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Self Nanoemulsifying Drug Delivery System of Candesartan Cilexetil with Improved Bioavailability

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ABSTRACT

Candesartan is an angiotensin II receptor blocker with inherent low bioavailability. The present studies entail the optimization and evaluation of self- nanoemulsifying drug delivery system (SNEDDS) of Candesartan in order to enhance its oral bioavailability. For this Capryol 90, Captex 500, Labrasol were used as oil, surfactant and co surfactant respectively. FTIR studies revealed no interaction among the drug and polymers used in the formulation. Based on the physicochemical parameters and in-vitro dissolution studies, F17 prepared with Smix (Surfactant: Co-surfactant) of 3:1 and Oil:Smix of 6:4, was found to be an optimum one. The formulation F17 was found to release 99.12 ± 5.10% drug at the end of one hour and scanning electron microscopic analysis showed nanosized particles. The droplet size of the optimized formulation was found to be 51.7 nm & Z-Average of 59.2 nm. The zeta potential of the optimized formulation (F17) was found to be -15.5 mV. The formulations were also found to be stable over a period of 6 months of testing. From *in vivo* bioavailability studies C_{max} of the SNEDDS 35.2 ± 0.02 ng/ml was significant (p<0.05) as compared to the pure drug suspension formulation 25.1 ± 0.03ng/ml. T_{max} of both SNEDDS formulation and pure drug suspension was 1.00 ± 0.03 h and 2.00 ± 0.01 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, $AUC_{0-\infty}$ infinity for SNEDDS formulation was higher (160.1±1.04ng. h/ml) than the pure drug suspension formulation 135.3 ± 2.02 ng.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 Candesartan from SNEDDS formulation as compared to the pure drug suspension formulation. The results from this study suggest the requirement for potential use of candesartan as self-nanoemulsifying drug delivery systems.

Keywords: Candesartan, Hypertension, SNEDDS, Caproyl 90, Captex, 500, Zeta Potential.

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INTRODUCTION

Lipid based drug delivery has become one of the promising technology over the past decade due to the multiple roles of lipids in enhancing oral bioavailability. The success of these lipid based systems may also be attributed to the development of novel

excipients with acceptable regulatory and safety profiles. [1] Depending upon the excipients and formulation techniques, a variety of systems such as physical mixture, liquid/ solid solutions, solid dispersions, self-micro and self-nanoemulsifying drug delivery systems (SMEDDS/ SNEDDS) may be developed. [2-3]

Candesartan cilexetil is a prodrug which has poor bioavailability because of poor solubility and belongs to BCS Class II. It is practically insoluble in water and has a partition coefficient of 9.8 at pH 7.4 (According to the BCS classification developed by Amidon in conjugation with FDA guidance). [4] Candesartan cilexetil is an antihypertensive drug used in the treatment of hypertension. Due to its low solubility, it is required to be administered in high doses for therapeutic effectiveness. [5] Candesartan has a low bioavailability of only 15% and is weakly acidic in nature. [6] Lipids are known to promote the transport of drugs through lymphatic route. [7] The possible mechanisms for this are increased transcellular absorption by increasing membrane fluidity, enhancing the paracellular transport by opening the tight junction, inhibiting the P-glycoprotein and cytochrome P450 to increase the intracellular concentration and formation of lipoprotein or chylomicron by phospholipid. [8] Hence, theoretically one can assume an increase in oral bioavailability of Candesartan cilexetil.

In the present study SNEDDS of candesartan cilexetil were formulated and evaluated for *in vitro* drug release, FTIR, zeta potential, Scanning electron microscopy and particle size.

MATERIALS AND METHODS

Candesartan cilexetil was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad. Caproyl 90, Labrafil 1944 Cs and Labrafil 2125 were procured from Gattefose France. Sesame oil was obtained from Dr Reddy's Laboratories, India. Peceol and Castor oil were obtained from Croda Chemicals. Capmul MCM C8 was obtained from Strides Arcolab, Bangalore, India. Brij 35 was procured from Ind Chem International c/o Abitec Corporation, USA. Glycerol and Captex 500 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. Tween 80 was obtained from Sigma Aldrich, USA. 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 Ethanol were procured from Abitec Ltd. Janesville.

Methods

Solubility studies

The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Candesartan. An excess amount (2 mg) of Candesartan was added into 2 ml of each excipients (Oils -Capmul MCM C8, Castor oil, Capryol 90, Peceol, and Sesame oil,). Surfactants – (Captex 500, Cremophor EL, Propylene glycol, Glycerol, Brij 35, Labrafil 2125 and Labrafil 1944Cs). Co-surfactants (Ethanol, Labrasol, Captex 200, Transcutol P, Tween 80, Lauroglycol 90 and PEG 400) and kept in mechanical shaker for 24 hours 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 257 nm. Concentration of dissolved drug was determined spectrophotometrically. [9]

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°C). 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. Pseudo ternary plots were constructed using Chemix software. 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. [10]

Visual observation

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°C using a magnetic stirrer. The tendency of formation of emulsion was observed. If the droplet spreads easily in water, it was judged as 'good' and judged as 'bad' when there was milky or no emulsion or presence of oil droplets. By performing this test one can assess the self-emulsification property of the final formulation. [11]

Development of SNEDDS formulation

Based on solubility studies, pseudo ternary phase diagram and visual observation, various formulations of Candesartan SNEDDS were prepared. Here, Capryol 90 was used as oil phase, Captex 500and Labrasol were used as surfactant and co-surfactant respectively. The composition was shown in Table 1. Candesartan was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40°C. 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 15 min and stored at room temperature.

Freeze Thawing (Thermodynamic Stability Studies)

Formulations were subjected to freeze cycle (-20°C for 2 days followed by 40°C for 2 days). Only stable

formulations were selected for further studies. [12] The main objective of this study was to evaluate the phase separation and effect of temperature variations on SNEDDS formulations.

Table 1: Formulation trials of Candesartan SNEDDS

Smix (Surfactant: Co-surfactant)	Oil : Smix	Formulation code	Drug Candesartan) (mg)	Oil (Capryol 90) (ml)	Surfactant (Capetex 500) (ml)	Co-surfactant (Labrasol) (ml)
1:1	1:9	F1	8	0.15	0.675	0.675
	2:8	F2	8	0.3	0.6	0.6
	3:7	F3	8	0.45	0.525	0.525
	4:6	F4	8	0.6	0.45	0.45
	5:5	F5	8	0.75	0.375	0.375
	6:4	F6	8	0.9	0.3	0.3
2:1	4:6	F7	8	0.6	0.6	0.3
	5:5	F8	8	0.75	0.5	0.25
	6:4	F9	8	0.9	0.4	0.2
	7:3	F10	8	1.05	0.3	0.15
	8:2	F11	8	1.2	0.2	0.1
	9:1	F12	8	1.35	0.1	0.05
3:1	2:8	F13	8	0.3	0.9	0.3
	3:7	F14	8	0.45	0.787	0.262
	4:6	F15	8	0.6	0.675	0.225
	5:5	F16	8	0.75	0.5625	0.187
	6:4	F17	8	0.9	0.45	0.15
	7:3	F18	8	1.05	0.3375	0.1125

Centrifugation

Centrifugation was performed at 3000 rpm for 5 minutes and observed for phase separation. Stable formulations without any phase separation were selected for further studies. [13]

% Transmittance measurement

Various SNEDDS formulations were reconstituted with distilled water and the percent transmittance was measured at 243 nm using UV spectrophotometer against water as a blank. [14]

Determination of drug content

SNEDDS equivalent to 2 mg of Candesartan were weighed accurately and dissolved in 100 ml of 0.1N HCl the solution was filtered, diluted suitable and drug content was analyzed at λ_{max} 257 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in SNEDDS}}{\text{Theoretical amount of drug in SNEDDS}} \times 100$$

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 Candesartan equivalent to 8 mg was filled in size "0" hard gelatin capsules. The dissolution media 0.1N HCl and temperature of the dissolution medium was maintained at 37°C 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 0.45µm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh medium. The concentrations were assayed spectrophotometrically at 257 nm.

Characterization of SNEDDS

FTIR studies

Analysis of Candesartan pure drug and physical mixtures of the drug with the excipients were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each the spectrum, 8 scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 400–4000 cm^{-1}. An FTIR-8400S Spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients.

Determination of droplet size

Photon correlation spectroscopy technique was used to determine the average droplet size of the optimized formulation. (Malvern Instrument UK). It is used to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement. [15]

Determination of Zeta Potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SNEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SNEDDS formulation was measured using a zeta meter system. 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. [16]

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was applied to assess the shape and surface morphology of microspheres. 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). [17]

Percent entrapment efficiency

The contents of free drug were separated from nanoemulsion by ultrafiltration at 3500 Da with centrifugation at 3000g for 5 to 10 minutes, followed by qualification using HPLC method. [18] The Entrapment Efficiency was calculated as follows.

$$\text{Entrapment Efficiency} = \frac{\text{Total amount of drug in SNEDDS}}{\text{Total weight of ingredients in nanoemulsion}} \times 100$$

Stability studies

Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated during stability testing. It was carried out at 40°C ± 2°C/75% RH ± 5% RH for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 90 and 180 days period according to ICH guidelines. [19]

Pharmacokinetic study

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°C, 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*. [20-21] 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 and each group contains 6 rats. 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 Candesartan (as such) made suspension with 0.5% methocel and second group was administered Prepared Candesartan SNEDDS diluted in 0.5% methanol by oral route at a dose of 10 mg/kg. Then, 500µL 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, 24 h 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 5 min to 10 minutes and stored frozen at −20°C until analysis. [22]

Determination of Candesartan in Rat plasma by HPLC method

Determination of Candesartan cilexetil and internal standard hydrochlorothiazide was carried out by using the chromatographic separation was achieved on Hibar C18 stationary phase (150 mm × 4.6 mm i.d; 5µ) with mobile phase containing phosphate buffer- acetonitrile (55:45) adjusted to pH 4.6 using ortho phosphoric acid was used and injection volume of 20µL, with a flow rate of 1.0 ml/min. and effluents were monitored at 244 nm. The retention times of Candesartan cilexetil and hydrochlorothiazide were 2.6 min and 3.6 min, respectively. [23]

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-t}), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$). AUC_{0-t} was calculated by the linear trapezoidal rule and $AUC_{0-\infty}$ from the following formula

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

Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA) was used to perform the pharmacokinetic parameters. All values are expressed as the mean ± SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was statistically significant.

RESULTS AND DISCUSSIONS

Solubility studies

In the case of SNEDDS initially preliminary solubility analysis was carried out to select the appropriate excipient from various (Oils - Capmul MCM C8, Castor oil, Capryol 90, Peceol, and Sesame oil,). Surfactants – (Captex 500, Cremophor EL, Propylene glycol, Glycerol, Brij 35, Labrafil 2125 and Labrafil 1944Cs); Co-surfactants (Ethanol, Labrasol, Captex 200, Transcutol P, Tween 80, Lauroglycol 90 and PEG 400). The solubility of pure drug was found to be 0.014 mg/ml in water. Based on drug solubility, Capryol 90, Captex 500, 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 (Tables 2, 3, 4 and Figures 1, 2, 3).

Table 2: Solubility studies of Candesartan in various oils

Oils	Solubility (mg/ml)
Capmul MCM C8	55.26 ± 1.05
Capryol 90	80.25 ± 3.28
Castor oil	69.12 ± 3.01
Peceol	45.29 ± 0.99
Sesame oil	60.93 ± 2.73

Table 3: Solubility studies of Candesartan in various surfactants

Surfactants	Solubility (mg/ml)
Glycerol	180.23 ± 2.56
Brij 35	105.1 ± 1.01
Labrafil 2125	120.88 ± 1.56
Labrafil 1944Cs	148.20 ± 1.99
Propylene glycol	98.26 ± 0.98
Captex 500	190.5 ± 2.78
Cremophor EL	152.9 ± 2.01

Table 4: Solubility studies of Candesartan in various co-surfactants

Co-surfactants	Solubility (mg/ml)
Ethanol	109.6 ± 1.99
Labrasol	156.5 ± 3.53
Captex 200	130.2 ± 2.89
Transcutol P	95.60 ± 1.58
Tween 80	120.1 ± 2.05
Lauroglycol 90	89.91 ± 0.85
PEG 400	140.25 ± 3.01

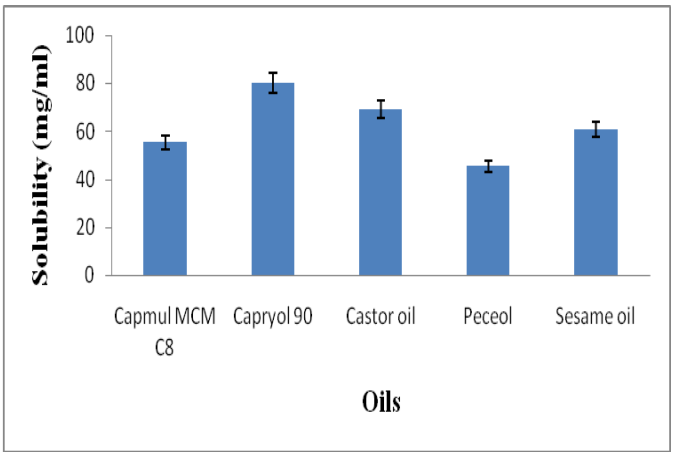

Fig. 1: Solubility studies of Candesartan in oils

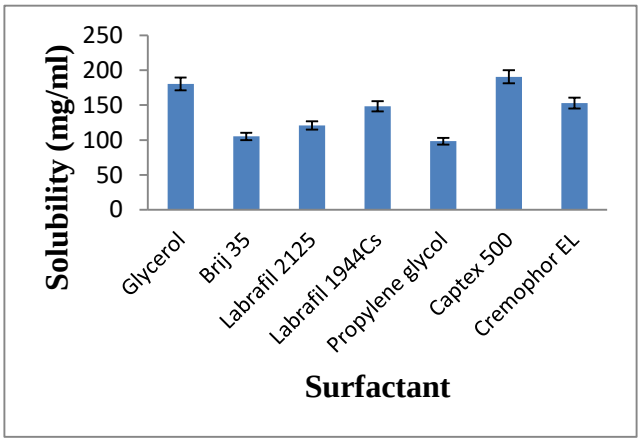

Fig. 2: Solubility studies of Candesartan in surfactant

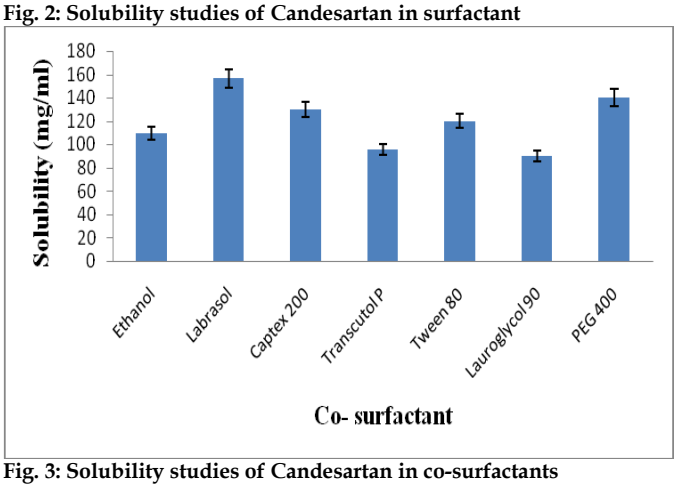

Fig. 3: Solubility studies of Candesartan in co-surfactants

Pseudo ternary phase diagram

From the solubility studies, Capryol 90, Captex 500 and Labrasol were selected as oil, surfactant and co-surfactant respectively. From the phase diagram indicated as 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).

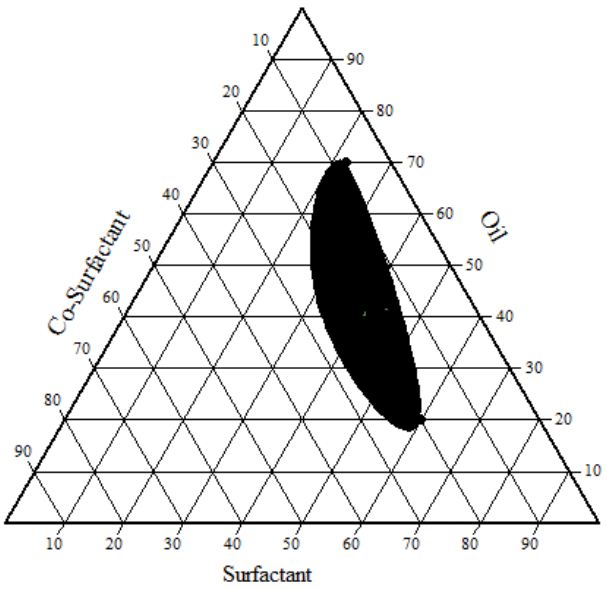

Fig. 4: Ternary phase diagram of Capryol 90, Captex 500 and Labrasol

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 2:8, 3:7, 5:5 and 6:4 of Smix 1:1 and 1:9, 4:6, 5:5, 8:2, 9:1 of Smix 2:1 and 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 5, 6 & 7 and Figures 5, 6).

Table 5: Visual observation test for Smix (Surfactant: Co-surfactant) ratio 1:1

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

Table 6: Visual observation test for Smix (surfactant: co-surfactant) ratio 2:1

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

Table 7: Visual observation test for Smix (surfactant: co-surfactant) ratio 3:1

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

Fig. 5: Visual observation test

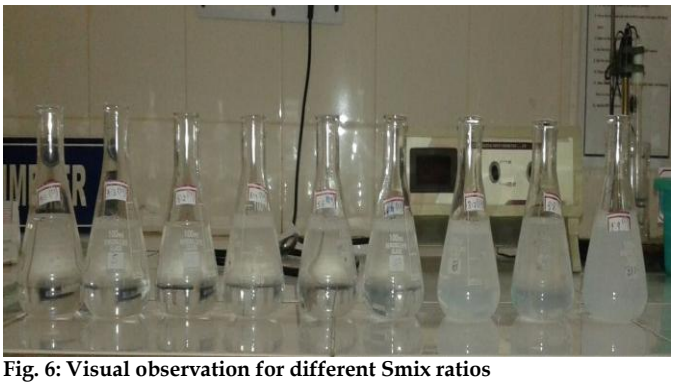

Fig. 6: Visual observation for different Smix ratios

Preparation of Candesartan SNEDDS

SNEDDS of Candesartan were prepared by using Capryol 90 (oil) Captex 500 (surfactant) and Labrasol (co-surfactant). In the present study, fifteen formulations were prepared (Table 8). All the formulations prepared were found to be clear and transparent. Pictorial representations of formulations F1 to F18 were shown in Figure 7.

Thermodynamic stability studies

In thermodynamic stability study, no phase separation and effect of temperature variations on prepared formulations were observed. 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 (Table 8).

Table 8: Thermodynamic stability studies of the formulations

Formulation code	Centrifugation	Freeze thaw method	
		-20°C for 2 days	+40°C for 2 days
F1	No phase separation	No change	No change
F2	No phase separation	No change	No change
F3	No phase separation	No change	No change
F4	No phase separation	No change	No change
F5	No phase separation	No change	No change
F6	No phase separation	No change	No change
F7	No phase separation	No change	No change
F8	No phase separation	No change	No change
F9	No phase separation	No change	No change
F10	No phase separation	No change	No change
F11	No phase separation	No change	No change
F12	No phase separation	No change	No change
F13	No phase separation	No change	No change
F14	No phase separation	No change	No change
F15	No phase separation	No change	No change
F16	No phase separation	No change	No change
F17	No phase separation	No change	No change
F18	No phase separation	No change	No change

Transmittance measurement

The visual observation of all the formulations was shown in Table 9. The clarity of nanoemulsions was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w microemulsion since water is external phase Formulation F 17 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 microemulsions. 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 are affected (Table 9).

Table 9: Visual observation and % Transmittance of different formulations

S. No.	Formulation Code	Visual observation	% Transmittance
1	F1	Turbid	75.27
2	F2	Turbid	88.72
3	F3	Slightly clear	90.27
4	F4	Slightly clear	92.88
5	F5	Turbid	66.22
6	F6	Transparent	86.77
7	F7	Transparent	93.57
8	F8	Turbid	86.37
9	F9	Transparent	95.34
10	F10	Slightly clear	88.52
11	F11	Transparent	94.28
12	F12	Transparent	97.31
13	F13	Turbid	88.79
14	F14	Slightly clear	91.27
15	F15	Transparent	95.37
16	F16	Transparent	94.37
17	F17	Transparent	98.37
18	F18	Slightly clear	90.11

Drug content of SNEDDS

Actual drug content of all 18 formulations are shown in table 10. The drug content of the prepared SNEDDS was found to be in the range of 88.27–98.77%. Maximum % drug content i.e. 98.77% was found in the formulation F17.

Table 10: % Drug Content for different formulations of Candesartan SNEDDS

S. No.	Formulation code	% Drug content
1	F1	90.79
2	F2	93.27
3	F3	89.79
4	F4	93.67
5	F5	88.27
6	F6	95.77
7	F7	97.22
8	F8	92.77
9	F9	96.11
10	F10	90.88
11	F11	92.77
12	F12	91.92
13	F13	94.22
14	F14	94.04
15	F15	95.00
16	F16	90.27
17	F17	98.77
18	F18	95.27

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 F17 was faster than other SNEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution (Tables 11, 12, 13 and Figures 7, 8, 9).

Table 11: Dissolution profiles of various formulations (F 1 to F6)

Time (min)	Dissolution media – 0.1N HCl (% drug release) Formulation Code F1 to F6 (1:1)						
	Pure drug	F1	F2	F3	F4	F5	F6
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	4.98 ± 0.02	15.12 ± 0.68	14.98 ± 0.66	14.03 ± 0.66	11.26 ± 0.52	13.25 ± 0.65	12.99 ± 0.62
5	7.28 ± 0.09	18.25 ± 0.98	19.46 ± 0.99	17.66 ± 0.97	18.01 ± 0.98	17.99 ± 0.97	16.25 ± 0.96
10	11.25 ± 0.52	25.89 ± 1.59	22.13 ± 1.25	20.19 ± 1.21	22.16 ± 1.25	25.16 ± 1.28	28.16 ± 1.39
15	14.68 ± 0.61	35.69 ± 2.35	39.15 ± 2.55	39.45 ± 2.56	33.25 ± 2.35	38.19 ± 2.45	35.19 ± 2.35
20	22.13 ± 1.25	59.56 ± 3.25	49.16 ± 2.98	48.99 ± 2.96	49.45 ± 2.98	48.67 ± 2.96	49.99 ± 2.98
25	29.27 ± 1.89	69.25 ± 3.59	65.16 ± 3.25	69.88 ± 3.45	55.12 ± 3.25	68.15 ± 3.45	65.25 ± 3.25
30	33.09 ± 2.20	72.19 ± 3.65	79.89 ± 3.98	79.98 ± 3.99	63.12 ± 3.35	74.22 ± 3.95	79.45 ± 3.99
45	39.98 ± 2.56	85.99 ± 4.86	86.16 ± 4.56	89.25 ± 4.99	75.16 ± 3.98	85.66 ± 4.59	86.22 ± 4.60
60	46.98 ± 2.98	93.12 ± 5.03	90.78 ± 5.01	92.25 ± 5.02	89.99 ± 4.99	91.88 ± 5.01	90.35 ± 5.00

Table 12: Dissolution profiles of various formulations (F 7 to F12)

Time (min)	Dissolution media – 0.1N HCl (% drug release) Formulation Code F7 to F12 (2:1)						
	Pure drug	F7	F8	F9	F10	F11	F12
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	4.98 ± 0.02	12.58 ± 0.54	8.78 ± 0.09	13.25 ± 0.60	11.85 ± 0.52	10.99 ± 0.45	9.98 ± 0.15
5	7.28 ± 0.09	14.98 ± 0.62	15.67 ± 0.68	18.36 ± 0.97	20.19 ± 0.99	18.15 ± 0.98	15.16 ± 0.68
10	11.25 ± 0.52	25.67 ± 1.59	22.15 ± 1.25	25.67 ± 1.56	22.98 ± 1.28	28.96 ± 1.99	28.18 ± 1.99
15	14.68 ± 0.61	39.15 ± 2.85	38.25 ± 2.84	39.16 ± 2.85	35.16 ± 2.45	34.12 ± 2.33	36.22 ± 2.46
20	22.13 ± 1.25	49.67 ± 2.98	50.12 ± 3.22	52.16 ± 3.18	42.98 ± 2.96	43.55 ± 2.97	44.25 ± 2.98
25	29.27 ± 1.89	55.12 ± 3.45	59.99 ± 3.45	55.89 ± 3.46	49.55 ± 2.98	54.16 ± 3.44	58.16 ± 3.33
30	33.09 ± 2.20	75.18 ± 3.92	65.36 ± 3.55	62.13 ± 3.51	52.18 ± 3.45	69.89 ± 3.58	69.28 ± 3.59
45	39.98 ± 2.56	80.19 ± 4.58	78.99 ± 3.95	75.99 ± 3.92	69.55 ± 3.58	79.99 ± 3.96	75.19 ± 3.92
60	46.98 ± 2.98	90.25 ± 5.01	85.19 ± 4.86	89.99 ± 4.99	80.1 ± 4.58	91.1 ± 5.02	92.19 ± 5.03

Table 13: Dissolution profiles of various formulations (F 13 to F18)

Time (min)	Dissolution media – 0.1N HCl (% drug release) Formulation Code F13 to F18 (3:1)						
	Pure drug	F13	F14	F15	F16	F17	F18
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	4.98 ± 0.02	10.12 ± 0.45	13.12 ± 0.56	12.11 ± 0.54	11.03 ± 0.52	17.98 ± 0.81	15.68 ± 0.65
5	7.28 ± 0.09	15.67 ± 0.68	18.25 ± 0.91	19.20 ± 0.92	14.12 ± 0.61	25.23 ± 1.89	21.05 ± 1.02
10	11.25 ± 0.52	29.12 ± 1.98	29.66 ± 1.98	23.52 ± 1.55	25.98 ± 1.80	38.65 ± 2.51	33.98 ± 2.21
15	14.68 ± 0.61	35.98 ± 2.25	48.12 ± 2.98	39.16 ± 2.56	32.15 ± 2.22	55.67 ± 3.45	45.19 ± 2.88
20	22.13 ± 1.25	49.17 ± 2.88	52.13 ± 3.46	58.12 ± 3.49	49.97 ± 2.98	69.03 ± 3.88	53.67 ± 3.45
25	29.27 ± 1.89	59.25 ± 3.56	65.03 ± 3.62	70.25 ± 3.99	65.11 ± 3.65	78.25 ± 4.01	68.13 ± 3.60
30	33.09 ± 2.20	65.12 ± 3.79	72.25 ± 3.91	72.19 ± 3.91	72.19 ± 3.92	80.11 ± 4.45	75.98 ± 3.99
45	39.98 ± 2.56	78.55 ± 4.02	81.98 ± 4.56	80.15 ± 4.25	79.89 ± 4.01	88.97 ± 4.72	82.09 ± 4.35
60	46.98 ± 2.98	89.28 ± 4.75	90.1 ± 5.01	91.25 ± 5.02	89.99 ± 4.75	99.12 ± 5.10	90.99 ± 5.01

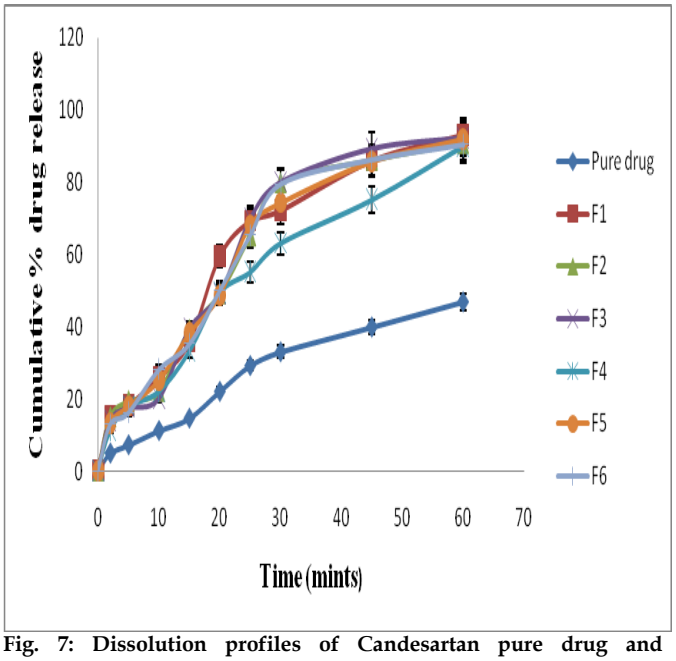

Fig. 7: Dissolution profiles of Candesartan pure drug and formulations (F1 to F6)

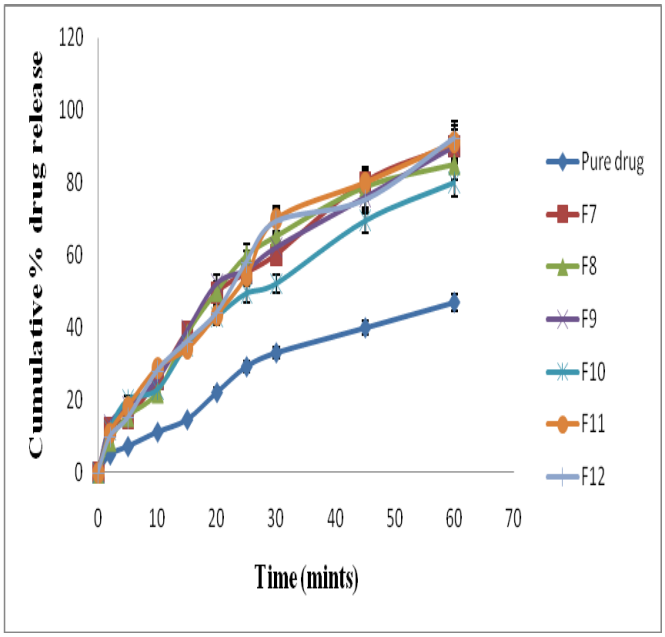

Fig. 8: Dissolution profiles of Candesartan pure drug and formulations (F7 to F12)

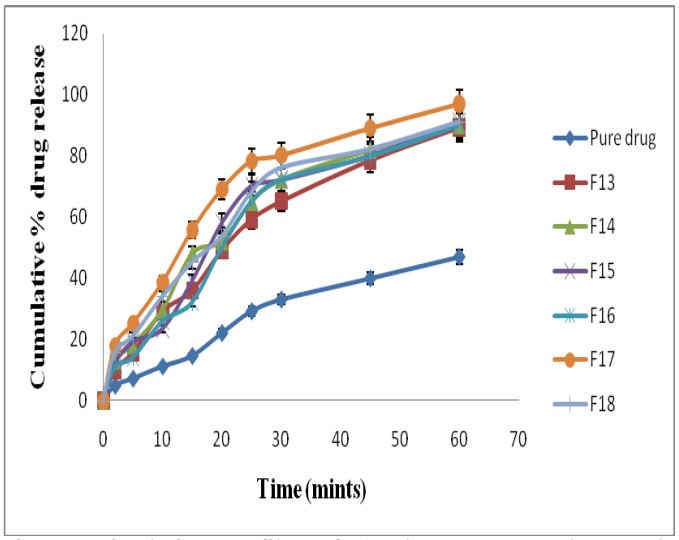

Fig. 9: Dissolution profiles of Candesartan pure drug and formulations (F13 to F18)

Interpretation of FTIR data

The FTIR spectra of optimized formulation were having similar fundamental peaks and pattern when compared with the pure drug (Figure 10). Thus there are no significant interactions among the drug and excipients. The optimized formulation of Candesartan spectrum was shown in Figure 11.

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 (F17) was found to be 51.7 nm & Z-Average of 59.2 nm indicating all the particles were in the nanometer range (Figure 12).

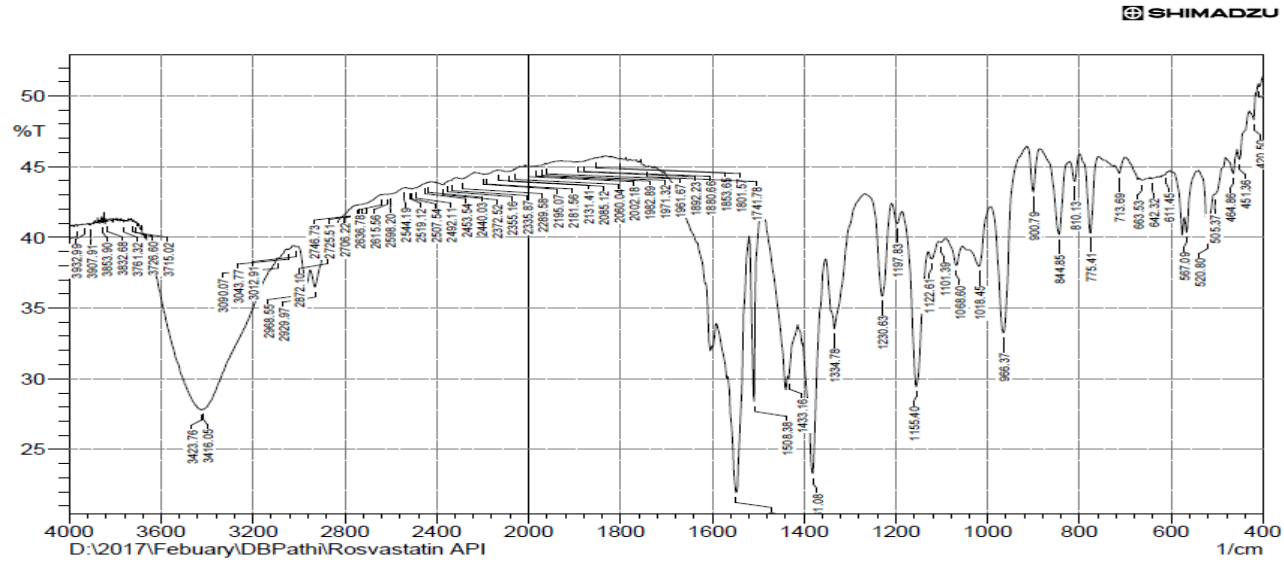

Fig. 10: FTIR Spectroscopy of Candesartan pure drug

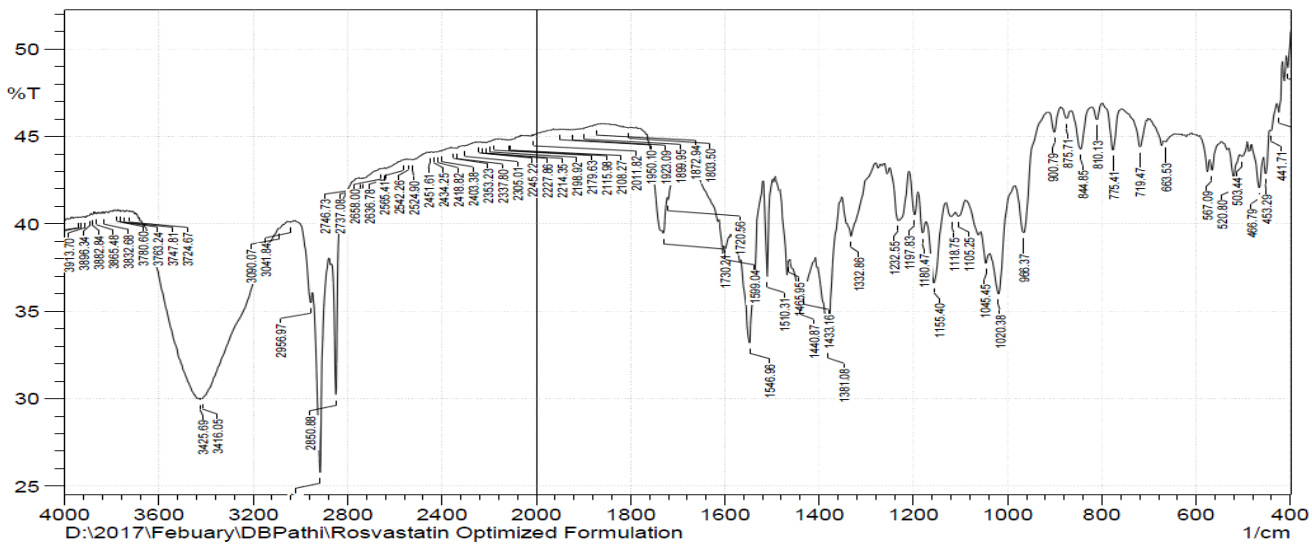

Fig. 11: FTIR Spectroscopy of Candesartan optimized formulation F17

201701102327004.nsz
Measurement Results

Date	:	10 January 2017 23:27:48
Measurement Type	:	Particle Size
Sample Name	:	5-Candesartan-SIZE
Scattering Angle	:	90
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	16376
Viscosity of the dispersion medium	:	2.032 mPa.s
Form Of Distribution	:	[Standard]
Representation of result	:	Scattering Light Intensity
Count rate	:	4216 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.45	7.1 nm	2.6 nm	7.6 nm
2	0.51	227.3 nm	558.1 nm	87.6 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	115.3 nm	413.2 nm	7.6 nm

Histogram Operations

Size (Median)	:	51.7 nm
Mode	:	7.6 nm
% Cumulative (1)	:	10.0 (%) - 4.8 (nm)
% Cumulative (2)	:	50.0 (%) - 51.7 (nm)
% Cumulative (3)	:	90.0 (%) - 194.7 (nm)
% Cumulative (4)	:	30.0 (%) - 7.7 (nm)
% Cumulative (5)	:	40.0 (%) - 9.6 (nm)
% Cumulative (6)	:	50.0 (%) - 51.7 (nm)
% Cumulative (7)	:	20.0 (%) - 6.2 (nm)
% Cumulative (8)	:	70.0 (%) - 97.4 (nm)
% Cumulative (9)	:	95.0 (%) - 305.4 (nm)
% Cumulative (10)	:	100.0 (%) - 8510.6 (nm)

Cumulant Operations

Z-Average	:	59.2 nm
PI	:	0.505

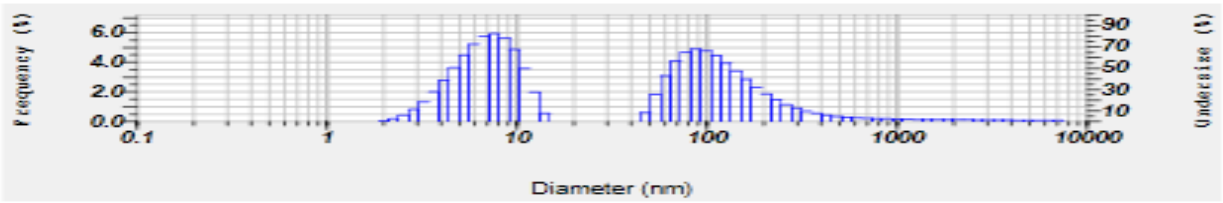

Fig. 12: Particle size analysis of optimized formulation F17

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UNIVERSITY COLLEGE OF TECHNOLOGY
Osmania University

5-Candesartan-ZETA.nzt
Measurement Results

File Name	:	5-Candesartan-ZETA.nzt
Date	:	10 January 2017 23:36:07
Measurement Type	:	Zeta Potential
Sample Name	:	5-Candesartan-ZETA
Material	:	
Cuvette type	:	Electrode Cell (Carbon, 6mm)
Temperature of the holder	:	25.0 deg. C
pH	:	---
Concentration	:	---
Conductivity	:	0.094 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-15.5 mV	-0.000120 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-15.5 mV
Electrophoretic Mobility mean	:	-0.000120 cm²/Vs

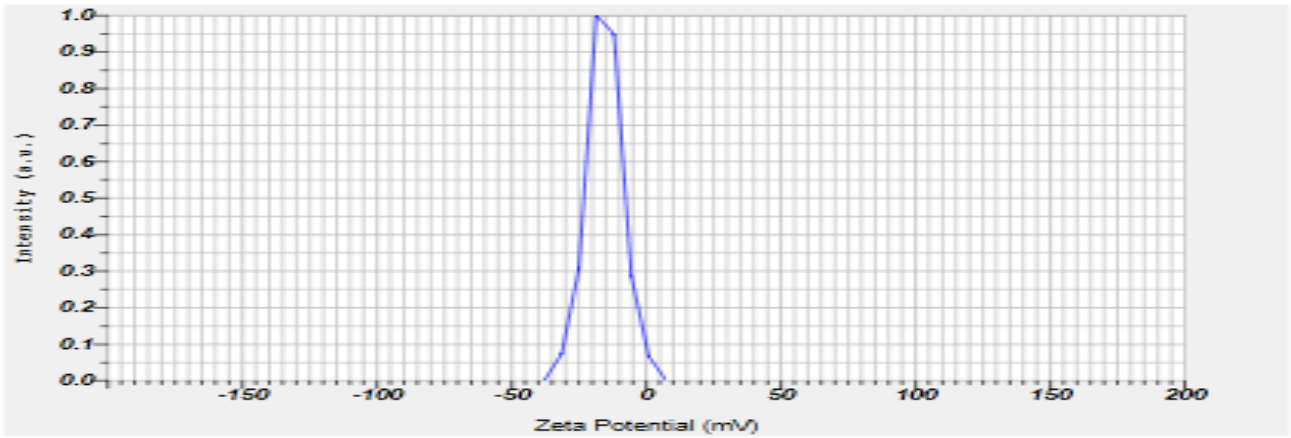
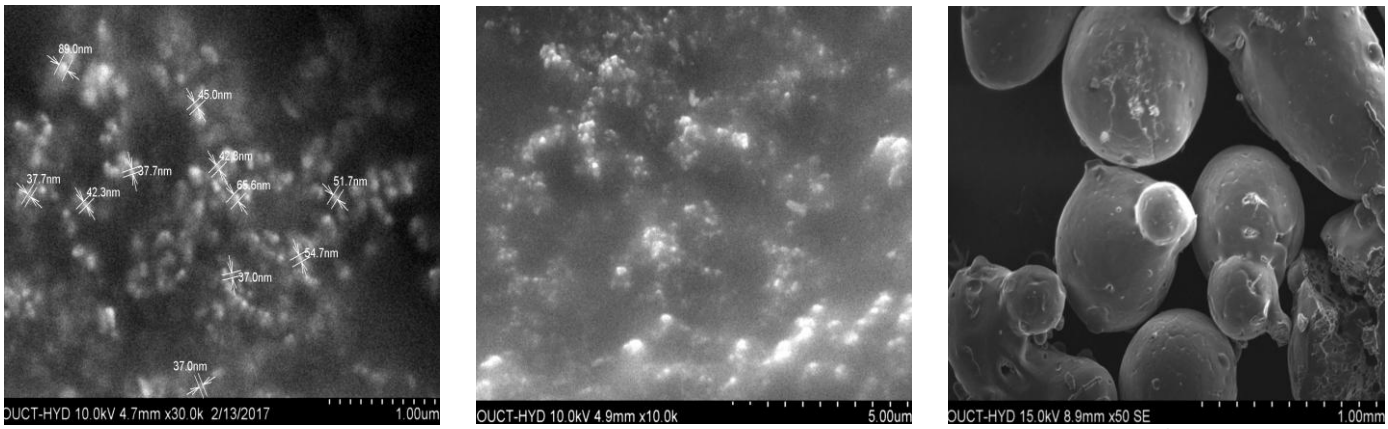


Fig. 13: Zeta potential of the optimized formulation F17



A B C

Fig. 14: Scanning electron microscopy of optimized Candesartan formulation F17

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 (F17) was found to be -15.5 mV which comply with the requirement of the zeta potential for stability (Figure 13).

SEM studies

Scanning electron microscope studies of optimized formulation of Candesartan (F17) revealed oval shaped globules. The size is within nanometers. There are clear liquid droplets without any pores (Figure 14).

Stability studies

The Candesartan SNEDDS F17 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 formulation 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.

Pharmacokinetic parameters comparison for pure drug suspension and SNEDDS

Figure 15 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Candesartan SNEDDS formulation as compared to Candesartan pure suspension. At all the indicated time points, the Candesartan plasma concentrations in rats treated with SNEDDS formulation was significantly higher than those treated with pure drug. Pharmacokinetic parameters of Candesartan after oral administration of the two formulations in Wistar rats are shown in Table 9. C_{max} of the SNEDDS 35.2 ± 0.02 ng/ml was significant (p<0.05) as compared to the pure drug suspension formulation 25.1 ± 0.03 ng/ml. T_{max} of both SNEDDS formulation and pure drug suspension was 1.00 ± 0.03 h and 2.00 ± 0.01 h, 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 (160.1 ± 1.04 ng. h/ml) than the pure drug suspension formulation 135.3 ± 2.02 ng.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 Candesartan from SNEDDS formulation as compared to the pure drug suspension formulation.

Table 9: Pharmacokinetic Parameters of Candesartan SNEDDS formulation and pure drug

Pharmacokinetic parameters	Candesartan Pure drug	Candesartan – SNEDDS Optimized Formulation
C_{max} (ng/ml)	25.1 ± 0.03	35.2 ± 0.02
AUC_{0-t} (ng. h/ml)	82.2 ± 1.02	104.4 ± 1.01
AUC_{0-inf} (ng. h/ml)	135.3 ± 2.02	160.1 ± 1.04
T_{max} (h)	2.00 ± 0.01	1.00 ± 0.03
$t_{1/2}$ (h)	4.52 ± 0.02	3.02 ± 0.02

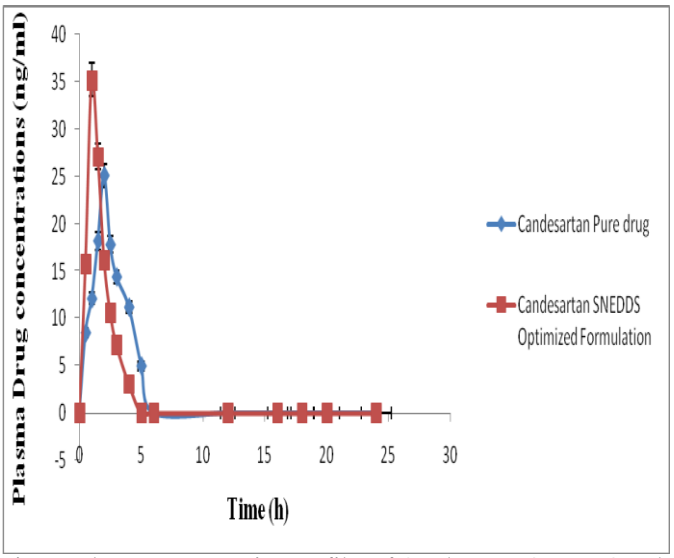


Fig. 15: Plasma concentration profiles of Candesartan SNEDDS and pure drug

In the present study, SNEDDS comprising of Capryol 90, Captex 500, 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. Nearly complete drug release was achieved from the formulation F17 which is significantly higher as compared to a conventional dosage form. From in vivo bioavailability studies C_{max} of the SNEDDS 35.2 ± 0.02 ng/ml was significant (p<0.05) as compared to the pure drug suspension formulation 25.1 ± 0.03 ng/ml. T_{max} of both SNEDDS formulation and pure drug suspension was 1.00 ± 0.03 h and 2.00 ± 0.01 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, $AUC_{0-\infty}$ infinity for SNEDDS formulation was higher (160.1 ± 1.04 ng.h/ml) than the pure drug suspension formulation 135.3 ± 2.02 ng.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 Candesartan from SNEDDS formulation as compared to the pure drug suspension formulation**.** The results from this study suggest the requirement for potential use of Candesartan as self-nanoemulsifying drug delivery systems. Thus the developed SNEDDS can be used as an effective approach for the management of hypertension with low drug dose.

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