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Research Article

Development of Candesartan Loaded Solid Lipid Nanoparticles by Box-Behnken Design

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ABSTRACT

The primary motive behind this the present investigation was to develop and optimize the solid lipid nanoparticles formulation of candesartan to enhance solubility and dissolution rate. The prepared SLNs, composed of precirol, poloxamer 188, soy lecithin, tween 80, were fabricated employing hot emulsification/ ultrasonication technique. Box-Behnken design was employed for 17 formulation batches in which 3 factors namely lipid, surfactant, and co-surfactant (precirol, poloxamer 188 and soy lecithin) tween 80 were tested at 3 levels of their concentration, *i.e.,* low, medium and high. The effect of different levels of factors was evaluated for the particle size, entrapment efficiency and % cumulative drug release. Kinetic model fitting for candesartan solid lipid nanoparticles (SLN) formulation was done to interpret the release rate from the SLN**.** Optimized formulation was subjected for fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM) and stability studies. The mean particle size, PDI, zeta potential, entrapment efficiency, content uniformity and in-vitro drug release of optimized candesartan-loaded SLNs (CD10) were found to be 135.38 ± 3.41 nm, 0.125 ± 0.04, -18.16 ± 2.89 mV, 86.4 ± 2.35%, 99.78 ± 2.54% and 98.91 ± 0.85% respectively. The release kinetics suggested that drug release followed zero-order and release was anomalous non-fickian diffusion super case II transport. FTIR studies revealed no incompatibility between drug and excipients, SEM images exhibited nanoparticles to be more porous and in a spherical shape. Stability studies indicated good stability of the formulation. The proposed way of SLN preparation could be considered a proper method for producing a candesartan-loaded colloidal carrier system.

Introduction

Aqueous solubility and first-pass metabolism are the major factors responsible for poor oral bioavailability of lipophilic drugs. Lipid-based formulations successfully reduced the inherent limitations of slow and incomplete dissolution of poorly water-soluble drugs by facilitating the formation of solubilized phase and further, improving the absorption.[1]

In controlled and targeted drug delivery, SLNs are emerging as alternative carriers to other colloidal drug systems. These are in submicron size range (50–1000 nm) and are made of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs. SLNs combines the advantage of different colloidal carriers, vesicular carriers, and polymeric carriers[2] as physiologically acceptable systems and impart the controlled release of drugs from lipid matrix.[3] Such a system enhances the lymphatic transport of the lipophilic drugs, irrespective of the route of administration, and therefore increases the systemic availability of drug molecules.

Candesartan cilexetil (CC) is an ester prodrug of candesartan, and a non-peptide angiotensin II type 1 (AT1) receptor antagonist, used to treat hypertension and heart failure. Candesartan cilexetil is BCS class II drug. To overcome hepatic first-pass metabolism and enhance oral bioavailability, lipid-based drug-delivery systems like solid lipid nanoparticles can be used. These systems enhance the lymphatic transport of the lipophilic drugs and therefore increase the bioavailability.[3] The present investigation was to develop and optimize the solid lipid nanoparticles formulation of candesartan to enhance solubility and dissolution rate by using Box-behnken design.[4]

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Material and Methods

Candesartan was procured from Hetero Drugs Ltd, Hyderabad. Precirol, poloxamer 188, soy lecithin, tween 80were purchased from Gattefosse, Mumbai. All the reagents used were of analytical grade.

Preparation of Candesartan Loaded Solid Lipid Nanoparticles

Candesartan-SLNs were prepared by hot emulsification/ ultrasonication method. Candesartan (16 mg) and precirol (%) were dissolved in a mixture of chloroform and methanol (1:1) (20 mL). The solvent was then completely removed using a rotary evaporator (temp, rpm). The drug-embedded lipid layer was melted by heating at 75°C. An aqueous phase was then formulated by dissolving the surfactant and co-surfactant (%), such as poloxamer 188 and soy lecithin in double-distilled water and adding it to the molten lipid phase. This was followed by homogenization for 3 minutes where coarse hot oil in a water emulsion was obtained, then ultrasonicated using a probe sonicator. Finally, the obtained hot nanoemulsion was allowed to cool to room temperature to achieve candesartan -SLNs. The impact of varying the critical parameter variables on the preparation of different formulations were shown in the following sections. The composition of formulation batches was given in Table 1.

Experimental Design

Box–Behnken Experiment Design

A BB design was employed for formulation batches (*i.e.,* 17 formulations) in which three factors namely lipid, surfactant (tween80), and co-surfactant (precirol, poloxamer 188, or soy lecithin), were tested at three levels of their concentration, i.e., low, medium and high. The effect of different levels of factors was evaluated at the particle size of resultant formulation, entrapment efficiency, and %cumulative drug release. The composition of formulation batches is given in Table 1.

Table 1: List of dependent and independent variables in box-behnken design

Independent variables			*Levels*		
Variable	*Name*	*Units*	*Low*	*Middle*	*High*
			(-1)	(0)	(+1)
X1	Precirol	%	6	8	10
X2	Poloxamer 188	%	2	4	6
X3	Soy lecithin	%	1	2	3

Dependent variable			*Goal*
Y1	Particle size	nm	Minimize
Y2	Entrapment Efficiency	%	Minimize
Y3	Drug release after 12Hrs	%	Maximize

Table 2 showed 17 randomized experimental runs for the selected independent variables, including five replicates at the center point (asterisk-marked) generated from three factors, three-level BBD, and their corresponding responses. Five replicates at the center point were taken in this study for a more uniform estimate of the prediction variance over the entire design space. The amount of drug added to the formulation was kept constant. Based on the boundary of the solid lipid nanoparticles domain, three levels of independent or formulation variables (amount of lipid, surfactant and co-surfactant) were identified: low (coded as–1), middle (coded as 0) and high (coded as +1), as shown in Table 1. The range for each independent variable was selected for solid lipid nanoparticles: that is, the amount of precirol (Lipid, X_1) was 6 to 8 %, the amount of poloxamer 188 (surfactant, X_2) was 2 to 6% and the amount of soy lecithin (co-surfactant, X_3) was 1 to 3% (Table 1).

The BBD matrix was generated using Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), and the same software analyzed the data. All responses were fitted to a second-order quadratic model by the Design Expert software. The composition of Candesartan SLNs formulation by Box Behnken Design was given in Table 2.

Optimization Using the Desirability Functions

To optimize multiple responses, they should be highly correlated with each other. It is unlikely that the values desirable to optimize one response's effect will have the same effect on the second response. Thus a conflict can occur between them. Hence, the most favorable compromising zone must be sought for each response without any bias. In the present study, all three responses were simultaneously optimized by a desirability function that uses the numerical optimization method introduced by Derringer and Suich in the Design-Expert software (Version 8.0, Stat-Ease Inc., Silicon Valley, CA, USA). Recently, the desirability function approach was reported in several articles to optimize multiple responses.[5]

Evaluation of Candesartan Loaded SLN

Measurement of Particle Size and Zeta Potential

The particle size and zeta potential of SLN were measured by photon correlation spectroscopy using a Zetasizer3000HSA (Malvern, UK). Zeta potential was measured out at room temperature and the electric-field strength was around 23.4 V/cm. Samples were diluted appropriately with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 50 to 200 for measurements, and the pH of diluted samples ranged from 7.0 ± 0.2.[6]

Drug Content

1-mL SLNs dispersion was taken into 100 mL volumetric flask, and the volume was made up of methanol. It was sonicated for 5 minute in a bath sonicator. Solution was

Table 2: Composition of candesartan SLN formulation by BBD

F.No	Candesartan (mg) mg	Precirol (%)	Poloxamer 188 (%)	Soy lecithin (%)	Tween 80 (ml)	Chloroform: Methanol (1:1)	Distilled Water (mL)
CD1	16	6	2	2	0.5	20	Q.S
CD2	16	10	2	2	0.5	20	Q.S
CD3	16	6	6	2	0.5	20	Q.S
CD4	16	8	6	1	0.5	20	Q.S
CD5	16	6	4	1	0.5	20	Q.S
CD6	16	10	4	1	0.5	20	Q.S
CD7	16	6	4	3	0.5	20	Q.S
CD8	16	10	4	3	0.5	20	Q.S
CD9	16	8	2	1	0.5	20	Q.S
CD10	16	10	6	2	0.5	20	Q.S
CD11	16	8	2	3	0.5	20	Q.S
CD12	16	8	6	3	0.5	20	Q.S
CD13	16	10	4	2	0.5	20	Q.S
CD14	16	8	4	1	0.5	20	Q.S
CD15	16	8	4	6	0.5	20	Q.S
CD16	16	6	4	2	0.5	20	Q.S
CD17	16	8	4	2	0.5	20	Q.S

filtered through Whatman filter paper (0.45 μ) and the filtrate was analyzed at a UV-visible spectrophotometer at 228 nm.[7]

Determination of Entrapment Efficiency (%)

Entrapment efficiency (EE%) was determined by measuring free drug concentration (unentrapped) in an aqueous medium as reported. The aqueous medium was separated by ultra-filtration using centrisart tubes (Sartorius, USA), which consists of filter membrane (M.wt. cut off 20,000 Da) at the base of the sample recovery chamber. About 1-mL of the formulation was placed in the outer chamber, and sample recovery chamber was placed on top of the sample and centrifuged at 4000 rpm for 15 minutes. The SLN and encapsulated drug remained in the outer chamber, and the aqueous phase moved into the sample recovery chamber through the filter membrane.[8] The amount of candesartan in the aqueous phase was estimated by HPLC method, and the entrapment efficiency was calculated by the equation:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Analyzed weight of drug in SLNs}}{\text{Theoretical weight of drug - loaded in the system}} \times 100$$

In-vitro Release Study

In vitro release study was performed in 0.1 N HCl (pH 1.2) using modified Franz diffusion cell and dialysis membrane having pore size 2.4 nm, molecular weight cut-off between 12,000 to 14,000 was used. The membrane was soaked in double-distilled water for 12 hours. SLN dispersion (2mL) was placed in the donor compartment, and the receptor compartment was filled with 50 mL of release media. During the experiments, the solution on receptor side was maintained at 37 ± 0.5°C and stirred at 50 rpm with magnetic stirring bars for 2 hours. Then, the pH was increased to pH 6.8 for the remaining 10 hours. An aliquot of the sample (5 mL) was taken from the dissolution medium at different time 0.5, 1, 2, 3, 4, 6, 8 and 12 hours time points, samples were withdrawn and analyzed by UV-visible spectrophotometer at 228 nm. Data obtained were fitted to various kinetic equations to find out the mechanism of Candesartan release from SLN.[9]

Kinetic Model Fitting

In vitro release data were fitted to various kinetic equations to find out the mechanism of candesartan release from SLN.[9] To elucidate the mode and drug release mechanism, the in vitro release study data were fitted into various kinetic models such as zero-order, first-order, Higuchi's, and Korsmeyer–Peppas model.[10]

Characterization of Optimized Formulation

FTIR Studies

An FTIR-8400S Spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients. Analysis of pure drug and physical mixtures of the drug with the excipients

were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra to remove the influence of residual moisture. For each spectrum, 8 scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 400 to 4000 cm^{-1}.

Scanning Electron Microscopy

Shape and surface morphology of microspheres was studied using scanning electron microscopy (SEM). The SNEDDS, after converting to emulsion, were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument HITACHI, S-3700N.

Stability Studies

Among all batches of candesartan loaded solid lipid nanoparticles were subjected to stability studies under guidelines of ICH stability protocol. The test specifications include a Temperature of 40°C ± 2°C and relative humidity of 75 ± 5% RH for a period of 6 months in the Humidity chamber (REMI, Mumbai). The specifications to be evaluated in the stability study period included particle size, entrapment efficiency, *in vitro* drug release.[11]

Results and Discussion

Physicochemical Evaluation Of SLNs

Developed candesartan SLNs were physic chemically evaluated in terms of drug content, mean particle size, entrapment efficiency, zeta potential, polydispersity index.

%Drug Content

The drug content for all formulation was within satisfactory limits and found to be between 95.56 and 99.78% along with the increase with increase in the surfactant concentration in all formulations (Table 3). The highest drug content was observed for CD10 formulation.

Polydispersity Index

The polydispersity index (PDI) is a marker of particle size distribution. Its value in case of submicron particles ranges from 0.115 to 0.185 indicates size homogeneity, while PDI greater than 0.3 results in heterogeneity. The polydispersity index of all SLNs was significantly varying from to as depicted in Table 3, indicating narrow size distribution which reveals the higher stability of candesartan solid lipid nanoparticles. Earlier studies reported similar findings on cyclosporine A incorporated cationic candesartan solid lipid nanoparticles for drug delivery. [12]

In Vitro Dissolution Testing Of Candesartan SLNs

The dissolution profiles of plain candesartan and candesartan SLNs formulation in simulated intestinal. To understand the release mechanisms of candesartan SLNs formulations, the drug release profiles of formulation CD10 were analyzed. As shown in Fig. 1 to 3, more than 85% of drug was dissolved and released from CD10 after 12 hours (98.91%). However, the marketed product (Candesar 16 mg tablet) showed only approximately 86% drug released after the same time period. This result suggested that the SLNs formulation significantly enhanced the dissolution of candesartan. The enhanced dissolution may be due

Table 3: Physico-chemical parameters of candesartan SLN of BBD formulation

F. No	*# Content uniformity (%)*	*% Entrapment Efficiency*	*Mean particle size (nm)*	*Zeta potential (-mV)*	*Polydispersity Index*
CDF1	97.45 ± 1.26	77.9 ± 1.46	188.51 ± 2.83	21.34 ± 4.83	0.165 ± 0.07
CDF2	96.33 ± 2.89	75.4 ± 1.57	145.30 ± 3.15	16.21 ± 4.55	0.185 ± 0.03
CDF3	98.12 ± 2.43	78.5 ± 1.65	136.22 ± 5.78	19.64 ± 2.17	0.146 ± 0.09
CDF4	98.18 ± 1.52	72.8 ± 2.36	165.35 ± 1.47	21.55 ± 4.61	0.156 ± 0.01
CDF5	97.51 ± 2.16	78.7 ± 1.19	170.17 ± 3.23	20.12 ± 2.35	0.127 ± 0.05
CDF6	97.73 ± 2.33	77.5 ± 1.54	223.16 ± 4.17	22.28 ± 2.44	0.187 ± 0.03
CDF7	98.44 ± 2.67	82.6 ± 2.88	157.58 ± 3.14	16.47 ± 4.35	0.115 ± 0.08
CDF8	97.23 ± 2.89	79.8 ± 1.72	166.21 ± 5.24	18.22 ± 5.43	0.171 ± 0.03
CDF9	97.11 ± 2.53	73.7 ± 1.57	142.50 ± 4.98	20.34 ± 2.57	0.156 ± 0.02
CDF10	99.78 ± 2.54	86.4 ± 2.35	135.38 ± 3.41	18.16 ± 2.89	0.125 ± 0.04
CDF11	96.88 ± 2.65	78.4 ± 1.23	168.53 ± 2.87	20.18 ± 1.31	0.175 ± 0.05
CDF12	96.23 ± 1.62	75.2 ± 2.91	141.12 ± 3.33	17.73 ± 2.32	0.128 ± 0.06
CDF13	95.56 ± 1.89	79.3 ± .2.84	156.31 ± 4.75	21.44 ± 4.32	0.139 ± 0.02
CDF14	97.01 ± 2.39	79.2 ± 1.70	213.46 ± 2.21	18.53 ± 5.78	0.162 ± 0.05
CDF15	96.78 ± 2.17	75.2 ± 2.67	138.61 ± 1.26	20.32 ± 1.55	0.178 ± 0.03
CDF16	96.45 ± 2.56	79.6 ± 1.42	189.26 ± 3.17	16.78 ± 4.15	0.145 ± 0.05
CDF17	97.26 ± 2.13	81.8 ± 2.18	175.33 ± 4.56	21.19 ± 3.67	0.169 ± 0.04

to the decrease in crystallinity and the increase in solubility of the drug. The increase in cumulative drug released is mainly attributed to rapid self-emulsification of the formulations due to instantaneous dispersion in the medium after dissolution of the capsule shell. As the amount of free energy required to form an emulsion is very low, this results in the spontaneous formation of an oil-water interface. This increases the water penetration of lipid droplets, resulting in disruption of the interface, decreasing the particle size and eventually increasing the release rate. From the relationship of formulation composition factors and the particle size, higher drug release rate correlates with a particle size that gives a larger surface area and subsequent water penetration. [13]

Kinetic Analysis of Candesartan Release Data

Drug release data for all SLN formulations and the marketed formulation was fitted into various kinetic equations to determine the order and mechanism of drug release (Table 4).

In vitro drug release order kinetics for optimized (CD10) formulation

From the results of Table 4, it was apparent that the regression coefficient value closer to unity in case of zero order plot

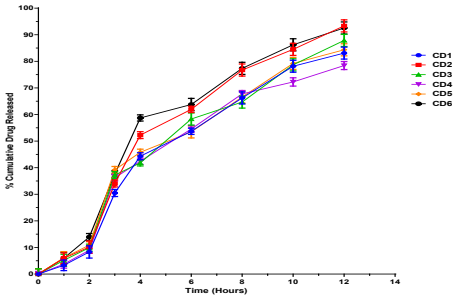

Fig. 1: *In vitro* drug released profile of prepared candesartan loaded solid lipid nanoparticles CD1-CD6

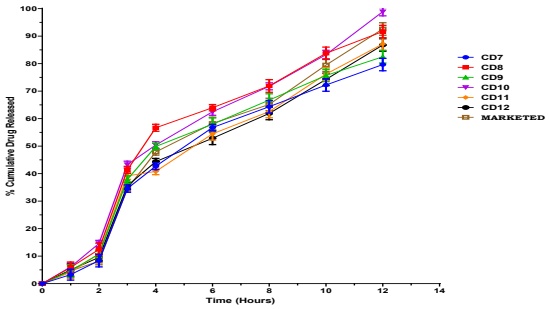

Fig. 2: *In vitro* drug released profile of prepared candesartan loaded solid lipid nanoparticles CD7-CD12

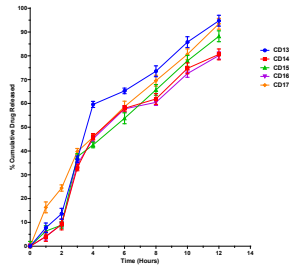

Fig. 3: *In vitro* drug released profile of prepared candesartan loaded solid lipid nanoparticles CD13-CD17

Table 4: Kinetic data of candesartan SLNs

S.No	*Formulation*	*Zero order (R^2)*	*First order (R^2)*	*Higuchi (R^2)*	*Korsmeyer-peppas (R^2)*	*Korsmeyer-peppas (n)*
1	CD1	0.915	0.792	0.758	0.881	1.050
2	CD2	0.841	0.743	0.812	0.865	1.018
3	CD3	0.835	0.838	0.744	0.913	1.041
4	CD4	0.928	0.792	0.739	0.823	1.036
5	CD5	0.913	0.783	0.803	0.811	1.029
6	CD5	0.882	0.763	0.811	0.879	1.040
7	CD6	0.830	0.743	0.728	0.804	1.022
8	CD7	0.916	0.813	0.786	0.865	1.041
9	CD8	0.908	0.764	0.821	0.889	1.028
10	CD10	0.935	0.865	0.843	0.822	1.062
11	CD11	0.912	0.811	0.817	0.922	1.038
12	CD12	0.896	0.823	0.733	0.859	1.013
13	CD13	0.931	0.804	0.749	0.886	1.025
14	CD14	0.886	0.822	0.826	0.875	1.034
15	CD15	0.911	0.856	0.789	0.913	1.017
16	CD16	0.837	0.779	0.815	0.883	1.028
17	CD17	0.875	0.796	0.822	0.871	1.020
18	Marketed	0.865	0.935	0.843	0.822	2.062

i.e., 0.952 for optimized SLN formulation (CD10), indicated that the drug release follows a zero-order mechanism. This data indicated lesser linearity when plotted by the first order equation. For marketed formulation the regression coefficient value closer to unity in the case of first order plot 0.935 indicated that the drug release followed a first-order mechanism.

Further the n value obtained from the Korsmeyer-Peppas i.e., 0.934 for optimized SLN formulation CD10 indicating non-Fickian (anomalous) transport thus, it is projected that it delivered its active ingredient by coupled diffusion and erosion.

Design of Experiment

About 17 experiments were performed according to experimental runs generated by BBD. All responses fitted into second order quadratic equations and the competence of model validated by analysis of variance (ANOVA) tests provided by Design- Expert software (Tables 5 to 8).

Response Surface Analysis

Stat-Ease Design Expert® software V8.0 was utilized to analyze data, get regression equation, regression coefficient and ANOVA.

Particle Size

The particle size of the nanoparticles was found to be in the range of 135 to 223 nm. The quadratic model generated revealed that the amount of precirol, amount of poloxamer 188 and amount of soy lecithin significantly influences the particle size (Table 6). The theoretical (predicted) and observed values were in reasonably good agreement. The respective contour plots are as shown in Figs. 4 and 5. The increase in the droplet size with concomitant increase in the amount of lipid (X1) or decrease in the amount of surfactant (X2) and vice versa has been reported in many papers pertaining to SLNs. This phenomenon may be explained because a higher proportion of surfactant (with simultaneously lower lipid amount) may provide

Table 5: Regression equations of the fitted models

Response	*Equation*
Particle Size (Y1)	$124.69 +84.23\ X1 - 19.48\ X2 - 13.15\ X3 -38.11X_1^2 + 23.59X_1X_3 +19.12\ X_2^2 -21.19\ X_2X_3 +15.36\ X_3^2$
Entrapment Efficiency (Y2)	$75.85+13.37X1+6.15X2+1.89X3+0.54X_1^2 -2.74X_1X_3-09.55\ X_2^2 -2.50\ X_2X_3 -3.44\ X_3^2$
% Cumulative drug released (Y3)	$78.67-4.52X1 +16.77X2-14.39X3+1.82X_1^2 -13.91X_1X_3+4.14\ X_2^2 -24.15\ X_2X_3 +3.53\ X_3^2$

Where Y_1, Y_2 and Y_3 are the predicted response and X_1, X_2 and X_3 are thecoded values of the test variables in respective concentrations.

Table 6: ANOVA of the quadratic model for the response particle size (Y1)

Source of variations	*Sum of squares*	*Degree of freedom*	*Mean squares*	*F-value*	*p-value Prob > F*	R^2
Model	2765.27	6	460.87	0.0185	< 0.05	
A-Amount of Precirol	88.12	1	88.12	0.0293	< 0.05	
B-Amount of Poloxamer 188	751.06	1	751.06	0.0345	< 0.05	
C-Amount of Soy lecithin	12.53	1	12.53	0.0256	< 0.05	
AB	2245.12	1	2245.12	0.0385	< 0.05	0.9995
AC	1993.45	1	1993.45	0.0147	< 0.05	
AB	1.16	1	1.16	0.0340	< 0.05	
Residual	3436.23	6	572.66			
Lack of Fit	3874.62	6	645.89	0.0263	< 0.05	

Table 7: ANOVA of the quadratic model for the response entrapment efficiency (%) (Y2)

Source of variations	*Sum of squares*	*Degree of freedom*	*Mean squares*	*F-value*	*p-value Prob > F*	R^2
Model	1287.88	6	214.56	0.0126	< 0.05	
A-Amount of Precirol	19.80	1	19.80	0.0187	< 0.05	
B-Amount of Poloxamer 188	62.31	1	62.31	0.0338	< 0.05	
C-Amount of Soy lecithin	35.12	1	35.12	0.0156	< 0.05	
AB	160.84	1	160.84	0.0256	< 0.05	0.9993
AC	180.10	1	180.10	0.0347	< 0.05	
AB	311.80	1	311.80	0.0240	< 0.05	
Residual	447.24	9	49.63			
Lack of Fit	253.17	6	42.16	0.0173	< 0.05	

closely packed interfacial surfactant film, thereby stabilizing the lipid droplets. This may also explain the significant interaction between lipid and surfactant.

Entrapment efficiency (%)

The entrapment efficiency (%) of the SLNs was in the range of 72.8% to 86.4%. The quadratic model generated revealed that the amount of precirol and poloxamer 188 have a significant influence on the entrapment efficiency (%) (Table 7). As seen, the theoretical (predicted) values and the observed values were in reasonably good agreement. The respective contour plots are as shown in Figs. 6 and 7.

Cumulative Percent Drug Release

The cumulative percent drug release in 12 hours from the SLNs was found to be in the range of 74.49 to 98.91%. The quadratic model generated revealed that the amount of precirol, poloxamer 188, and Soy lecithin significantly influences particle size. The theoretical (predicted) values and the observed values were in reasonably good agreement, as seen. The mathematical model generated for percent drug released in 12 hours (Y3) was significant with F-value of 0.0418, implying the model is significant (Table 8).

The respective contour plots are as shown in Figs. 8 and 9. The amount of surfactant was mainly responsible for the cumulative percentage of drugs released from the formulation. The increase in cumulative drug release was mainly attributed to rapid self-emulsification of the formulations due to instantaneous dispersion in the medium after dissolution of the capsule shell. As the amount of free energy required to form an emulsion is

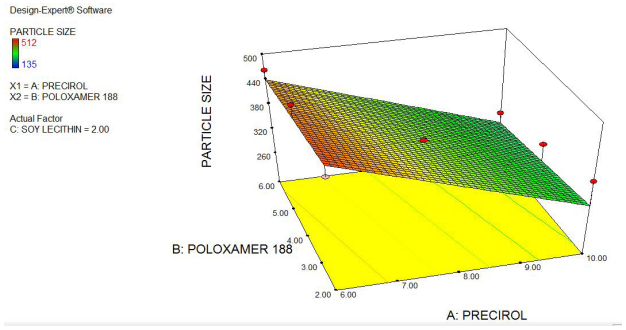

Fig. 4: Response 3D surface plot showing the influence of amount of Precirol and amount of Poloxamer 188 on particle size fixed level of C

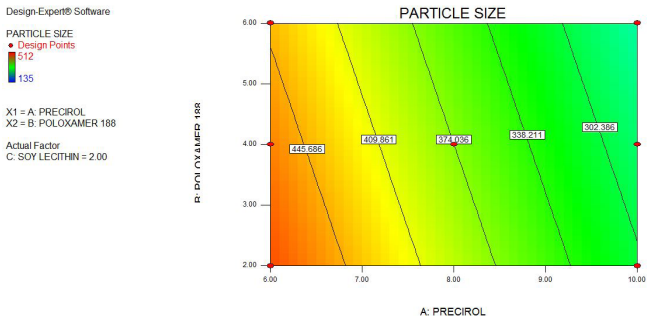

Fig.5: Contour plot showing the influence of amount of precirol and amount of poloxamer 188 on particle size fixed level of C

Table 8L: ANOVA of the quadratic model for the response Cumulative percent drug released (Y3)

Source of variations	*Sum of squares*	*Degree of freedom*	*Mean squares*	*F-value*	*p-value Prob > F*	R^2
Model	1439.07	6	239.83	0.0418	< 0.05	0.9998
A-Amount of Precirol	30.77	1	30.77	0.0167	< 0.05	
B-Amount of Poloxamer 188	11.45	1	11.45	0.0298	< 0.05	
C-Amount of Soy lecithin	89.17	1	89.17	0.0395	< 0.05	
AB	41.38	1	41.38	0.0143	< 0.05	
AC	78.57	1	78.57	0.0139	< 0.05	
AB		1	63.70	0.0261	< 0.05	
Residual		9	100.22			
Lack of Fit		6	111.16	0.0356	< 0.05	

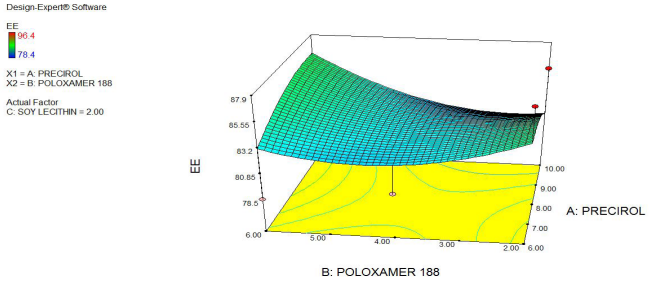

Fig. 6: Response 3D surface plot showing the influence of amount of precirol and amount of poloxamer 188 on entrapment efficiency (%) fixed level of C

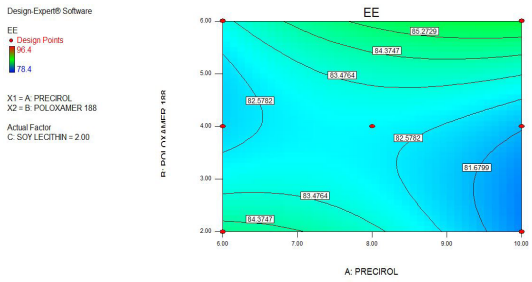

Fig. 7: Contour plot showing the influence of amount of Precirol and amount of Poloxamer 188 on Entrapment Efficiency (%) fixed level of C

Table 9: Optimized values obtained by the constraints applies on Y1, Y2 andY3

		Predicted values						
Independent variable	*Nominal values %*	*Particle size (nm) (Y1)*	*Entrapment efficiency (%) (Y2)*	*%CDR (Y3)*	*Batch*	*Particle size) (nm) (Y1*	*Entrapment efficiency (%) (Y2)*	*Percent drug release in 12 hours (Y3)*
Precirol (X1)	10	135	96.4	98.91	1	137	96.2	97.67
Poloxamer 188 (X2)	06				2	138	96.0	98.24
Soy lecithin (X3)	02				3	136	96.5	98.18

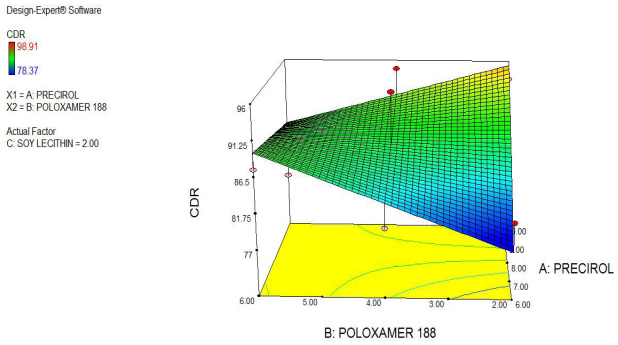

Fig. 8: Response 3D surface plot showing the influence of amount of precirol and amount of poloxamer 188 on cumulative % drug released fixed level of C

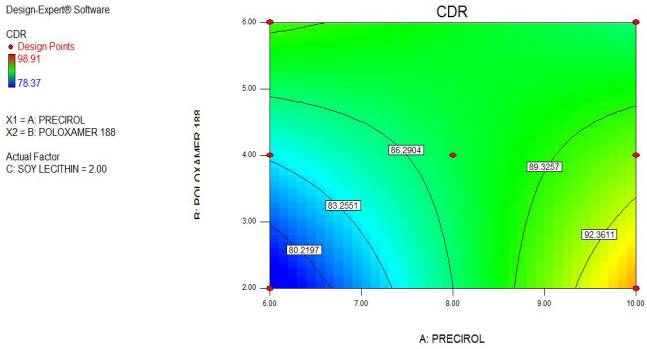

Fig. 9: Contour plot showing the influence of amount of precirol and amount of poloxamer 188 on cumulative % drug released fixed level of C

Table 10: Stability studies of optimized formulation

Retest time for optimized formulation (CD10)	*Particle size (nm)*	*Entrapment efficiency (%)*	*In-vitro drug release profile (%)*	*Drug content (%)*
0 days	135.38 ± 3.41	86.4 ± 1.17	99.91 ± 1.06	99.78 ± 2.3
30 days	135.38 ± 2.74	96.4 ± 1.09	99.84 ± 1.23	99.68 ± 2.9
60 days	136.10 ± 3.13	96.4 ± 1.15	98.72 ± 1.15	99.56 ± 2.7
120 days	136.18 ± 2.55	96.3 ± 1.11	97.92 ± 1.20	99.54 ± 1.4
180 days	136.23 ± 2.78	96.2 ± 1.05	96.68 ± 1.13	99.54 ± 1.6

Values are expressed in mean ± SD :(n=3)

very low, this results in the spontaneous formation of a lipid–water interface. This increases the water penetration of lipid droplets, resulting in disruption of the interface, decreasing the particle size and eventually increasing the release rate. It was also seen that the addition of the co-surfactant further improved the cumulative percentage of drug released. This phenomenon might be due to the penetration of the co-surfactant into the surfactant monolayer interface, which further enhances the self-emulsification performance of SLNs.

Optimization by Desirability Function

An optimization process was undertaken with desirability function to optimize the three responses simultaneously. The responses: particle size (Y1), entrapment efficiency (%) (Y2), and cumulative percentage of drug released in 12 hours (Y3) were transformed into the desirability scale, respectively. Y1 and Y2 had to be minimized, while Y3 had to be maximized. For individual desirability function, Y_{max} and Y_{min} were taken as the highest objective function (D) was calculated by Equations for each response. Finally, the global desirability value was calculated by combining the individual desirability function as the geometric mean by an extensive grid search and feasibility search over the domain by the Design-Expert software. The maximum function value was obtained at X1:10, X2:06 and X3:02. To confirm the model adequacy for prediction, three batches of formulations under the optimum composition were prepared, and the three responses were evaluated for each formulation. The results are s hown in Table 9. The model was validated since a fine agreement existed between the predicted and observed results. It can be seen that the experimental values were in very close agreement with the predicted values, indicating the success of the Box–Behnken design combined with a desirability function for the evaluation and optimization of SLNs formulations.

Characterization of Candesartan SLN Optimized Formulation

FTIR Studies

The FTIR spectrum of pure candesartan (Fig. 10) exhibited N-H stretching vibrations at 3726.6, 3689.95 cm^{-1}, the C-N stretching modes were assigned at 1388.79 cm^{-1}, phenyl CH stretching vibrations occur above 3000 cm^{-1} and are typically exhibited as multiplicity of weak to moderate bands compared with the aliphatic CH stretching. For the candesartan pure drug compound, this calculation gave CH stretching vibrations of the phenyl rings at 3246.31 and 3122.86 cm^{-1}. The aliphatic CH stretching vibrations were at 3101.64, 3066.92, and 3032.2 cm^{-1}. Fig. 11 presented similar prominent peaks in optimized formulation same as that of pure drug and these results indicated the absence of any chemical interactions between the drug candesartan and used excipients in the formulation.

Particle Size

The particle size of the optimized SLN formulation of candesartan (CD10) was 135.38 nm as shown in Fig. 12A.

Zeta Potential

The zeta potential of the optimized SLN formulation of candesartan (CD10) is -18.16mV as shown in Fig. 12B.

SEM studies

SEM photographs for optimized formulation of candesartan CD10 are shown in Figs. 13A–B. Spherical particles were observed with drug particles incorporated in lipid matrix. The surface of the drug appeared to be porous in

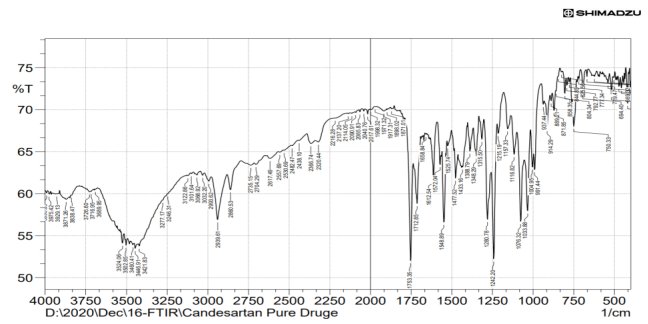

Fig. 10: FTIR spectrum of pure drug candesartan

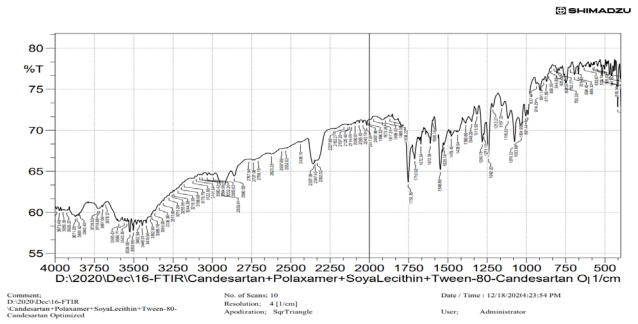

Fig. 11 : FTIR spectrum of candesartan optimized formulation (CD10)

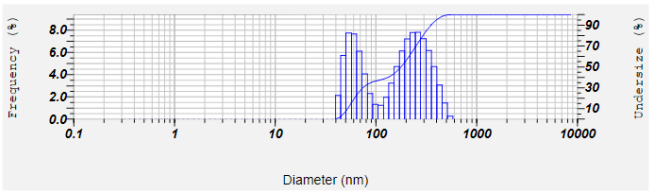

Fig. 12A: Particle size of optimized solid lipid nanoparticles of candesartan (CD10) formulation

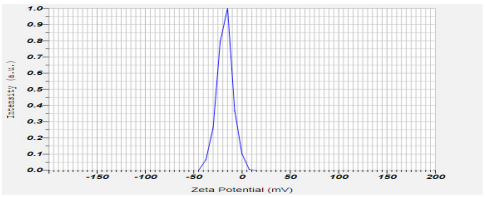

Fig. 12B: Zeta potential of optimized solid lipid nanoparticles of Candesartan (CD10) formulation

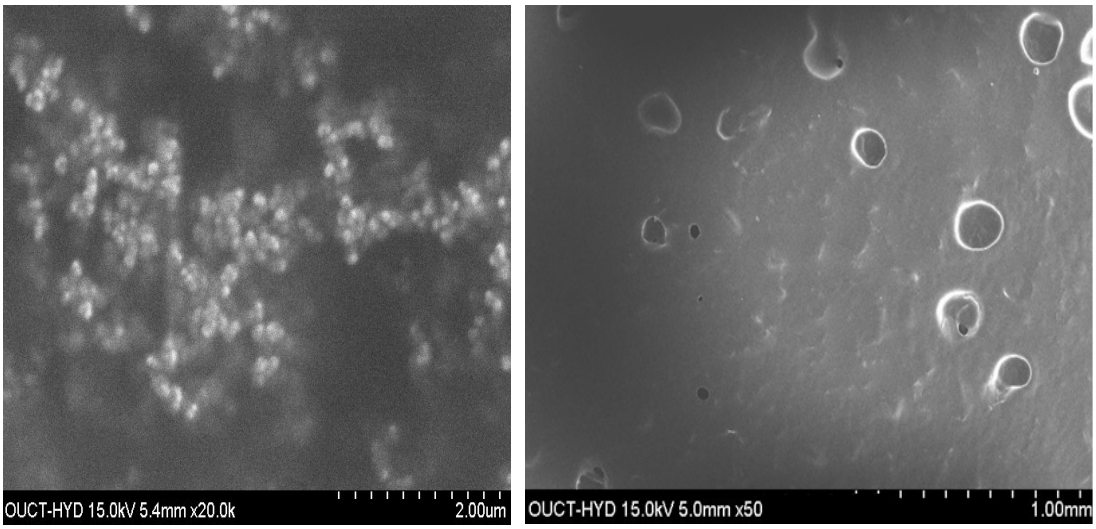

Fig. 13 A & B: SEM images of solid lipid nanoparticles of candesartan (CD10)

nature in candesartan optimized formulation solid lipid nanoparticles (CD10). The results could be attributed to dispersion of the drug in the polymer's molten mass, which leads to the sustained release of the drug upto 12 hours.

Stability Studies

Optimized formulation CD10 was loaded for stability studies for 6months as per ICH guidelines and formulation was found to be stable. There was no significant change in particle size, entrapment efficiency, *in-vitro* release and drug content observed at 40°C ± 2°C/75% RH ± 5% and at 25°C ± 2°C/60% RH ± 5% the values are shown in Table 10.

Conclusion

The present work prepared the SLNs containing candesartan to employ the modified emulsification/ ultrasonication method. A BBD evaluated the influence of independent variables on the particle size, PDI, and zeta potential. Subsequently, the formulation parameters were statistically optimized. Out of all formulations the mean particle size, PDI, zeta potential, entrapment efficiency (EE), content uniformity and in-vitro drug release profile of optimized candesartan-loaded SLNs (CD10) were found to be 135.38 ± 3.41nm, 0.125 ± 0.04, -18.16 ± 2.89mV, 86.4 ± 2.35%, 99.78 ± 2.54% and 98.91 ± 0.85%, respectively. The release kinetics suggested that drug release followed zero order and release from optimized formulation was anomalous non-fickian diffusion super case II transport. FTIR studies revealed no incompatibility between drug and polymers found, SEM images exhibited nanoparticles to be more porous and in spherical shape. The formulation was stable for 6 months. The physicochemical characteristics of the designed formulation revealed that SLNs could be regarded as an appropriate colloidal carrier system, because they showed small particle size with a spherical shape, narrow size distribution, suitable zeta potential, and other desirable physicochemical properties, including high values for EE% and drug content. The solid lipid nanoparticles exhibited a prolonged release of candesartan.

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