**INTRODUCTION**

Candesartan cilexetil (CDC), widely used as a drug of choice to treat hypertension, is a prodrug hydrolyzed to candesartan during absorption from the gastrointestinal tract [1, 2]. The use of a prodrug form increases the bioavailability of candesartan. Despite this, absolute bioavailability is relatively poor at 15% (tablets) to 40% (solution). CDC is a selective AT1 subtype angiotensin II receptor antagonist known to be important in cardiovascular regulations and demonstrate the highest potency among the angiotensin receptor blockers used in the treatment of hypertension [3-5]. However, due to very poor solubility of CDC within the physiological pH range, it shows incomplete intestinal absorption and very low systemic exposure after oral administration [6, 7]. In this work, CDC-loaded solid lipid nanoparticle (SLN) were designed to improve the oral bioavailability. SLNs were designed by modified emulsification ultrasonication method, and the physicochemical properties were characterized. From time to time, many new formulations have been fabricated to improve the oral bioavailability of CDC including new solid dosage forms [6], nanoparticles [8-10], niosomes [11], nanoemulsion [12], nanosuspension [13], SMEDDS [7], and solid dispersion [14] and many more. From the literature review the SLN is also reported for CDC [15, 16], but stearic acid-based SLN of CDC and modified emulsification- ultrasonication method of preparation is not reported anywhere. Stearic acid is GRAS status lipid known to be very safe and widely used in food, drugs, and cosmetics [17-20]. Hence, stearic acid-based SLN of CDC offers very safe formulation, which could be successful to improve its oral bioavailability issues and in vivo performance. SLNs are lipid nanoparticles of size 10–1000 nm, reported to be a suitable carrier system for pharmaceuticals with various benefits including the lymphatic absorption, hence, bypass hepatic metabolism [21, 22], and the potential of controlled/sustained release of incorporated compounds due to solid matrix which helps in maintaining therapeutic concentration over a longer period of time [23]. They are also recommended for the protection of labile drugs against chemical degradation [24, 25]. Due to nano size, SLN possesses unique properties and reported to have features like enhancement in bioavailability [26, 27]. SLNs have received considerable interest due to their ability to overcome the limitations of previous colloidal carriers [28-31] and offer an alternative to the polymeric/metal nanoparticles [32]. They are supposed to be identical to oil/water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by the solid lipid [21, 22]. SLNs can be prepared from biodegradable and non-toxic lipid components such as fatty acids, mono-, di-, and tri-glycerides, and phospholipids which are normal constituents of the human body and are thus biocompatible [33]. SLNs can efficiently incorporate lipophilic as well as hydrophilic drugs in the lipid matrix [34-36].

**MATERIAL AND METHODS**

**Materials**

Candesartan cilexetil, widely used as a drug of choice to treat hypertension, is a prodrug hydrolyzed to candesartan during absorption from the gastrointestinal tract [1, 2]. The use of a prodrug form increases the bioavailability of candesartan. Despite this, absolute bioavailability is relatively poor at 15% (tablets) to 40% (solution). CDC is a selective AT1 subtype angiotensin II receptor antagonist known to be important in cardiovascular regulations and demonstrate the highest potency among the angiotensin receptor blockers used in the treatment of hypertension [3-5]. However, due to very poor solubility of CDC within the physiological pH range, it shows incomplete intestinal absorption and very low systemic exposure after oral administration [6, 7]. In this work, CDC-loaded solid lipid nanoparticle (SLN) were designed to improve the oral bioavailability. SLNs were designed by modified emulsification ultrasonication method, and the physicochemical properties were characterized. From time to time, many new formulations have been fabricated to improve the oral bioavailability of CDC including new solid dosage forms [6], nanoparticles [8-10], niosomes [11], nanoemulsion [12], nanosuspension [13], SMEDDS [7], and solid dispersion [14] and many more. From the literature review the SLN is also reported for CDC [15, 16], but stearic acid-based SLN of CDC and modified emulsification- ultrasonication method of preparation is not reported anywhere. Stearic acid is GRAS status lipid known to be very safe and widely used in food, drugs, and cosmetics [17-20]. Hence, stearic acid-based SLN of CDC offers very safe formulation, which could be successful to improve its oral bioavailability issues and in vivo performance. SLNs are lipid nanoparticles of size 10–1000 nm, reported to be a suitable carrier system for pharmaceuticals with various benefits including the lymphatic absorption, hence, bypass hepatic metabolism [21, 22], and the potential of controlled/sustained release of incorporated compounds due to solid matrix which helps in maintaining therapeutic concentration over a longer period of time [23]. They are also recommended for the protection of labile drugs against chemical degradation [24, 25]. Due to nano size, SLN possesses unique properties and reported to have features like enhancement in bioavailability [26, 27]. SLNs have received considerable interest due to their ability to overcome the limitations of previous colloidal carriers [28-31] and offer an alternative to the polymeric/metal nanoparticles [32]. They are supposed to be identical to oil/water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by the solid lipid [21, 22]. SLNs can be prepared from biodegradable and non-toxic lipid components such as fatty acids, mono-, di-, and tri-glycerides, and phospholipids which are normal constituents of the human body and are thus biocompatible [33]. SLNs can efficiently incorporate lipophilic as well as hydrophilic drugs in the lipid matrix [34-36].

**Method of preparation of SLN**

SLNs were prepared by “modified emulsification-ultrasonication method” [37, 38]. Briefly, the lipid phase was prepared by dissolving stearic acid and the drug in 10 ml of methanol and heating at 70°C (temperature above melting point of the lipid). An aqueous solution of Poloxamer 188 heated up to the same temperature of lipid phase was added slowly to the lipid phase along with continuous stirring to form a pre-emulsion. The pre-emulsion formed was stirred at 10,000 rpm for 10 min using high-speed homogenizer (REMI). Then, the dispersion was ultrasonicated for 10 min to reduce the size to nanoscale using Probe Sonicator (PGI 750F). Further, this dispersion
was poured into cold (1–4°C) water and stirred with a magnetic stirrer to recrystallize the SLNs. The SLN dispersions were freeze-dried (VERTIN) and stored under refrigerator conditions. The design of experiments (DOE) was applied to optimize the lipid and surfactant concentration and to select the best formulation [39]. The thirteen batches of SLN were prepared by varying of lipid concentration ($X_1$) and surfactant concentration ($X_2$) using central composite design as shown in Table 1a and b, and the best formulation was optimized in terms of entrapment efficiency.

$$Y = 92.82 + 3.90X_1 + 2.02X_2 + 1.76X_1X_2 − 3.58X_1^2 − 0.62X_2^1$$

Statistical validity of the polynomials was established, three-dimensional response surface plots describing entrapment efficiency and desirability in Fig. 1a and b, constructed based on the modal polynomial functions using design expert software. The effect of lipid and surfactant concentration on the entrapment efficiency was studied with the help of these plots. On the basis of these results, SLN3 was optimized as the best formulation as it showed maximum entrapment efficiency of 78.28%.

Table 1a: Composition of SLNs using central composite design

<table>
<thead>
<tr>
<th>Variables</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$ (lipid)</td>
<td>−1 0 1 −1.41 1.41</td>
</tr>
<tr>
<td>2% (w/v)</td>
<td>3% (w/v)</td>
</tr>
<tr>
<td>$X_2$ (poloxamer 188)</td>
<td>0.5% (w/v) factor</td>
</tr>
</tbody>
</table>

Table 1b: Combination levels of independent variables and the outcomes of response encapsulation efficiency

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Independent variable</th>
<th>Dependent variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>SLN 1</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 2</td>
<td>3</td>
<td>0.7414</td>
</tr>
<tr>
<td>SLN 3</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>SLN 4</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 5</td>
<td>1.585</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 6</td>
<td>3</td>
<td>0.4585</td>
</tr>
<tr>
<td>SLN 7</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 8</td>
<td>4.414</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 9</td>
<td>4</td>
<td>0.7</td>
</tr>
<tr>
<td>SLN 10</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 11</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>SLN 12</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>SLN 13</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

SLN: Solid lipid nanoparticle

Fig. 1: Three-dimensional response surfaces for solid lipid nanoparticles: (a) Entrapment efficiency (b) desirability, (c) particle size, (d) zeta potential
Particle size and zeta potential determination
The zeta potential measurements were performed using Malvern Zetasizer Nano ZS, Malvern Instruments (Malvern, UK). The particle size analysis was performed by photon correlation spectroscopy (PCS). The PCS yields the mean diameter of the SLNs and polydispersity index (PDI) as a measure of the width of particle size distribution.

Transmission electron microscopy (TEM) of SLN
The shape and surface morphology of prepared SLNs was examined by TEM (Morgagni 268; Phillips, Holland). Samples were stained with phosphotungstic acid (PTA, 2%), spread on a gold grid, and examined for shape and morphology.

Drug entrapment
The percentage of drug entrapped was calculated by centrifugation method. A fixed volume (1 ml) of SLN was dissolved in methanol and centrifuged at 18,000 rpm at temperature 4°C using cooling centrifuge (REMI) for 30 min, and the supernatant was decanted without disturbing the SLN pellets. The samples were filtered and analyzed spectrophotometrically for CDC using Shimadzu ultraviolet (UV)-1800 spectrophotometer. The percentage drug entrapment was calculated using following formula:

\[
\text{Entrapment efficiency} = \frac{\text{total drug} - \text{free drug}}{\text{total drug}} \times 100
\]

In vitro release study
In vitro release studies of optimized CDC-loaded SLN and CDC suspension, at equivalent amounts, were carried out using dialysis bag method. The samples were taken in the dialysis membrane (Himedia) and placed in a beaker containing 100 ml of simulated intestinal fluid (without enzymes) which acted as receptor compartment. Previously, the dialysis membrane was soaked in distilled water for about 12 h. The beaker was placed over a magnetic stirrer (100 rpm) and maintained at 37±2°C. An aliquot of 1 ml of the receptor fluid was withdrawn at predetermined time intervals and replaced with fresh volumes. The samples were filtered through a 0.22 µm membrane filter (millipore) and analyzed after suitable dilution at λmax 255 nm against simulated intestinal fluid as blank using Shimadzu UV-1800 spectrophotometer. The percentage drug entrapment was calculated by centrifugation method. The samples were weighed accurately (5 mg) in aluminium pans and heated at a predefined rate of 10°C/min over the temperature range of 20 and 30°C in nitrogen atmosphere. Thermal data analyses of DSC thermograms were conducted using TA instruments universal analysis 2000 software (version: 4.5A). The scans were recorded, and plots between heat flow (w/g) and temperature (°C) were obtained.

Differential scanning calorimeter (DSC) studies
DSC thermograms of CDC, lipid, and CDC-SLN3 were recorded on a Q20 DSC (TA systems, USA). Samples were weighed accurately (5 mg) in aluminium pans and heated at a predefined rate of 10°C/min over the temperature range of 20 and 30°C in nitrogen atmosphere. Thermal data analyses of DSC thermograms were conducted using TA instruments universal analysis 2000 software (version: 4.5A). The scans were recorded, and plots between heat flow (w/g) and temperature (°C) were obtained.

PXRD studies
Powder X-ray diffractometer (Bruker D8) was used to get diffraction patterns and to find crystalline/amorphous nature of SLN. PXRD studies were performed for CDC and SLN powder by exposing them to CuKα radiation (50 kv, 34 mA) and scanned from 3 to 45° 2θ values at a scan step of 0.02° and step time of 3’s/min.

In vivo pharmacokinetic studies in rats
The experimental protocol was approved by the Institutional Animal Ethical Committee and conducted according to the guidelines of “Committee for the Purpose of Control and Supervision of Experiments on Animals.” A single dose in vivo study was designed, and adult male Wistar rats (200–250 g) were used for the study. The animals were divided into two groups (n=6). Group I was administered CDC suspension and Group II administered CDC-SLN (10 mg/Kg body weight) orally using oral feeding cannula [40,41]. The animals were anesthetized and blood samples (0.5 ml) were withdrawn at different time intervals from retro-orbital sinus into heparinized microcentrifuge tubes containing 50 μl of heparin per ml of blood [27]. Plasma was separated by centrifuging the blood samples at 15,000 rpm for 10 min at 4°C and stored at –20°C until further analysis. To 150 μl of plasma, 300 μl of acetonitrile (deproteinizing agent) was added and the dispersion was vortexed for 5 min. The samples were then centrifuged at 15,000 rpm for 5 min at 4°C. The supernatant was decanted and filtered (0.2 µm membrane filters) and 20 µl of each sample was injected into HPLC column. Simultaneously calibration curve was plotted by spiking known concentration of drug into the rat plasma over the concentration range of 1–5 μg/ml.

A simple and precise RP-HPLC method using HPLC (Shimadzu) equipped with C18 column was developed for the analysis of CDC content in plasma.

Stability studies
The optimized SLN formulations were subjected to stability testing at refrigerated temperature (2–8°C) and at 25±2°C/60±5% RH conditions in a stability chamber (REMI) for 3 months. The parameters used to access the stability of SLNs were Variations in pH of formulation, particle size and PDI values, zeta potential, and drug entrapment efficiency.

Statistical analysis
All the results were statistically analyzed through one-way ANOVA using Sigma Stat Software. The data presented are mean ± standard error (n=3) at p<0.05 as significant value.

RESULTS
Particle size and zeta potential
The results of particle size of optimized formulation along with PDI value and zeta potential values are shown in Fig. 1c and d, respectively. The globule size and PDI of SLN3 are found to be 197.9 nm and 0.690, respectively. Zeta potential is used to predict long-term stability of the prepared dispersions. The zeta potential value was observed to be -21.3 mV; the negative values of zeta potential are due to the carbonyl group of stearic acid.

TEM analysis
The nanosized SLNs spherical in shape are shown in TEM images at various resolutions in Fig. 2a and b. The size of SLNs measured by TEM analysis is smaller than that measured by PCS method. This discrepancy could be due to difference in principles of measurements, measuring conditions, and technology applied in this technique. This may be explained in terms of a unique arrangement of these small particles as observed under TEM. The size measurements with a Zetasizer are expected to be biased in such cases, wherein a particle appearing to be single is actually a cluster of much smaller particles [27].

Drug entrapment
The entrapment efficiency which is ratio of the drug encapsulated to that of total drug loaded was calculated for all the thirteen SLN formulations, and the values are as shown in the Table 1b, these values were also compared with the predicted values depicted from DOE. The total drug content of 88.33±1.23% and entrapment efficiency of 78.28±1.91% were calculated for optimized formulation.

Percentage drug release studies
The cumulative percentage release of CDC from the optimized SLN and CDC suspension at equivalent amounts is as shown in Fig. 2c. CDC-SLN released 60.43% of drug in comparison to 17.11% released by CDC suspension in 24 h (p<0.05). The SLN formulation showed initial fast release followed by comparatively slower release up to 66.09±0.01% lasting up to 24 h. The initial fast release may be explained in terms of free drug present on the surface of SLN molecules. The further slow release is due to diffusion of the entrapped drug from the solid matrix of the lipids. Zero order; first order, Korsmeyer–Peppas, and Higuchi mathematical models for data fitting for drug release from the SLN matrix were applied. From regression analysis, the highest value of
regression coefficient ($r^2=0.960$) for Higuchi model was obtained, and hence, the best fit for release profile of CDC for SLN is explained in terms of Higuchi model.

**DSC studies**
A sharp endothermic peak at 176.7°C was observed for CDC in Fig. 3a. The endothermic peak for main component stearic acid was observed at 58.67°C in Fig. 3b. The characteristic peaks was appeared in physical mixture of CDC and stearic acid, but no peak was observed in lyophilized SLN in Fig. 3c, which confirmed that no crystal of CDC existed in SLN. This indicated the molecular dispersion of CDC in SLN and successful encapsulation of drug in lipid matrix.

**PXRD studies**
The characteristics sharp peaks of CDC in XRD patterns was appeared at $2\theta$ values of 9.8, 17.1, 18.7, 19.2, 21, 23.2, and 24.5° as shown in Fig. 3d and e. All these characteristics peaks existed in physical mixture confirm the crystallinity of the components. However, a broad peak was observed in the diffraction pattern of CDC-SLN. Hence, PXRD results confirmed that CDC was encapsulated in lipid matrix in molecular or amorphous form.

**Pharmacokinetic studies in rats**
The plasma concentration of CDC was monitored by HPLC method, and $AUC_{0-t}$ was calculated. The plasma drug concentration versus time profile is presented in Fig. 4 and pharmacokinetic parameters are summarized in Table 2. The pharmacokinetic parameters were calculated using non-compartmental analysis with NCSS software. The experimental results indicated that the pharmacokinetic parameters in rats after oral administration of CDC-SLN were significantly improved than that of free drug suspension. Most importantly, $AUC_{0-t}$ of CDC-SLN was significantly enhanced over 3-folds than that of free drug suspension ($p<0.05$). Furthermore, the $C_{max}$ of CDC-SLN was remarkably improved over 3-folds in comparison to drug suspension ($p<0.05$). In addition, the time to $C_{max}$ ($T_{max}$) of CDC from SLN and drug suspension was 3 h and 8 h, respectively, indicating that SLN could be absorbed more rapidly in the form of SLN.

**Stability studies**
The stability estimation results (Table 3) showed that changes found in any of assessed parameters at low temperature conditions were less than that observed at room temperature. The increase in particle sizes and small changes in zeta potential values observed at room temperature conditions are attributed to aggregation of particles. The reduction in percentage drug entrapment values at different time intervals at 25±2°C is explained in terms of some drug expulsion from solid lipid matrix on aging.
DISCUSSION

The SLN-based drug delivery is reported to provide many advantages in drug delivery. The poorly soluble CDC is reported with low oral bioavailability. In this research, SLN of CDC was investigated and found that SLN could enhance the oral absorption of CDC compared with free drug suspension. CDC-SLN with total drug content of 88.33±1.23% and entrapment efficiency of 78.28±1.91%, with an average particle size of 197.9 nm and zeta potential value -21.3 mV, was prepared by “modified emulsification-ultrasonication method.” The high entrapment efficiency is attributed to high affinity of lipophilic CDC toward the lipid stearic acid, and the maximum stabilization provided by the surfactant poloxamer 188 to the lipid core. CDC-SLN released 60.43% of drug in comparison to 17.11% released by CDC suspension in 24 h (p<0.05). The in vivo pharmacokinetic results indicated that the oral pharmacokinetic parameters were improved; AUC was increased up to 3-folds in comparison to free drug suspension. The better pharmacokinetic profile achieved with CDC-SLN is attributed to 197.9 nanometric size of SLNs. Furthermore, the intestinal lymphatic absorption of SLN through oral lymphatic region, hence avoiding the first pass metabolism, could be important to enhance oral bioavailability [27, 42-44]. The drug is absorbed better in the form of

Table 2: Pharmacokinetics parameters for CDC and SLN in rats

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Drug</th>
<th>SLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (mg/ml. h)</td>
<td>7.6355</td>
<td>24.7385</td>
</tr>
<tr>
<td>C_{max} (mg/ml)</td>
<td>0.713</td>
<td>2.356</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

CDC: Candesartan cilexetil, SLN: Solid lipid nanoparticle
SLN due to non-polar core and polar head orientation toward aqueous phase. The mean residence time for CDC-loaded SLN was significantly higher than that of free CDC, depicting sustained release over a long period. Hence, these \textit{in vivo} results confirmed that SLN formulation could be successful in improving the oral bioavailability of poorly soluble CDC, and controlled release over an extended period of time is achieved. DSC and PXRD results confirmed molecular encapsulation of CDC in lipid matrix of stearic acid.

CONCLUSIONS

The present work mainly involved the development and characterization of CDC-loaded SLN, using stearic acid as main encapsulating lipid, stabilized with poloxamer 188. The “modified emulsification-ultrasonication technique” was successful to prepare SLNs with high entrapment efficiency and satisfactory drug loading. Our approach showed that the release profile can be sustained up to 24 h in comparison to pure drug. Hence, SLNs of CDC could be a novel formulation in treatment of hypertension due to more release of drug from solid matrix, more stability of encapsulated drug, and sustained release patterns over an extended period of time. SLN formulations can provide constant and prolonged therapeutic effects as evident from \textit{in vivo} pharmacokinetic results. Hence, these systems offer the possibility to develop well-tolerated oral drug delivery formulations.

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AUTHOR’S CONTRIBUTION

This research work is done by the authors, and the authors are responsible for the content and writing of the paper.

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

27. Bhandari R, Kaur IP. Pharmacokinetics, tissue distribution and relative

Table 3: Stability studies of SLN 3 at different temperatures

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial 0 day</th>
<th>After 30 days</th>
<th>After 60 days</th>
<th>After 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5±3°C</td>
<td>7.4±0.41</td>
<td>7.3±0.44</td>
<td>7.2±0.74</td>
<td>7.2±0.58</td>
</tr>
<tr>
<td>25±2°C</td>
<td>7.2±0.23</td>
<td>7.2±0.69</td>
<td>7.1±0.85</td>
<td>7.0±0.55</td>
</tr>
<tr>
<td>Particle size (nm)</td>
<td>197.9</td>
<td>439.6</td>
<td>531.6</td>
<td>736.5</td>
</tr>
<tr>
<td>25±2°C</td>
<td>197.9</td>
<td>661.4</td>
<td>713.2</td>
<td>841.6</td>
</tr>
<tr>
<td>Drug entrapment (%)</td>
<td>100</td>
<td>98.45±0.87</td>
<td>96.69±0.23</td>
<td>97.1±0.44</td>
</tr>
<tr>
<td>25±2°C</td>
<td>100</td>
<td>94.52±0.89</td>
<td>93.65±0.76</td>
<td>91.4±0.20</td>
</tr>
</tbody>
</table>

SLN: Solid lipid nanoparticle.


