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In Vivo Evaluation of A Self Nanoemulsifying Drug Delivery System for Lercanidipine HCL

J. Venkateswara Rao[*1], T Rama Mohan Reddy[2]
1.Research Scholar, Mewar University, Chittorgarh, Rajasthan, India.
2.Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India

ABSTRACT

The present study aimed at improvement of solubility and bioavailability of Lercanidipine HCl using self-nanoemulsifying drug delivery systems (SNEDDS). The extent of self-emulsification was checked with various oils with suitable surfactants and co-surfactants. The final optimized formulation contained Caproyl 90, Tween 80 and Labrosol as oil, surfactant and co-surfactant respectively. Based on Lercanidipine solubility analysis, ternary phase diagrams were constructed for optimizing the system. The formulations were evaluated for solubility, droplet size determination, zeta potential and stability studies. The droplet size was found to be 5.1 nm & Z-Average of 14.6 nm. The zeta potential of the optimized formulation (F16) was found to be -19.7 mV. In vitro drug release from SNEDDS was significantly higher than pure drug. From in vivo bioavailability studies the optimized formulation was exhibited a significantly greater C_{max} (56.2±0.04ng/ml) than the pure drug suspension (35.1±0.03ng/ml). $AUC_{0-\infty}$ for SNEDDS formulation was higher (190.5±2.04 ng.h/ml) than the pure drug suspension 145.7±2.02ng.h/ml. Statistically, AUC_{0-t} of the SNEDDS formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. The study demonstrated that SNEDDS was a promising strategy to enhance the solubility and oral bioavailability of Lercanidipine.

Keywords: Lercanidipine, Hypertension, Labrosol, Bioavailability, Self-nano emulsifying drug delivery system.

*Corresponding Author Email venkateswararao.j@gmail.com
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INTRODUCTION

Poor solubility of the drug molecules is difficult to formulate by applying conventional approaches as they pose problems such as slow onset of action, poor oral bioavailability, lack of dose proportionality, failure to achieve steady state plasma concentration, and undesirable side effects, thus resulting in over or under medication and poor patient compliance[1]. These challenges can be overcome by applying self-nanoemulsifying systems that offer benefits like reduction in dose frequency, lowering of dose size, site specific targeting, enhanced permeability, and improvement in oral bioavailability[2]. Nanotechnology is a promising strategy in the development of drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility, low permeability, inadequate bioavailability, and other poor biopharmaceutical properties[3]. SNEDDS formulations for poorly water-soluble drugs have shown considerable increase in solubility and bioavailability[4].

Lercanidipine is an anti-hypertensive agent used in patients with high blood pressure, chronic stable angina pectoris and prinzmetal's variant angina. It is a dihydropyridine calcium channel blocker used alone or along with angiotensin-converting enzyme inhibitor (ACE Inhibitor) to treat hypertension and angina together[5]. Anti-hypertensive drugs help in preventing the cardiovascular morbidity and mortality. Lercanidipine also has nephroprotective effect[6].

Lercanidipine has a plasma half-life of about 8-10 hours[7]. It has a high membrane partition coefficient and remains at the membrane for a long period of time, thus providing long lasting effect at the receptor and membrane levels[8]. Lercanidipine is a drug with very poor water solubility and bioavailability. To increase the solubility and oral absorption of crystalline Lercanidipine HCl, SNEDDS technique is employed to change its form. The solubility of Lercanidipine HCL is 0.05189±0.0023 mg/ml[9]. In the present study SNEDDS of Lercanidipine were formulated and evaluated for, zeta potential, Scanning electron microscopy, particle size and *in vivo* bioavailability studies.

MATERIALS AND METHOD

Lercanidipine HCl was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad. Caproyl 90 and Gelucire 44/14 were procured from Gattefose France. Peceol and Castor oil were obtained from Croda Chemicals. Capmul MCM C8 was obtained from Strides Arcolab, Bangalore, India. Glycerol and Tween 80 were obtained from Loba Chemie Pvt Ltd, Mumbai. Propylene Glycol was obtained from Suvidinath Laboratories, Baroda, India. Cremophor EL was obtained from Signet Chemicals Corporation Pvt. Ltd. Mumbai, India. Transcutol P and Labrasol were procured from

Gattefosse India. PEG 400 was obtained from Otto Chemie Pvt. ltd. Mumbai, India. Lauroglycol 90 was obtained from Ranbaxy Laboratories India. Captex 200 and Caprol PGE- 860 were procured from Abitec Ltd. Janesville. Span 20 was obtained from Sigma Aldrich, USA. Miglyol 810 N was procured from Sassol and Crodamal GTCC from Dr Reddy's Laboratories, India. Capmul PG 12 EP/NF was obtained from Ind Chem International c/o Abitec Corporation USA.

Solubility Studies

The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Lercanidipine. An excess amount (10 mg) of Lercanidipine was added into 2 ml of each excipient (Oils - Capmul MCM C8, Castor oil, Peceol, Crodamal GTCC, and Capryol 90). Surfactants – (Tween 80, Transcutol P, Propylene glycol, Glycerol, Capmul PG - 12EP/NF, Gelucire 44/14, Miglyol 810 N). Co-surfactants (Caprol PGE -860, Labrasol, Captex 200, Span 20, Cremophor EL, Lauroglycol 90, PEG 400) and kept in mechanical shaker for 24hrs and centrifuged at 10,000 rpm for 20 min using a centrifuge. Supernatant was filtered through membrane filter using 0.45µm filter disk. Filtered solution was appropriately diluted with methanol, and UV absorbance was measured at 238 nm. Concentration of dissolved drug was determined spectrophotometrically[10].

Pseudo Ternary Phase Diagram

To determine the concentration of components for the existing range of SNEDDS, pseudo ternary phase diagram was constructed using water titration method at ambient temperature (25^0C). Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratio (1:1, 2:1, 3:1). Oil and surfactant/co-surfactant mixture (Smix) were mixed thoroughly in different volume ratios 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) w/w for all the three Smix ratios 1:1,2:1, 3:1. The mixture of oil, surfactant and co-surfactant at certain ratios were titrated with water by drop wise addition under gentle agitation. Deionized water was used as diluting medium and added into the formulation. The proper ratio of one excipient to another in the SNEDDS formulation was analyzed. Pseudo ternary plots were constructed using Chemix software[11].

Visual Observation

A visual test to assess the self-emulsification properties was modified and used in the present study. With the use of this method, a predetermined volume of mixture (0.2 ml) was added to 300 ml of water in a glass beaker under stirring and temperature was maintained at 37^0C using a magnetic stirrer. The tendency of formation of emulsion was observed. If the droplet spreads easily in water was judged as 'good' and judged as 'bad' when there was milky or no emulsion or presence of oil droplets[12].

Development of SNEDDS Formulation

A series of SNEDDS formulations for Lercanidipine were prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Here, Capryol 90 was used as oil phase Tween 80 and Labrasol were used as surfactant and co-surfactant respectively. 10 mg of Lercanidipine was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40ºC. The surfactant and co-surfactant were added to the oily mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15mins and stored at room temperature until its use in subsequent studies (Table 1).

Table 1: Formulation trials of liquid SNEDDS

Smix (Surfactant :Co-surfactant)	Oil:Smix	Formulation code	Drug(Lercanidipine) (mg)	Oil(Capryol 90) (ml)	Surfactant(Tween 80) (ml)	Cosurfactant (Labrasol) (ml)
1:1	4:6	F1	10	0.6	0.45	0.45
	5:5	F2	10	0.75	0.375	0.375
	6:4	F3	10	0.9	0.3	0.3
	7:3	F4	10	1.05	0.225	0.225
	8:2	F5	10	1.2	0.15	0.15
	9:1	F6	10	1.35	0.075	0.075
2:1	1:9	F7	10	0.15	0.9	0.45
	2:8	F8	10	0.3	0.8	0.4
	3:7	F9	10	0.45	0.7	0.35
	4:6	F10	10	0.6	0.6	0.3
	5:5	F11	10	0.75	0.5	0.25
	6:4	F12	10	0.9	0.4	0.2
3:1	3:7	F13	10	0.45	0.787	0.262
	4:6	F14	10	0.6	0.675	0.225
	5:5	F15	10	0.75	0.562	0.187
	6:4	F16	10	0.9	0.45	0.15
	7:3	F17	10	1.05	0.337	0.112
	8:2	F18	10	1.2	0.22	0.075

Freeze Thawing (Thermodynamic Stability Studies)

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variations on SNEDDS formulations.

Freeze Thawing

The main objective of this study was to evaluate the phase separation and effect of temperature variations on SNEDDS formulations. Formulations were subjected to freeze cycle (-20°c for 2days followed by 40°C for 2days) and stable formulations were further studied[13].

Centrifugation

Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies[14].

% Transmittance Measurement

The percent transmittance of various SNEDDS formulations was measured at 238 nm using UV spectrophotometer keeping water as a blank[15].

Determination of Drug Content

SNEDDS equivalent to 2mg of Lercanidipine were weighed accurately and dissolved in 100 ml 0.1N Hcl. The solution was filtered, diluted suitable and drug content was analyzed at λ_{max} 238 nm against blank by UV spectrometer.

***In - Vitro* Dissolution Studies**

The release of drug from liquid SNEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. SNEDDS of Lercanidipine (equivalent to 2 mg of Drug) was filled in size "0" hard gelatin capsules. The dissolution media is 0.1N HCl and temperature of the dissolution medium was maintained at 37^0C operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals 2, 5, 10, 15, 20, 25, 30, 45, and 60 mins and filtered through 0.45-µm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh medium. The concentrations were assayed spectrophotometrically at 238 nm.

Characterization of SNEDDS

Determination of Droplet Size

The average droplet size of Lercanidipine SNEDDS formulations were determined by Photon correlation spectroscopy (Malvern Instrument UK) able to measure sizes between 10 and 5000 nm[16].

Determination of Zeta Potential

The SNEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta-potential of the resulting micro emulsion was determined using a Zetasizer[17].

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[18].

Percent Entrapment Efficiency

The contents of free drug were separated from nano emulsion by ultrafiltration at 3500 Da with centrifugation at 3000g for 5 to 10 minutes, followed by quantification using HPLC method[19].

Stability Studies

Stability testing was conducted at 40˚C ± 2˚C/75% RH ± 5% RH for 3 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 60 and 90 days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated[20].

Pharmacokinetic study of Lercanidipine

Animals:

Healthy Wistar rats were (Weighing 150-180 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee (**IAEC NO: IAEC/1657/CMRCP/T2/Ph D-16/70).**

Study Design:

Rats were divided in to two groups at random. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group was administered with pure Lercanidipine (as such) made suspension with 0.5% methocel and second group was administered Prepared Lercanidipine SNEDDS diluted in 0.5% methocel by oral route at a dose of 10mg/kg. Then, 500 µL blood samples were collected from the femoral artery at certain times 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 5, 6, 8, 12, 16, 20, 24h post dose and transferred into Eppendorf tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at −20°C until analysis.

Determination of Lercanidipine in Rat plasma by HPLC method[21]

Determination of Lercanidipine and internal standard Atenolol was carried out by using Symmetry C8 (4.6×250 mm, 5µm) column with the ACN/phosphate buffer (60:40, v/v, pH 3.6) as mobile phase, at a flow rate of 0.5ml/min. Detection was carried out at 235 nm. The retention time of Lercanidipine and Atenolol (internal standard) were found to be 5.97 and 2.27 min respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max} and $t_{½}$ values, area under plasma concentration–time curve from zero to the last sampling time ($AUC_{0\text{-}t}$), area under plasma concentration–time curve from zero to infinity ($AUC_{0\text{-}\infty}$). $AUC_{0\text{-}t}$ was calculated by the linear trapezoidal rule and $AUC_{0\text{-}\infty}$ from the following formula

$$AUC_{0-\infty} = AUC_{0-t} + C_t / K_E$$

RESULTS AND DISCUSSION

Solubility Studies

Preliminary solubility analysis was carried out to select the appropriate excipient from various (Oils - Capmul MCM C8, Castor oil, Peceol, Crodamal GTCC, and Capryol 90). Surfactants – (Tween 80, Transcutol P, Propylene glycol, Glycerol, Capmul PG -12EP/NF, Gelucire 44/14, Miglyol 810 N). Co-surfactants (Caprol PGE -860, Labrasol, Captex 200, Span 20, Cremophor EL, Lauroglycol 90, PEG 400). The solubility of pure drug was found to be 0.05189±0.0023 mg/ml. Based on drug solubility, Capryol 90, Tween 80, Labrasol, were selected as oil, surfactant and co-surfactant respectively. The drug solubility values of these polymers were found to be highest when compared with the pure drug and other polymers. (Figure 1, 2 & 3).

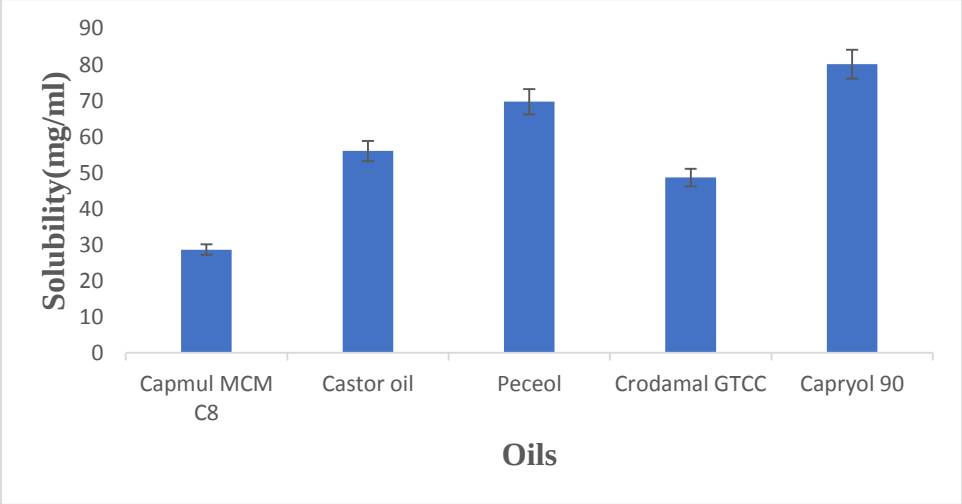

Figure 1: Solubility studies of Lercanidipine in oils

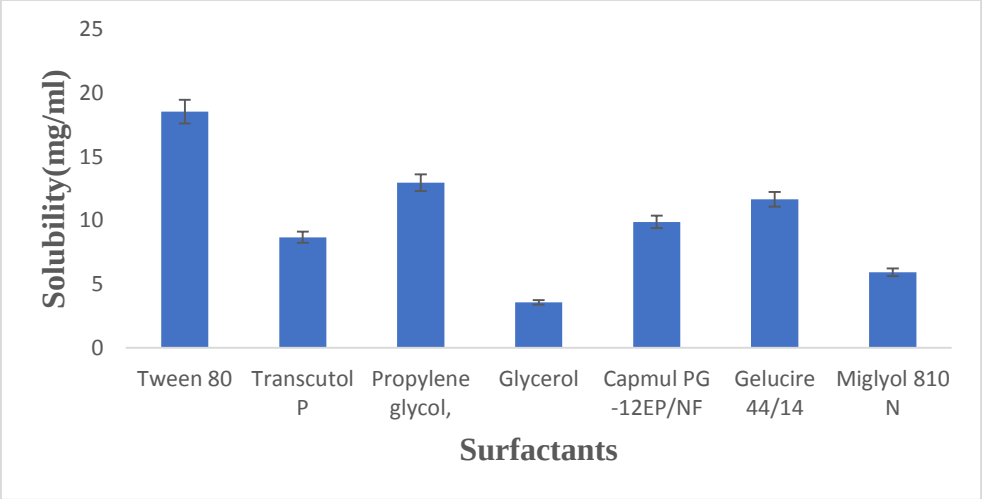

Figure 2: Solubility studies of Lercanidipine in surfactant

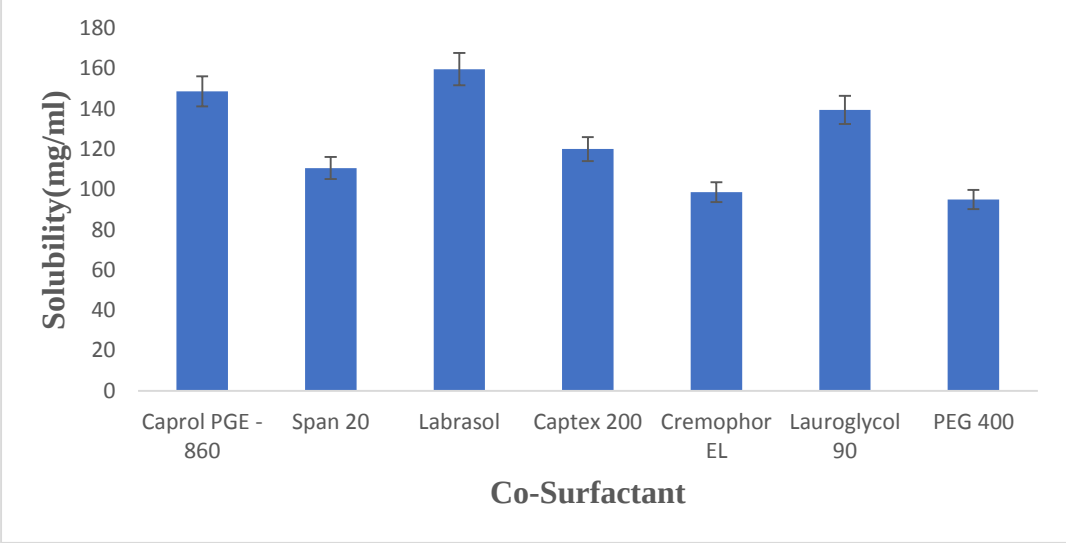

Figure 3: Solubility studies of Lercanidipine in co-surfactants

Pseudo Ternary Phase Diagram

From the solubility studies, Capryol 90, Tween 80 and Labrasol were selected as oil, surfactant and co-surfactant respectively. From the phase diagram (Figure 4) it was observed that self emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased.

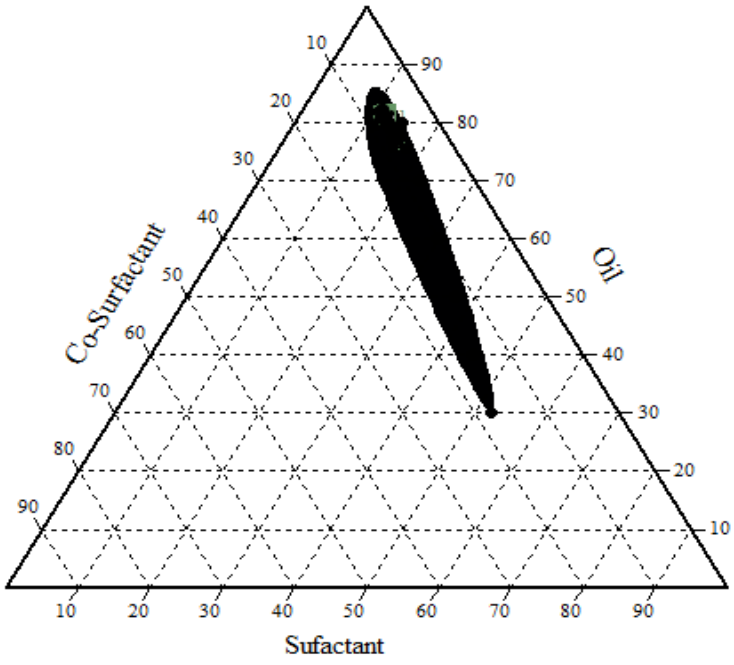

Figure 4: Ternary phase diagram of Capryol 90, Tween 80, Labrasol

Visual Observation

With the use of visual observation method, the tendency of formation of emulsion was observed. Visual observation test was performed for different ratios by keeping the surfactant and co-surfactant ratio (Smix) as 1:1, 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of micro-emulsion. Ratios 4:6, 5:5, 6:4 and 7:3 of Smix 1:1 and 1:9, 2:8, 3:7, 4:6, 5:5 of Smix 2:1 and 9:1, 2:8, 3:7, 4:6, 5:5 of Smix 3:1 showed rapid formation of micro emulsion within a minute having a clear appearance. Therefore, these ratios were selected for the formulation of SNEDDS. (Tables 2, 3, 4)

Table 2: Visual observation test for Smix (Surfactant: Co-surfactant) ratio 1:1

Oil: Smix	Time of self-emulsification (min)	Grade
1:9	<1	I
2:8	<1	I
3:7	<2	III
4:6	<1	I
5:5	<2	I/ II
6:4	<1	I
7:3	<1	I
8:2	<2	I/ II
9:1	<2	III

Table 3: Visual observation test for Smix (surfactant: co-surfactant) ratio 2:1

Oil: Smix	Time of self emulsification (min)	Grade
1:9	<2	III
2:8	<1	I
3:7	<2	I/ II
4:6	<2	I/ II
5:5	<1	I
6:4	<2	III
7:3	<1	I
8:2	<2	I/ II
9:1	<2	III

Table 4: Visual observation test for Smix (surfactant: co-surfactant) ratio 3:1

Oil: Smix	Time of self emulsification (min)	Grade
1:9	<2	I/ II
2:8	<2	I/ II
3:7	<1	I
4:6	<1	I
5:5	<1	I
6:4	<2	III
7:3	<1	I
8:2	<2	III
9:1	<2	I

Thermodynamic Stability Studies

No phase separation and effect of temperature variations on prepared formulations were observed during thermodynamic stability studies. There was no change in the visual description of samples after centrifugation freeze-thaw cycles. Formulations which are thermodynamically stable only those were selected for further characterization.

% Transmittance Measurement

The clarity of microemulsion was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w microemulsion since water is external phase Formulation F16 has % transmittance value greater than 98%. These results indicate the high clarity of microemulsion. In case of other systems %T values were less than 99% suggesting less clarity of microemulsion. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T.

Drug Content of SNEDDS

The drug content of the prepared SNEDDS was found to be in the range of 88.37 – 98.63 %. Maximum % drug content i.e. 98.63% was found in the formulation F16.

***In-Vitro* Dissolution Studies of SNEDDS**

The faster dissolution from SNEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The release from liquid SNEDDS formulation F16 was faster and higher than other SNEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution. (Figure 5, 6 and 7).

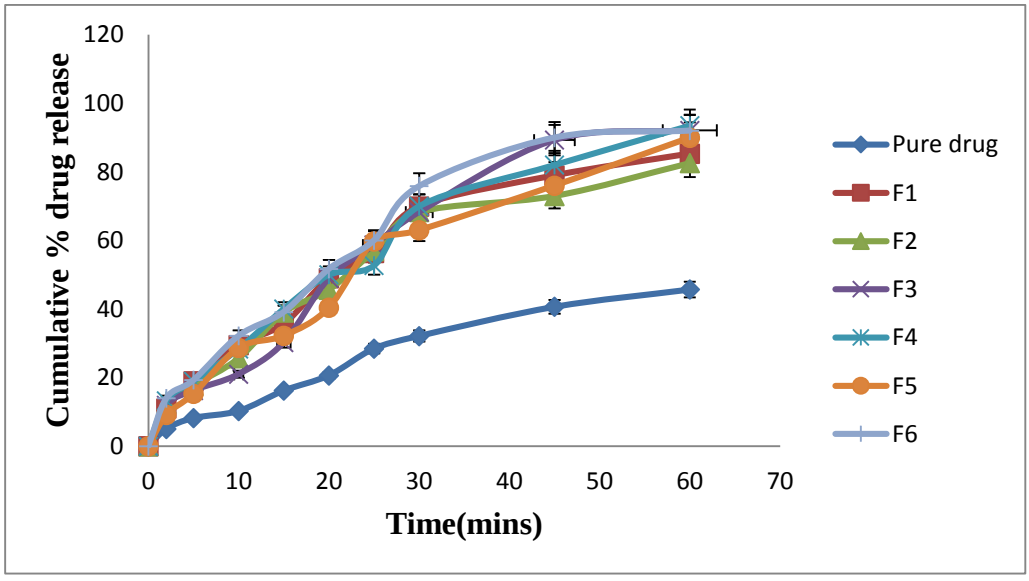

Figure 5: Dissolution profiles of Lercanidipine pure drug and formulations (F1 to F6)

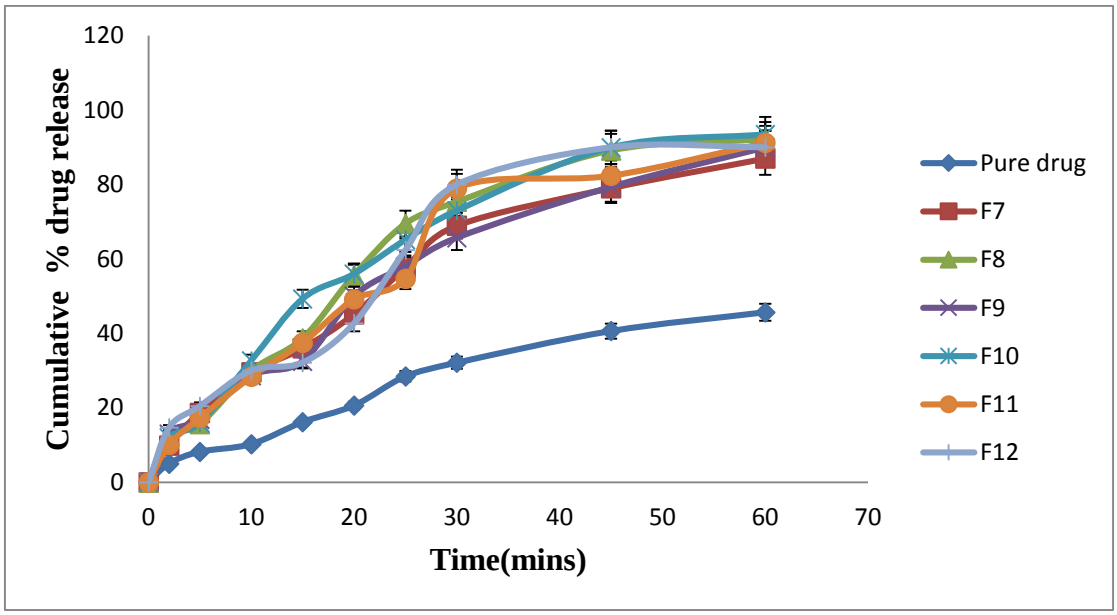

Figure 6: Dissolution profiles of Lercanidipine pure drug and formulations (F7 to F12)

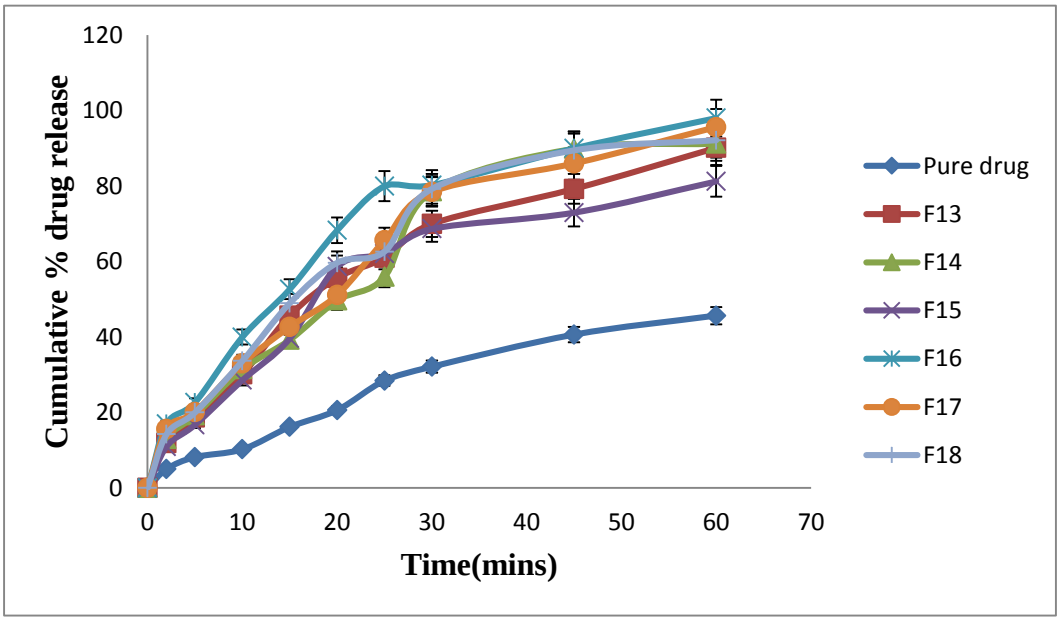

Figure 7: Dissolution profiles of Lercanidipine pure drug and formulations (F13 to F18)

Particle Size Analysis of SNEDDS

Droplet size determines the rate and extent of drug release as well as drug absorption. Smaller the particle size, larger the interfacial surface area which may lead to more rapid absorption and improved bioavailability. SNEDDS with a mean droplet size below 200 nm exhibit excellent bioavailability. The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The

particle size of the optimized SNEDDS formulation (F16) was found to be 5.1 nm & Z-Average of 14.6 nm indicating all the particles were in the nanometer range. (Figure 8).

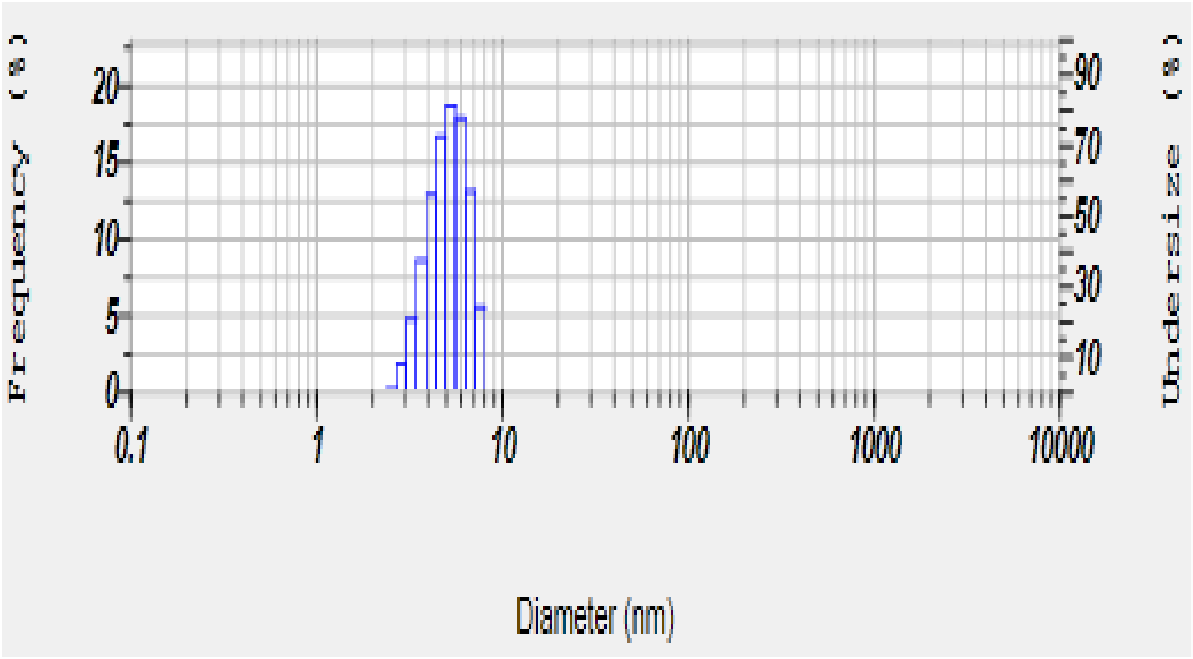

Figure 8: Particle size analysis of optimized formulation F16

Zeta Potential of SNEDDS

Zeta potential is responsible for the degree of repulsion between adjacent, similarly charged, dispersed droplets. A zeta potential value of ±30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SNEDDS formulation (F16) was found to be -19.7 mV which comply with the requirement of the zeta potential for stability. (Figure 9)

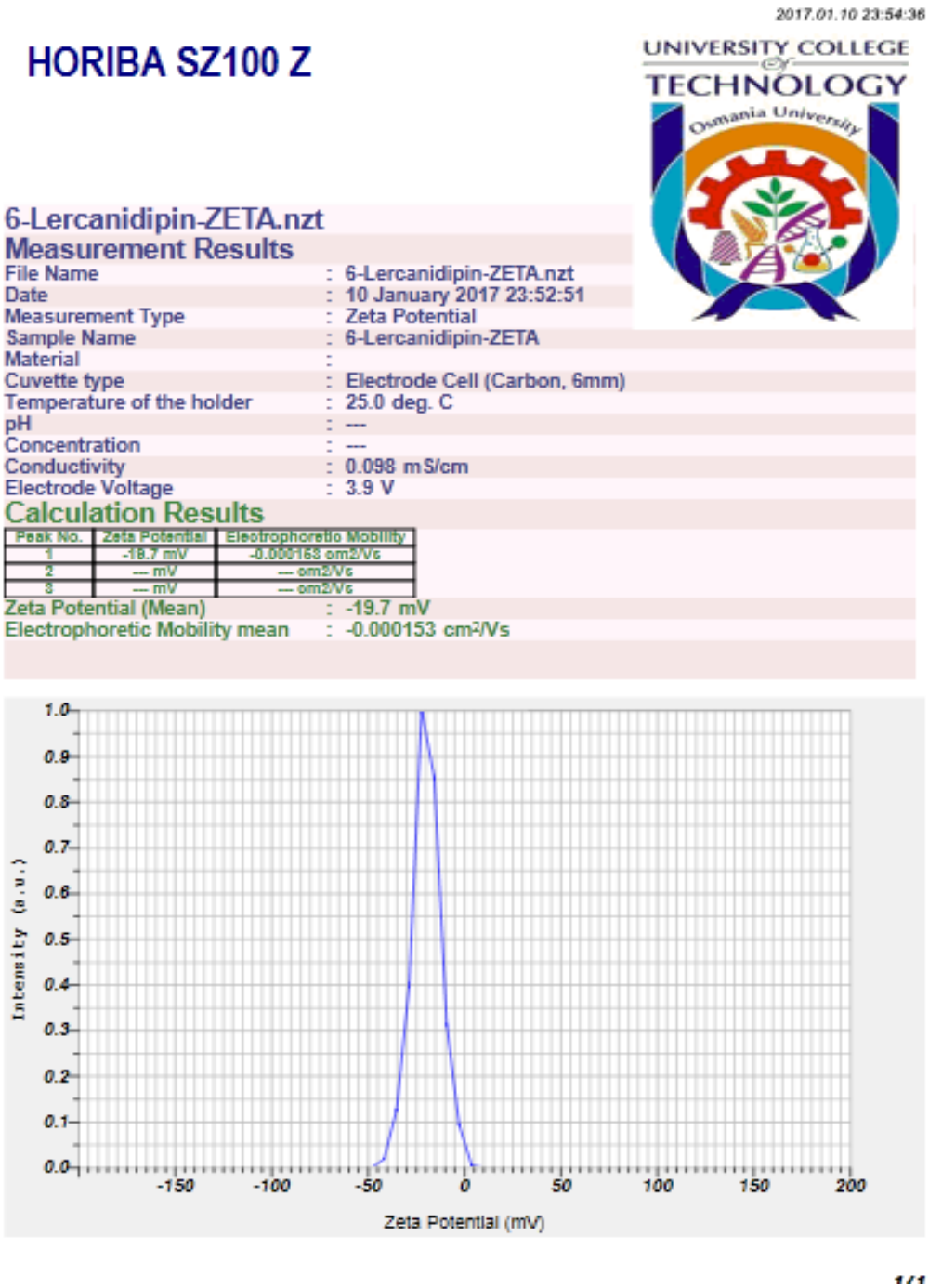

Figure 9: Zeta potential of the optimized formulation F16

Scanning Electron Microscopy (SEM) For Rosuvastatin SNEDDS

Scanning electron microscope studies of optimized formulation of Lercanidipine (F16) revealed oval shaped globules. The size is within nanometers. There are clear liquid droplets without any pores (Figure 10).

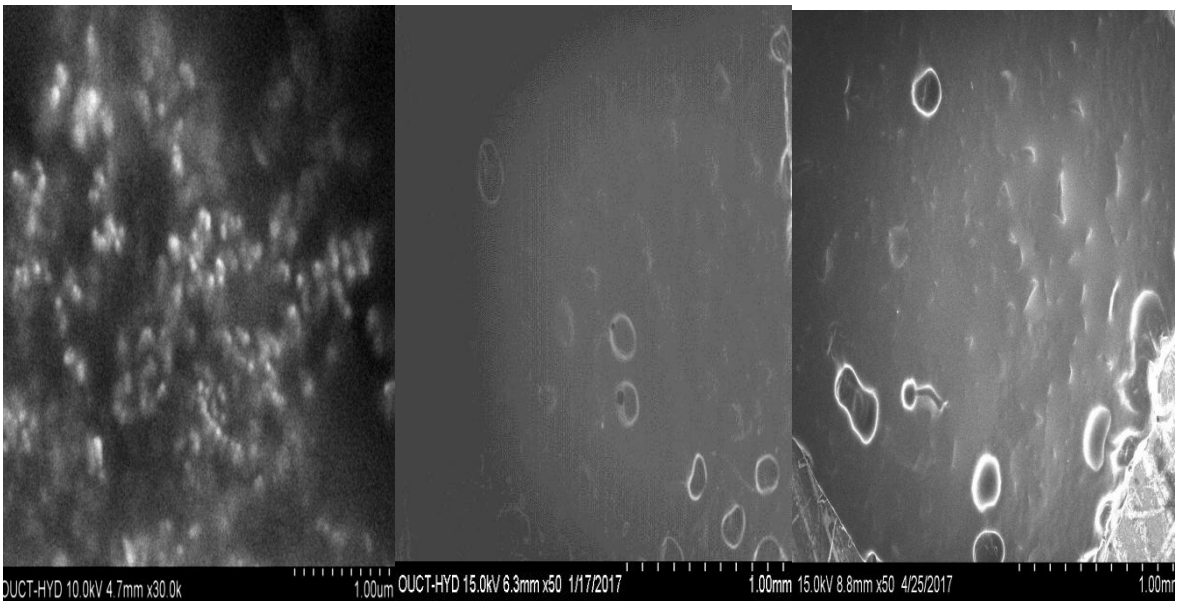

Figure 10: Scanning Electron Microscopy of Lercanidipine optimized formulation (F16)

Stability Studies

The Lercanidipine SNEDDS F16 formulation was filled in hard gelatin capsules as the final dosage form and subjected to stability studies for 6 months. There was no significant change in the drug content and drug release. It was also seen that the formulations were compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance, or micro emulsifying property.

***In vivo* bioavailability studies:**

The results of plasma concentration–time curve in Wistar rats after a single oral dose of Lercanidipine SNEDDS formulation as compared to Lercanidipine pure suspension are shown in Figure 11. At all the indicated time points, the Lercanidipine plasma concentrations in rats treated with SNEDDS formulation was significantly higher than those treated with pure drug. Pharmacokinetic parameters of Lercanidipine after oral administration of the two formulations in Wistar rats are shown in Table 5.

Table 5: Pharmacokinetic Parameters of Lercanidipine SNEDDS formulation and pure drug

Pharmacokinetic parameters	Lercanidipine Pure drug	Lercanidipine SNEDDS
C_{max} (ng/ml)	35.1±0.03	56.2±0.04
AUC_{0-t} (ng. h/ml)	102.2±1.02	154.4±2.01
AUC_{0-inf} (ng. h/ml)	145.7±2.02	190.5±2.04
T_{max} (h)	1.50±0.03	1.00±0.01
$t_{1/2}$ (h)	3.50±0.02	2.62±0.02

C_{max} of the SNEDDS 56.2±0.04ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 35.1±0.03ng/ml. T_{max} of both SNEDDS formulation and pure drug suspension was 1.00±0.01 and 1.50±0.03h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for SNEDDS formulation was higher (190.5±2.04ng. h/ml) than the pure drug suspension formulation 145.7±2.02ng. h/ml. Statistically, AUC_{0-t} of the SNEDDS formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of Lercanidipine from SNEDDS formulation as compared to the pure drug suspension formulation.

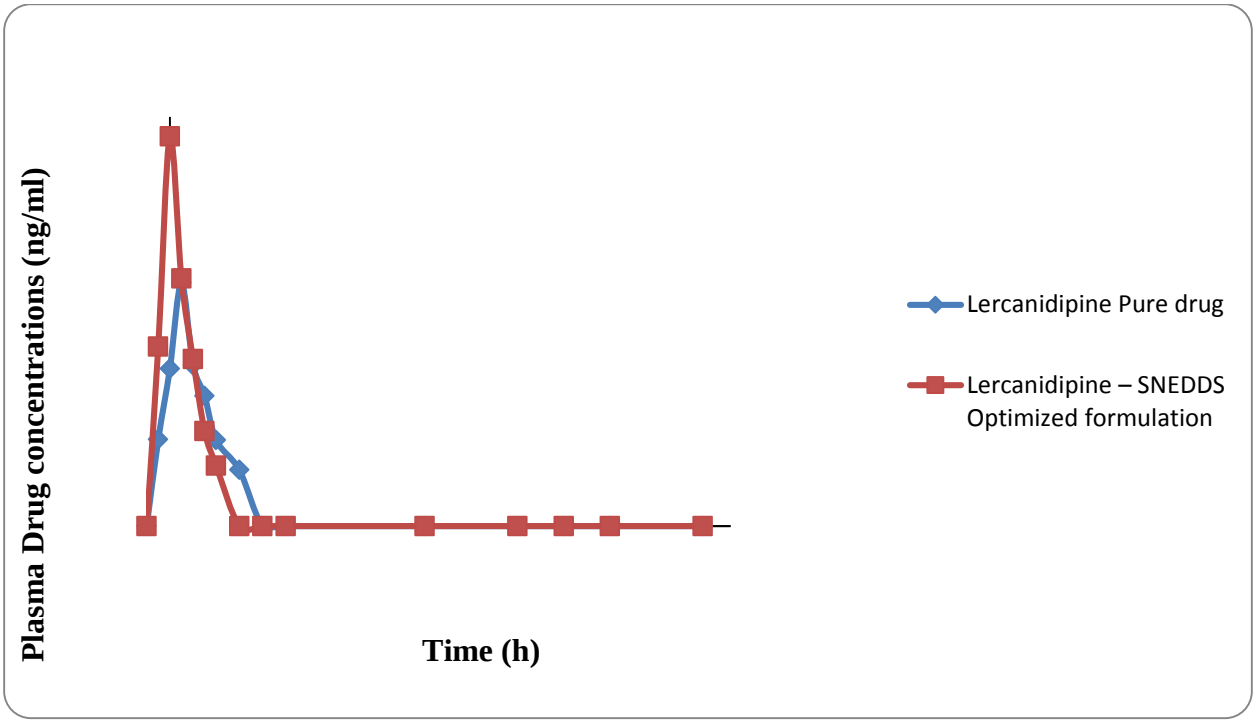

Figure 11: Plasma concentration profiles of Lercanidipine SNEDDS and pure drug

Pharmacokinetic parameters comparison for pure drug suspension and SNEDDS

CONCLUSION

SNEDDS of Lercanidipine comprising of Capryol 90, Tween 80 and Labrasol were prepared for enhancing the dissolution and bioavailability of candesartan. SNEDDS were optimized based on the optimum globule size, increased dissolution and drug release. Close to complete drug release was achieved from the formulation F16 which is significantly higher as compared to that of pure drug.

From In vivo bioavailability studies, the pharmacokinetic parameters in rats indicated that compared to the pure drug, the optimized SNEDDS formulation significantly improved the oral bioavailability of Lercanidipine. Therefore, from our results the study suggests that the Lercanidipine loaded self-nanoemulsifying formulation has a great potential for clinical application. The current investigation of nano emulsion may serve as a promising approach for the formulation development of poorly soluble drug Lercanidipine. Thus, the developed SNEDDS can be used as an effective approach for the management of hypertension with relatively low drug dose with higher solubility and bioavailability.

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