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FORMULATION AND INVITRO EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

The present Researchwork was aimed at enhancing the solubility of Candesartan cilexetil by formulating SEDDS (self-emulsifying drug delivery system) evaluating its in vitro potential. The solubility of Candesartan cilexetil was determined in various vehicles. Various combinations of oils (arachis oil, Sunflower oil, coconut oil and soybean oil), surfactant (Cremophor Rh 40), and co-surfactant (polyethylene glycol 400) were used to formulate SEDDS. Pseudo ternary phase diagram was used to evaluate the effective emulsification area. SEDDS formulations were tested for emulsifying properties and the resultant emulsions were evaluated for clarity, precipitation, particle size distribution and drug release studies. Optimization of the formula and screening was done based on characteristics of resultant emulsions. The optimized formulation composed of soybean oil (45%), Cremophor Rh 40 (50%), and polyethylene glycol 400 (05%) and showed complete drug release in 60 minutes which was far better than that of plain drug.

KEYWORDS: Candesartan cilexetil, SEDDS, pseudo ternary phase diagrams, angiotensin-receptor blocker, BCS class II drug.

INTRODUCTION

The oral route has been the major route of drug delivery for the chronic treatment of many diseases. However, oral delivery of 50% of the drug compounds is hampered because of the high lipophilicity of the drug itself. Nearly 40% of new drug candesartan exhibit low solubility in water, which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality.[1] Efforts are going on to enhance the oral

bioavailability of lipophilic drugs in- order to increase their clinical efficacy.[2] Candesartan cilexetil is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. Candesartan cilexetil is administered orally as the prodrug which is rapidly converted to its active metabolite, candesartan, during absorption in the gastrointestinal tract. It is classified as BCS (bio-pharmaceutical classification system) class II drug, which means it has high permeability and poor water solubility.Candesartan cilexetil lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough.[3] Candesartan is available in various doses (2 mg, 4 mg, 8 mg, 16 mg, and 32 mg). For our study we selected 8 mg as the working dose to limit the total formulation volume. The main objective of the study was to enhance the solubility of Candesartan by formulating an optimal SEDDS formulation and to evaluate various in-vitro characteristics.

MATERIALS AND METHODS

Candesartan cilexetil and cremophor RH 40 was obtained as a gift sample from Aurobindo Pharmaceuticals gift sample (Hyderabad). Soybean oil, Arachis oil, Arachis oil, Sunflower oil, Coconut oil., AOS products Pvt (Ghaziabad, UP, INDIA), Polyethylene glycol (PEG 400),Sodium lauryl sulphate, Hydrochloric acid, Sodium chloride, Methanol was purchased from S.D. fine Chem-limited (Mumbai, India) Other ingredients and excipients used were of analytical grade.

Determination of melting point

Melting point of the drug sample was determined by using melting point apparatus.[4]

Solubility analysis of drug

The solubility of Candesartan cilexetil in oils, surfactant, and co-surfactant was determined in triplicates. 5mL of each of the selected components were added to each cap vial containing an excess of the drug. Mixing of the systems was performed using a vortex mixture. Formed suspensions were shaken on a mechanical rotary shaker at 25°C for 48 hours. On completion of shaking, each vial was centrifuged at 3000 rpm for 5 minutes. Supernatant liquid was filtered and the amount of drug solubilized was quantified by UV spectrophotometer using respective components as blank.

Determination of absorption maxima (λ_{max}) of candesartan cilexetil

Preparation of 0.1N HCl

8.5 mL of concentrated HCl was taken into 1000mL volumetric flask and the volume made up to the mark with distilled water.

Preparation of stock solution

Standard stock solution of Candesartan cilexetil was prepared by dissolving 10mg of Candesartan cilexetil in 10mL of the solvent system to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken and diluted up to 10mL by using 0.1N HCl to produce a concentration of 100µg/mL, was the standard stock solution.

Preparation of working standard solution and determination of λmax

From the above stock solution, 1ml was pipetted into a 10mL volumetric flask and the volume made up to the mark with 0.1N HCl to produce concentration of 10µg/ml. This 10µg/mL sample was scanned in UV-VIS Spectrophotometer (Elico, India) in the range 200-400nm using 0.1N HCl as a blank. The wavelength at which maximum absorbance occurred was considered as the λ_{max} of the drug is found to be 290nm. The procedure was performed in triplicates to confirm reproducibility.

Construction of calibration curve

The construction of calibration curve was done by taking the above prepared stock solution and diluting it to produce solutions of various concentrations ranging from 2-12µg/ml. The absorbance of each sample was recorded at 290nm against 0.1N HCl as blank by UV spectrophotometer. Graph of concentrations of drug versus absorbance was plotted. The linear regression analysis was done on absorbance data points. To confirm the reproducibility procedure was performed in triplicates.[6]

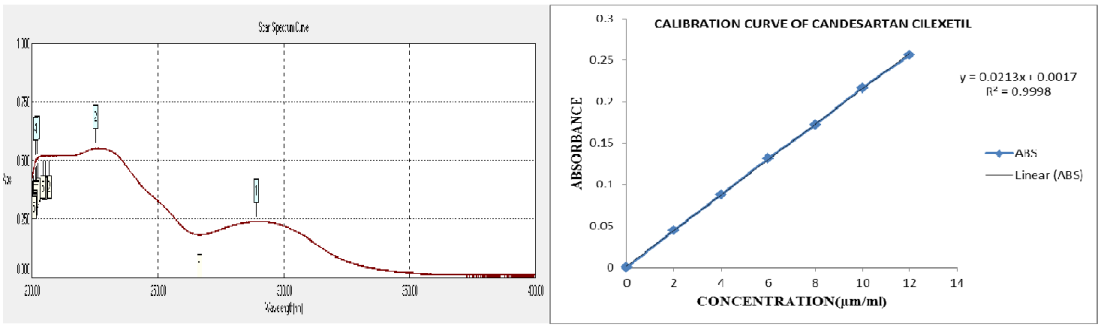


Figure 2: A) Spectram of candesartan celexitile B) Calibration curve of Candesartan cilexetil Comparision.

Construction of pseudo-ternary phase diagram

A series of self-emulsifying systems were prepared with varying volume percentages of oil from 25 to 60%, surfactant (SA) from 25 to 75%, co-surfactant (Co-S) from 0 to 15% and drug was maintained constant at a concentration of 16mg/mL at room temperature. Above mixture (1ml) was gently mixed with 100mL of distilled water in a glass beaker at room temperature. The tendency to emulsify spontaneously and the progress of emulsion globules spread were visually assessed. This provides the information about the region where the system could emulsify effectively. On identification of the effective region of emulsification, a pseudo-ternary phase diagram was constructed using software, ProSim Ternary Diagram.[7]

Formulation and development studies

In all the formulations, the amount of candesartan cilexetil was kept constant (i.e. 16mg/mL). A series of SEEDS were formulated with varying concentrations of oil (25-95%), surfactant (05-70%), and co-surfactant (0 and 5%). Accurately weighed quantity of drug was dispersed in required quantity of soybean oil in a glass beaker. To this, required quantities of Cremophor Rh 40 and PEG-400 were added under gentle stirring and sonication until Candesartan cilexetil has completely dissolved. From the final formulations 1mL was filled in hard gelatin capsules to produce unit solid dosage form of 16mg Candesartan cilexetil. Various compositions of SEDDS were given in the table.2.

Evaluation of SEDDS

Assessment of self-emulsification time: Evaluation of the self-emulsifying properties of SEDDS formulations was performed by visual assessment. The time taken by the formulation to disperse was noted by drop wise addition of the SEDDS (100 µL) in 20mL/250mL/900mL of 0.1N HCl and in distilled water in triplicates in a glass beaker at 37°C ± 0.5°C. The contents were stirred using magnetic stirrer at 100 rpm.

Globule size distribution analysis The globule size measured in triplicates by optical microscopic Method using a stage micrometer to confirm accuracy.

$Xg = 10 X [ni X \log Xi/N]$

Where,

Xg	=	Geometric mean diameter
Ni	=	Number of globules in range
Xi	=	The mid-point of range
N	=	Total number of globule

Cloud point determination: Cloud point temperature indicates the maximum temperature where the formulation could be stable. Each formulation was diluted with 0.1N HCl and heated in a water bath with gradual increase in temperature. Corresponding cloud point temperatures were noted when the formulations turned cloudy.[8]

Robustness to dilution: Robustness to dilution provides information about the stable dilution ranges of the formulations. Robustness to dilution of the selected formulations was assessed in triplicates by diluting the formulation in a ratio of 1:50 and 1:1000-folds with 0.1N HCl. The diluted emulsions were stored for 24 hours and monitored for physical changes such as precipitation or phase separation.[9]

Thermodynamic stability studies: The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SEDDS formulation.

Heating cooling cycle: Six cycles between refrigerators temperature 4°C and 45°C with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

Centrifugation: Formulations that passed heating cooling cycles were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

Freeze-thaw cycling: Three freeze thaw cycles between -10°C and +25°C with storage at each temperature for not less than 48hr was done for the formulations those which passed centrifugation.[10]

Percentage of drug solubilized in the formulation: A sample equivalent to 16mg of Candesartan cilexetil was taken in a 100mL volumetric flask and the volume was made up to the mark with 0.1N HCl. The solution was mixed well. From this solution 1ml was pipetted out into a 10mL volumetric flask and made up to 10mL with 0.1N HCl. The resulting solution was filtered and the absorbance of the filtrate was measured at 290mn.

In-vitro drug release studies: The dissolution studies were performed using USP dissolution apparatus XXIV type-I at 100 rpm. Dissolution studies of each formulation was performed with six capsules filled with SEDDS using 0.1 N HCl as dissolution medium at 37°C ± 0.5° C. 10mL aliquots of the medium were collected and replaced with equal volumes of fresh

medium at pre-determined time intervals. The collected samples were analysed spectrophotometrically using UV spectrophotometer at 290nm against 0.1N HCl as blank.[11]

Methodology of SEM analysis: The surface morphology of Solid SEDDS, drug, and polymer was analysed in an Dual Beam Electron Microscope (SEM). The samples were fixed on an aluminium stub using a double sided carbon adhesive tape and were made electrically conductive by coating with palladium under vacuum. An accelerating voltage of 5 kV was used to visualize the samples.[12,13,14,15,16]

RESULTS AND DISCUSSION

Determination of melting point: Melting point of the drug sample was determined by using melting point apparatus. The melting point of drug was found to be 161˚C.

Solubility analysis of the drug: Solubility of the drug in various oils, surfactant and co-surfactant was quantified by UV spectrophotometer. The solubility data obtained results Candesartan cilexetil, PEG 400, Soybean oil, Sunflower oil, Arachis oil, Coconut oilwas found to be having solubility of 36.5 ± 0.058mg/mL, 31.9 ± 0.042mg/mL, 5.81 ± 0.002mg/mL, 1.04 ± 0.07, 0.89 ± 0.022mg/mL, 0.71 ± 0.021mg/mL respectively.

Fourier Transform Infrared Spectrophotometry (FTIR) studies: Infrared spectra of pure drug and mixtures of drug and excipients of SEDDS were obtained by using FTIR – spectrophotometer and observed peak are carboxylic acid 1740.87,ethoxy group is 1167, C-N is 1250, C=N 1316.81 and N-H is 2927.50 observed.

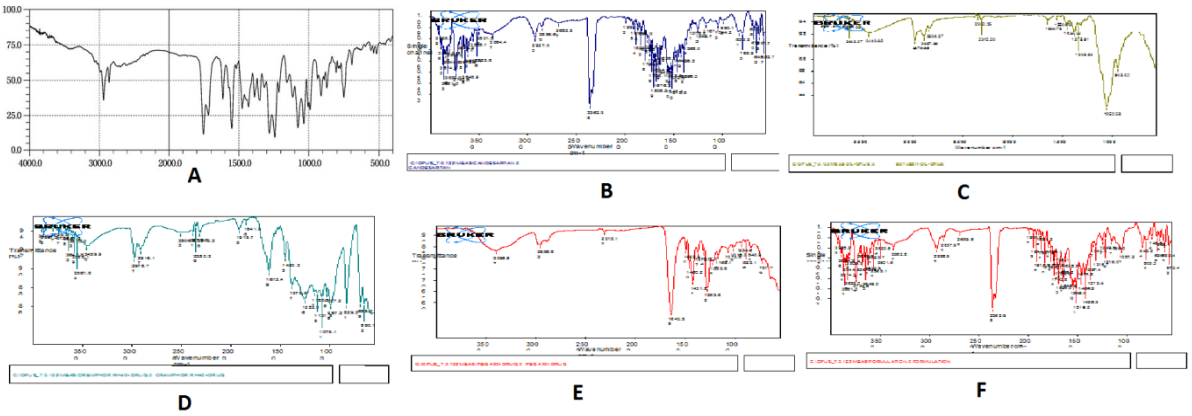


Figure 1: FTIR spectrum ofA) Standard Candesartan cilexetil[5] B) Candesartan cilexetil C) Candesartan cilexetil with soybean oil D) Candesartan cilexetil with Cremphor RH 40E) Candesartan cilexetil with PEG 400F) Formulation.

Construction of pseudo-ternary phase diagram: Pseudo-ternary phase diagram the shaded part represents the prominent region of emulsification and the yellow colored points represent the formulated SEDDS which form the most effective region of SEDDS formation. The constructed pseudo ternary phase diagram was shown in the figure.3A.

Formulation and development of Sedds: Soybean oil was selected for preparation of SEDDS among the other oils based on data obtained from solubility studies. Cremophor Rh 40 and PEG 400 acts as surfactant and co-surfactant respectively in the formulation. Formulations were made by varying the ratios of oil (25-95%), surfactant (5-70%) and co-surfactant (0-5%) based on the data obtained from the pseudo ternary phase diagram.

Evaluation of SEDDS

Assessment of self-emulsification time: In SEDDS surfactant system reduces the interfacial tension between oil and aqueous phases resulting in easy dispersion and formation of o/w emulsion.

Globule size distribution analysis: Globule size distribution following self-emulsification is a critical factor to evaluate self-emulsifying systems in-vitro. The globule sizes given in the table. 2.

Cloud point determination: The cloud point is the temperature above which the formulation clarity turns into cloudiness. The cloud points of different formulations were given in the table. 2.

Robustness to dilution: The SEDDS formulation were found to be stable after dilution up to 1:50 and 1:1000 with 0.1N HCl, there were no signs of drug precipitation and phase separation in formulations S8 to S15. The data was represented in the table.2.

Thermodynamic stability study: Thermodynamic stability study was designed to identify and avoid the metastable SEDDS formulations. No phase separation and precipitation indicates that formulations are stable and thus metastable formulations have been avoided. The formulations S10 to S15 showed good stability to thermodynamic stability test conditions. The data was represented in the table.2.

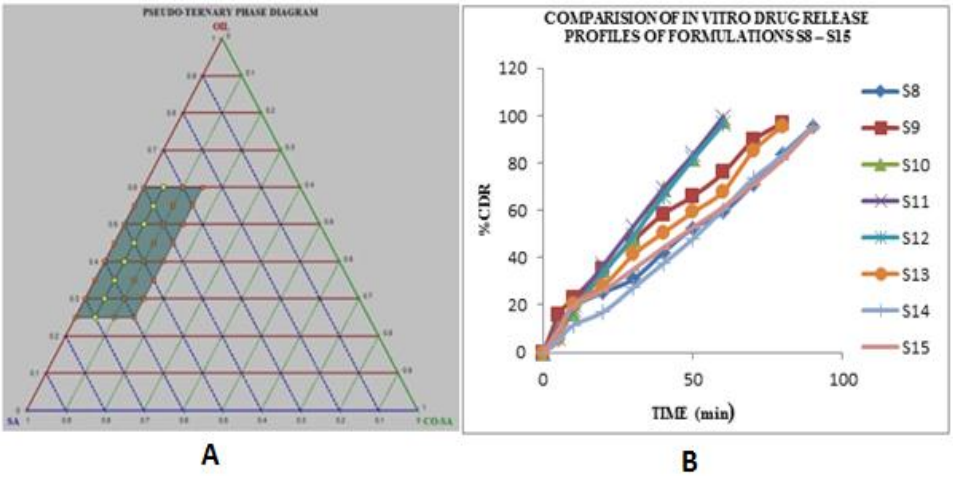
Determination of percentage of drug solubilized in the formulation: This test confirms the actual amount of the drug present in the formulation out of the total amount added. The drug content should be in the range of 100±5%. The results have been tabulate in the table.2.

In- vitro drug release studies: The in vitro dissolution has been performed for those formulations whose percentage drug content was above 95%. The data was represented in the table.1 and figure 3B. Candesartan cilexetil in respectively in 60min.

Table 1: Comparison of in vitro drug release data of formulated SEDDS.

Formulation code	%CDR in time (min)								
	10	20	30	40	50	60	70	80	90
S8	19.09± 0.54	25.44± 2.56	30.99± 1.49	42.24± 0.93	52.65± 1.58	59.31± 0.72	71.05± 1.63	83.93± 1.65	95.59± 0.37
S9	22.83± 1.69	35.36± 1.78	47.31± 1.08	58.46± 1.62	66.04± 1.2	75.85± 1.69	89.56± 1.37	96.03± 0.68	-
S10	16.75± 0.35	34.88± 0.68	48.22± 1.94	68.97± 0.75	82.20± 2.75	97.10± 0.58	-	-	-
S11	20.43± 1.16	36.86± 1.44	53.18± 504	69.29± 1.52	83.59± 1.73	98.04± 0.25	-	-	-
S12	18.08± 3.98	33.39± 1.01	49.29± 0.85	66.04± 1.02	81.77± 1.99	96.44± 1.89	-	-	-
S13	20.37± 7.82	28.16± 1.62	41.23± 2.74	50.57± 1.13	59.31± 1.72	68.01± 1.96	85.03± 2.04	95.75± 0.44	-
S14	11.57± 0.88	17.12± 0.77	27.31± 268	37.60± 1.27	48.06± 1.91	60.38± 1.62	73.45± 2.16	83.32± 1.5	95.43± 0.33
S15	19.84± 1.37	26.19± 2.43	35.15± 44	44.22± 2.06	52.91± 1.31	61.29± 1.49	70.89± 1.56	81.67± 1.13	95.00± 0.57

All values expressed as Mean±SD, n = 3



A B

Figure 3: A) Pseudo-ternary phase diagram showing region of effective emulsificationB) Comparision of in vitro drug releaseprofiles of all formulations(S8-S15).

From the in-vitro dissolution profile, formulations S10 and S11 were found to have similar release pattern for initial 60min. To compare, dissolution of these two formulations was repeated by analyzing aliquots at time intervals of 5, 10, 20, 30, 40, 50, 60min. It was observed that the formulation S11 has released 98.04% of drug in 60min whereas formulation S10 released 97.10 in 60 min.

SEM Analysis results: Scanning electron microscopy reveals the morphology of solid SEDDS. From the figure Figure.5, drug appeared to be made of smooth crystal structures. Similar observations about drug micrographs were made by other researchers. Polymer appears to be round (shape rayali) particles of size of spears approximately 72nm.

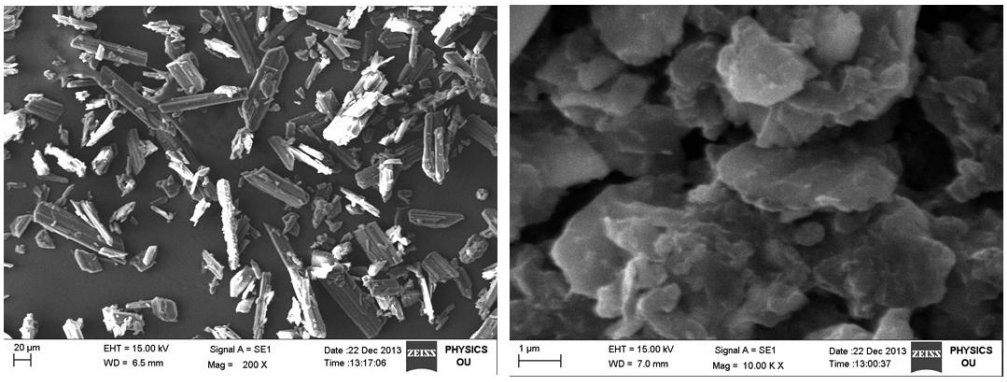


Figure 5: Candesartan cilexetil Pure drug and Formulation.

Table 2: evaluation data of formulated SEDDS.

Formulation code	Oil : SA : CO-SA (%v/v)	Self-emulsification time (sec)	Globule size distribution (nm)	Cloud point (^{0}C)	Robustness to dilution	Thermodynamic stability	% Drug solubilized
S1	95:05:00	95±1.98	486.5±0.28	52±0.08	-	-	51.37±3.12
S2	90:05:05	86±1.52	480.1±0.18	55±3	-	-	58.85±1.52
S3	85:10:05	80±0.57	440.3±0.32	56.4±2.8	-	-	66.4±1.81
S4	80:15:05	74±1.15	390.2±0.09	57.9±0.7	-	-	74.67±2.27
S5	75:20:05	64±1.52	330.9±0.13	59.7±1.5	-	-	79.85±3.50
S6	70:25:05	60±2	240.3±0.13	60.0±0.6	-	-	84.62±0.26
S7	65:30:05	55±1	189.34±0.6	63.3±1.2	-	-	87.31±0.59
S8	60:35:05	40±0.57	139.5±0.32	65.7±1.5	+	-	96.25±0.15
S9	55:40:05	35±0.57	98.95±0.27	69.31±28	+	-	97.73±2.3
S10	50:45:05	31±0	81.09±0.28	76±3	+	+	98.76±1.94
S11	45:50:05	30.33±0.6	72.47±0.16	79.7±1.5	+	+	99.62±1.22
S12	40:55:05	34.33±1.5	77.62±0.44	72.3±1.5	+	+	96.97±3.3
S13	35:60:05	38.67±1.6	89.48±0.05	68±2	+	+	98.02±2.5
S14	30:65:05	45±1.73	93.93±0.09	67.3±2.5	+	+	97.83±1.02
S15	25:70:05	51.3±2.08	107.5±0.28	66.3±1.5	+	+	98.69±0.97

All values expressed as Mean ± SD, n = 3

+ = No phase separation and drug precipitation.

- = Phase separation and precipitation

CONCLUSION

Candesartan cilexetil an angiotensin receptor antagonist considered as BCS class II drug due to its low solubility and high permeability. Bioavailability is major factor responsible for the pharmacological action of any drug and solubility is the limiting step for bioavailability of Candesartan cilexetil. Hence, the present work focused on the formulation of this active pharmaceutical ingredient (API) as Self-emulsifying Drug Delivery System (SEDDS) because of the advantages of SEDDS that they increase solubility and permeability which in turn increases bioavailability. The optimized formulation, S11 got dispersed in 30.33 ± 0.57sec which had globule size distribution of 72.47 ± 0.16 nm and cloud point temperature of 79.67 ± 1.53^{o}C. The formulation was stable on subjecting to robustness to dilution and thermodynamic stability test. The percentage drug content of the formulation was found to be 99.62 ± 1.22% and released 98.04 ± 0.04% of drug in 60min during in vitro drug release studies. The results showed that the optimized formulation was effective than that of plain drug and the marketed tablet, Candesar. Hence, solubility of Candesartan cilexetil has been enhanced by formulating Self-emulsifying Drug Delivery System.

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