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FORMULATION AND EVALUATION OF CANDESARTAN CILEXETIL LOADED SOLID LIPID NANOPARTICLES WITH IMPROVED BIOAVAILABILITY

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Keywords:

Entrapment efficiency, % Loading, Particle size, Solid lipid nanoparticles, Zeta potential

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ABSTRACT: In this present work, BCS class II Candesartan celexitil, as an angiotensin receptor blocker used mainly for the treatment of high blood pressure and congestive heart failure, was successfully loaded in the solid lipid nanoparticles (SLNs) by using hot high-speed homogenization method. The SLN formulations were prepared and optimized by Box Benhken design study. The SLN was characterized by Nanophox size analyzer and Delsa nano C zetameter. The SLN was also evaluated for particle size, zeta potential, entrapment efficiency, drug loading, surface morphology, and *in-vitro* dissolution study. The average particle size, zeta potential, percentage entrapment efficiency, and drug loading were found to be 177nm, -8.23 mV, 95.72 %, and 8.23 %, respectively. The dissolution study of SLN showed 92.07 % released in six hours compared to 46.89% release from the pure drug, which indicates a significant improvement in the bioavailability. The release of the drug from SLN showed zero-order kinetics. The stability study was also carried out and found to be stable after three months.

INTRODUCTION: Candesartan cilexetil is mostly used for the treatment of heart failure and hypertension. It is an ester prodrug of candesartan, a selective AT_1 subtype angiotensin receptor antagonist. After oral administration, it is completely bio-activated by ester hydrolysis in the gastrointestinal tract. However, it shows very low aqueous solubility and first-pass metabolism within the physiological pH range, which leads to incomplete intestinal absorption [1]. Candesartan cilexetil comes under the BCS II class drug (*i.e.*, low solubility and high permeability). It may be used alone or in combination with other antihypertensive agents.

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CC is effective and safe under the dosages of 4 mg to 32 mg [2] and can be administrated once or twice daily with a total dosage range. The drug's half-life is 5.1 h, and the absolute bioavailability is 15% for tablet dosage form; it can be increased in case of suspension up to 40 % [3]. Solid lipid nanoparticle is the most popular and alternative drug delivery system of traditional polymeric nanoparticles to improve oral bioavailability of poorly water-soluble drug SLN are sub-micron colloidal carriers having size (50-1000nm), and are composed of physiological lipid.

SLN has properties such as large surface area, small particle size, vary high drug loading capacity, SLN as an alternative system can enhance the solution and permeability of lipophilic drugs; they may increase the drug absorption [4]. Therefore, the main objective of the present work was to formulate stable CC-SLN with improved bioavailability.

MATERIALS AND METHODS:

Materials: Candesartan cilexetil was obtained as a gift sample in India. Gelucire 50/13, Labrasol, was a gift sample from Gattefose, Mumbai. Tween 80, Stearic acid, Glyceryl monostearate, Tween 20 were purchased from SD fine chemicals, Pune, India

Preparation of CC-SLNs: CC-SLN were formulated using gelucire 50/13 as solid lipid and Tween 80 as a surfactant, lipid was melted at 60℃, and an aqueous phase containing surfactant was heated at the same temperature, the drug is then added to molten lipid phase, the aqueous phase was then added slowly to lipid phase and continuously stirring carried out. This pre-emulsion was homogenized at 6000 RPM for 5 min, and temperature at 70 ℃ was maintained to a homogenized mixture; then CC loaded SLN was cooled down by keeping it at room temperature [5].

Physicochemical Characterization of CC-SLNs: The morphology of CC – SLN was examined using scanning electron microscopy (SEM, JEOL, 5400, Japan), and the sample was coated by gold ion and the coating was performed for 5-6 min and the sample was analyzed at 1,000 and 2,500 X^3. The particle size of CC- SLN's was measured by photon cross-correlation spectroscopy (PCS) using a particle size analyzer (Nanophox, Symphtech, Germany). The particle size analysis data were evaluated using the polydispersity index. Zeta potential was analyzed by zetameter, and values were calculated using Smoluchowski equation [6]. Differential Scanning Calorimetry (DSC 1, Star^e System, Metter Toledo) was used for a thermogram of pure Candesartan Cilexetil indium as reference material sample (3mg) was placed in an aluminum pan, and the pan was sealed by using a crimper. The heating rate of the sample and reference material was 10 ℃/min from room to 250 ℃ [6].

Drug Entrapment Efficacy and Drug Loading: Entrapment efficacy of Candesartan cilexetil was subjected to check the amount of drug entrapped in the lipid. Optimized SLN formulation was evaluated by adding 1ml of SLN in 10 ml methanol, and further, it was centrifuged at 10,000 rpm for 25min. The supernatant was segregated and filtered by Whatman filter paper dilutions were prepared using methanol. Dilutions were analyzed for % entrapment efficacy by using UV-Visible analysis at 254.4 nm. The %EE and drug loading of Candesartan cilexetil in the SLN form were calculated using equation [7].

$$\% EE = W_{\text{initial drug}} - W_{\text{free drug}} / W_{\text{inital drug}} \times 100$$

$$\% DL = \text{Total Drug} - \text{Free Drug} / \text{Total Lipid} \times 100$$

Formulation Optimization: Box- Behnken Statistical design with 3-factor, 3-level, and 17 runs were employed for the optimization study using Design-Expert software (Design Expert, version 11, Stat- Ease Inc., and Minneapolis, USA). The Box-Behnken design explains the main effect interactions effects of the independent variables on the formulation characteristics. The present study's objective function was selected to maximize entrapment efficiency and drug loading while minimizing particle size. The box-Behnken design was specifically selected because it requires a small number of runs than the central composite design, in the case of three or four variables [8-9]. The design was applied to study the effect of concentration of Gelucire 50/13, the concentration of Tween 80, and homogenization time on the formulation. The amount (mg) lipid phase, Gelucire 50/13(A) and the amount (ml) of surfactant, Tween 80 (B), Homogenization time (min) (C) were selected as independent variables in this study. These three factors were evaluated at 3 levels as lower, middle, and higher levels with coding -1, 0, and +1, respectively. Levels of A were selected as 150 mg, 250 mg, 350 mg, for B levels selected were 0.25 ml, 0.50 ml and 0.75ml, for C levels time was selected at 5, 10 & 15 (min). The dependent or response variables included particle size (R_1), % entrapment efficiency (R_2), and % Drug loading (R_3).

TABLE 1: THE LEVELS OF EXPERIMENTAL FACTORS

Factor	Level		
	-1	0	+1
A: Solid Lipid (Gelucire 50/13) (mg)	150	250	350
B: Surfactant (Tween 80) (ml)	0.25	0.50	0.75
C: Homogenization time (min)	5	10	15

***In-vitro* Drug Release Studies:** Candesartan cilexetil release study from optimized SLNs formulation was carried out using Franz Diffusion Cell. Freshly prepared 6.8 pH phosphate buffer was placed in receptor compartment at 34 ℃±0.5 ℃ for

6 h medium was continuously stirred at 400 rpm. The sample was withdrawn at the predetermined time interval of 6hrs and replaced with the same volume, then estimated by UV Spectroscopy method [9]. The same method was used to study the release from the pure drug.

Release Kinetics Study: Optimized batch was evaluated to study the release mechanism of the formulation. The formulation was essential to check drug release for different kinetics such as; Zero-order, First-order, Higuchi, Hixon Crowell cube root, and Korsmeyer-Peppas models, and the best fit model was selected [10].

Accelerated Stability Study as Per ICH Guidelines: Stability study was performed as per ICH guidelines Q1A (R2) for the optimized batch to determine the effect of the presence of formulation additives on the stability of the drug and to determine the physical stability of the formulation under accelerated storage conditions. The optimized batch was subjected to temperature and humidity conditions of 40±1°C/ 75% RH. Samples were withdrawn at the end of 0, 30, 60, and 90 days and evaluated for particle size, % entrapment efficiency, active drug content, and appearance [12].

RESULTS AND DISCUSSION:

Physicochemical characterization of SLNs: SEM images for candesartan cilexetil loaded SLN show the spherical shape of SLN. This indicates SLNs prepared by the high-speed homogenization method were monodispersed. Images show that candesartan cilexetil loaded SLN is in the nanosized range. The particle size of CC- SLNs was found to be 177.12 nm, along with polydispersity index of 0.2922. Zeta potential was negative for Candesartan Cilexetil loaded solid lipid nanoparticles due to the ester group of gelucire 50/13 and insufficiency of counterions for neutralization within the electrically double diffuse layer.

Tween 80 provides additional stearical stabilization to the nanoparticles. Candesartan Cilexetil loaded solid lipid nanoparticles have the potential of physical stability as they have a zeta potential of -8.13 mV. DSC studied the thermal behaviour of Candesartan Cilexetil. The characteristic endothermic peak of the pure drug was observed at 175.76℃, corresponding to its melting point, which indicated the purity of the sample. Thermograms of CC-SLN exhibited an endothermic peak at 107 ℃. Hence, it could be concluded that the drug was entrapped into solid lipid.

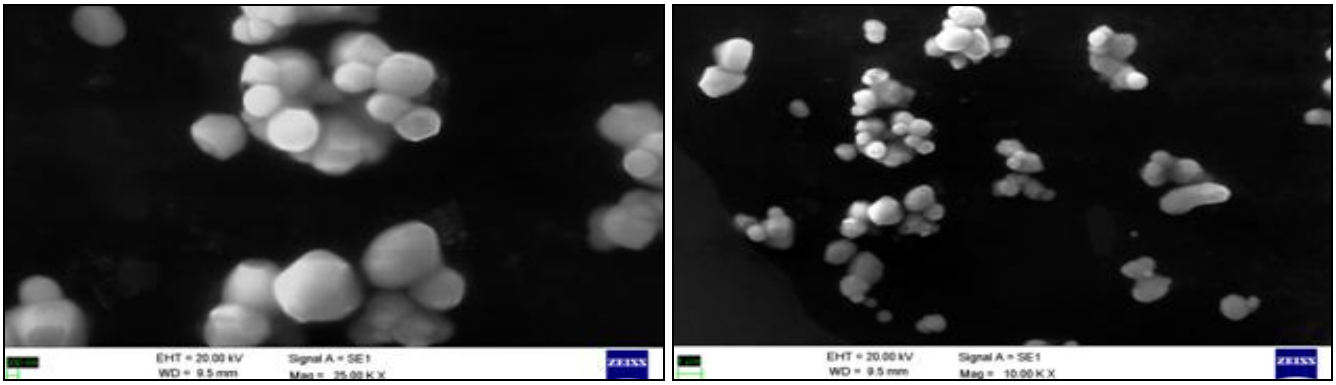

FIG. 1: SCANNING ELECTRON MICROSCOPY OF CC-SLN AT 1000 AND 2500 X

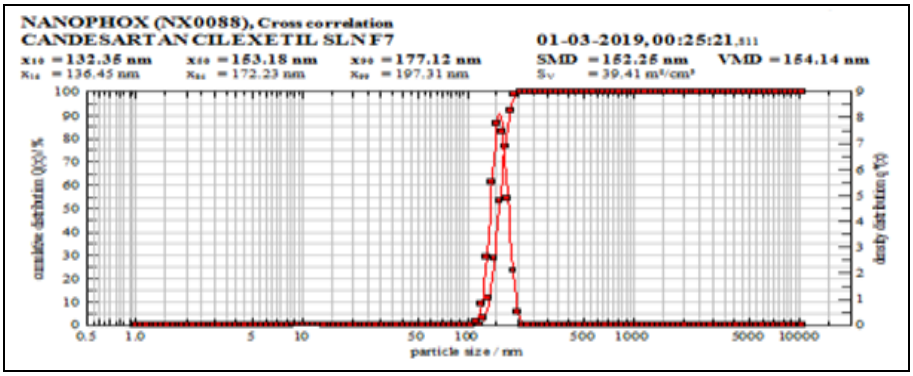

FIG. 2: PARTICLE SIZE OF CC-SLN

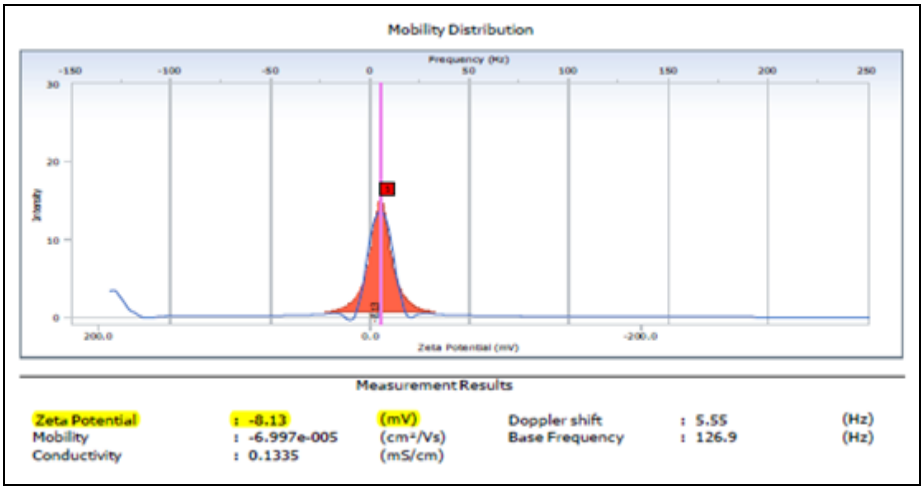

FIG. 3: ZETA POTENTIAL OF CC-SLN

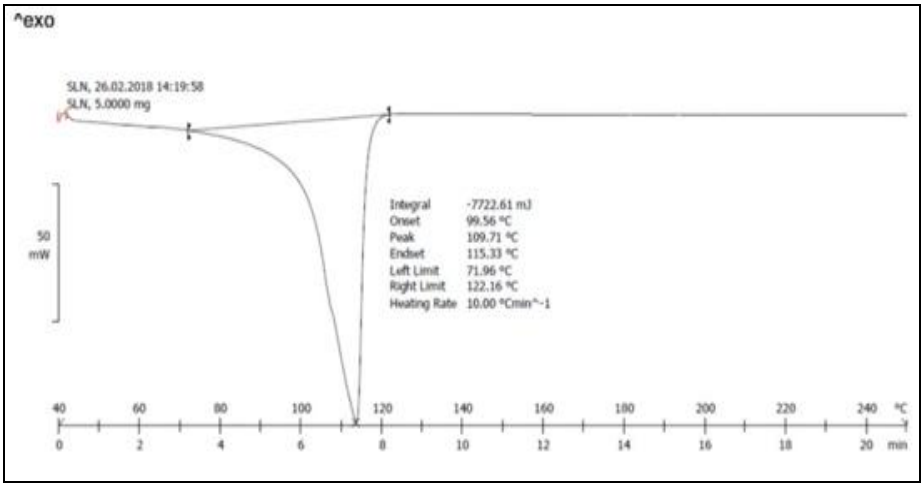

FIG. 4: DSC THERMOGRAM OF CC-SLN

Drug Entrapment Efficiency and Drug Loading: The experimental data showed that CC-SLNs have high drug entrapment efficiency of 95.72 % and drug loading of 8.23%. As a result, it was evident that SLNs prepared by the hot homogenization method could achieve encapsulation.

Formulation Optimization: Design expert software (Design Expert 11) was used for the optimization of the Batch no.7. The optimized CC-SLN was composed of Gelucire 50/13 (solid lipid), Tween 80 (surfactant). The CC-SLN was optimized by a three factor-three level Box-Benhken Design,17 batches were run, and the results are suggested by the software such as particle size,% entrapped efficacy, and % drug loading is given [13]. The particle size (R_1), % entrapped efficacy (R_2), and % drug loading (R_3) were found to be in the range of 177.12nm, 95.72%, and 8.23%, respectively. The p-value is an analysis of variance (ANOVA) was found less than 0.05% and f value obtained was within the limit, that was significant for in case of the quadratic model, such that every model terms was significant; hence factor (A, B and C) were found significant and responses R_1, R_2 and R_3 are shown in eqation -

$$\text{Particle Size } (R_1) = +9.44 - 149.44A + 128.90B - 249.83AC + 52.94BC + 266.37A^2 + 0.3487B^2 + 237.42C^2$$

$$\%\text{ Entrapment Efficiency } (R_2) = +94.16 + 0.9788A + 0.4625B - 0.7963C - 1.68AB - 1.12AC + 3.71BC + 0.4600A^2 - 2.37B^2 - 2.33C^2$$

$$\%\text{ Drug Loading } (R3) = +4.76 + 0.3063A + 0.0438B - 0.2750C - 0.1000AB - 0.5375AC + 0.1475BC + 2.22A^2 - 0.8175B^2 - 0.3700C^2$$

The optimised formulation F7 showed maximum % entrapment efficiency, % drug loading as well as desired particle size. The optimized SLN formulation consist of Drug (10mg), solid lipid (350mg), Surfactant (500mg) and water (10 ml). The Entrapment represent the predicted Regression Cofficient R^2 (-0.2413) value that was close to adjusted R^2 (0.8227).

Particle size reflected the predicted R^2 (-0.9636) and adjusted R^2 (0.7195). % Drug loading there predicted R^2 (-0.5432) and the adjusted R^2 (0.7795). Hence, the results obtained from an experimental method were accurate and reproducible for SLN formulation.

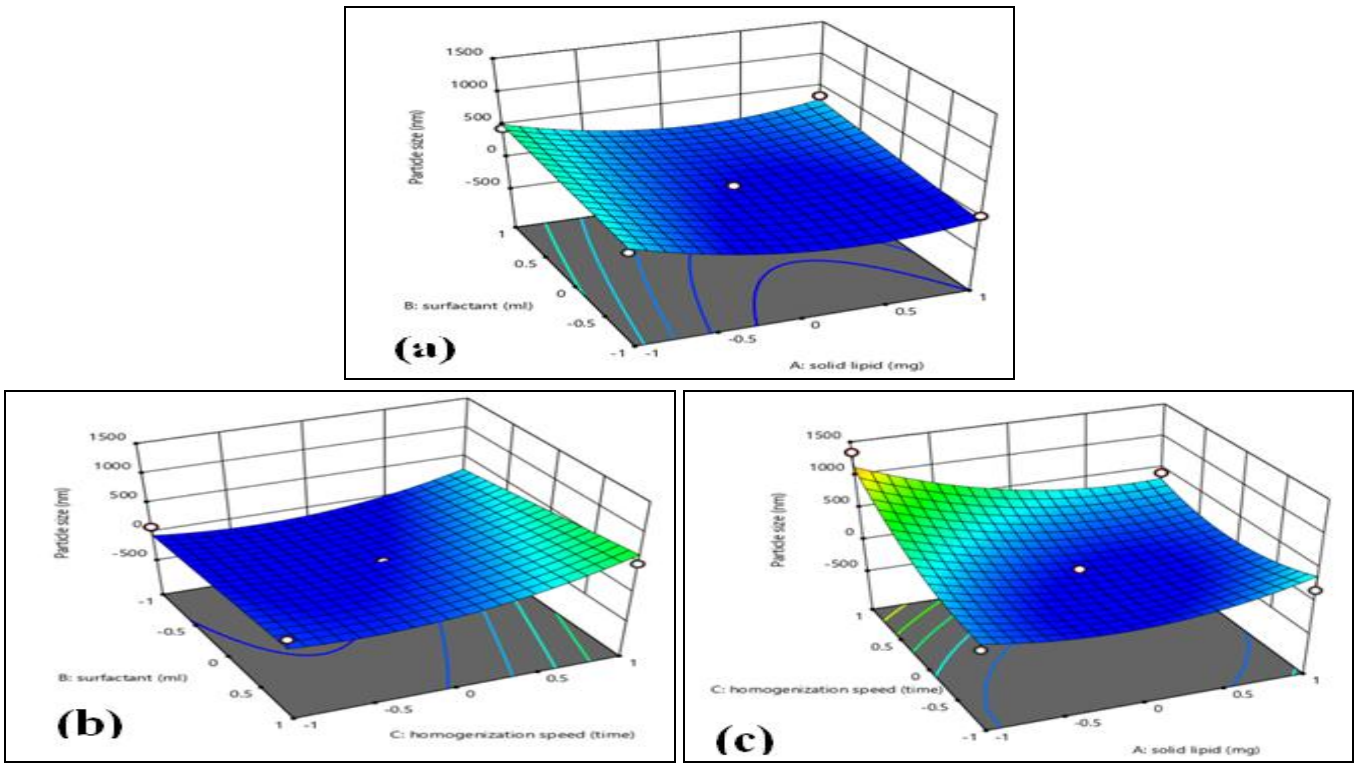

FIG. 5: 3D RESPONSE SURFACE PLOTS FOR THE EFFECT OF (A) SOLID LIPID AND SURFACTANT, (B) HOMOGENIZATION TIME AND SURFACTANT, (C) SOLID LIPID AND HOMOGENIZATION TIME ON PARTICLE SIZE

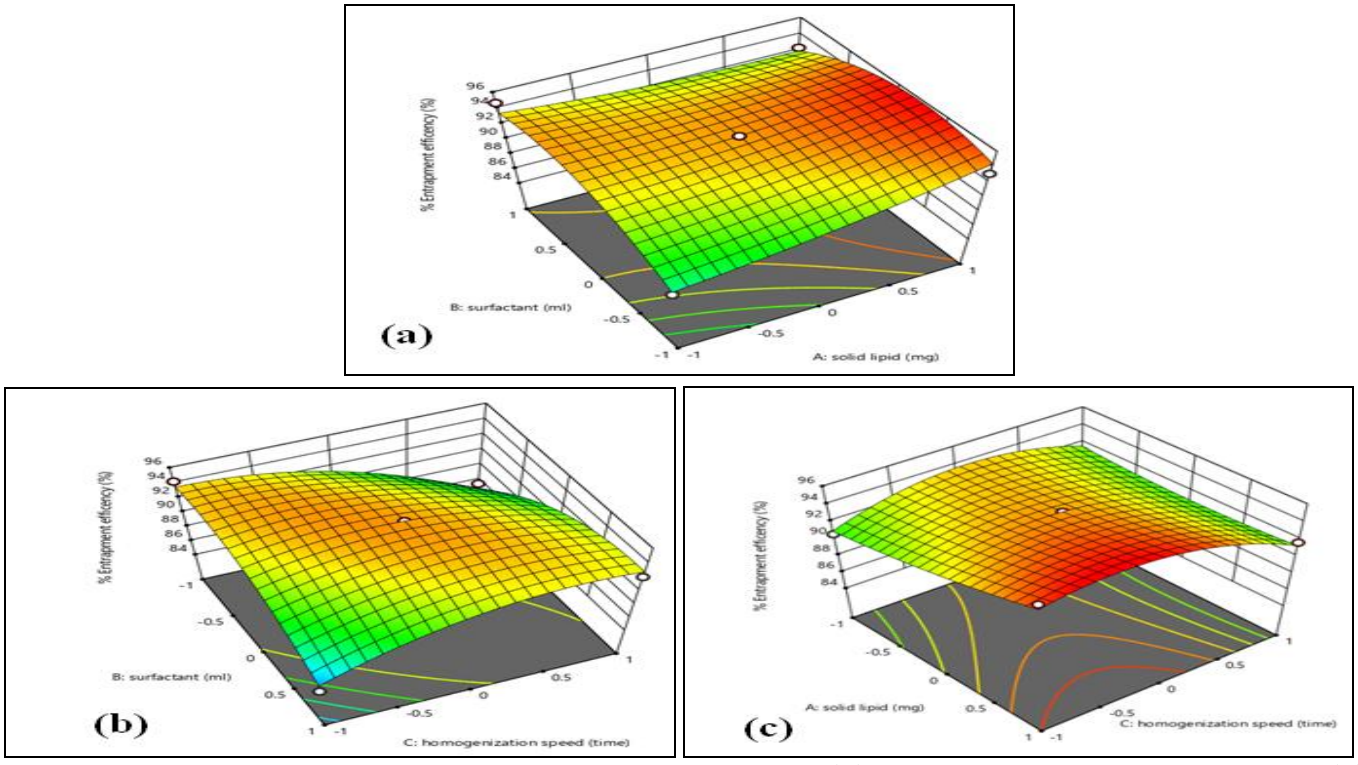

FIG. 6: 3D RESPONSE SURFACE PLOTS FOR THE EFFECT OF (A) SOLID LIPID AND SURFACTANT, (B) HOMOGENIZATION TIME AND SURFACTANT, (C) SOLID LIPID AND HOMOGENIZATION TIME ON % ENTRAPMENT EFFICIENCY

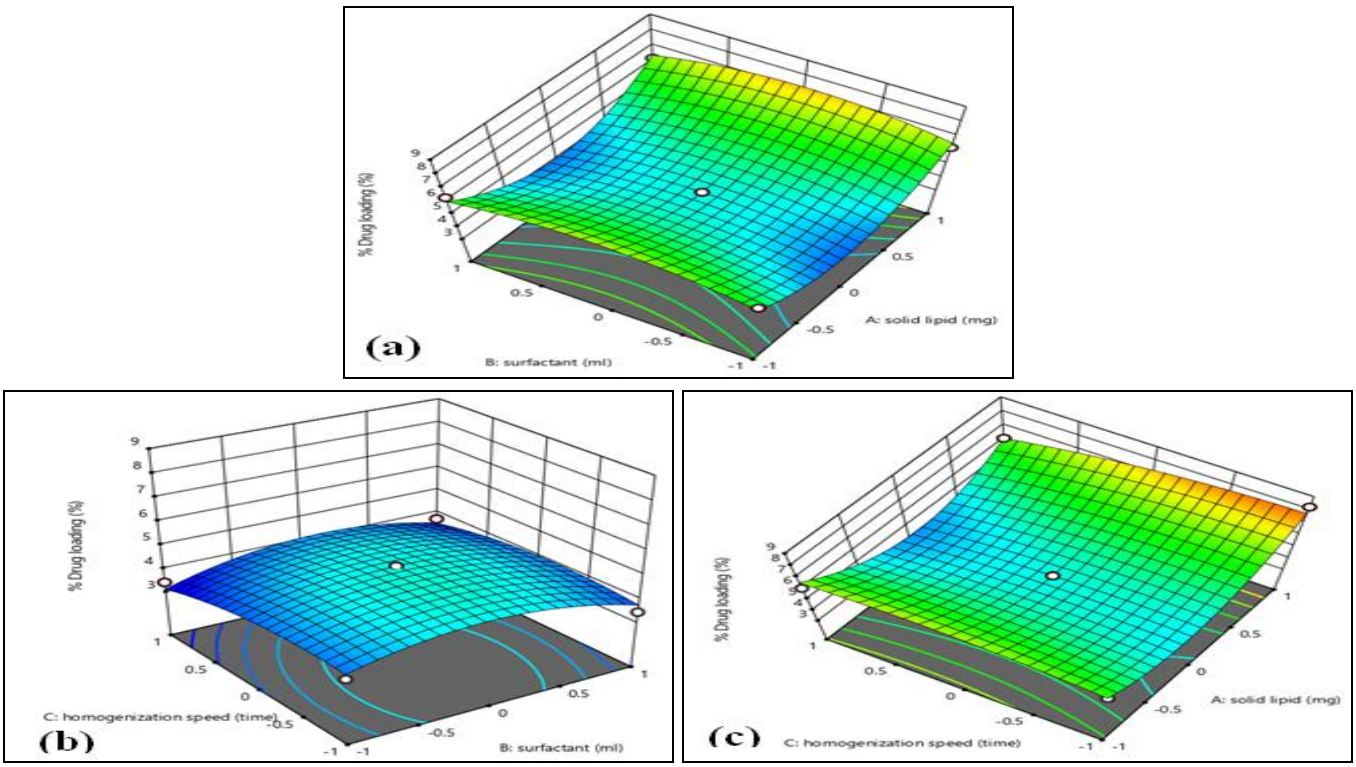

FIG. 7: 3D RESPONSE SURFACE PLOTS FOR THE EFFECT OF (A) SOLID LIPID AND SURFACTANT, (B) HOMOGENIZATION TIME AND SURFACTANT, (C) SOLID LIPID AND HOMOGENIZATION TIME ON % DRUG LOADING

***In-vitro* Drug Release Study:** Candesartan Cilexetil release from SLNs *In-vitro* drug release study for batch F7 was carried out. The results for % drug release are given in fig. *In-vitro* drug release of Candesartan Cilexetil from SLN through the dialysis membrane revealed for plain CC suspension for 6 hr with 46% and CC-SLN for 6 hr with 92.07%. Type of lipid matrix and its concentration also pronounced effect on drug release. The higher amount released from Gelucire 50/13 particles may also reflect the smaller particle size of SLNs as the mean globule particle size was well within the nanometer range.

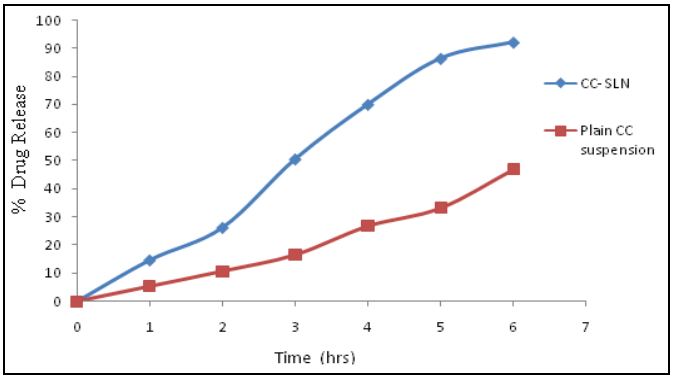

FIG. 8: % DRUG RELEASE OF CC-SLN AND PLAIN CC SUSPENSION IN 6.8 PH PHOSPHATE BUFFER AT TIME INTERVAL

TABLE 2: DATA FOR KINETIC MODELS

Model	R^2
Zero-order	0.9843
First order	0.7615
Higuchi Model	0.8959
Peppas-Korsmeye	0.7318
Hixon-Crowell Model	0.8192

Release Kinetic: The release data was fitted to different kinetic mathematical models: zero-order, which describes the release rate as independent of drug concentration.

The first order describes that the release rate depends on drug concentration.

Higuchi is based on the Fick's law of diffusion, and Korsemeyer-Peppas is based on the Quasi Fickian diffusion mechanism.

The determination coefficient (*R2*) was used as an indicator for the best fitting of the data for each model and plots are depicted.

It was evident from data that the Zero-order model was best fitted for Candesartan cilexetil release from SLN with a higher correlation coefficient (*R2*=0.9843).

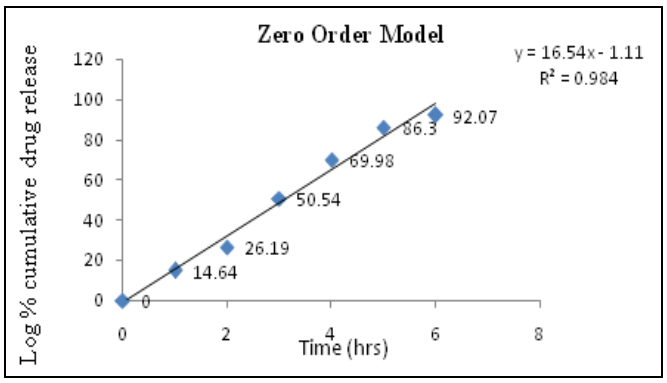

FIG. 9: ZERO-ORDER KINETICS OF DRUG RELEASE FOR OPTIMIZED BATCH

Accelerated Stability Study: Optimized batch of SLN was subjected to stability testing for 3 months in order to check the possibility of drug degradation or any possible development of instability in the formulation. The results suggest that there was no significant difference in drug content values and the formulation's physical characteristics also remained unchanged, suggesting that formulation was stable under given conditions.

CONCLUSION: Candesartan cilexetil loaded solid lipid nanoparticles were prepared by a high-speed homogenization method to enhance the oral bioavailability and aqueous solubility. The particle size of optimized CC-SLN was found to be 177.12nm. The components were found to be compatible during DSC- excipient studies.

In-vitro drug release of optimized batch F7 SLN formulation showed 92.07% at 6 h. This confirms the increase in the *in-vitro* bioavailability due to the nanosize of the SLN, which might result in improved absorption. The release date of the optimized batch was studied for various release kinetics models such as zero order, first order, Higuchi, Hixon - Crowel cube root, and Korsmeyer - Peppas model, which gave R^2 as 0.9843, 0.7615, 0.8959, 0.8192, and 0.7318, respectively.

Depending upon R^2values Zero-order model was suggested maximum with R^2 = 0.9843. Stability data of optimized batch F7 of Candesartan cilexetil loaded SLNs revealed that there were no changes observed in appearance and drug content, suggesting that Candesartan cilexetil loaded SLN is stable at 40ºC/75% RH. Thus, results reveal that the oral drug delivery system for Candesartan cilexetil can be developed further.

Optimized CC-SLN form can be evaluated further for *in-vivo* bioavailability studies.

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CONFLICTS OF INTEREST: The authors confirm that this article content has no conflicts of interest.

REFERENCES:

1. Ahmed A, Ahmed N, Allam and Ossama Y: Preparation, characterization and *ex-vivo in-vivo* assessment of candesartan cilexetil nanocrystals *via* solid dispersion technique using an alkaline esterase activator carrier. Drug Development and Industrial Pharmacy 2019; 45(7): 1140-1148
2. Kamalakkaman V, Puratchikody A and Ramanathan L: Development and characterization of controlled release polar lipid microparticles of candesartan cilexetil by solid dispersion. Research in Pharmecutical Science 2013; 2: 125-136.
3. Fahim J, Sayyed S, Laxman T and Bhupa K: Design and development of liquisolid compact of candesartan cilexetil to enhance dissolution. Journal of Pharmaceutical Research 2013; 7: 381-388.
4. Dudhipalla N and Veerabrahma K: Candesartan cilexetil loaded nanodelivery systems for improved oral bioavailability. Therapeutic Delivery 2017; 2: 79-88.
5. Fakhar D, Zeb A and Shah K: Development, *in-vitro* and *in-vivo* evaluation of ezetimibe-loaded solid lipid nanoparticles and their comparison with marketed product. Journal of drug delivery science and technology 2019; 51; 583-590.
6. Bagul U, Pisal V and Solanki N: Current status of solid lipid nanoparticles: A review. Modern Applications of Bioequivalence and Bioavailability 2018; 3(4): 1-10.
7. Gamal A: Ciprofloxacin controlled-solid lipid nanoparticles: Characterization, *in-vitro* release,and antibacterial activity assessment. BioMed research international 2017; 4: 1-9.
8. Mohd Y and Sara US: Preparation and optimization of haloperidol loaded solid lipid nanoparticles by Box Behnken design. Journal of Pharmacy Research 2013; 551-558.
9. Guney G, Kutlu H M and Genc L: Preparation and characterization of ascorbic acid loaded solid lipid nanoparticles and investigation of their apoptotic effects. Colloids and surfaces B: Biointerface 2014; 121: 270-280.
10. Dudhipalla N and Veerabrahm K: Candesartan cilexetil loaded solid lipid nanoparticle for oral delivery: characterisation, pharmacokinetic and pharmacodynamic evaluation. Drug Delivery 2016; 23: 395-404.
11. Vakilinezhad M, Tanha S, Montaseri H and Dinarvand R: Application response surface method for preparation, optimization, and characterization of nicotinamide loaded solid lipid nanoparticles. Advanced Pharmaceutical bulletin 2018; 8 (2): 245-256.

12. ICH Harmonised tripartite guideline, Stability testing of new drug substances and products Q1A (R2), current step 4 version 2003; 3.
13. Shah D, Gupta D and Shah Y: Effect of lipid and surfactant concentration on cefpodoximeproxetil solid lipid nanoparticle. European JBPS 2017; 4(9): 817-823.

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