Original Article

Quality by Design: Optimization of Letrozole Solid Lipid Nanoparticle for Breast Cancer

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ABSTRACT

Aim: Hormone responsive breast cancer is the most prevalent cancer worldwide. Letrozole is a third-generation aromatase inhibitor widely used for the treatment of advanced breast cancer. The primary objective of the present work is to develop and optimize an injectable solid lipid nanoparticle incorporating letrozole to circumvent the side-effects of a marketed conventional formulation and thereby improve patient compliance. **Materials and Methods:** Emulsification solvent evaporation and melt dispersion techniques were used for formulating the said solid lipid nanoparticles. Quality by design concept was used for the development and optimization of formulation and process variables. **Results:** The optimum level selected is 60 mg lipid, 30 mg surfactant, and co-surfactant, 15000 psi HPH pressure, and 15 HPH passes. **Conclusion:** Glyceryl dibehenate was selected as suitable lipid based on solubility and partition coefficient. With an increase in lipid content there is increase in particle size and PDI and decrease in entrapment efficiency. Higher surfactant and co-surfactant concentrations result in lower particle size, PDI, higher zeta potential, and lower entrapment efficiency. An increase in HPH pressure reduced particle size and PDI up to a certain level, however, the increase in HPH pressure from 15000 to 20000 psi increased particle size. An increase in the number of HPH passes reduces particle size and PDI. The drug release mechanism for LTR-SLN was found to follow the first order and higuichi model.

Keywords: Solid lipid nanoparticle, Letrozole, Ishikawa diagram, Plackett burman design, Central composite design, Mathematical modeling.

INTRODUCTION

Cancer is one of the major reasons of mortality across the world. Breast cancer is the most leading type of cancer in females taking a significant toll on life across the globe.[1] Hormone receptor breast cancer is the most prevalent type of breast cancer that requires hormones for growth.[2] Hormone deprivation using aromatase inhibitors is recommended for the treatment of estrogen receptor positive breast cancer. The mechanism of action of an aromatase inhibitor is to block aromatase from producing estrogen.[3-4] Letrozole is a third-generation non-steroidal aromatase inhibitor widely used for the prophylaxis of hormone receptor positive breast cancer.[5-6] Letrozole is approved and marketed as a conventional tablet dosage form. A major barrier of conventional chemotherapy is poor specificity, side effects, and drug resistance leading to a reduction in the therapeutic window. Hence a novel delivery system is recommended for the delivery of letrozole.[7]

Solid lipid nanoparticles (SLN) were utilized as an alternate drug delivery system to traditional carrier systems. SLN were developed combining advantages and nullifying disadvantages of colloidal carrier such as liposomes, emulsions, polymeric nanoparticles.[8] Solid lipid nanoparticle is an aqueous colloidal dispersion consisting of solid biodegradable lipid as matrix and stabilized with the aid of surfactant.[9]

Pharmaceutical development of novel solid lipid nanoparticles includes complex

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procedures that involve various formulation (critical material attributes) and process (critical process parameter) variables and their interaction that can have an impending effect on the CQAs (critical quality attributes) of the pharmaceutical drug product.[10]

The traditional approach for formulation development and optimization involves modulating one factor/ variable/ at a time keeping other variables constant OVAT (one variable at a time) or OFAT (one factor at a time) or COST (changing one single variable/factor at a time) or shotgun approach.[8] This empirical approach suffers some loopholes such as uneconomical (may lead to unnecessary runs/batches), time-consuming, not suitable when all variables change simultaneously, fails to establish a cause-effect relationship, etc.[11] To alleviate these pitfalls a systematic approach i.e QbD (quality by design) is recommended during development of drug product from concept to commercialization. With the development of modern concepts such as quality by design, there shall be a significant shift in paradigm from empirical approach to scientific and risk-based approach.[12] Recently various regulatory agencies across the world have also started emphasizing the firms to submit the dossier with the inclusion of QBD-based development and optimization. International conference of harmonization (ICH) guideline Q8, Q9, Q10 also recommends the use of Qbd tools to design a quality product.[13]

The present study aims at developing and optimizing solid lipid nanoparticles of letrozole (LTR-SLN) using the quality by design (QbD) principle. Design of experiments (DOE) using various statistical models (screening using plakett burman and optimization using the central composite design) and risk assessment using Ishikawa/fishbone diagram were selected for drug product designing.

MATERIALS AND METHODS

Materials

Letrozole was obtained as a generous gift sample from beta drug, baddi, India. Glyceryl dibehenate (Stelliesters Dbhg) was procured from Stearineriedubois, france; Poloxamer 188 from BASF, germany; soya lecithin from lipoid GmbH, switzerland; disodium hydrogen phosphate from merck millipore, germany; sodium dihydrogen phosphate from merck millipore germany and trehalose from hayashibara co ltd, japan. Emprove grade chloroform and ethanol are procured from merck millipore, germany. Analytical grade methanol, orthophosphoric acid, hydrochloric acid used for analysis were procured from sigma aldrich, germany. Phosphate buffered saline (PBS) was procured from sigma aldrich, germany.

Methods

Selection of lipid

The selection of lipid plays a pivotal role in the formulation of stable solid lipid nanoparticles.[14] The selection of lipid is based on solubility,[15-16] and partition co-efficient of a drug in lipid matrix,[17-18] Various lipids (glyceryl monostearate, glyceryl dibehenate, glyceryl monooleate, glyceryl palmiostearate, trimyristin, tripalmitin, and cetyl palmitate) were evaluated to select the most promising lipid matrix for solid lipid nanoparticles of letrozole.

a) Solubility: The drug was dissolved in 1g of melted lipid at a temperature of 5°C above melting point of lipid to form a clear solution. The higher the drug solubility in lipid, the higher is the entrapment efficiency.[10]
b) Partition co-efficient: Higher partition co-efficient indicates slower drug release. Partition coefficient and drug release are inversely related.

10 mg drug was dissolved in 1g lipid. 1 mL of hot water for injection was added and shaken for 30 min. The sample was cooled to room temperature and centrifuged to separate the aqueous phase. Aliquots of the sample were collected from the aqueous phase to determine % drug content.

Selection of Surfactant

Surfactants (ionic, non-ionic, and amphoteric) are required to stabilize the solid lipid nanoparticle and prevent aggregation by reducing the interfacial tension between two phases (hydrophilic and lipophilic).[19-20] The non-ionic surfactant is mostly preferred for parenteral dosage form owing to its less toxicity and better stability.[15] Poloxamer 188 is the most preferred surfactant (non-ionic) for stabilization of solid lipid nanoparticles as it provides steric stabilization along with electrostatic stabilization.[21]

Amphoteric surfactants are preferred as co-surfactants as they show characteristics of anionic and cationic surfactants depending on pH. Lecithin is selected as a co-surfactant for the present study.[21]

Preparation of SLN

The solid lipid nanoparticle of letrozole was prepared by two methods (Method I and II).

Method I (emulsification and solvent evaporation technique)[22]

Letrozole, glyceryl dibehenate, and soya lecithin were dissolved in chloroform and ethanol (90:10). Poloxamer

188, disodium hydrogen phosphate, sodium dihydrogen phosphate was dissolved in water. The aqueous phase was added to the lipid phase and subjected to homogenization using silverson homogenizer at 10,000 rpm for 30 min. The residual organic solvent present in nano-emulsion was removed by using inert nitrogen gas at 2bar at 35°C. The particle size of the emulsion was reduced by passing the emulsion through a high-pressure homogenizer at 15000 psi for 15 passes. Trehalose was added as a cryoprotectant to nano-emulsion and subjected to lyophilization.

Method II (melt dispersion technique)[23]

Glyceryl dibehenate was melted by heating at 70°C. Letrozole was added to melted lipid under stirring to dissolve. Soya lecithin, poloxamer 188, disodium hydrogen phosphate, sodium dihydrogen phosphate was added to the aqueous phase heated to the same temperature as the lipid phase. The aqueous phase was added to the lipid phase and subjected to homogenization using silverson homogenizer at 10,000 rpm for 30 min. The particle size reduction of the emulsion was done using a high-pressure homogenizer at 15000 psi for 15 passes. Trehalose was added to nano-emulsion and stirred to form a homogeneous suspension and subsequently lyophilized.

Risk identification: using the Fishbone Diagram

Fishbone/Ishikawa diagram was established as a risk assessment tool to identify potential formulation and process variables that could have an impact on CQA of LTR-SLN i.e particle size, PDI, zeta potential, and entrapment efficiency.[24]

Experimental Design

Optimization of LTR-SLN was carried out in two stages. At the first stage screening of different independent variables which has a significant effect on dependent variables/CQAs was carried out. In the second stage, the optimum level of these screened independent variables was determined.[25]

Screening of Formulation and Process Variable

The formulation and process variable which can have an impact on LTR-SLN were selected for screening using plackett burman's design. Based on risk assessment and feasibility studies nine factors were selected at two levels. Twelve trials were executed. Factors along with levels used for the study are presented in Table 1. Randomized and blocking design of experiments using statistical software Minitab® 19 is presented in Table 2. Average particle size (Y1), PDI (Y2), zeta potential (Y3), and entrapment efficiency (Y4) were selected as CQA/dependent variables for the study.[26] Statistical analysis was carried out to establish the significant effect of independent factors/variable (X1-X9) on the dependent variable (Y1-Y4).

Table 1: Screening design -Factors with their level.

Factors	Levels	
	Low	High
X1: Amount of lipid (mg)	40	80
X2: Amount of surfactant (mg)	10	50
X3: Amount of co-surfactant (mg)	10	50
X4: Number of HPH Passes	5	15
X5: HPH Pressure (Psi)	5000	10000
X6: High shear Rpm	5000	10000
X7: High Shear time (minutes)	10	30
X8: Amount of trehalose	10	50
X9: Method	Method I	Method II

Table 2: Screening study design.

Trials	X1	X2	X3	X4	X5	X6	X7	X8	X9
F1	40	10	0	5	10000	5000	10	10	I
F2	80	50	0	15	10000	5000	10	50	II
F3	80	10	0	5	5000	10000	30	10	II
F4	40	50	0	5	10000	10000	30	50	I
F5	40	50	50	15	10000	10000	30	10	II
F6	40	10	50	15	5000	5000	30	50	I
F7	80	50	0	15	5000	5000	30	10	I
F8	40	50	50	5	5000	5000	10	10	II
F9	80	10	50	5	10000	5000	30	50	II
F10	80	50	50	5	5000	10000	10	50	I
F11	40	10	0	15	5000	10000	10	50	II
F12	80	10	50	15	10000	10000	10	10	I

Optimization Study

Post-screening and identification of critical formulation and process variable which has a significant impact on average particle size (Y1), PDI (Y2), zeta potential (Y3), and entrapment efficiency (Y4) were further included in the optimization study. Optimization of the independent variable is carried out using face centered central composite design. The central composite design is usually preferred for five variables due to the reduced number of experiments.[27] The selected design is a two-level factorial half fraction having 16 cube points and 10 axial points. Four factors (X6-X9) used in the screening study were kept constant in the optimization study as per levels presented in Table 3. These independent variables were found to be statistically insignificant during the analysis of the plackett burman screening

Table 3: Fixed level of factors to be used in central composite design.

Factors	Fixed Level
X6: High shear Rpm	10000
X7: High Shear time (minutes)	30 min
X8: Amount of trehalose	50 mg
X9: Method	Method II

Table 4: Optimization design-factors with their level.

Factors	Levels		
	Low	Medium	High
X1: Amount of lipid (mg)	40	60	80
X2: Amount of Surfactant (mg)	10	30	50
X3: Amount of Co-Surfactant (mg)	10	30	50
X4: Number of HPH Passes	5	10	15
X5: HPH Pressure (Psi)	10000	15000	20000

design indicating that the level of these variables will not have any significant effect on the dependent variable. Thirty-two trials were executed using five independent variables (X1-X5). Independent factors along with their level are presented in Table 4. Randomized, blocking central composite study design is presented in Table 5. The formulations were evaluated for Y1 (average particle size), Y2 (PDI), Y3 (zeta potential), and Y4 (entrapment efficiency). The formulation and process variable were optimized wrt independent variables (X1-X5) and design space was established.

Design Space

The design space was established,[28] for LTR-SLN by plotting overlaid contour plot using Minitab® 19. An overlaid contour plot is plotted using a pair of an independent variables while other variables are held at a constant value. The target response is selected as not more than 200 nm for particle size, not less than 85% for entrapment efficiency, more negative than -30 for zeta potential and PDI within a suitable range.

Verification of Selected Level

Response surface optimizer was used to select the combination of the optimum variable to achieve target response. Based on the desirability approach each response is associated with a value between 0 to 1. Desirability function (d-value) close to 1 indicates desirability/closeness of selected response to predefined target value.[29]

Table 5: Optimization using central composite design.

Trials	X1	X2	X3	X4	X5
F1	60	30	50	10	15000
F2	40	30	30	10	15000
F3	80	10	50	15	10000
F4	80	50	50	5	10000
F5	80	10	10	5	10000
F6	60	30	30	10	15000
F7	80	50	10	15	10000
F8	40	50	50	5	20000
F9	60	30	30	5	15000
F10	40	50	50	15	10000
F11	40	50	10	5	10000
F12	60	30	10	10	15000
F13	60	30	30	10	15000
F14	80	10	50	5	20000
F15	60	30	30	15	15000
F16	80	30	30	10	15000
F17	80	50	10	5	20000
F18	40	10	50	15	20000
F19	60	30	30	10	20000
F20	80	50	50	15	20000
F21	40	10	10	15	10000
F22	40	50	10	15	20000
F23	80	10	10	15	20000
F24	40	10	50	5	10000
F25	40	10	10	5	20000
F26	60	30	30	10	15000
F27	60	30	30	10	10000
F28	60	50	30	10	15000
F29	60	10	30	10	15000
F30	60	30	30	10	15000
F31	60	30	30	10	15000
F32	60	30	30	10	15000

Characterization

Particle size and PDI

The particle size and PDI were determined by dynamic light scattering (DLS) technique using particle size analyzer malvern zetasizer (Nano-ZS). The solid lipid nanoparticle was diluted (1: 10) with water for injection for the determination of particle size.

Zeta Potential

The solid lipid nanoparticle was diluted with water for injection and analyzed for zeta potential using malvern zetasizer (Nano-ZS).

Entrapment Efficiency

Entrapment efficiency (EE%) was determined using an indirect technique by determining the amount of un-entrapped (free) drug in supernatant liquid by ultrafiltration technique using amicon@ ultra 15 molecular cut off 10 KD (merck Millipore). The drug formulation was added to the upper chamber of the amicon tube and subjected to centrifugation at 4000 rpm for 12 min. The concentration of un -entrapped drug present at the bottom of the tube was collected and analyzed for drug content by RP-HPLC using C_{18} column and methanol and orthophosphoric acid as mobile phase. The amicon tube was washed with water for injection before use to remove traces of glycerin.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Amount of unentrapped drug}}{\text{Total amount of drug}} \times 100$$

In-vitro Release Study

An *in-vitro* release study was carried out for LTR-SLN using an open-loop USP type IV flow-through cell. The accelerated dissolution medium selected is phosphate buffer saline (PBS) and 0.1N hydrochloric acid. The dissolution is carried out at a flow rate of 8 ml/min at 37°C using 1mm glass beads. Aliquots of the sample were collected at a predefined interval and analyzed for drug content using RP-HPLC at 240 nm using C_{18} column and methanol and orthophosphoric acid as mobile phase.

RESULTS

Selection of Lipids

The solubility and partition coefficient results are depicted in Figures 1 and 2.

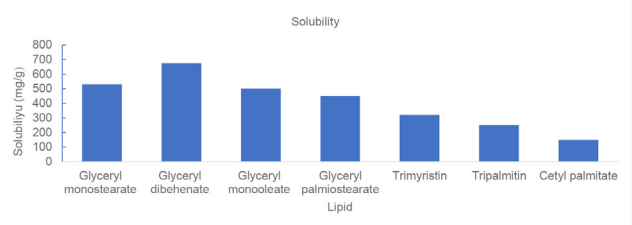

Figure 1: Solubility in different lipids.

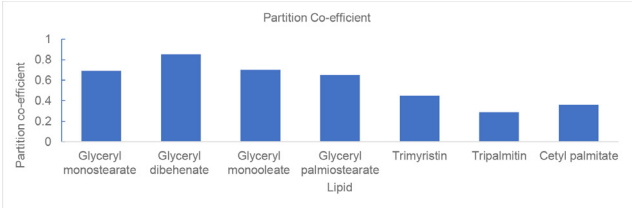

Figure 2: Partition coefficient in different lipid.

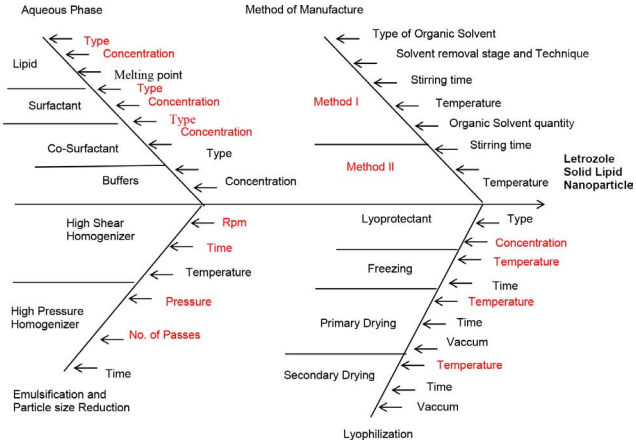

Figure 3: Risk Assessment using Fish bone diagram.

Table 6: Plackett burman design results.

Trials	Y1	Y2	Y3	Y4
F_1	190	0.21	-8	85
F_2	158	0.22	-17	90
F_3	250	0.27	-10	96
F_4	159	0.16	-19	80
F_5	105	0.12	-33	75
F_6	130	0.15	-28	78
F_7	172	0.23	-22	91
F_8	125	0.17	-35	73
F_9	160	0.2	-30	88
F_{10}	147	0.16	-36	83
F_{11}	211	0.23	-11	84
F_{12}	131	0.18	-27	85

Y_1: Average particle size; Y_2: PDI; Y_3: zeta potential; Y_4: entrapment efficiency.

Risk assessment

The fishbone diagram along with potential risk factors is presented in Figure 3.

Screening

The results of twelve trials carried out using 9 independent variables are presented in Table 6. Statistical analysis of the model is presented in Table 7.

Optimization Study using Central Composite Design

The results of optimization study are presented in Table 8 and correlation coefficient and P-value presented in Table 9.

Based on R^2, $R^2_{predicted}$, $R^2_{adjusted}$ value of different models (Linear, linear+ square. linear +interaction and quadratic), the quadratic model was selected for response Y1, Y2, Y4 and linear model for Y3.

Table 7: Statistical Analysis.

Factors	Y1		Y2		Y3		Y4	
	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
X1	9.08	0.024	0.01833	0.016	0.667	0.465	4.833	0.008
X2	-18.08	0.006	-0.01500	0.024	-4.00	0.033	-2.00	0.042
X3	-27.58	0.003	-0.02833	0.007	-8.500	0.008	-3.667	0.013
X4	-9.42	0.021	-0.00333	0.293	-0.000	1.00	-0.167	0.733
X5	10.08	0.019	0.01000	0.051	-0.667	0.465	0.167	0.733
X6	6.58	0.052	-0.00500	0.168	0.333	0.698	-0.167	0.733
X7	0.25	0.874	-0.00333	0.293	-0.667	0.465	0.667	0.257
X8	-1.58	0.375	-0.00500	0.168	-0.500	0.571	-0.167	0.733
X9	5.75	0.054	0.01000	0.0501	0.333	0.698	0.333	0.515

X_1: lipid concentration; X_2: surfactant concentration; X_3: co-surfactant concentration; X_4: number of passes; X_5: HPH pressure; X_6: High shear rpm; X_7: High shear mixing time; X_8: Trehalose concentration; X_9: Method.
Y_1: Average particle size; Y_2: PDI; Y_3: zeta potential; Y_4: entrapment efficiency.

Table 8: Central composite design results.

Trials	Y1	Y2	Y3	Y4
F1	115	0.14	-39	87
F2	118	0.12	-34	78
F3	123	0.26	-36	95
F4	128	0.24	-43	80
F5	145	0.28	-18	98
F6	125	0.16	-35	91
F7	124	0.25	-31	96
F8	230	0.45	-44	70
F9	138	0.17	-33	90
F10	110	0.13	-43	69
F11	139	0.19	-29	74
F12	130	0.16	-28	94
F13	126	0.14	-32	90
F14	315	0.57	-35	94
F15	105	0.15	-34	92
F16	118	0.22	-33	97
F17	345	0.61	-31	94
F18	310	0.56	-37	73
F19	254	0.54	-36	90
F20	218	0.57	-45	83
F21	115	0.25	-21	78
F22	279	0.45	-29	71
F23	310	0.61	-19	97
F24	142	0.23	-37	70
F25	308	0.65	-22	77
F26	127	0.14	-35	90
F27	100	0.17	-34	91
F28	115	0.16	-39	86
F29	133	0.21	-27	93
F30	131	0.15	-34	95
F31	128	0.16	-36	94
F32	126	0.15	-36	95

Y_1: Average particle size; Y_2: PDI; Y_3: Zeta potential; Y_4: Entrapment efficiency.

Average particle size, PDI, zeta potential and entrapment efficiency can be determined using the following regression equation (Equation I-IV).

$$PSD = 412.4 + 0.07\,X1 + 0.440\,X2 + 1.215\,X3 - 1.59\,X4 - 0.05459\,X5 + 0.0075\,X1*X1 + 0.0225\,X2*X2 + 0.0187\,X3*X3 + 0.260\,X4*X4 + 0.000002\,X5*X5 + 0.00609\,X1*X2 - 0.01422\,X1*X3 - 0.0956\,X1*X4 + 0.000029\,X1*X5 - 0.03328\,X2*X3 - 0.0369\,X2*X4 - 0.000092\,X2*X5 + 0.0344\,X3*X4 - 0.000093\,X3*X5 + 0.000002\,X4*X5 \quad \textbf{(I)}$$

$$PDI = 1.7296 - 0.00348\,X1 - 0.010060\,X2 - 0.002560\,X3 - 0.01870\,X4 - 0.000193\,X5 + 0.000015\,X1*X1 + 0.000090\,X2*X2 + 0.000002\,X3*X3 + 0.000433\,X4*X4 + 0.000000\,X5*X5 + 0.000066\,X1*X2 + 0.000009\,X1*X3 + 0.000087\,X1*X4 + 0.000000\,X1*X5 + 0.000009\,X2*X3 - 0.000012\,X2*X4 - 0.000000\,X2*X5 + 0.000137\,X3*X4 - 0.000000\,X3*X5 - 0.000000\,X4*X5 \quad \textbf{(II)}$$

$$\text{Zeta Potential} = -15.03 + 0.0139\,X1 - 0.2278\,X2 - 0.3639\,X3 - 0.0333\,X4 - 0.000067\,X5 \quad \textbf{(III)}$$

$$\text{Entrapment Efficiency} = -3.9 + 1.881\,X1 + 0.448\,X2 + 0.069\,X3 + 1.414\,X4 + 0.00194\,X5 - 0.01058\,X1*X1 - 0.00558\,X2*X2 - 0.00308\,X3*X3 - 0.0293\,X4*X4 - 0.000000\,X5*X5 - 0.00266\,X1*X2 - 0.00234\,X1*X3 + 0.00312\,X1*X4 - 0.000001\,X1*X5 - 0.00234\,X2*X3 - 0.00187\,X2*X4 - 0.000001\,X2*X5 + 0.00437\,X3*X4 + 0.000008\,X3*X5 - 0.000068\,X4*X5 \quad \textbf{(IV)}$$

The effect of significant independent variables (X1, X2, X3, X4 and X5) and their interaction on dependent response average particle size (Y1), PDI (Y2), zeta

Table 9: Statistical Analysis.

Factors	Y1		Y2		Y3		Y4	
	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
X1	4.17	0.05	0.03	0.00	0.28	0.50	9.67	0.00
X2	-11.83	0.00	-0.03	0.00	-4.56	0.00	-2.89	0.00
X3	-11.33	0.00	-0.02	0.00	-7.28	0.00	-3.22	0.00
X4	-10.89	0.00	-0.01	0.00	-0.17	0.68	0.39	0.37
X5	80.17	0.00	0.17	0.00	-0.33	0.42	-0.11	0.79
X1*X1	2.99	0.59	0.01	0.24	-	-	-4.23	0.00
X2*X2	8.99	0.12	0.04	0.00	-	-	-2.23	0.07
X3*X3	7.49	0.19	0.00	0.86	-	-	-1.23	0.30
X4*X4	6.49	0.25	0.01	0.04	-	-	-0.73	0.53
X5*X5	61.99	0.00	0.19	0.00	-	-	-1.23	0.30
X1*X2	2.44	0.27	0.03	0.00	-	-	-1.06	0.03
X1*X3	-5.69	0.02	0.00	0.07	-	-	-0.94	0.06
X1*X4	-9.56	0.00	0.01	0.00	-	-	0.31	0.49
X1*X5	2.94	0.19	0.00	0.51	-	-	-0.06	0.89
X2*X3	-13.31	0.00	0.00	0.07	-	-	-0.94	0.06
X2*X4	-3.69	0.11	0.00	0.51	-	-	-0.19	0.68
X2*X5	-9.19	0.00	-0.01	0.01	-	-	-0.06	0.89
X3*X4	3.44	0.13	0.01	0.00	-	-	0.44	0.34
X3*X5	-9.31	0.00	0.00	0.07	-	-	0.81	0.09
X4*X5	0.06	0.97	0.00	0.51	-	-	-1.69	0.00

X1: lipid concentration; X2: surfactant concentration; X3: co-surfactant concentration; X4: number of passes; X5: HPH pressure; Y1: average particle size; Y2: PDI; Y3: zeta potential; Y4: entrapment efficiency.

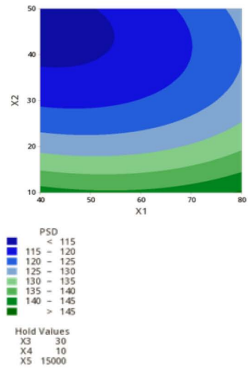

Figure 4a: Contour Plot PSD Vs X2 X1.

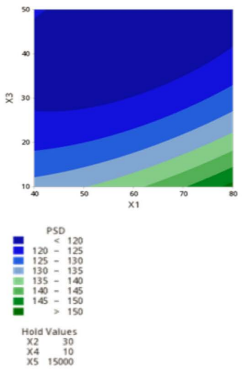

Figure 4b: Contour Plot PSD Vs X3X1.

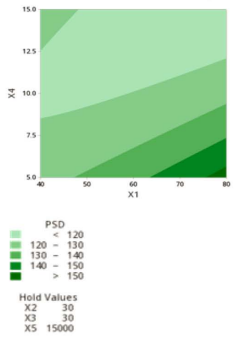

Figure 4c: Contour Plot PSD Vs X4 X1.

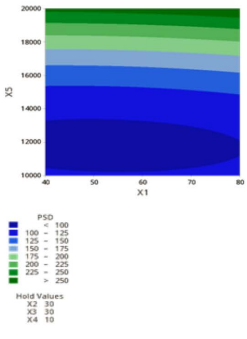

Figure 4d: Contour Plot PSD Vs X5X1.

Figure 4: Contour plot of significant factors on Average Particle size.

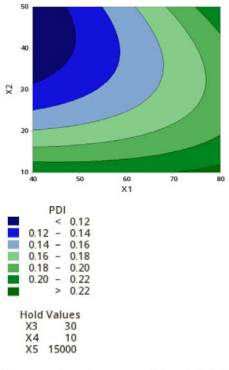

Figure 5a: Contour Plot PDI Vs X2 X1.

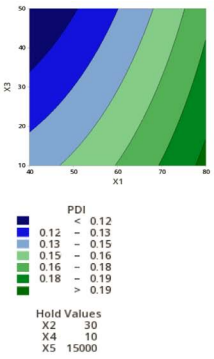

Figure 5b: Contour Plot PDI Vs X3X1.

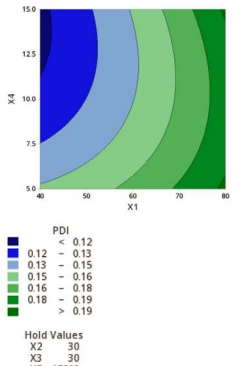

Figure 5c: Contour Plot PDI Vs X4 X1.

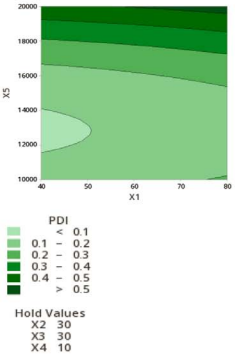

Figure 5d: Contour Plot PDI Vs X5X1.

Figure 5: Contour plot of significant factors on PDI.

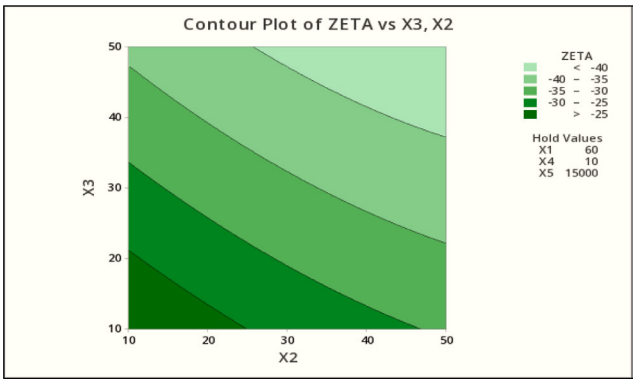

Figure 6: Contour plot of significant factors on Zeta potential.

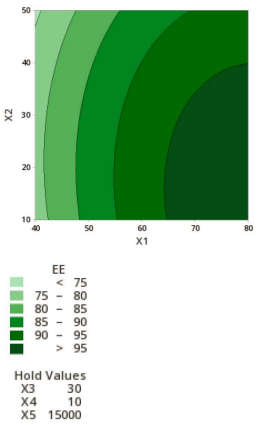

Figure 7a: Contour Plot EE Vs X2 X1.

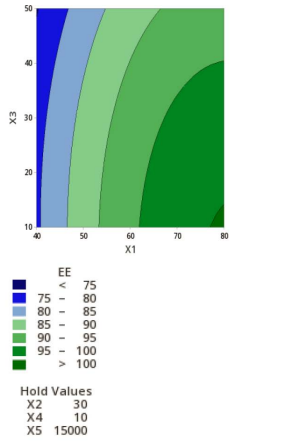

Figure 7b: Contour Plot EEVs X3X1.

Figure 7: Contour plot of significant factors on Entrapment Efficiency.

potential (Y3) and entrapment efficiency (Y4) is plotted graphically. Contour plots were plotted to study the relation between two independent factors and response factors keeping the other three variables constant as depicted in Figure 4 to 7.

Design Space

The shaded area in the plot determines the area of compromise where the predicted response is not within a predefined target. The non-shaded area defines the design space. The overlaid contour plot is presented in Figure 8.

Verification of Levels of Selected variables

Hence based on optimization design the following level of the independent variable is selected as mentioned in Table 10. The composite D value was found to be 0.94 which was closer to 1 for batches manufactured using optimum level as Table 11.

Batches were manufactured using a selected optimum level of the independent variable and predicted and

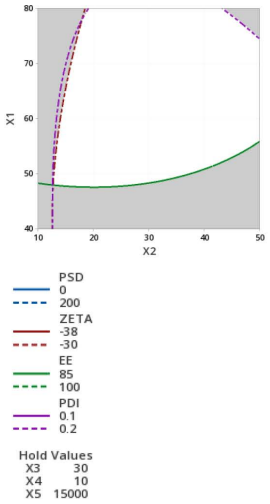

Figure 8a: Overlaid Contour Plot.

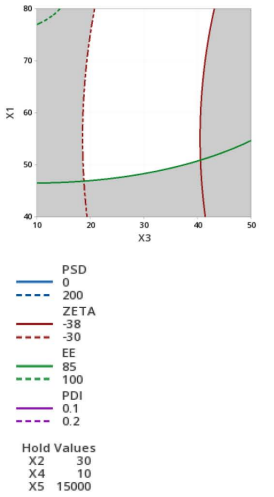

Figure 8b: Overlaid Contour Plot.

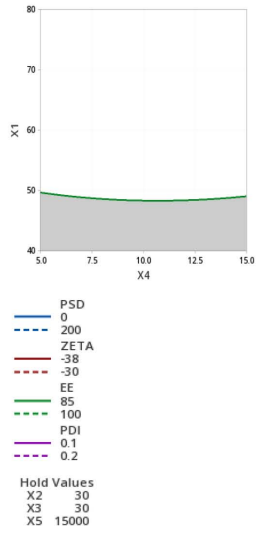

Figure 8c: Overlaid Contour Plot.

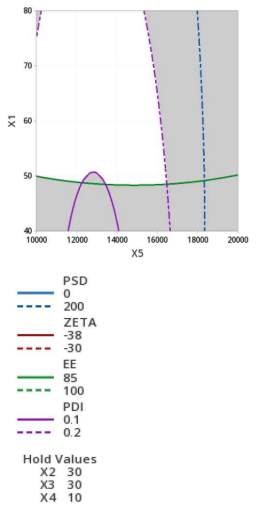

Figure 8d: Overlaid Contour Plot.

Figure 8: Overlaid Contour plot.

Table 10: Optimum Formulation level.

Sl. No	Independent Variable	Optimum Level
1	Lipid concentration (X1)	60 mg
2	Surfactant Concentration (X2)	30 mg
3	Co-Surfactant Concentration (X3)	30 mg
4	Number of Passes (X4)	15
5	HPH Pressure (X5)	15000 Psi

Table 11: Table Verification.

Response	Predicted	Observed (Average of triplicate batches)
Particle size	105.84	106.0
PDI	0.19	0.193
Zeta Potential	-33.10	-32.3
Entrapment Efficiency	93.84	94.2

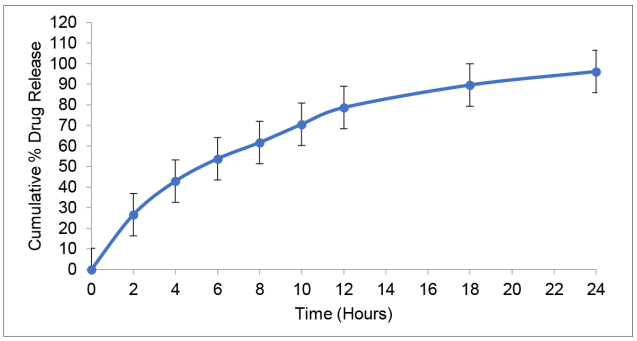

Figure 9: *In-vitro* drug release.

Table 12: Kinetic Model.

	Zero order	First order	Korsmeyer peppas	Higuichi
R^2	0.8421	0.9949	0.7376	0.9852

observed responses (Particle size, PDI, Zeta Potential, and Entrapment Efficiency) are presented below.

In-vitro Drug Release

The optimized formulation was analyzed for an *in-vitro* release study. Cumulative % drug release is plotted vs time as depicted in Figure 9.

The obtained *in-vitro* drug release data were subjected to different mathematical models such as Zero order reaction, First Order reaction, Korsmeyer -Peppas, and Higuichi Model. R^2 value is presented in Table 12.

DISCUSSION

Glyceryl dibehenate has higher solubility (Figure 1) and higher partition co-efficient as compared to other evaluated lipids (Figure 2). Glyceryl dibehenate is a mixture of various esters of behenic acid and-glycerol. Lipids consisting of different chain lengths result in imperfect crystal lattices providing more space for drug loading and thereby improve drug loading and reduce drug expulsion.[30] Glyceryl dibehenate has GRAS status and is suitable for use in parenteral drug delivery. Hence Glyceryl dibehenate is selected as suitable lipid for the formulation of LTR -SLN.

The inclusion of co-surfactant along with surfactant results in lower particle size and PDI as compared to the use of only surfactant (Table 5). This can be confirmed from the fact that the addition of co-surfactant (lecithin) along with surfactant (poloxamer) provides additional better coverage to solid lipid nanoparticles and prevents particle aggregation,[22] thereby reducing particle size and PDI.

A quality by design approach was used for the optimization of LTR-SLN. Ishikawa /fishbone diagram was used for risk identification (Figure 3). Plackett Burman's design was used for screening and optimization of independent variable was central composite design.

In the screening study, lipid concentration (X1), surfactant concentration (X2), co-surfactant concentration (X3), Number of passes (X4) and HPH pressure (X5) will have a significant contribution on Average particle size (Y1) and PDI (Y2) as the p-value is less than 0.05 (Table 7). Similarly, surfactant concentration (X2) and co-surfactant concentration (X3) will have a significant contribution to zeta potential (Y3); Lipid concentration (X1), surfactant concentration (X2), and co-surfactant concentration (X3) will have a significant contribution to entrapment efficiency (Y4). High shear rpm (X6), high shear mixing time (X7), trehalose concentration (X8), and method (X9) do not have a significant effect on average particle size, PDI, zeta potential, and entrapment efficiency indicated by p-value greater than 0.05. Hence these parameters are kept constant in optimization study.

A higher coefficient of determination (R^2) value advocates good correlation and model fit. Higher $R^2_{adusted}$ value and closer quantitatively to R^2 indicates the significance of the model.[31] A good correlation between predicted value and observed value is indicated by higher $R^2_{predicted}$.[31] Hence the selected model is suitable for optimization of formulation and process variables used for designing of LTR-SLN.

In a statistical analysis, positive sign of co-efficient indicates synergistic effect (independent variable is directly proportional to the response). Similarly, a negative sign of co-efficient indicates antagonist effect (independent variable bears an inverse relationship with response).[31]

The coded independent variable (X1) bears a positive sign whereas (X2), (X3), (X4) and (X5) bears a negative sign for particle size and PDI. An increase in lipid content increases particle size as viscosity increases thereby increasing surface tension. Higher surface tension can cause collision /aggregation of particles leading to higher particle size and PDI.[31-32] Increase in surfactant and co-surfactant concentration leads to decrease in the particle size and PDI. Higher surfactant and co-surfactant concentrations stabilize the surface of particles. The SLN particles needs to be stabilized to prevent particle aggregation during homogenization due to hydrophobic interaction.[33] An increase in HPH pressure from 10000 psi to 15000 psi results in a decrease in particle size and however further increase in HPH pressure from 15000 psi to 20000 psi results

in an increase in particle size and PDI. This can be inferred that with an increase in HPH pressure there is an increase in cavitation force and particle collision due to an increase in kinetic energy.[34] This increased force can destabilize the protective surfactant layer formed on the particle thereby leading to particle aggregation/ coalescence resulting in increased particle size.[35] An increase in the number of passes from 5 to 15 results in lower particle size and PDI due to an increase in cavitation force and longer exposure time.

Coded independent variable (X2) and (X3) bears a negative sign for zeta potential. The zeta potential tends towards the negative side with an increase in surfactant and cosurfactant concentration.[36]

Coded independent variable (X1) bears a positive sign and (X2) and (X3) bear a negative sign on entrapment efficiency. Higher lipid content leads to enhancement of entrapment efficiency. This is due to the fact that increase in lipid content increases viscosity leading to faster solidification of SLN and there by prevents/ reduce drug diffusion.[36] An increase in surfactant and co-surfactant concentration leads to a decrease in entrapment efficiency. Increased partitioning of drug from internal to external phase increases solubilization of drug in external phase leading to reduction in drug entrapment.[37]

The observed values of drug product CQA determined using an optimum level of the independent variable are closer to the predicted value (Table 11). The composite desirability value is closer to 1 which indicates the goodness of fit of the selected model.

R^2 value determined for mathematical modeling of *in-vitro* release study was found to fit significantly for first-order reaction (R^2 = 0.99) and higuichi (R^2 =0.98) based on closeness of R^2 value to 1.[38] Hence the release of LTR-SLN follows first-order drug release (drug release rate is proportional to the concentration of drug) and higuichi model (drug release follows both dissolution and diffusion mechanism). The release exponent (n) higher than 0.89 indicates that formulation follows super case II non-fickian diffusion release mechanism.[39-40]

CONCLUSION

LTR–SLN was developed using emulsification solvent evaporation and melt dispersion techniques. Glyceryl dibehenate was selected as suitable lipid based on solubility and partition coefficient. Inclusion of co-surfactant along with surfactant is necessary to reduce particle aggregation and hence a combination of surfactant and co-surfactant is required for stabilization of formulation. Quality by design concept was used for selection and optimization of critical formulation (CQA) and process variables (CPP). Risk assessment was done to select the variable which will have an impact on CQAs of drug products such as particle size, PDI, zeta potential, and entrapment efficiency. Plackett burman design was selected for initial screening and central composite design for optimization of formulation and process variables.

High shear rpm, high shear mixing time, trehalose concentration, and manufacturing method did not have a significant effect on CQAs as observed in Plackett Burman design and the levels were fixed at 10000 rpm, 30 min, 50 mg, and method II respectively. The optimum level of factors as established by central composite design are 60 mg lipid, 30 mg surfactant, 30 mg co-surfactant,15000 Psi HPH pressure and 15 HPH passes. Design space was established. The observed values of CQAs for batches manufactured using optimized parameters were close to statistical predicted values of CQAs and desirability (d value) was close to 1 indicating the suitability of the selected model. *In-vitro* release study of the optimized formulation was carried out using USP type IV (flow through cell). The obtained results were subjected to various mathematical modeling for determining the mechanism of drug release. The drug release from follows a first-order and higuichi release (dissolution and diffusion i.e non-fickian Super case II transport mechanism).Hence it can be concluded that the optimized formulation and process parameters can be utilized to design a quality drug product meeting its predefined specification as per recommendation of various regulatory guidelines for global market.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LTR-SLN: Letrozole Solid Lipid Nanoparticle; **SLN:** Solid Lipid Nanoparticle; **Qbd:** Quality by design; **CQA:** Critical Quality Attributes; **PSD:** Particle size Determination; **PDI:** Polydispersity Index; **HPH:** High-Pressure Homogenizer; **psi:** Pounds per square inch; **Mg:** Milligram; **Rpm:** Revolutions per minute.

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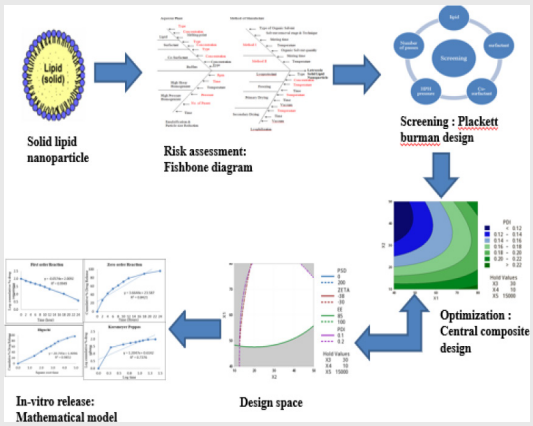
PICTORIAL ABSTRACT



SUMMARY

The objective of the present study was to develop and optimize solid lipid nanoparticle of letrozole. Ishikawa/ fish bone diagram was used for risk assessment to identify variables which can an impact on CQA of drug product. Plackett burman design was used for screening and central composite design was used for optimization of independent variable. The optimum level of factors as established by central composite design are 60 mg lipid, 30 mg surfactant, 30 mg co-surfactant,15000 Psi HPH pressure and 15 HPH passes. USP Type IV (Flow through cell) was used for carrying out *in-vitro* release study of drug product. The dissolution results were subjected to various mathematical models to determine mechanism of drug release. The drug release from follows a first-order and higuichi release (dissolution and diffusion i.e non-fickian Super case II transport mechanism).

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