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Optimization And Development Of Candesartan Cilexetil Loaded Solid Lipid Nanoparticle for The Treatment of Hypertension

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Abstract:
Candesartan is an angiotensin-receptor blocker (ARB) that is used to treat hypertension. However, poor aqueous solubility and poor oral bioavailability has limited therapeutic applications of Candesartan. We formulated solid lipid nanoparticles of Candesartan cilexetil by using high speed homogenization followed by probe sonication. The solid lipid nanoparticles were evaluated for particle size analysis, entrapment efficiency, zeta potential, and in vitro drug release. The resultant solid lipid nanoparticles had a mean size of 87.7nm and entrapment efficiency of 80.46 %. Zeta potential was found to be -31 mV, it shows that the formulation is stable. The SEM studies indicated smooth and spherical shape nanoparticle. The in vitro drug release study by dialysis method indicated that drug entrapped in the solid lipid nanoparticle remains entrapped at acidic pH and in phosphate buffer of pH 6.8, drug is released in a controlled manner for a prolonged period of time as compared to plain drug and marketed preparation. All these findings reinforce the fact that Candesartan solid lipid nanoparticles are promising novel delivery system.

Keywords: Solid lipid nanoparticles, candesartan, oral bioavailability, lymphatic transport

INTRODUCTION

Hypertension, which had affected more than quarter of world's adult population in 2000 i.e. nearly 1 billion and will be increased to 29% i.e. 1.56 billion by 2025[1]. The pathophysiology of hypertension is defined as a lasting elevation of blood pressure to ⩾140/90 mmHg [2]. It is a major cause for both cardiovascular and cerebrovascular morbidity and mortality [3].

Oral route is the most preferred route for drug administration due to greater ease of administration, negligible pain, high patient compliance and no needle based injuries. However, newer formulations and dosage forms are unable to deliver via oral drug delivery systems due to low drug solubility, poor GI absorption, and metabolism related issues, continuous fluctuation drug plasma levels and variability due to food effects which may compromise the conventional dosage delivery system [4, 5].

Recently, solid lipid nanoparticles (SLNs) have gained increased attention because of their unique structure and properties, such as good biocompatibility, protection for the incorporated compound against degradation, good stability, good tolerability, improved therapeutic effect, lower cytotoxicity, controlled release of drugs and best production scalability [5-8]. Solid lipid nanoparticles (SLNs) are promising carriers for oral delivery of lipophilic as well as hydrophilic drug candidates [9, 10]. SLN gives targeted delivery and enhanced oral bioavailability. When encapsulated in lipid-base vehicles poorly water soluble drugs shows enhanced bioavailability [11-13]. SLN has combine advantages of colloidal drug carrier systems such as liposomes, polymeric nanoparticles and emulsions and also avoid the drawbacks associated with respective carrier system [14, 15]. The mechanism of bioavailability enhancement by SLNs is due to adhesive properties that make them adhere to gut wall and release the drug exactly where it should be absorbed [16]. Lymphatic uptake was found to be the major absorption

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mechanism for drugs encapsulated in SLN [17]. One of the prominent advantages of SLNs prepared by high pressure homogenisation is its ability for production at industrial scale up [18]. Lipid nanoparticles fulfill essential prerequisites required for entering the market with a new formulation. These prerequisites are low cost production, clinical and large-scale production facilities, and accepted status of excipients [19].

A number of large clinical trials have recently showed that angiotensin II type 1 receptor blockers (ARBs) are equal to or more effective than conventional antihypertensive treatments in protecting against organ damage due to hypertension [20, 21].

Candesartan is a selective AT1 subtype angiotensin II receptor antagonist. Candesartan cilexetil (CC) is widely used for the treatment of hypertension and heart failure in clinical application. After oral administration it undergoes rapid ester hydrolysis to convert to the active Candesartan during absorption in the gastrointestinal (GI) tract [22, 26]. It does not affect other hormone receptors or ion channels and known to be very important in cardiovascular regulation. Moreover, CC demonstrates the highest potency among the angiotensin receptor blockers used in the treatment of hypertension. The major drawback of Candesartan cilexetil as an oral dosage form is its very low aqueous solubility and first-pass metabolism which affect the therapeutic application and efficacy. Based on its solubility and absorption characteristics, Candesartan cilexetil was classified in the Biopharmaceutics Classification System (BCS) as a class II drug. In clinical application, it shows favorable safe profiles, including no adverse effects on heart rate, cardiac output, and renal function; no impairment of cerebrovascular regulation or of glucose or lipid metabolism; and no clinically significant alterations in laboratory measurements of serum chemistry, hematology and urine analysis. In addition, CC also shows other advantages including reduction in left ventricular hypertrophy and micro-albuminuria, improvement in coronary vasomotion, and preservation or improvement in renal function. However, CC shows very poor solubility within the physiological pH range, which could result in incomplete intestinal absorption and very low systemic exposure after oral administration have an oral bioavailability of about 15%. Candesartan cilexetil is a highly lipophilic compound. Therefore, it is necessary to find a new approach to enhance the oral bioavailability of CC [20, 27- 29].

In this work we designed and developed CC-loaded SLNs (CLNs) to improve the oral bioavailability. CC-SLNs were prepared by high speed homogenization followed by probe sonication method. Optimisation of SLNs batches were carried out using Box-behnken design. Freeze dried optimised batch then evaluated by particle size, EE, zeta potential, XRD, DSC and SEM. In vitro drug release studies were performed using pure drug, marketed preparation and optimised batch of CLNs.

MATERIAL AND METHODS

Candesartan cilexetil was kindly gifted by one of the leading pharmaceutical company in India. Glyceryl monostearate (GMS), Span 20, Tween 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of analytical or high-performance liquid chromatography (HPLC) grade purchased from Loba Chemie Pvt. Ltd., Mumbai.

Preparation of SLN:

Solid lipid nanoparticles were prepared by ***high speed homogenization followed by probe sonication method*** [30]. The drug Candesartan cilexetil and lipophilic surfactant (Span 20) were added in melted solid lipid (GMS). The aqueous surfactant (Tween 80) solution was prepared separately in a beaker. Both mixtures were heated in controlled temperature water bath (at 50C higher than melting point of lipid to avoid solidification of lipid). The aqueous surfactant solution was added in lipidic mixture at same temperature. The mixture was homogenized using high speed homogenizer (Speed controller, Bio Lab) at 4000 rpm for 30 min. Then the mixture was subjected to probe sonication. The final

product obtained was cooled at room temperature to get the solid lipid nanoparticles[31, 32].

Experimental design:

To design the formulation of lipid based nanoparticles, it was essential to recognise the parameters in the formulation as these variables can affect the properties of desired formulation. Various batches of CC loaded SLNs were planned based on the Box behnken design, to study the effect of different variables on its properties. The choice of lipid was done on the basis of solubility of CC in the lipid. Aqueous phase surfactant and lipid phase surfactant were selected on the basis of stability of dispersion prepared by using different surfactants. Three factors, the drug: lipid ratio (X1), surfactant (aqueous phase) concentration (X2) and sonication time (X3) were used in the design and the responses were the average particle size (PS) (Y1) and % Entrapment Efficiency (EE) (Y2). These three factors that might affect the designed characteristic of nanoparticle formulation were varied over three levels (Table 1) and arranged according to a Box- Behnken experimental design (Table 2)[33].

Table 1: independent variables and their selected levels for formulation of solid lipid nanoparticle

Factors	Coded levels			Dependent Variables
	-1	0	+1	
Drug: Lipid ratio (X_1)	1:10	1:20	1:30	1. Particle size (Y_1) 2. Entrapment efficiency (Y_2)
Concentration of aq. surfactant (X_2) (%)	1%	5%	10%	
Sonication Time(X_3)(min)	5	10	15	

Evaluation of Solid Lipid nanoparticle (Table 3):

Particle size

The mean particle size and particle size distribution of drug loaded solid lipid nanoparticle was determined by Horiba SZ-100 nanoPartica Dynamic Light Scattering (DLS) system, at room temperature. All the samples were diluted with double distilled water to get a suitable concentration for examination and every sample was measured in triplicate. For the measurement the laser obscuration range was maintained between 1-5 %.

Entrapment Efficiency [34]

The entrapment efficiency of prepared SLN was calculated by centrifugation method. About 5 ml of dispersion of solid lipid nanoparticle was taken in centrifuge tube and further it was centrifuged in cooling centrifuge (REMI-C24 BL. Remi Elektrotechnik ltd., India) at 15,000 rpm for 40 mins. After centrifugation the supernatant was removed and diluted with appropriate solvent. The concentration of drug (free drug) in supernatant layer was determined by using UV-VIS Spectroscopy.

The entrapment efficiency (EE) is calculated by using following formula :

$$EE = \frac{Winitial\ drug - Wfree\ drug}{Winitial\ drug} \times 100$$

Where,

W*initial* drug= Weight of initial drug added into the formulation.

W*free* drug= Weight of free drug into the formulation.

Freeze drying of optimized batch of SLN [32]

The optimized SLN dispersion was further freeze dried at pressure lower than 0.5 mBar and temperature of about -390C. The mannitol (3% solution) was added in SLN dispersion as a lyoprotectant so as to avoid lysis of nanoparticles. The sample was first subjected to overnight deep freezing in Deep freezer (REMI, RQV-200 PLUS) and then it was freeze dried in Freeze dryer ALPHA 1-2 LD PLUS, MARTIN CHRIST.

Evaluation of Freeze dried optimized batch of SLN

Zeta Potential

The zeta potential of Candesartan cilexetil-loaded SLN was measured by Horiba SZ-100 nano particle analyzer.

Laser Doppler Micro-electrophoresis was used to measure zeta potential. An electric field was applied to a solution of molecules or a dispersion of particles, which then move with a velocity related to their zeta potential. This velocity was measured using laser interferometric technique which enables the calculation of electrophoretic mobility, and from this, the zeta potential and zeta potential distribution.

Differential Scanning Calorimetric (DSC) study

The DSC thermogram of Candesartan cilexetil- loaded SLNs was recorded by using a differential scanning calorimeter (PerkinElmer 4000) equipped with a computerized data station. The sample (appox. 1mg) was weighed and heated in a closed pierced aluminum pan at a scanning rate of 10°C/min between 30- 300°C and 20 ml/min of nitrogen flow.

Stability studies [35]

Stability studies were carried out on reconstituted dispersion of SLN. Batch R5 (SLNs with good particle size and entrapment efficiency) was further analyzed for stability after storage for 1 month. The samples were analyzed for its particle size and entrapment efficiency on 15th day and 30th day after storage at 2-80C.

In-vitro drug release study

The in vitro drug release from Candesartan cilexetil-loaded SLN in phosphate buffer pH 6.8 was examined by the dialysis method. In brief, SLN dispersion (containing 2 mg of Candesartan cilexetil) was added to the dialysis bag (molecular weight cutoff 12000) and the dialysis bag was placed into 200 ml dissolution medium with stirring rate of 50 rpm at 37 0C. One ml of dispersions was withdrawn and fresh release medium at equal volume was added quickly to maintain the sink condition. The samples were analyzed by UV-VIS Spectrophotometry. Each experiment was performed in triplicate.

$$PS=+190.99+85.60*A+12.56*B+1.14*C-22.95*AB+1.15*AC+54.42*BC \quad \ldots\ldots \quad (i)$$

$$EE=+80.10+0.30*A+0.39*B+1.83*C-0.025*AB+4.92*AC-3.05 \quad \ldots\ldots \quad (ii)$$

X-ray Diffraction Study

Candesartan cilexetil- loaded SLNs was studied for X-ray diffraction spectra. The powder X ray diffraction patterns was recorded using an X-ray Diffractometer (Bruker D8 advance) with 2.2 KW copper as an anode material and dermic X-ray tube as a source. The sample was analyzed using the 2 angle of 3-30° using lynux eye detector and filtered using Ni filter.

Scanning Electron Microscopy

SEM analysis of the optimized SLN formulation was carried out to understand the morphology of SLN. Freeze dried solid lipid nanoparticles of CC were suitably diluted and a drop of nanoparticle formulation was placed on sample holder and air dried. Then the sample was observed at accelerating voltage of 15 000 volts at various magnifications. Imaging was carried out in high vacuum.

Table 2: A Box- Behnken Experimental Design Layout

Run	Formulation Code	Coded Levels (Independent variables)		
		X1	X2	X3
6	R_1	1	0	-1
8	R_2	1	0	1
4	R_3	1	1	0
2	R_4	1	-1	0
7	R_5	-1	0	1
10	R_6	0	1	-1
3	R_7	-1	1	0
5	R_8	-1	0	-1
11	R_9	0	-1	1
1	R_{10}	-1	-1	0
12	R_{11}	0	1	1
9	R_{12}	0	-1	-1

Table 3: Particle size and entrapment efficiency of batches

Formulation Code	Particle size	Entrapment efficiency
R_1	285.1	72.93
R_2	300.4	85.7
R_3	276.5	80.9
R_4	281.2	80.6
R_5	98.4	75.6
R_6	126.9	82.3
R_7	179.7	80
R_8	87.7	82.5
R_9	109.4	85.5
R_{10}	92.6	79.6
R_{11}	227.3	80.6
R_{12}	226.7	75

RESULTS

Experimental design and statistics

In this study, candesartan cilexetil loaded SLNs were prepared by high speed homogenisation followed by probe sonication method using GMS as a lipid, tween 80 aqueous surfactant and span 20 as lipophilic surfactant.

In order to quantify the effect of formulation variables on the response parameters, it was necessary to construct a mathematical model which would help in predicting values of response parameters at any selected values of formulation variables within the boundaries of the design. It may happen that the levels of formulation variables which are intermediate between the selected levels may yield optimum formulation. Design Expert 7.1 software was used to generate a mathematical model (Table 2) for each response parameter and the subsequent statistical analysis. The equation **(i)** was the polynomial equation of particle size variable obtained from ANOVA.

The Model F-value of 12.73 implies that 2FI model is significant. There is only a 0.67% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.006, less than 0.050 indicate model terms are significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 9.143 indicates an adequate signal. This model can be used to navigate the design space. The equation **(ii)** was the polynomial equation of entrapment efficiency variable obtained from ANOVA.

The Model F-value of 18.31 implies that 2FI model is significant. There is only a 0.29% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.002, less than 0.050 indicate model terms are significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 14.524 indicates an adequate signal. This model can be used to navigate the design space.

Three dimensional response surface plots for each response parameter were constructed to study the effects of both formulation variables simultaneously and behavior of the system. Fig. 1 shows response surface plot for particle size. It can be observed from the figure that drug: lipid ratio had positive effect on particle size i.e. particle size increased with increase in drug: lipid ratio. Least particle size was observed at the lowest level of drug:lipid ratio. Concentration of aqueous surfactant had opposite effect on particle size. It can be observed from Fig.1 that particle size increased with increase in concentration of aqueous surfactant. Fig. 2 indicates that the drug: lipid ratio had a greater impact on particle size compared to concentration of aqueous surfactant.

Fig. 2 shows simultaneous effect of concentration of aqueous surfactant and drug: lipid ratio on entrapment efficiency. The value of EE was maximum when both formulation variables were employed at their highest level. The reasons can be attributed to the maximum amount of lipid present for entrapment of the drug. At high lipid levels, concentration of aqueous surfactant had more effect on EE but at low lipid level the increase in concentration of aqueous surfactant have little effect on EE. This may be due to low lipid content for the encapsulation of drug.

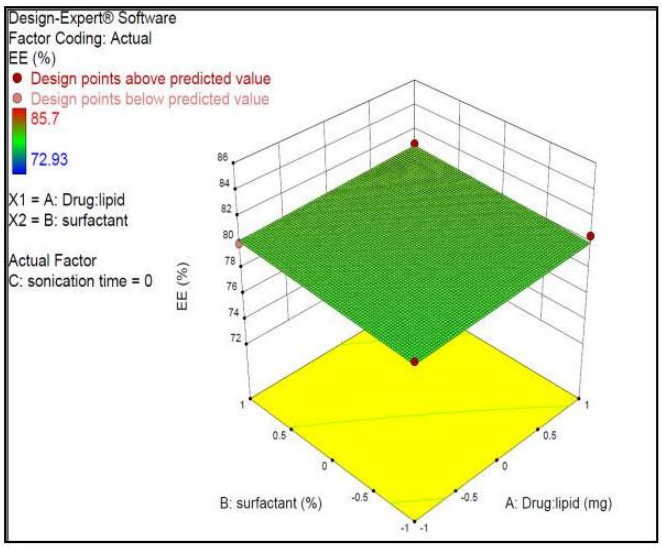

Fig 1. Three dimensional response surface plot for particle size

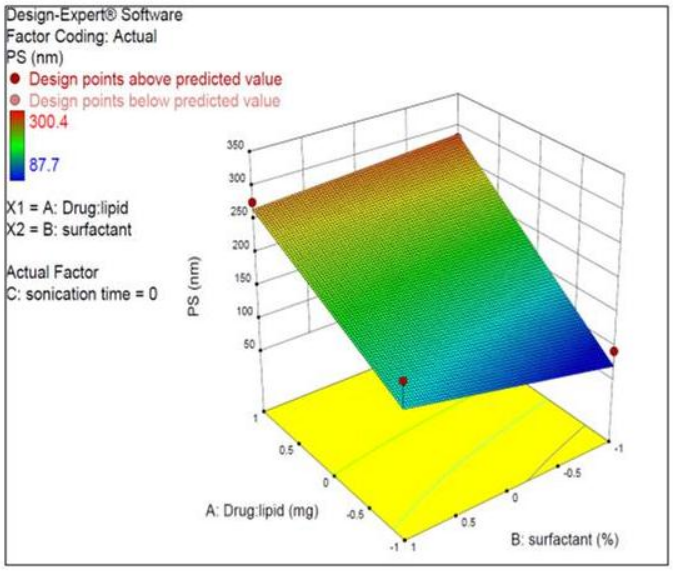

Fig 2. Three dimensional response surface plot for entrapment efficiency

Measurement of particle size and entrapment efficiency

Batch 5 (R5) was suggested as optimized batch based on optimum particle size and entrapment efficiency. Batch was formulated by high speed homogenization followed by probe sonication. The batch S5 was evaluated for its particle size and entrapment efficiency.

Freeze drying of optimized batch of SLN

The optimized batch S5 of lipid nanoparticles was subjected to freeze drying. The 3% mannitol solution was added into dispersion of lipid nanoparticles to avoid the lysis of nanoparticles.

Particle size:

The batch R5 of solid lipid nanoparticle was formulated and evaluated for particle size. The particle size of optimized batch is found to be 87.7 nm (Fig 3).

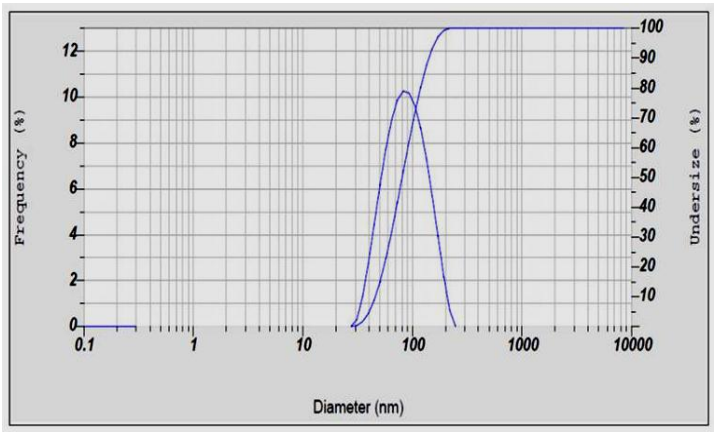

Fig 3. Particle size distribution curve of optimized batch

Entrapment efficiency

Entrapment efficiency (% EE) is expressed as fraction of drug incorporated into formulations relative to the total amount of drug used. Determination of % entrapment efficiency is an important parameter in case of lipid nanoparticles as it may affect the drug diffusion. The entrapment efficiency of optimized batch was calculated by centrifugation method and it is found to be 80.46%.

Zeta potential

Zeta potential value of optimized batch is found to be -31.00 mV (Fig. 4). It refers to the surface charge of the particles and indicates the degree of repulsion between close and similarly charged particles in the dispersion. The repulsion force prevents aggregation of the particles. Therefore, zeta potential is a useful parameter to predict the physical stability of the SLN dispersions. The repulsive interactions will be larger between the particles as the zeta potential increases or decreases, leading to the formation of more stable particles with a

more uniform size distribution. Result of zeta potential measurement showed that surface charge of optimized batch is consistently negative which indicates that the SLN dispersion is stable.

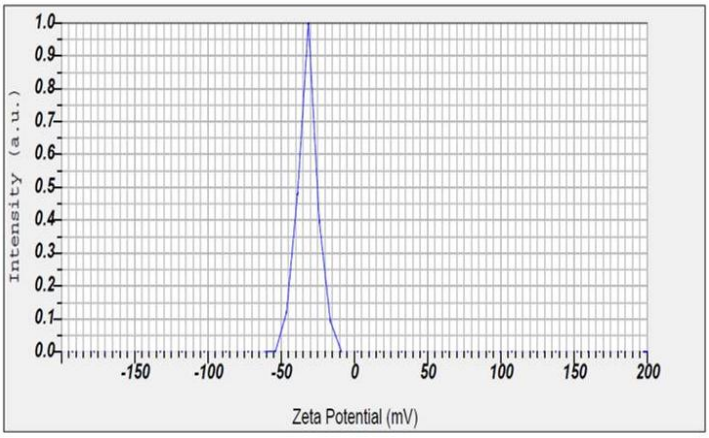

Fig. 4: Zeta potential of optimized batch

Differential Scanning Calorimetric study

The DSC thermogram of Candesartan cilexetil, GMS, physical mixture of Candesartan cilexetil and GMS and Candesartan SLN is shown in Fig.5.

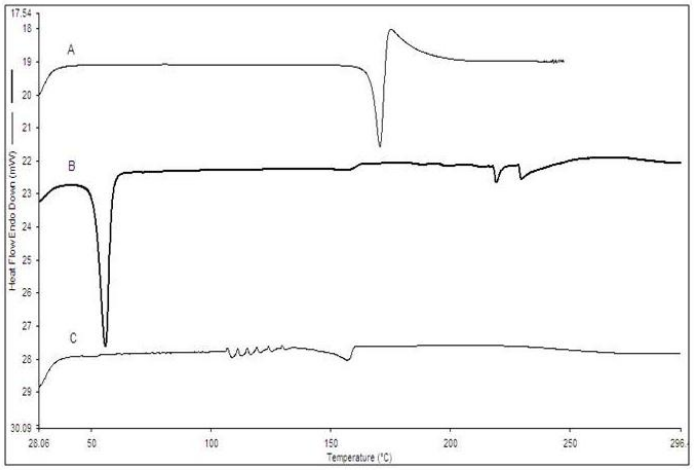

Fig. 5: DSC thermogram of Candesartan (A), physical mixture of Candesartan and GMS (B), Candesartan - SLN(C)

The peak of Candesartan cilexetil is completely absent in lyophilized Candesartan- SLN, while it is clearly evident in physical mixture of Candesartan cilexetil and glyceryl monostearate. It has been reported that when the Candesartan cilexetil does not show its endothermic peak in the nanoparticulate formulation, it is said to be in the amorphous state. Hence, it could be concluded that the drug is present in the amorphous phase and may have been homogeneously dispersed in the lipid nanoparticles.

X-ray Diffraction Study

From the X-ray diffraction data (Fig. 6), it is clear that pure Candesartan cilexetil (A) showed highly crystalline nature with principal peak at 2θ value of 9.9, 17.26, 18.7, 19.28, 20.32, 23.26, and 25.14 degrees. The characteristic peaks existed at 2θ values of 9.94, 17.3, 19.62, 23.24 degrees in PM (B), which confirmed the crystallinity of the components, whereas the Candesartan- SLN (C) formulation showed deformed peak for Candesartan cilexetil, indicating its presence in amorphous or molecular dispersion state. The extra peaks at 2θ value in XRD of Candesartan SLN may be due to presence of GMS in the formulation.

Scanning electron microscopy

Fig. 7 shows the SEM image of optimized batch of SLNs almost spherical shaped and smooth surfaced on scale of 4µm, 2µm, 500nm and 300nm. Increased particle size and agglomeration may be due to lyophilisation process.

Table 4: Stability profile of SLN

Samples/Parameters	SLN
Particle size (nm)	
Fresh	88.46
15th day	89.23
1 month	91.12
Entrapment efficiency (%)	
Fresh	80.46
15th day	79.52
1 month	76.87

Stability study

The formulation batch R5 was studied for its parameters like particle size and entrapment efficiency after storage for 1 month. An increasing trend of particle size and decreasing trend of drug encapsulation efficiency (EE) was observed with storage time at 2- 80c (table 4).

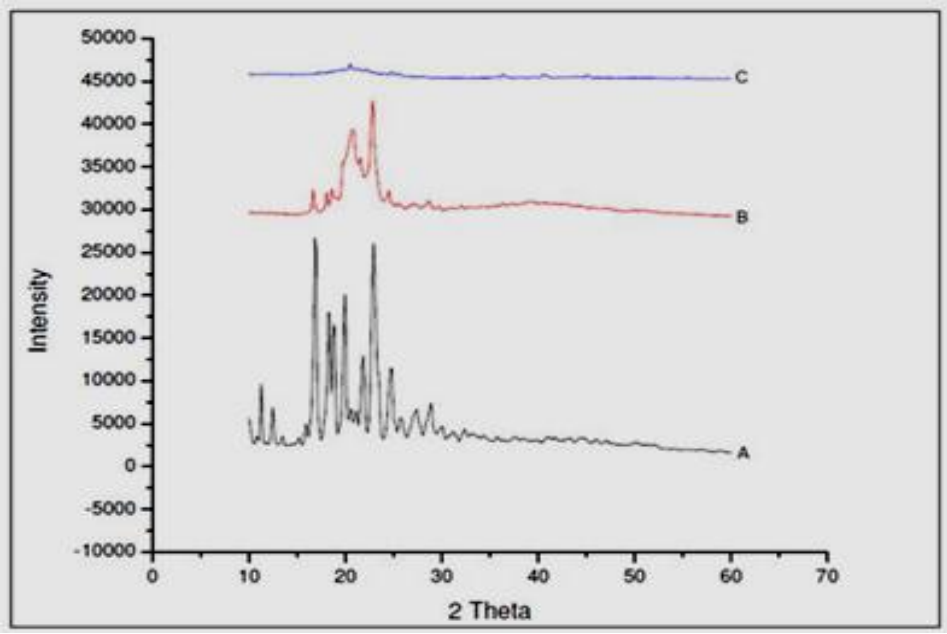

Fig. 6: XRD crystallograph of Candesartan (A), physical mixture of Candesartan and GMS (B), Candesartan- SLN(C)

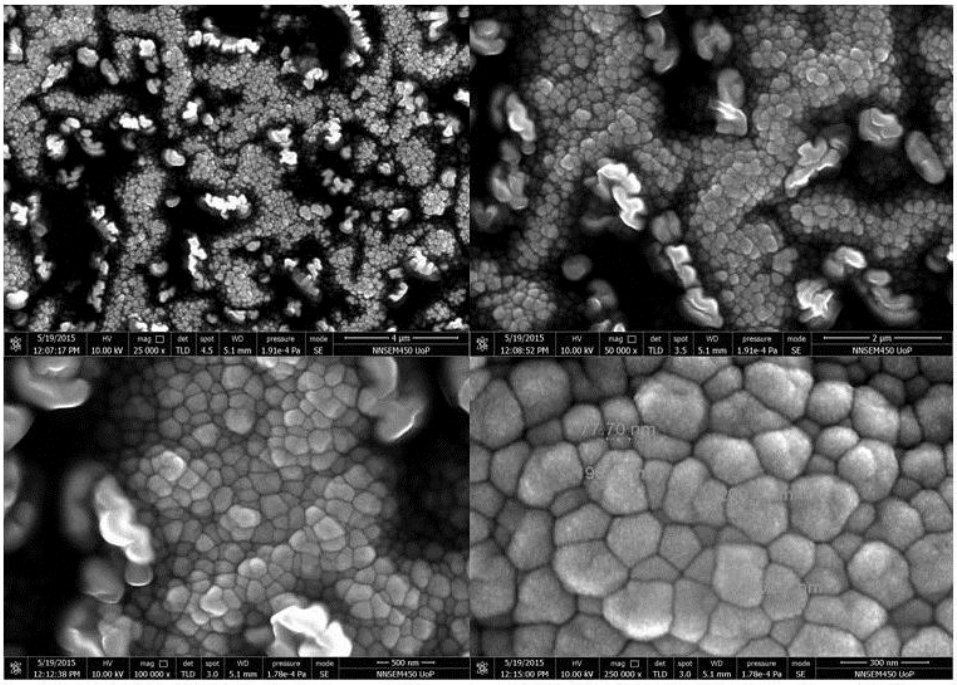

Fig. 7: SEM images of optimized batch of SLNs

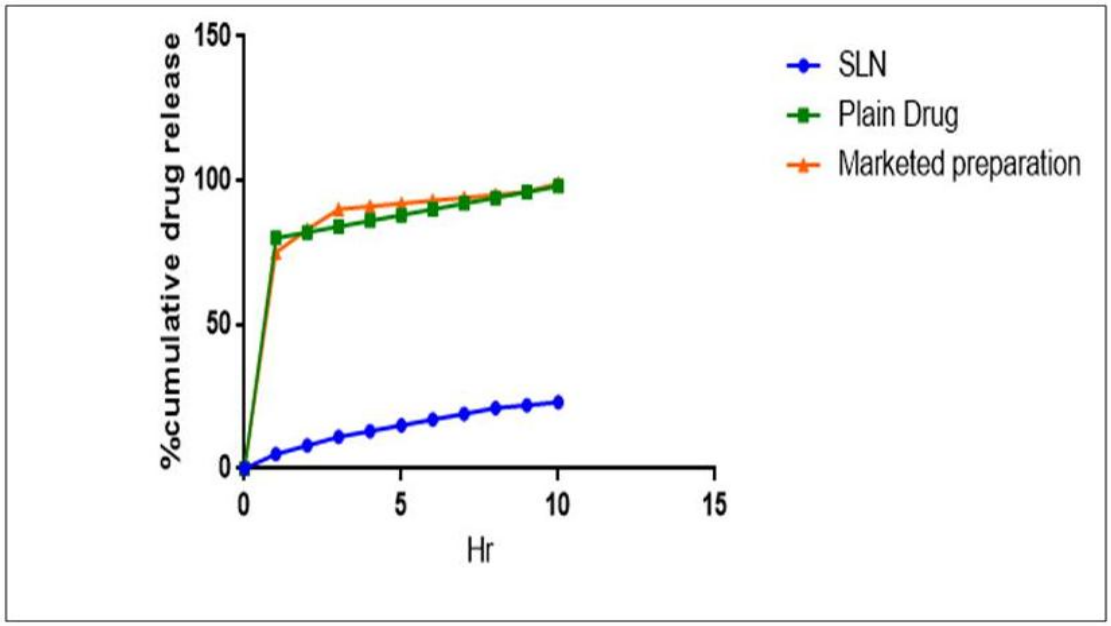

Fig. 8: In vitro drug release from plain Candesartan suspension, Candesartan-SLNs and marketed preparation in phosphate buffer pH 6.8

In-vitro drug release study

The in vitro drug release from SLNs vs. plain drug suspension and marketed preparation (Atacand® 8 mg tablet) were plotted against time as shown in Fig.8. The total drug release from SLN in phosphate buffer pH 6.8, in 10 hours was 23.96% while drug release in case of plain drug suspension and marketed formulation showed 80% release during first 4 hrs. Low release of CC from SLNs could mainly result from the poor aqueous solubility. It was reported that SLNs could be absorbed into blood or lymph after duodenal administration to rats66. Therefore, the low in vitro release of CC from SLNs implied that SLNs could be absorbed through the enterocytes after oral administration, which was beneficial to achieve the desired therapeutic effect.

DISCUSSION

Hypertension, which had affected more than quarter of world's adult population in 2000 i.e. nearly 1 billion and will be increased to 29% i.e. 1.56 billion by 2025. It is a major cause for both cardiovascular and cerebrovascular morbidity and mortality. The pathophysiology of hypertension is defined as a lasting elevation of blood pressure to ⩾140/90 mmHg.

Candesartan is a selective AT1 subtype angiotensin II receptor antagonist. Candesartan cilexetil (CC) is widely used for the treatment of hypertension and heart failure in clinical application. After oral administration it undergoes rapid ester hydrolysis to convert to the active Candesartan during absorption in the gastrointestinal (GI) tract. It is a BCS class II drug so the major drawback of Candesartan cilexetil as an oral dosage form is its very low aqueous solubility and first-pass metabolism which affect the therapeutic application and efficacy.

The present research work was aimed towards formulation of Candesartan cilexetil loaded solid lipid nanoparticle. As solid lipid nanoparticles got absorbed by lymphatic pathway due to stimulation of chylomicron formation by lipids, it bypasses the liver and hence first pass metabolism. In this way, by incorporating Candesartan cilexetil in SLNs we can enhance the oral bioavailability of drug.

For formulation of Candesartan cilexetil SLN, different solid lipids and surfactants were tried. The selection of lipids and surfactants were done on the basis of saturation solubility studies. Compatibility study between drug and different excipients were performed by FTIR and DSC. High speed homogenization followed by probe sonication was the method of preparation of SLN. The Box behnken design was applied to formulation of SLN for preparing different batches of SLN with varying concentration of drug: lipid ratios and surfactants. The selection of optimized batch of SLNs was done by analysing the optimum size and entrapment efficiency. The optimized batch further characterized and evaluated for its particles size, entrapment efficiency, zeta potential, SEM, DSC, XRD, In vitro drug release.

The particle size and entrapment efficiency of optimized batch of SLN was found to be 87.7 nm and 80.46 % respectively. The zeta potential was -31.00 mV which indicates that dispersion is stable. The in vitro drug release study was done by dialysis method by using phosphate buffer pH 6.8 as dissolution medium. The Candesartan cilexetil SLN releases only 20.96 % drug in 10 hours which reveals that drug is releasing in a sustained manner in systemic circulation.

CONCLUSION

Conclusively, Candesartan cilexetil loaded solid lipid nanoparticle consisting of GMS as the solid lipid, span 20 as the lipophilic surfactant and tween 80 as the hydrophilic surfactant was successfully developed with an optimum particle size and entrapment efficiency which gives a sustained release of drug from the formulation into the systemic circulation, through lymphatic pathway thus surpassing first pass metabolism. Results from stability studies confirmed the stability of the developed formulation. Thus, the study shows that the Candesartan cilexetil SLN formulation can be an effective oral drug delivery system with enhanced oral bioavailability.

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