: CMAs and CPPs affecting CQAs

Critical Quality Attributes (CQAs)	Critical Material Attributes (CMAs)	Critical Process Parameters (CPPs)
Particle Size	Lipid Type	Ultrasonication Time
% Encapsulation Efficiency	Lipid Concentrations	Process Temperature
Poly Dispersibility Index (PDI)	Solid Lipid/Liquid Lipid ratio	–
Zeta Potential	Surfactant Concentration	–
Drug Release	Surfactant Type	–

Table 3: Design of the 2^3 full factorial design with three centre points DoE for NLC-RH

Factors: Critical Formulation and Process Variables (Independent)		Levels		
		-1	0	+1
A	Solid/Liquid Lipid Ratio (X1)	3:7	5:5	7:3
B	Surfactant Concentration (%) (X2)	5	7.5	10
C	Ultrasonication Time (min) (X3)	10	15	20
	Responses (Dependent)	Goal	Acceptance Criteria	
Y1	Particle Size (Y1)	Minimize	NMT 150 nm	
Y2	Entrapment Efficiency (%EE) (Y2)	Maximize	To achieve as maximum as 100%	

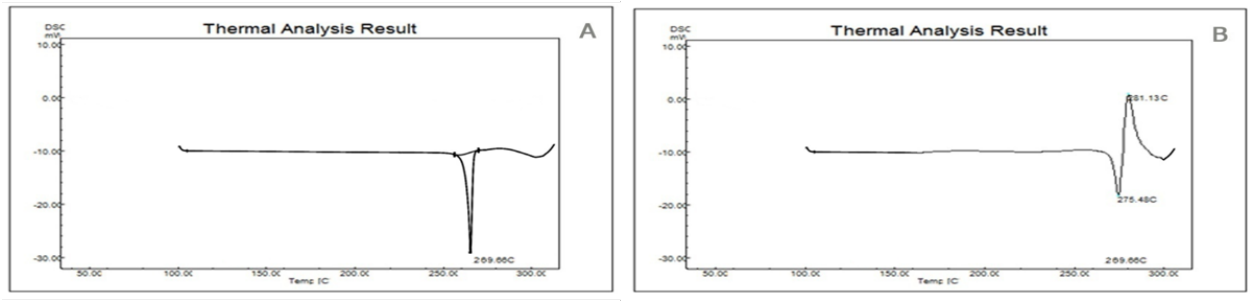


Figure 3: DSC Image of A: Raloxifene HCl; B: Optimized formulation (NLC-RH2)

Table 4: Experimental Results of the DOE to Study Critical Formulation Variables and Critical Process Parameter for NLC-RH

Run No.	Batch No.	Type	X1: The ratio of solid/ liquid lipid	X2: Level of Surfactant	X3: Ultrasonication time	Y1: Particle Size (nm)	Y2: Entrapment Efficiency (%)
1	NLC-RH1	Centre	0	0	0	455.3	60.23 ± 1.05
2	NLC-RH2	Factorial	+1	+1	+1	278.2	88.07 ± 0.84
3	NLC-RH3	Factorial	+1	-1	-1	365.7	72.10 ± 1.32
4	NLC-RH4	Factorial	+1	-1	+1	300.5	75.53 ± 0.93
5	NLC-RH5	Factorial	-1	-1	+1	538.1	51.03 ± 1.10
6	NLC-RH6	Centre	0	0	0	462.2	61.87 ± 1.23
7	NLC-RH7	Factorial	+1	+1	-1	352.2	81.90 ± 1.14
8	NLC-RH8	Factorial	-1	+1	+1	505.4	56.80 ± 1.39
9	NLC-RH9	Factorial	-1	+1	-1	648.6	54.10 ± 0.80
10	NLC-RH10	Centre	0	0	0	441.2	62.77 ± 0.99
11	NLC-RH11	Factorial	-1	-1	-1	603.3	41.47 ± 0.70

Table 5: Composition of DoE trials of Prepared NLC-RH

DoE Trials	Drug (mg)	Solid Lipid (stearic acid in mg)	Liquid Lipid (MCT 65/35 in mg)	Total Lipid Weight (mg)	Solid lipid: Liquid Lipid Ratio	Surfactant (Polysorbat 80 in mg)	Ultrasonication time (min)
NLC-RH1	60.0	150.0	150.0	300.0	5:5	25.0	15
NLC-RH2	60.0	210.0	90.0	300.0	7:3	36.0	20
NLC-RH3	60.0	210.0	90.0	300.0	7:3	18.0	10
NLC-RH4	60.0	210.0	90.0	300.0	7:3	18.0	20
NLC-RH5	60.0	90.0	210.0	300.0	3:7	18.0	20
NLC-RH6	60.0	150.0	150.0	300.0	5:5	25.0	15
NLC-RH7	60.0	210.0	90.0	300.0	7:3	36.0	10
NLC-RH8	60.0	90.0	210.0	300.0	3:7	36.0	20
NLC-RH9	60.0	90.0	210.0	300.0	3:7	36.0	10
NLC-RH10	60.0	90.0	210.0	300.0	3:7	18.0	15
NLC-RH11	60.0	150.0	150.0	300.0	5:5	25.0	10

Note: Amount of Purified water taken was 450 ml as an aqueous phase. In all the formulations, 0.5% of Lecithin was used as the lipophilic excipents.

Table 6: Characteristic Peak

Characteristic peak	Range	Drug (Raloxifene)	Formulation batch
OH Phenolic stretch	3200-3600	3410.97	3410.97
Keto (C=O) stretch	1700-1725	1703.84	1737.96
Aliphatic C-H stretch	2850-3000	2916.58	2917.27
Aromatic C-H stretch	3050-3150	3133.26	3145.41
Ether C-O stretch	1000-1300	1168.77	1169.18

Table 7: Release kinetics and correlation coefficients

F-Code	Mathematical Models (Release kinetics)			
	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Peppas (R^2)	n
NLC-RH2	0.9872	0.6892	0.9522	0.9910	0.4976

Table 8: Design points of the optimized formulation by experimental design

	Particle Size	
Independent Variable	Coded values	Actual values for coded values
X_1	+1	7:3 (Solid Lipid/Liquid Lipid Ratio)
X_2	-0.2	7 %
X_3	+1	15 min
	Encapsulation Efficiency	
Independent Variable	Coded values	Actual values for coded values
X_1	+1	7:3 (Solid Lipid/Liquid Lipid Ratio)
X_2	-0.2	7 %
X_3	0	15 min

Table 9: Statistics of Model

Statistical terms	Particle size	% Entrapment Efficiency
Standard Deviation	10.69	1.15
C.V %	2.38	1.80
R2	0.9984	0.9986
Adj R2	0.9928	0.9938
Adeq R2	38.255	44.999

Table 10: Comparative Values of Predicted and Observed values of responses

Variables	Predicted response	Observed response	Predicted error %
Y1	150	146	-2.67
Y2	86	89	+3.488

verified (FTIR BRUKER) over a wavelength range of 4000-400cm^{-1} at resolutions of 4cm^{-1} sample were directly placed on the probe and spectra were recorded.

The FTIR spectra of pure Raloxifene Hydrochloride and mixture of all the ingredients used in preparations of NLCs were observed to find any interactions between the drug and excipients.

Construction of calibration curve of RH

For spectral scanning, 100 mg of RH drug was readily dissolved in 100 ml of a suitable solvent. One ml of prepared stock solution was further diluted to 100 ml with the same solvent (10 μg/ml) and finally scanned for maximum absorbance using UV spectrophotometer (UV-1800 SHIMADZU) in the range from 200 to 400 nm, maximum absorbance was found at 286 nm was 0.998, for methanol as a solvent, the standard graph of RH in Methanol has also shown a line of good fit over a concentration range of 0 to 10 μg/ml.

The drug was analyzed spectrophotometrically at 286 nm, with an R^2 value of 0.998.

Formulation Studies

Preparation of NLC-RH

The Raloxifene Hydrochloride (RH) loaded NLCs were formulated by using high-speed homogenization followed by ultrasonication. The lipid and aqueous phases were prepared separately. Lipid phase consists of solid lipid (stearic acid), liquid lipid (MCT 55/45) and 0.5% lecithin as the lipophilic emulsifier, while the aqueous phase consisted of

hydrophilic emulsifier (Tween 80) dissolved in distilled water. RH was dissolved in MCT and then mixed with other lipid phase components. All components of the lipid phase were heated separately to 10°C above solid lipid transition temperatures for 10 min before mixing. The aqueous phase was added drop-wise to the molten lipid phase and mixed using a high-speed homogenizer (Janke & Kunkel, GmbH, Staufen, Germany) at 10,000 rpm for 10 min. The mixture was further treated using a probe-type sonicator (ultrasonic processor, GE130, probe CV18, Newtown, CT). The resultant emulsions were adsorbed on to the granular colloidal silicon dioxide and cooled at room temperature. The NLC adsorbed powder was compressed into tablets.

Formulation Optimization of RH-NLC as per Design of Experiments (DoE)

The formulation that meets the desired CQAs is the ultimate goal of all formulation development activities. The formulation components of NLC-RH that have a high potential to impact the drug product CQAs are the ratio of solid lipid/liquid lipid ratio, the surfactant concentration (polysorbate 80) and the ultrasonication time as shown in Table 3. The water quantity, the temperature has a low to medium risk to impact the CQAs. As Raloxifene Hydrochloride is a nanoparticulate formulation, particle size is the most critical quality attributes to study. In addition to this, the encapsulation efficiency (%) of the Raloxifene HCl in nanostructured lipid particles is also considered as the dependent variable.

Therefore, particle size (PS) and % entrapment efficiency (% EE) was selected as an output response. Hence, to achieve optimum performance, the formulation design space has to be established within which all the CQAs are met.

To achieve this, a 2^3 full factorial design with three centre point study was used to optimize the levels of solid/liquid lipid ratio, Surfactant concentration and ultrasonication time to establish the formulation and process space.

Statistical design and analysis were conducted using the software Design Expert 7.0. (trial version). The trials were conducted randomly in the order given by the software to avoid any intentional error. An overview of different formulations is done by changing the ratio of solid/liquid lipids, surfactant concentration and ultrasonication time is shown in below Table 4.

Statistical Analysis

A full factorial design was used to study the dependence between the formulation variables (independent variables: X_1, X_2, X_3) and dependent variables (Malvey *et al.*, 2020) (Responses: Y_1, Y_2) the following generation of the polynomial equation from software and experimental values are incorporated into it, as shown in Table 5.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$$

Where Y represents the overall predicted response (Particle size, entrapment efficiency) β_0 is the overall intercept, β_1 β_2 β_3 are individual intercept of the main effect of X_1, X_2, X_3. The ANOVA studies are generated from the software to evaluate the p-values, F-ratio, and lack of fit. To evaluate the fitting extent of the experimental data regression coefficient ($R^{2)}$ and adjusted R^2 were determined. 3D and counterplots prediction was made to run further experimental studies for estimation of model accuracy.

Characterization of NLC-RH

Particle size analysis

The particle size was determined by laser particle size analyzer (Zetatrac 10.6.2, Microtrac Inc.) using the distilled water as a dispersant.

The dispersed nanoparticle was added to the sample dispersion unit containing stirrer. The average particle size was measured after experimenting triplicate.

Zeta potential

The prepared NLC were carried out for Zeta potential estimation by using binocular microscopy (Model CH20iBIMF), particle size was recorded by calibrations of stage micrometre division and eyepiece micrometre division.

Further particle analysis was carried out by using Zeta sizer (Malvern, Model: Nano ZS90). The Average size of NLCs was expressed in d. nm, and a charge is measured.

Drug encapsulation efficiency (%)

Drug encapsulation efficiency (EE) was conducted through an indirect method where an aliquot (2 ml) of RH-loaded NLCs was centrifuged at 100,000 RPM for 2h at 4 ^{0}C using a Beckman Optima™ Ultracentrifuge (Optima™ XL, Indianapolis, IN).

The proportion of free drug (RH) supernatant fluid was measured spectrophotometrically (Shimadzu, the model UV-1800 PC, Kyoto, Japan) at 286 nm against the blank. The following equation then calculated the encapsulation efficiency:

$$EE\% = (Da - Df \,/\, Da) * 100$$

Where EE % is the percentage of encapsulation efficiency, Da is the amount of drug added during preparation of NLCs and Df is the amount of free drug present in the supernatant fluid after centrifugation.

Surface morphology study (SEM)

Scanning electron microscopy (Zeiss, TIFR, Mumbai) of an optimized formulation of NLC-RH was evaluated to find the particle size and surface topography.

The photographs were taken using an SEM under magnification of 10 KX–12 KX.

In vitro drug release

In vitro diffusion studies were carried out by using two open-ended dialysis tubes with an artificial membrane using an optimized formulation of NLC-RH. The prepared Raloxifene Hydrochloride NLCs were re-dispersed in 5 ml of USFDA's OGD (Office of Generic Drugs) recommended media, *i.e.*, 0.1% polysorbate 80 in purified water and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 50 ml of 0.1% polysorbate 80 in water. The receptor medium was agitated continuously using a magnetic bead under magnetic stirrer, and the temperature was maintained at 37 ± 0.5 °C. 0.5 ml sample of receptor compartment was taken at predetermined time intervals for 1 hr and each time replaced with 0.5 ml of fresh media. The amount of drug released was determined spectrophotometrically at 286 nm.

Differential scanning calorimetry (DSC)

It is based on the principle of measurement of heat flow in and out of sample and reference for the period of the controlled temperature cycle, merely 5-10 mg sample was sealed in aluminium pan followed by heating at the rate the of 20 °C/min over the temperature range of 10-200 °C under liquid nitrogen flow rate 40 ml/min and thermogram was obtained. The thermal behaviours of pure drug substance are applied.

RESULTS AND DISCUSSION

The purpose of this study was to develop lipid-based NLCs of raloxifene hydrochloride for the treatment of osteoporosis. NLC-RH was prepared by emulsification using high-speed homogenization followed by ultrasonication. A 2^3 full factorial design with three centre point was employed.

Drug polymer interaction study

Fourier transform infrared spectroscopic (FTIR) analysis was used to identify any interaction between the molecules used in the preparation of formulation. The FTIR studies show that there are no interactions between the molecules and the preparations of NLCs, as shown in Figure 1 and Figure 2, corresponding values are plotted in Table 6.

Results of DSC

As DSC thermogram is shown in Figure 3, piercing endothermic peak at 278.48 °C was observed in the finalized formulation, corresponding to melting point observed in DSC of Pure drug was 289.88 °C as shown in Figure 3, but the slight difference to pure form of the drug in the image view.

SEM (Scanning Electron Microscopy)

The NLCs are observed under scanning electron microscopy. A topographical characterizing technique shows detailed surface examination at the molecular level. The optimized formulation is represented in Figure 4.

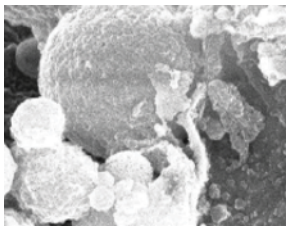


Figure 4: SEM images of optimized NLCs

Particle Size (PS) and Zeta Potential

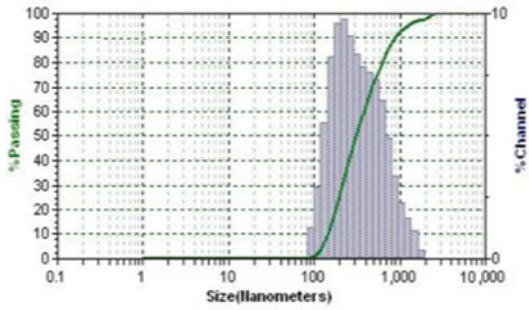
The prepared NLC-RH showed particle size between 278.2 and 648.6 nm. The average diameter and zeta potential of optimized formulation were determined by photon correlation spectroscopy (PCS) at room temperature. The average particle size of optimized formulation was in the range of 278.2 nm with a maximum intensity of 536.0 nm particles. It shows the broader range of particle size distribution from 1635 nm to 72.30 nm. The value greater than +25 mV or less than –25 mV has a high degree of stability. Zeta potential was found to be 0.52 mV for optimized formulation. Thus it suggests that possible aggregation of particles as shown in Figure 5 along with values. The final equation was generated in the coded form by the software was given below from 3D graphs, as shown in Figure 6.

$$Y_1 = +448.63 - 124.88\,A - 2.87B - 43.38\,C - 5.87AB + 8.63AC - 10.88\,BC + 8.62ABC$$

The F-value of 448.63 implies the model is significant; the p-value is less than 0.0500 indicate model terms are significant. In this case, AC, ABC are significant model terms. The remaining model terms are not significant due to its values greater than 0.05.

Entrapment Efficiency (EE)

Table 5 shows the percentage entrapment efficiency of formulations. The entrapment efficiencies of NLC-RH ranged from 41.47 ± 0.70% to 88.07 ± 0.84%. The results of the study demonstrated that the encapsulation efficiency of NLC-RH was affected



Mobility	0.04u/ s/ V/ cm
Zeta Potential	0.52 mv
Charge	0.001 fC
Polarity	Positive
Conductivity	44 uS/ cm

Figure 5: Particle size distribution and zeta potential of NLC-RH2

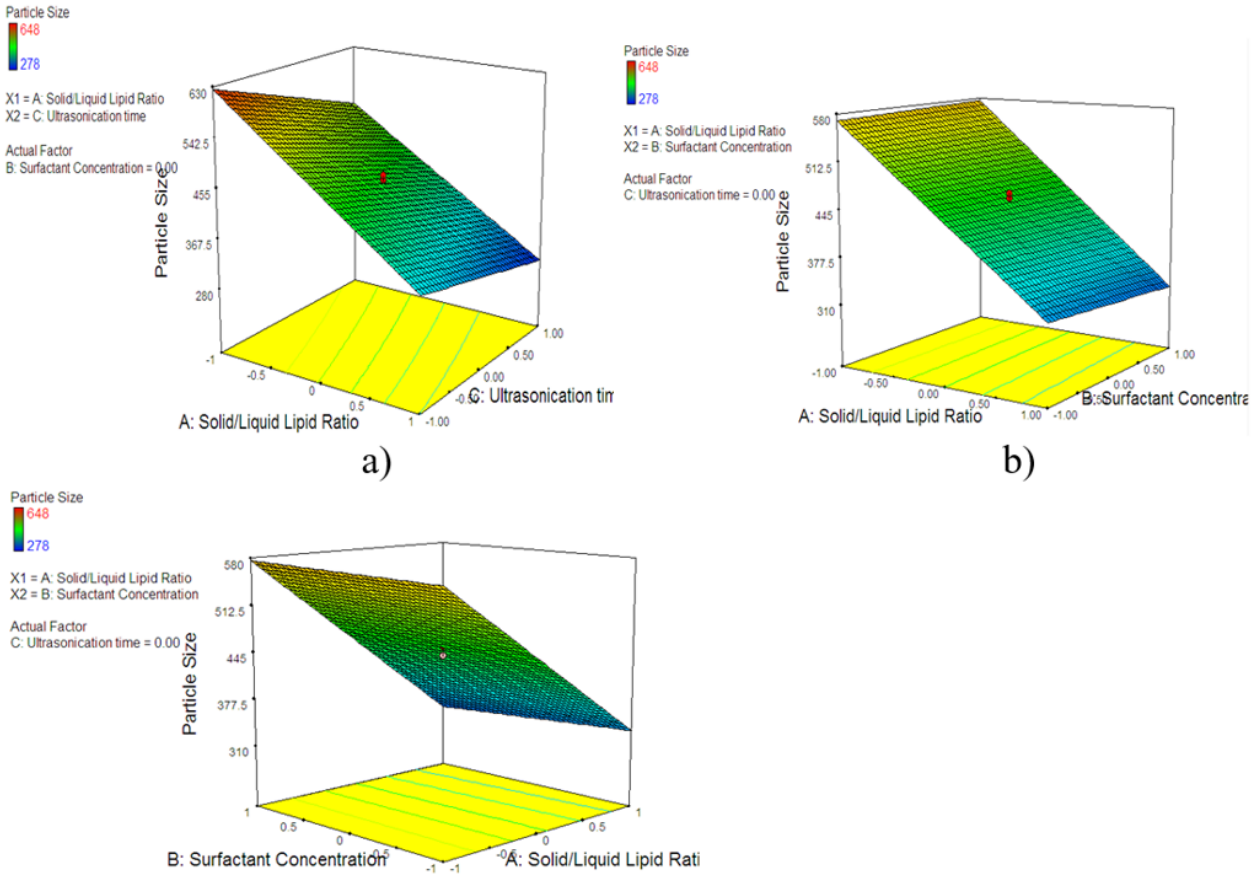


Figure 6: 3D images of contour plots for particle size

by the concentration of solid lipid/liquid lipid proportion and surfactant concentration. The maximum percentage of drug entrapment was obtained for the formulation NLC-RH2. The increase in the concentration of solid lipid and surfactant significantly increases percentage entrapment. However, it was observed during formulation development study, the further increase in stearic acid. The final equation was generated in the coded form by the software was given below 3D graphs, as shown in Figure 7.

$$Y_2 = +65 + 14.25A + 5.25B + 2.75C + 0.50AB - 0.50AC - 0.50BC + 1.25\,ABC$$

The obtained F value is 65, which show the model is significant. The model terms A, B, C is less than 0.05, which indicates these models are significant.

In-vitro drug release studies

In-vitro release studies were performed in 0.1% polysorbate 80 in purified water. A slow release of Raloxifene up to 12 hrs was observed. DSC results indicated that the Raloxifene entrapped in the NLCs. The Scanning electron microscope (SEM) has revealed that the NLCs are obtained as a spherical-shaped carrier system.

The highest correlation coefficients were obtained for the Korsmeyer-Peppas model, to follow Non-

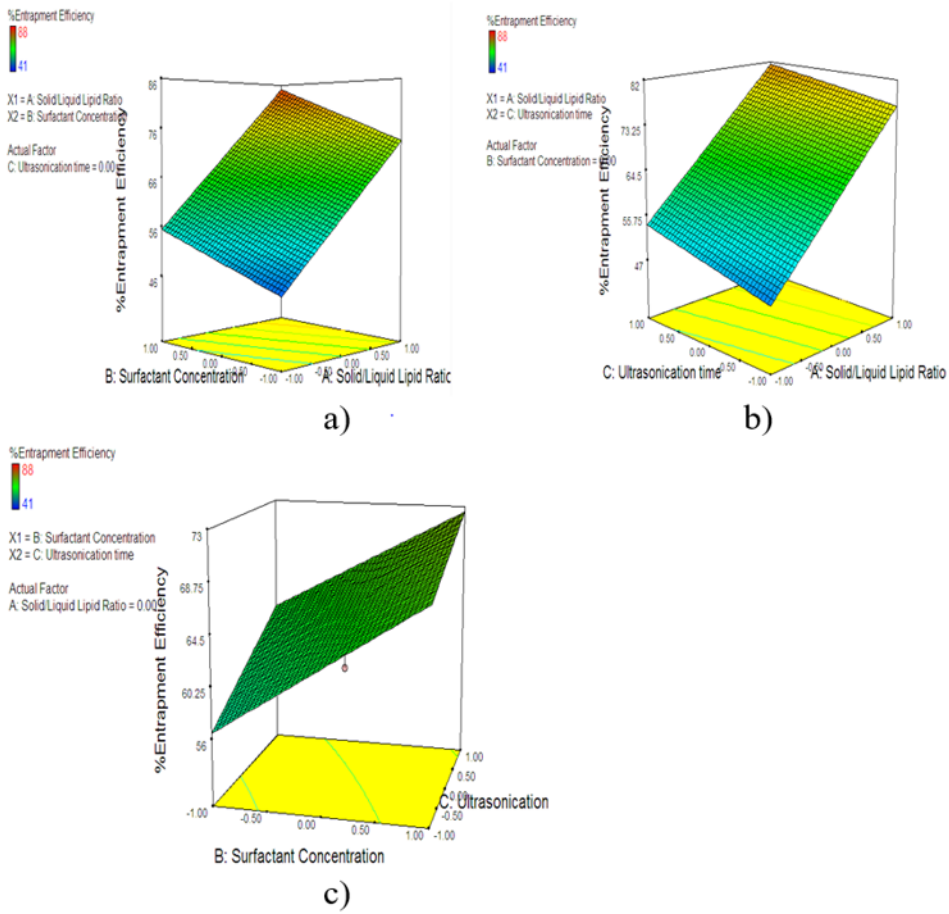


Figure 7: 3D images of contour plots to %EE responses

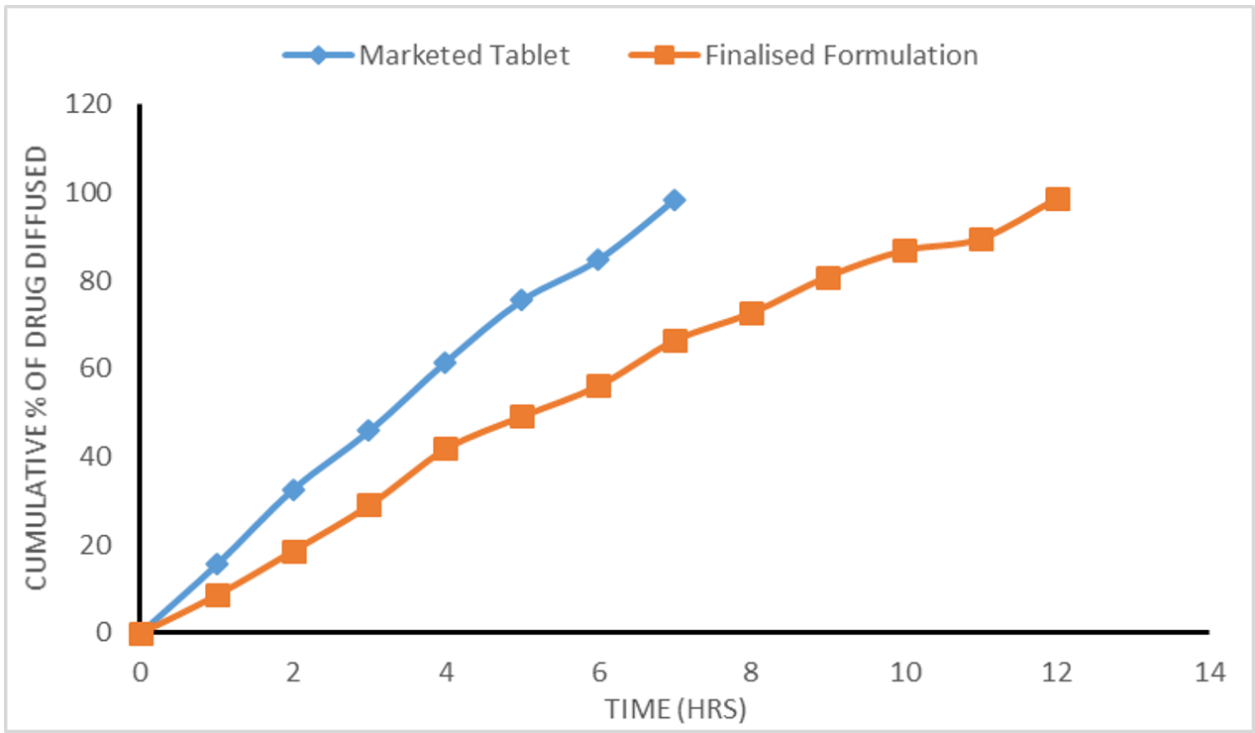


Figure 8: The comparative Dissolution profile of marketed Tablet and NLCs compressed tablet

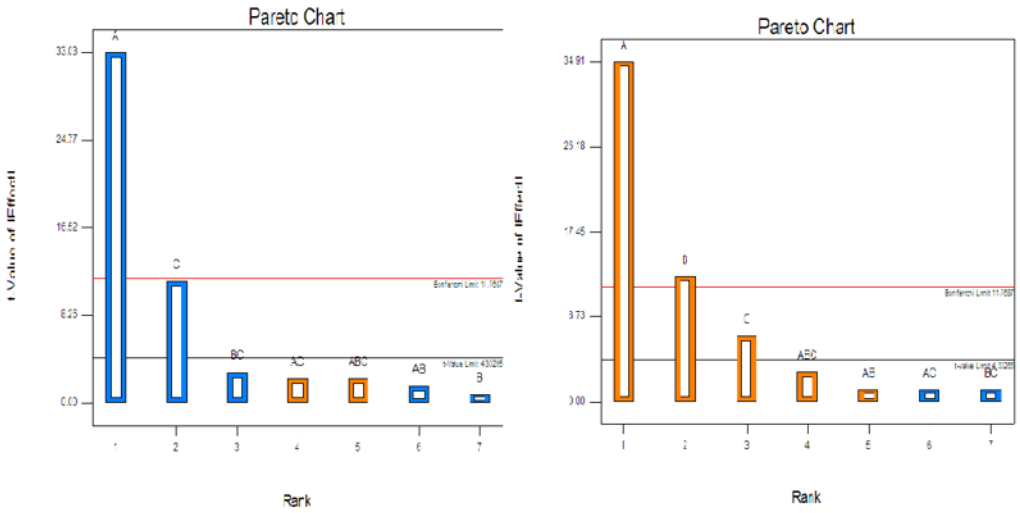


Figure 9: Pareto chart for PS and EE

Fickian diffusion for the drug release, as shown in Table 7.

The results demonstrated that raloxifene hydrochloride NLCs with such ingredients could be an emerging delivery method for the long-term treatment of osteoporosis.

Marketed Formulation

The obtained dissolution graph of NLCs is comparted with the marketed formulation and found to be sustained release of medication without sudden increased in concentration, as shown in Figure 8.

Statistical Analysis

The experiments results were determined by the half-normal plot and Pareto chart to find significant effects of X_1 X_2 X_3 variables. The higher t-values observed in the pereto chart was for particle size and entrapment efficiency, as shown in Figure 9. The results indicate that the concentration of solid/liquid lipid ratio and surfactant exhibits the highest effect in altering the particle size and encapsulation efficiency as compared to the time required for sonication. The experimental data were analyzed and fitted to various models (liner, interactive and quadratic) the results showed the liner order model exhibited highest regression R^2 hence linear model was adopted to fit the experimental data and to find an interaction between formulation variable and dependent variables.

ANOVA analysis depicted that developed linear model was highly significant with low P-value which is less than 0.05 with the well fit was checked by R^2 value for particle size and entrapment efficiency (0.9984), (0.9986).

Further, more, a high degree precision of experiment was indicated by low values of coefficient of variation CV= 2.38% the adequate precession measures signal to noise ratio, and a ratio greater than four is desirable to navigate design space for the constrain selected as shown in Table 9, An Studentized residual versus the experimental run and residual *versus* run was constructed to ensure the obtained data.

The relationship between encapsulation efficiency and formulation variable model graphs, mainly 3D graphs and contour plots were generated to assess the individual ad interactive effect on the response.

Optimization and validation

Regression model developed in the study is to found optimum formulation with the desired value from 3D graphs. A coded value was found to X_1=+1, X_2= -0.2, X_3= 0 the corresponding experimental parameters for X_1=7:3, X_2 = 7%, X_3= 15 minutes, were respectively under the optimum condition the experimental predicted particle size was 150 nm as shown in Table 8.

However, considering the optimum condition for X_2 can be modified as the coded value of +1 that is 7.5 % of surfactant concentration, the statistical conclusion was given for the model to prove the model is the best fit, as shown in figure 9.

To compare the predicted results with experimental values additional data experiment was performed in triplicate under the new experimental condition the particle size found to be 146 microns and well-matched with the predicted response of 150 microns, the percentage error was found to be -2.67 which, is less than 5 % and it falls in an acceptable limit.

Likewise, for encapsulation efficiency the experimental value found to be 89% and well-matched with predicted response 86%, the percentage error

was found to be +3.488 which is less than 5 %, and it falls in the acceptable limit, as shown in Table 10.

CONCLUSION

The present research paper establishes a successfully optimized composition of nanostructured lipid carriers of raloxifene hydrochloride, designed by QbD approach and optimized by DoE tool. The optimized composition was derived from the initial risk assessment, preformulation studies and using the design of experiment (DoE) concept. The initial risk assessment was carried out to identify the critical formulation variables (CFVs) and critical process parameters (CPPs) that have a most likely impact on the critical quality attributes (CQAs). Based on the initial risk assessment, two CQAs, namely particle size and percentage entrapment efficiency, were found to be the most critical attributes for the NLC-RH. Besides, the CQAs that are found to be of medium impact are Zeta potential and Dissolution rate. The CFVs found to be the solid lipid/liquid lipid ratio and surfactant concentration and the most critical CPP found to be the ultrasonication time. The 2^3 full factorial design studied the impact of CFVs and CPP on the CQAs of the drug product with three centre points. The optimized composition of NLC-RH was found to be with solid to liquid lipid ration of 7:3, with 7 % surfactant concentration and 15 minutes of ultrasonication time. Future development of NLC-RH would be facilitated by *an in-vivo* performance with enhanced practical understanding. Based on these above results, it can be concluded that a promising Raloxifene HCl loaded NLC could be developed, which can be a potential alternative therapy for osteoarthritis for extended period of time compared to conventional preperation.

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Conflict of interest

The author has no conflict of interest in publishing this article in this journal.

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