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Novel Combination for Self-Nanoemulsifying Drug Delivery System of Candesartan Cilexetil

Safaa F. Mekhilef [*, 1] and Ahmed A. Hussein [*]

* Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Solubility problem of many of effective pharmaceutical molecules are still one of the major obstacle in the formulation of such molecules. Candesartan cilexetil (CC) is angiotensin II receptor antagonist with very low water solubility and this result in low and variable bioavailability. Self- emulsifying drug delivery system (SEDDS) showed promising result in overcoming solubility problem of many drug molecules. CC was prepared as SEDDS by using novel combination of two surfactants (tween 80 and cremophore EL) and tetraglycol as cosurfactant, in addition to the use of triacetin as oil. Different tests were performed in order to confirm the stability of the final product which includes thermodynamic study, determination of self-emulsification time, particle size and zeta potential measurement, and *in-vitro* drug release. The results showed that the particle size of the best formula was 13.3 nm and zeta potential of -37.45 mV with approximately 100% release after 45 minutes These results suggest that the preparation of CC as SEDDS with the use of the above combination of surfactant and cosurfactant is a promising maneuver for oral delivery of CC in order to improve its bioavailability.

Key words: Candesartan cilexetil, tween 80, cremophore EL, triacetin, self-emulsifying.

خليط جديد من مادتين لتقليل الشد السطحي وماده مساعده للشد السطحي لمستحلب نانوي ذاتي لعقار الكانديسارتان سيلاكسيتيل

صفاء فاخر مخيلف [*،١] و احمد عباس حسين [*]

[*]فرع الصيدلانيات ، كلية الصيدلة ، جامعة بغداد ، بغداد ،العراق .

الخلاصه

لاتزال مشكله الذوبانيه للعديد من الادويه واحده من اهم المعوقات التي تحول دون تحضيره على شكل مستحضرات صيدلانيه. عقار الكانديسارتان سيلاكسيتيل هو عقار يعمل على غلق مستقبلات الانجيوتنسين (نمط II) ذات النوع (AT 1) ولديه ذوبانيه قليله جداً في الوسط المائي والذي نتج عنه توافر حيوي قليل ومتذبذب. المستحلابات النانونيه الذاتيه أظهرت نتائج مشجعه في محاوله التغلب على مشكله الذوبانيه للعديد من العقارات. كانديسارتان سيلاكسيتيل حُضر بشكل مستحاب نانوي ذاتي بأستخدام خليط جديد يتكون من مادتيين لتقليل الشد السطحي (التوين ٨٠ والكريموفور EL) والتتراكلايكول كماده مساعده على تقليل الشد السطحي, بألاضافه الى أستخدام زيت الترايستين.

تم أجراء العديد من الاختبارات لغرض اثبات ثباتيه المنتج النهائي والتي تتضمن: الاستقرار الحراري, توزيع حجم الجزيئات, جهد زيتا, ومستوى التحرر الدوائي.

النتائج اظهرت انه حجم الجزيئات للصيغة المختاره هو (١٣,٣) نانومتر, وجهد زيتا (37.45-) ملي فولت, وتقريبا ١٠٠% تحرر للدواء خلال ٤٥ دقيقه. هذه النتائج تقترح انه تحضير عقار الكانديسارتان سيلاكسيتيل بشكل مستحلب نانوي ذاتي بأستخدام الخليط المذكور يعتبر طريقه واعده لأستخدامه في شكل صيدلاني يعطى عن طريق الفم من أجل زياده التوافر الحيوي.

الكلمات المفتاحيه: كانديسارتان سيلاكسيتيل, توين ٨٠, كريموفور EL, تراسيتين, مستحلب ذاتي.

Introduction

Oral rout still represent the most convenient and acceptable mean for administration of drug molecules to patients since it is associated with high rate of patient compliance in one hand and economic and flexible dosage design in others, that is why more than 70% of total dosage forms utilized by humans are tablets[1,2].

One of the most important prerequisite requirements of drug molecules to be available for systemic absorption is aqueous solubility since that is the nature of GIT fluid. Then when the drug molecules become solubilized, it has to pass the biological membrane in order to reach to systemic circulation [2].

Food and drug administration (FDA) classifies drug molecules to belong to one of four categories based on their aqueous solubility and ability to pass through biological membrane, termed as permeability. This classification system is called Biopharmaceutical Classification System (BCS) [3].

[1]Corresponding author E-mail: safaafakher@yahoo.com
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Drug molecules that belong to class II have problem in bioavailability mainly due to low aqueous solubility. In this class the rate limiting step is dissolution process and so choosing of suitable drug delivery and appropriate additives is crucial to overcome this major obstacle and improve the fraction that will reach to systemic circulation [4].

Many approaches were developed in order to overcome this issue with variable degree of success, from these approaches solid self-emulsifying drug delivery system (SSEDDS) is extensively tried.

SEDDS is type of lipid based drug delivery system and it is isotropic mixture consist of oil, surfactant and/or co-surfactant or co-solvent that has the ability to form o/w micro or nano-emulsion when mix with water upon slight agitation [5].

The simplicity of production and stability of the final product makes SEDDS more attractive to the formulators than ordinary emulsion[6].

SEDDS is preconcentrate that usually filled in either soft or hard gelatin capsule. The agitation that produced by action of GIT peristalsis along with aqueous media is sufficient for emulsification process to complete. In addition to enhance drug solubilization, drug release and absorption also improve since, drug is already dissolved and, upon emulsification, it will produce very fine particle with large surface area [7].

This system has many advantages, say: Improve patient compliance and palatability since the final product filled into unit dosage form as capsule, [8] protection of drug molecules from in-vivo hazard condition, [9] enhanced bioavailability through improving the solubilization process, [10]quicker onset of action as the time required reaching tmax is much less in many literatures that compare SEDDS with other conventional dosage form, [11] and predictable therapy due to reduced variability including food effects [12].

SEDDS has some drawbacks that limit its wider applications, from these limitations stability issues, low drug loading and volatile oil migration to gelatin capsule shell are the most frequent [13].

Candesartan cilexetil (CC) is a selective, reversible, competitive angiotensin II receptor-1 antagonist, and it's used for the management of hypertension, heart failure, and myocardial infarction. It is also used in patients with impaired left ventricular systolic function, either when ACE inhibitors are not tolerated, or in addition to ACE inhibitors [14, 15].

CC is a prodrug ([16], and following oral administration, CC undergoes hydrolysis at the ester link to form the active drug, candesartan, which is achiral. Candesartan contains two acidic functional groups: a carboxyl and tetrazole moieties (pKa = 5.3 for either). It is a colorless to off-white crystals or powder, with melting point range of (160-175 °C), and it is sparingly soluble in alcohol and practically insoluble in water with log partition coefficient (log P 7.43). It belongs to class II of BCS and has molecular weight of 610.66 Dalton [17, 18].

Materials and Methods

Materials

Candesartan cilexetil powder was purchased from Shenzhen Nexconn Pharmatechs, LTD, China, triacetin®, cremophor EL, Cremophor RH 100, Tetraglycol, labrafil®,labrafac CC,labrafac PG, Maisine 35-1, Miglyol 810, Miglyol 812, were purchased from hyper-chem LTD CO, China,tween 20 was purchased from SCRC, China, tween 40 was purchased from Avondale Lab, England , tween 60 was purchased from CP, China, tween 80 was purchased from Pure Chemistry, Germany, polyethylene glycol 200 (PEG 200) was purchased from BDH limited poole, England, potassium dihydrogen phosphate (KH2PO4), disodium hydrogen phosphate (Na2HPO4), and hydrochloric acid (HCl) were purchased from Thomas Baker, India.

Screening of SEDDS formulation components

Saturation solubility studies

Excess of CC was added to each vehicle (oils, surfactants and cosurfactants) and left in the water bath shaker for 72 hour (hr) under constant vibration to prepare a saturated solution. After this period, the solutions were filtered through a 0.45 micrometer "µm" millipore filter. Samples were suitably diluted with ethanol and analyzed by UV/Vis spectrophotometer at λ max of CC. Three measurements were accomplished for each sample to calculate the solubility of CC [19].

Construction of pseudo-ternary phase diagrams

After screening various oils, surfactants and cosurfactants, SEDDS were formulated with triacetin as oil, tween 80 as surfactant, and tetraglycol as co-solvent. The boundaries of the phase diagram designated the three components of the system. One of the axes represents the aqueous phase, the second axis for the oil and the third axis representing the surfactant with co-surfactant mixture. Pseudo-ternary phase diagrams of oil, surfactant with co-surfactant mixture and the aqueous phase were constructed using the aqueous titration method [20].

Preparation of CC SEDDS and formulation optimization

Self-emulsifying drug delivery system (SEDDS) liquid formulations of CC were prepared using triacetin, tween 80 and/or cremophore El and tetraglycol in ratios (1:1, 1:2, 1:3, 1:4) and oil: Smix at 1:9 ratios or 2:8 ratios (table 1). Preparation includes mixing CC with

triacetin oil in a screw-capped glass vial at the concentration of (8 mg/0.1 ml), and then heated in a water bath at (50-60 °C) for 20 min. to facilitate homogenization [21].

The components were mixed by vortex mixing for 5 min to obtain a clear, uniform mixture and again cooled to room temperature followed by equilibrating the mixture on a sonicator at room temperature for 15 min, after that the formulations were kept under visual observation for at least 48 hr. and examined for any signs of turbidity or phase separation prior to droplet size distribution studies [22].

Table 1.Components and percentage of SEDDS of CC.

Formula no.	Smix ratio	Oil:Smix ratio	Triacetin %(w/w)	Tween 80 %(w/w)	Cremophor EL %(w/w)	Tetraglycol %(w/w)
F-1	1:1	2:8	20	20	20	40
F-2	1:1	1:9	10	22.5	22.5	45
F-3	2:1	2:8	20	26.7	26.7	26.67
F-4	2:1	1:9	10	30	30	30
F-5	3:1	2:8	20	30	30	20
F-6	1:1	2:8	20	30	10	40
F-7	1:1	1:9	10	33.75	11.25	45
F-8	2:1	2:8	20	40.05	13.35	26.6
F-9	2:1	1:9	10	45	15	30
F-10	3:1	2:8	20	45	15	20
F-11	1:1	2:8	20	10	30	40
F-12	1:1	1:9	10	11.25	33.75	45
F-13	2:1	2:8	20	13.35	40.05	26.6
F-14	2:1	1:9	10	15	45	30
F-15	3:1	2:8	20	15	45	20

Characterization of the optimized formulation

Thermodynamic study

It includes the following tests:

1. Centrifugation Test: The SEDDS formulations were centrifuged at 3500 revolution per minute "rpm" for 30 min. These formulations that overcome this test and maintain a monophasic state were taken for heating/cooling cycle's test [23].

2. Heating/Cooling Cycles Test: Six cycles were performed in this test. Formulations were kept in refrigerator temperature of 4 °C for 48 hr and then in oven temperature of 45 °C for also 48 hr in each cycle. The formulations that pass this test were subjected to freezing-thawing cycle's test [23].

3. Freezing/ Thawing Cycles Test: Formulations were kept at a temperature (–21) for overnight and then kept at room temperature (+25 °C) until they were melted completely. Formulations that remain clear and not separate were selected for further studies [24].

Determination of self-nanoemulsification time

The nanoemulsification time of CC SEDDS was determined using USP type II dissolution apparatus. About 0.1 ml quantity of each formulation was added to 200 ml of 0.1 N HCl at 37°C. The samples were gently stirred at 50 rpm and visually monitored (i.e., until a transparent homogenous system was seen) to determine the time (min) for complete nanoemulsification according to the visual observation criteria for SEDDS formation (table 2). Upper limit for formation of good (transparent) SEDDS was set as one min, since when emulsification occurs slowly in more than one min, milky nanoemulsion with dull appearance will be formed [25].

Table 2. SEDDS visual observation grades [26]

Grade	Time required for nanoemulsion formation	Appearance
A	Within 1 min	Clear or slightly bluish
B	Within 1 min	Bluish white
C	Within 2 min	Bluish white, similar in appearance to milk
D	Longer than 2 min	Dull, ash emulsion, slightly oily appearance
E	Longer than 2 min	Poor or minimal emulsification , large oil droplets present on the surface

Particle size distribution and polydispersity index measurement

Particle size distribution and polydispersity index (PDI) measurements were performed for CC SEDDS formulations by using particle size analyzer instrument. [27] About 0.1 ml of each CC SEDDS was added to 200 ml of pH 0.1N at 50 rpm and 37°C, and then sample was taken from the resultant emulsion and filtered through 0.45 µm filter syringe and immediately measured [28].

Zeta potential measurement

Zeta potential determination was measured by zeta sizer instrument which relied on measuring electrophoretic mobility in micrometer per second (µm/second) and converting it to zeta potential by in–built software using Helmholtz-Smoluchowski equation. Positive particles with zeta potentials above (+30 mV) or negative particles with zeta potentials lower than (-30mV) are normally considered stable. Samples were prepared by the same method of measuring the particle size [29, 30].

Robustness to dilution

Robustness to dilution was evaluated by diluting all SEDDS formulations 100 and 1000 times with different dissolution media which were: 0.1N HCl (pH 1.2), Phosphate buffer (pH 6.8) and water. The diluted products were stored for 24 hr and monitored for any signs of phase separation or drug precipitation. Formula which give neither drug precipitation nor phase separation and are thus, said to be "robust" to dilution [29].

In vitro drug release study of CC SEDDS

The in vitro release of liquid SEDDS filled in hard gelatin capsule was performed in 900 ml of 0.5% tween 20 in 0.1 N HCl (pH 1.2), and the temperature was maintained at 37˚ C with paddle operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals of 5, 10,15,30,45 and 60 min. Aliquot were analyzed after filtration through Whatman filter paper (No.41), spectrophotometrically at 255 nm [31].

In vitro drug release kinetics study of CC SEDDS

To study the kinetics and mechanism of CC release from various NE formulations, data obtained from in vitro drug release study was plotted in various mathematical models including: zero order, first order, Higuchi's model and Korsmeyer's model [32].

Results and Discussion

Screening of SEDDS formulation components

Suitable vehicles with maximal solubilizing potential for the drug under investigation is crucial to achieve optimum drug loading, avoid precipitation of the drug on dilution in the gut lumen and finally minimize the final volume of SEDDS [33].

According to the saturation solubility study (table 3) triacein showed the highest solubility capacity for CC (9.37 mg/ml), and that is why it was selected for preparation of CC SEDDS. Triacetin is considered nontoxic and nonirritant when used as excipient in oral pharmaceutical dosage form[33]. Tween 80 and cremophore EL on other hand showed the highest solubility capacity in respect to surfactants. The high hydrophilic lipophilic balance (HLB) (≥ 12) is prerequisite to achieve o/w SEDDS, and since the HLB for tween is 15 and that of cremophore EL is 12, so both of them considered in this study.Finally transcutol P and tetraglycol showed excellent solubilizing capacity for CC with (142.94 mg/ml) for transcutol P and (173.4) for tetraglycol. Cosurfactants with low lipophilicity have a faster and better ability to emulsify an oil-surfactant mixture that is in contact with water, and since the log P-values of tetraglycol and transcutol P are -1.34 and -0.43, respectively [34], then tetraglycol was selected to be the cosolvent that was used in this study.

Table 3. Saturation solubility study for different oils, surfactants, and cosolvents with respect to CC.

Oil	Solubility(mg/ml)	SD
Labrafac CC	1.71	±0.87
Labrafac PG	1.22	±0.55
Labrafil CS	2.56	±0.56
Maisine 35-1	2.21	±0.96
Triactin	9.37	±0.79
Miglyol 810	1.21	±0.56
Miglyol 812	2.73	±0.37
Olive oil	5.71	±1.07
sunflower oil	7.93	±0.89
Surfactant		
Tween 20	4.23	±1.99
Tween 40	7.95	±1.02
Tween 60	10.73	±0.79
Tween 80	32.44	±0.75
Cremophore EL	29.81	±1.11
Cremophore RH	25.33	±2.27
Cosolvent		
PEG 200	27.33	±1.23
PEG 300	31.21	±1.09
PEG 400	48.58	±2.21
PEG 600	72.44	±1.45
Transcutol P	142.94	±2.91
Tetraglycol	173.4	±2.94

Pseudo-ternary phase diagram construction

The results for pseudo-ternary phase diagrams were illustrated in figures 1 - 3. Mixing of tetraglycol with tween 80 and cremophore EL to produce Smix was resulted in producing clear, slightly yellowish, low viscous solution.

Within the nano areas, only gentle magnetic stirring leads to formation of nanoemulsion, and this is related to action of the surfactant(s) used (tween 80 and/or cremophore EL in our study). Surfactant(s) localized on the surface of the NE droplets reducing the interfacial free energy and providing a mechanical barrier to coalescence resulting in a spontaneous dispersion [35].

Mixture of surfactants showed wide nano region and among the three different ratios that were used, (1:1, 3:1, and 1:3 of tween 80 and cremophore EL), (1:3 and 3:1) showed the bigger nano region.

These results are expected as the phase behavior is largely influenced by the size of the molecule of the oil used. triacetin oil has a smaller size molecules compared to that of the surfactants used, and so high degree of oil penetration was expected to occur in the interfacial surfactants layer forced by the large entropy of the nanosystem. A distinct central core, which greatly disrupts the packing of the surfactants molecules in this region, could be formed leading to destabilization of the nanoemulsion resulting in reduction in its existence with different oil: Smix ratios [36].

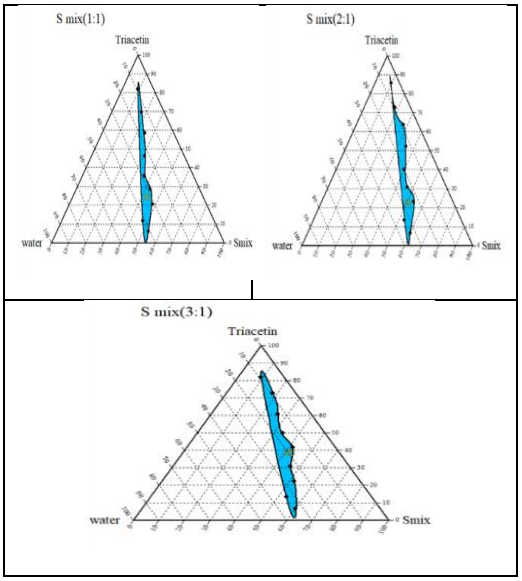


Figure 1. Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of triacetin oil (oil), tween 80 and cremophore EL (1:1) ratio (surfactant mixture), tetraglycol (co-surfactant) at different Smix ratios (1:1, 2:1, and 3:1) at 25 °C.

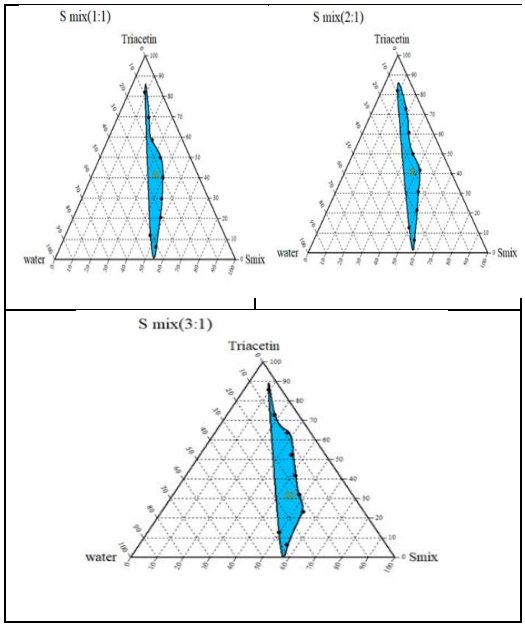
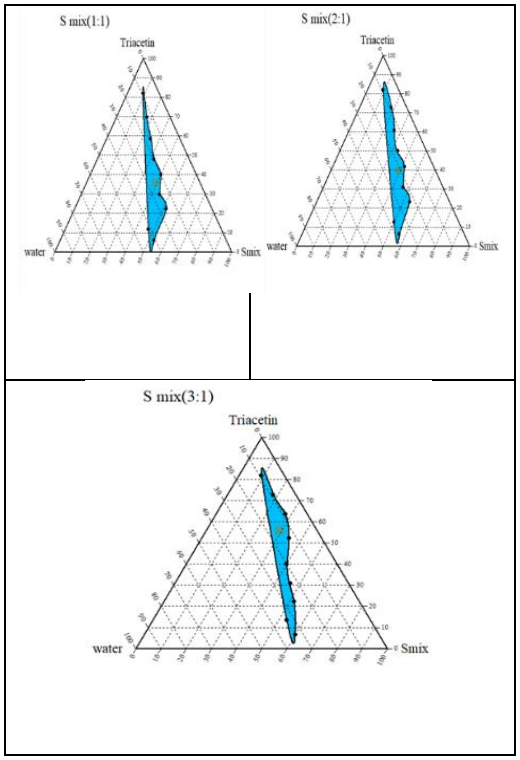


Figure 2. Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of triacetin oil (oil), tween 80 and cremophore EL (3:1) ratio (surfactant mixture), tetraglycol (co-surfactant) at



different Smix ratios (1:1, 2:1, and 3:1) at 25 °C.

Figure 3. Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of triacetin oil (oil), tween 80 and cremophore EL (1:3) ratio (surfactant mixture), tetraglycol (co-surfactant) at different Smix ratios (1:1, 2:1, and 3:1) at 25 °C.

Evaluation of prepared CC SEDDS

Thermodynamic study

Thermodynamic study was done for all SEDDS formulas, and all formulas pass successfully through these extreme conditions as no phase separation and/or flocculation was observed. The result of this study illustrated in table 4 and this indicates that sudden change in temperature has on effect on the entropy of the system [37].

Table 4. Result of thermodynamic study for all CC. formulations

Formula no.	Centrifugation test	Heating-cooling cycles test	Freeze-thawing cycles test
F-1	Pass	Pass	Pass
F-2	Pass	Pass	Pass
F-3	Pass	Pass	Pass
F-4	Pass	Pass	Pass
F-5	Pass	Pass	Pass
F-6	Pass	Pass	Pass
F-7	Pass	Pass	Pass
F-8	Pass	Pass	Pass
F-9	Pass	Pass	Pass
F-10	Pass	Pass	Pass
F-11	Pass	Pass	Pass
F-12	Pass	Pass	Pass
F-13	Pass	Pass	Pass
F-14	Pass	Pass	Pass
F-15	Pass	Pass	Pass

Determination of Self-Nanoemulsification Time

The ability of efficient self-emulsification is essential for SEDDS since the emulsification process is considered the rate limiting step for drug absorption, and this efficiency could be estimated through measuring the rate of emulsification time which was done by visual observation, considering one minute as maximum time for emulsification process to complete [38].

The rate of emulsification depends on degree of interfacial tension reduction, phase transition and on surfactant concentration. [39] The result of this test is shown in table 5 and it showed that all the formulas pass this test successfully with grade A result.

Lowest time of emulsification was achieved with combination of surfactants with high percentage of cremophore EL and this can be attributed to ability of them in reducing the interfacial tension and thus excess diffusion of the aqueous phase into the oil occurs, causing interfacial disruption and discharge of droplets into the bulk aqueous phase [40].

Table 5. Grade and emulsification time of SEDDS of CC.

Formula no.	Grade	Emulsification Time (sec.)
F-1	A	27
F-2	A	26
F-3	A	27
F-4	A	19
F-5	A	20
F-6	A	24
F-7	A	23
F-8	A	29
F-9	A	29
F-10	A	24
F-11	A	33
F-12	A	31
F-13	A	16
F-14	A	16
F-15	A	15

Particle Size distribution and polydispersity index measurement

The rate and extend of drug release in addition to drug absorption mainly depends on the particle size of SEDDS (table 6). Therefore, particle size determination is an essential factor for SEDDS. In most of the cases as the concentration of surfactant increased the surfactant concentration the mean droplet size decreased and this could be attributed to the stabilization of the oil droplets as a result of localization of the surfactant molecules at the oil/water interface [41].

Reading of these results explicated that surfactants mixture (tween 80+cremophore EL) in 1:3 ratio is best combination since it produced smallest particle size among all other maneuvers. Also the results showed that as the concentration of the surfactants mixture increase in the formula, the resultant particle size decreased. In addition to that results showed that as the ratio of cremophore EL increased in the mixture of surfactants smaller particle size was produced, so cremophore EL has better emulsifying capacity than tween 80.

There is no direct correlation between HLB value of the surfactants mixture and the resultant particle size and PDI, since the smallest particle size was produced with surfactants mixture that had lowest HLB value 13 among other used surfactants mixture ratios. On the other hand the obtained results were opposite to what was stated by Osterag F. et al and Qian C. et al, small molecular weight non-ionic surfactants are more efficient in producing smaller particle size, since the molecular weight of tween 80 (1310 g/ mol.) lower than that of cremophore EL (1630 g/ mol.) but cremophore EL showed better emulsification capability and lower particle size [42, 43].

The obtained result could be attributed to the theory that stats that molecular characteristics of surfactant can significantly influence the formation of SEDDS [44, 45].

The molecular geometry of a surfactant can be described by its surfactant packing parameter (CCP), and this differ from one surfactant to another and the result of differences in surfactant packing parameters influence the interfacial characteristics of the surfactant such as the curvature of the monolayer, which is generated by surfactant molecules spontaneously associate with each other in water and has a curvature allowing the most efficient packing of the surfactant molecules [46].

The change in spontaneous curvature of a surfactant during the emulsification process has been recognized to be a key factor for the nanoemulsion formulation, so there is an intermediate packing parameter which could be expected to impact the formation of fine oil droplets, and it seems that the packing parameter of cremophor EL may be closer to this intermediate value than tween 80 [47].

Results obtained for PDI values of formulations found to be less than 0.5 (figure 4) which indicates the uniformity of droplet size distribution and homogeneity of the formed dispersion [48, 49].

Table 6. Particale size and polydispersity index for CC formulations

Formula number	Partical size(nm)	Polydisersity index (PDI)
F-1	68.7	0.373
F-2	46.2	0.337
F-3	59.9	0.15
F-4	21.8	0.252
F-5	21.2	0.259
F-6	71.4	0.09
F-7	95.7	0.015
F-8	87.9	0.27
F-9	77.7	0.231
F-10	21.5	0.255
F-11	42.7	0.336
F-12	34.3	0.313
F-13	24.4	0.263
F-14	19.9	0.229
F-15	13.3	0.267

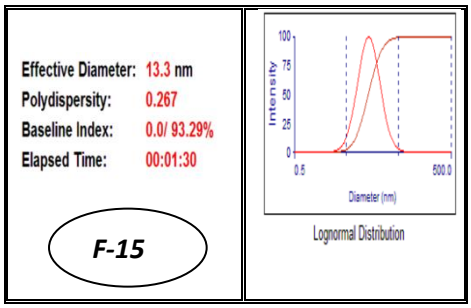


Figure 4. Particle size and polydispersity index for F- 15.

Measurement of zeta potential

Colloidal system stabilizes itself by combination of steric and electrostatic repulsion between particles. Steric stabilization can be achieved by thick layers of surfactant, which can prevent the coalescence between oil and aqueous droplets, additionally; the stability of the nano-droplets within the storage time could be due to the steric repulsion of the surfactant molecules in this system rather than electrostatic repulsion [50, 51].

Tween 80 and tetraglycol are both non-ionic and so, they will not contribute in any charge for the nano-particles [52].

The negative charge of the SEDDS droplets (table 7) was probably due to ionization of the free fatty acids present in cremophor EL , in addition, it was deduced that increasing HLB value leads to an increase in zeta potential due to increase hydrophilic property of the system. This could be attributed to an enhancement in the number of negatively charged hydroxyl groups of water with the increase in HLB value resulted in the observed increase in the zeta potential negativity [53].

Table 7. Zeta potential values of CC SEDDS formulations.

Formula no.	Zeta potential (mV)
F-1	-4.09
F-2	-8.91
F-3	-0.92
F-4	-3.6
F-5	-12.66
F-6	-16.07
F-7	-8.04
F-8	-0.88
F-9	-10.7
F-10	-0.36
F-11	-28.9
F-12	-16.21
F-13	-21.9
F-14	-30.89
F-15	-37.54

Robustness to dilution

All formulations did not showed any signs of phase separation or drug precipitation after 24 h and 48h storage. This result proved that dilution of liquid SEDDS did not change the rigidity of surfactants layer at the nano-droplet interface, since dilution may cause desorption of surfactants molecules from the nano-droplet surface which acts to maintain its water phase concentration equivalent to its CMC so that preserve the solubility of CC and prevent phase separation [54, 55].

In vitro drug release study

The result elicited that all SEDDS formulations were found to release nearly 100% of the drug at the end of 60 min (figures 5-7) and there is no significant difference in dissolution through fifteen formulations of CC SEDDS ($p>0.05$). One other hand there was a significant difference in dissolution of these formulations compared with marketed CC tablet and this difference in dissolution results due to limited surface area exposed of the marketed CC oral tablet to dissolution media compare with that offered by SEDDS [56]. Also the result showed that there is a direct relationship between the particle size of the formulation and the percentage of cumulative drug release after 60 min, as the particle size of the formula decreased the percent of cumulative release increased and this related to increase the exposed surface area to the dissolution media with reduction of globule size [57].

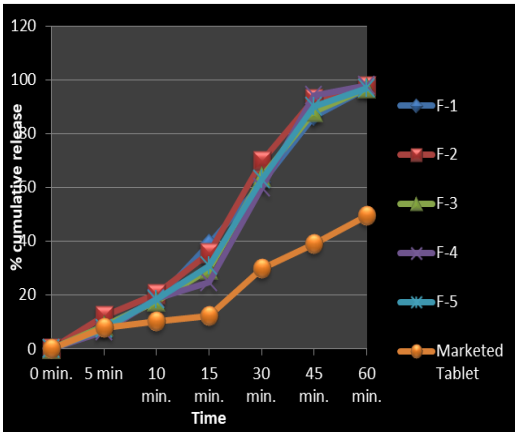


Figure 5. Comparative dissolution profile for formulas (F-1 to F-5) and marketed tablet of CC. in 0.1N HCl (pH 1.2) with 0.5 % tween 20 at 37 °C.

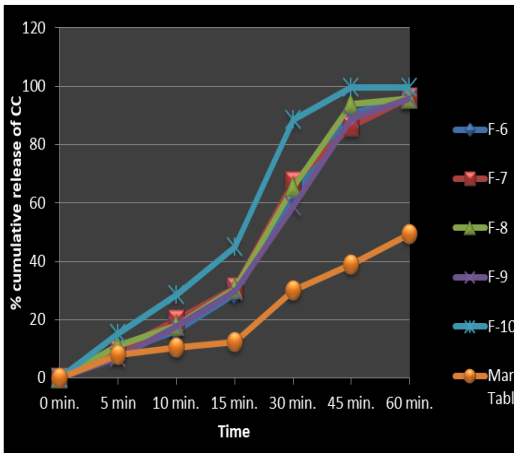


Figure 6. Comparative dissolution profile for formulas (F-6 to F-10) and marketed tablet of CC. in 0.1N HCl (pH 1.2) with 0.5 % tween 20 at 37 °C.

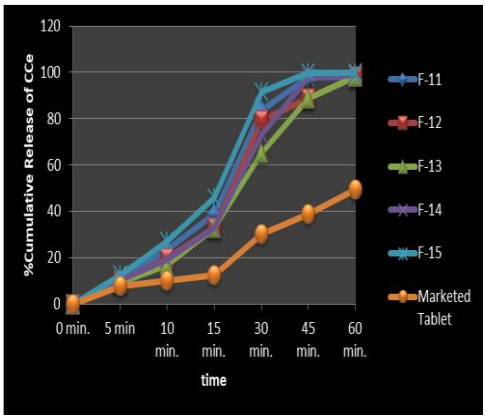


Figure 7. Comparative dissolution profile for formulas (F-11 to F-15) and marketed tablet of CC. in 0.1N HCl (pH 1.2) with 0.5 % tween 20 at 37 °C.

In vitro drug release kinetics study

The result of release kinetic of all CC SEDDS formulations illustrated in table 8. The best fit (highest R2 value) was found to be Korsmeyer-peppas equation and n value of above 0.5 and below 0.89 which indicated that the release mechanism was anomalous and the release was ruled by both diffusion of the drug and dissolution/ erosion.

Table 8. Release kinetic of CC SEDDS formulations.

Formula no.	Zero-order	First-order	Higuchi	Korsmeyer	n	Release mechanism
F-1	0.966	0.949	0.946	0.981	0.86	anomalous
F-2	0.953	0.969	0.945	0.972	0.86	anomalous
F-3	0.972	0.955	0.933	0.99	0.87	anomalous
F-4	0.968	0.939	0.912	0.993	0.85	anomalous
F-5	0.969	0.961	0.933	0.99	0.86	anomalous
F-6	0.969	0.961	0.924	0.99	0.87	anomalous
F-7	0.965	0.968	0.942	0.984	0.87	anomalous
F-8	0.956	0.95	0.929	0.981	0.87	anomalous
F-9	0.975	0.957	0.929	0.992	0.86	anomalous
F-10	0.887	0.935	0.938	0.946	0.84	anomalous
F-11	0.906	0.945	0.928	0.97	0.84	anomalous
F-12	0.931	0.956	0.928	0.98	0.85	anomalous
F-13	0.969	0.94	0.936	0.987	0.86	anomalous
F-14	0.943	0.938	0.927	0.981	0.85	anomalous
F-15	0.875	0.91	0.925	0.956	0.83	anomalous

Conclusion

The new formulations (SEDDS) are a promising approach for the formulation of CC. The oral delivery of water-insoluble drugs like CC may be possible by using SEDDS approach, which has been showed to be significantly improving oral bioavailability with future development of this technology. These current results demonstrated that SEDDS containing 20% w/w triacetin (oil), 15% w/w, tween 80 (surfactant), 45% cremophore EL (surfactant) and 20% w/w tetraglycol (co-surfactant) was successfully developed with an increased solubility, increased dissolution rate of a poorly water-soluble drug (CC) when compared to all

other formulations of SEDDS and marketed form of the drug. The result from the thermodynamic stability studies confirms the stability of the all developed formulation. Thus, the study confirms that the SEDDS of CC can be used as a possible alternative drug delivery to traditional oral formulations of CC with improved solubility and drug release.

References

1. Haritha B, A Review on Evaluation of Tablets. Journal of Formulation Science & Bioavailability. 2017; 1 (1): 1-5.
2. Hetal P. T., Jagruti L. D., Influence of excipients on drug absorption via modulation of intestinal transporters activity. Asian Journal of Pharmaceutics. 2015; 9:69-82.
3. Kunde S.D., Bhilegaonkar Sh., Godbole A.M.,Gajre P., Biopharmaceutical Classification System: A Brief Account. International Journal of Research Methodology. 2015; 1(1):20-46.
4. Rohan Ghadi R., Dand N., BCS class IV drugs: Highly notorious candidates for formulation development. Journal of Controlled Release.2017; 248: 71–95.
5. Thakare P., Mogal V., Borase P., Dusane J., Kshirsagar S., A review on self-emulsified drug delivery system. Journal of pharmaceutical and biological evaluations.2016; 3(2): 140-153.
6. Mistry R., Sheth N., a review: self-emulsifying drug delivery system. International Journal of Pharmacy and Pharmaceutical Sciences.2011; 3(2): 23-28.
7. Zanchetta B., Chaud M. and Santana M.H., Self-Emulsifying Drug Delivery Systems (SEDDS) in Pharmaceutical Development. Journal of Advanced Chemical Engineering. 2015; 5(3): 1-7.
8. Khan AW, Kotta S, Ansari SH, Sharma RK, and Ali J. Potentials and challenges in self nanoemulsifying drug delivery systems. Expert Opinion on Drug Delivery. 2012; 9:1305-1317.
9. Patel P.A., Chaulang G.M., Akolkotkar A., et al. Self-Emulsifying Drug delivery system: A Review. Res. J Pharm and Tech. 2008; 1: 313- 323.
10. Barakat N.S. Enhanced Oral bioavailability of Etodolac by SEDDS: In vitro and in vivo evaluation. J. Pharm. Pharmacol., 2010; 62:173-180.
11. Taha E, Ghorab D, Zaghloul A. Bioavailability assessment of vitamin a self nanoemulsified drug delivery systems in rats: a comparative study. Medical Principles and Practice. 2007; 16: 355-359.
12. Perlman M.E., Murdande S.B., Gumkowski M.J., et al. Development of a self-emulsifying formulation that reduces the food effects for torcetrapib. Int. J. Pharm., 2008; 351: 15-22.
13. Tarate B., Chavan R. and Bansal A., Oral Solid Self-Emulsifying Formulations: A Patent Review. Recent Patents on Drug Delivery & Formulation. 2014; 8 (2): 1-18.
14. Royal Pharmaceutical Society of Great Britain. British national formulary 73. London: Royal Pharmaceutical Society; 2016. Notes on drugs and Preparations.120-123.
15. Bandyopadhyay S, Bhuria L, Lal D. An explicit review on quantitative estimation of candesartan cilexetil employing various analytical techniques. Pharma Anal Acta. 2013; 4(6):1-7.
16. Beale JM, Block J, Hill R. Organic medicinal and pharmaceutical chemistry.12th ed. Philadelphia: Lippincott Williams and Wilkins; 2010. Chapter 18. Drugs acting on the renal system. 613.
17. Darwhekar GN, Jain DK, Chouhan J. Biopharmaceutical classification of candesartan and candesartan cilexetil. Asi J Pharm and Life Sci. 2012; 2(2):295-302.
18. Gleiter CH., Christine Jägle CH., Gresser U.,and Mörike K., Candesartan. Journal of Cardiovascular Drug Reviews, 2004; 22(4): 263-284.
19. United States Pharmacopeial Convention. The official compendia of standards. United States Pharmacopeia 30/National Formulary 25. USA: Rockville; 2007.Monographs.1191-1193.
20. Selvam PR, Kulkarni PK, Dixit M. Preparation and evaluation of self-nanoemulsifying formulation of efavirenz. Ind J of Pharm Edu and Res. 2013; 47(1):47-54.
21. Yasser M, Gad S, El-sayed M, Ghorab M. The effect of converting liquid valsartan SNEDDS into solid SNEDDS using different solid carriers on its performance. Int J Bio and Pharm Res. 2013; 4(12):1015-1026.
22. Wais M, Samad AB, Khale AN, Aqil MO, Khan M. Investigation of nanoemulsion system for transdermal delivery of glibenclamide. Int J Pharm and Pharm Sci. 2012; 4(4):482-487.
23. Sureshkumar R, Gowthamarajan K, Bhavani P., Nanoemulsion for lymphatic absorption: investigation of fenofibrate nanoemulsion system for lymphatic uptake. Int J ChemTech Res. 2015; 7(2):832-841.
24. Yadav PS, Yadav E, Verma A, Amin S. Development, characterization, and pharmacodynamic evaluation of hydrochlorothiazide loaded self-

nanoemulsifying drug delivery systems. The Scientific World J. 2014; 2014:1-10.

25. Shukla SN, Modi DC, Shah DP. A review on solid self-nanoemulsifying drug delivery system: an approach for bioavailability enhancement. World J Pharm and Pharm Sci. 2016; 5(5):302-316.

26. Tiwari SB, Amiji MM. Improved oral delivery of paclitaxel following administration in nanoemulsion formulations. J. nanosci and nanotechnol. 2006; 6(8):1-7.

27. Vishwakarma A., Nimisha M., Srivastava R. and Srivastava D., preparation and evaluation of self emulsifying drug delivery system of rosuvastatin calcium. International Journal of Pharma and Bio Sciences. 2016; 7(1): 117 – 137.

28. Gupta AK, Mishra DK, Mahajan SC. Preparation and in-vitro evaluation of self emulsifying drug delivery system of antihypertensive drug valsartan. Int J Pharm and Life Sci. 2011; 2(3):633-639.

29. Sengel CT, Sezgin Z, Badilli U. Preparation of polymeric nanoparticles using different stabilizing agents. J Fac Pharm Ankara. 2009; 38(4):257-268.

30. Shukla J., and patel Sh., Formulation and evaluation of self micro emulsifying system of candesartan cilexetil. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2(4): 143-146.

31. Kumar M, Pathak K, Misra A., Formulation and characterization of nanoemulsion-based drug delivery system of risperidone. Drug Development and Industrial Pharmacy. 2009; 35(4):387-395.

32. Sohn Y., Lee S., Lee G., Na Y., Kim S., Seong I., et al., Development of self-microemulsifying bilayer tablets for pH-independent fast release of candesartan cilexetil. Journal of Pharmazie. 2012; 67: 917–924.

33. Florence A.,Attwood D., physiochemical principle of pharmacy, 4th edition, Pharmaceutical press, Royal pharmaceutical society of Great Britain. 2006. P: 178-228.

34. Yeom D., Song Y., Kim S., Lee S., Kang M., Lee S., et al., Development and optimization of a self-microemulsifying drug delivery system for atorvastatin calcium by using d-optimal mixture design. International Journal of Nanomedicine. 2015; 10: 3865–3878.

35. Souto EB, Nayak AP, and Murthy RS., Lipid nanoemulsions for anti-cancer drug therapy. Die Pharmazie-An Int J. Pharmaceutical Sciences. 2011; 66(7):473-478.

36. Reddy BV, and Navaneetha K., Formulation and evaluation of orodispersible tablets of candesartan. Pharm Innov J. 2015; 4(1):25-32.

37. Ashok K., Kuldeep S., Murugesh K., Sriram R., and Ramesh M., Formulation and development of an Albendazole self-emulsifying drug delivery system (SEDDS) with enhanced systemic exposure. Acta Pharm. 2012; 62: 563–580.

38. Naseem A., Sushama T., Roop K., and Manu J., Self-nanoemulsifying lipid carrier system for enhancement of oral bioavailability of Etoposide by P-Glycoprotein modulation: In-vitro cell line and in-vivo pharmacokinetic investigation. J. Biomed. Nanotechnol. 2013; 9: 1216–1229.

39. Ebrahimi P, Ebrahim B, Pourmorad F, and Honary S., Ferulic acid lecithin-based nano-emulsions prepared by using spontaneous emulsification process. Iran J Chem and Chem Eng. 2013; 32(3):17-25.

40. Kallakunta V., Bandari S., Jukanti R., and Veerareddy P., Formulation and evaluation of oral self-emulsifying powder of lercanidipine hydrochloride. Journal of Powder Technol. 2012; 221: 375–382.

41. Balakumar K., Raghavan Ch., Selvan N., and Rahman S., Self emulsifying drug delivery system: Optimization and its prototype for various compositions of oils, surfactants and co-surfactants. Journal of pharmacy research. 2013; 6: 510-514.

42. Ostertag F., Weiss J., and McClements D., Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. J. Colloid Interface Sci., 2012; 388: 95–102.

43. Qian C., and McClements D., Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. Food Hydrocolloids. 2011; 25: 1000–1008.

44. Guttoff M., Saberi AH., and McClements D., Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. Journal of Food Chem., 2015; 171: 117–122.

45. Saberi AH., Fang Y., and McClements D., Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. J. Colloid Interface Sci., 2013; 391: 95–102.

46. Zeng L., Xin X., and Zhang Y., Development and characterization of promising Cremophor EL-stabilized o/w nanoemulsions containing short-chain

alcohols as a cosurfactant. Journal of RSC Adv., 2017; 7: 19815–19827.

47. Prajapati ST. Formulation and evaluation of self-nanoemulsifying powder of ezetimibe. J Pharma Sci and Drug Des. 2014; 1(1):1-8.

48. Honary S, and Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1). Trop J Pharm Res. 2013; 12(2):255-264.

49. Swarbrick J. Encyclopedia of pharmaceutical technology. 3rd ed. New York: Informa Healthcare; 2002. Dispersed system. 4120-4127.

50. Sari TP, Mann B, Kumar R, Singh RR, Sharma R, and Bhardwaj M. Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocolloids. 2015; 43:540-546.

51. Sohn Y., Lee S., Lee G., Na Y., Kim S., Seong I., et al., Development of self-microemulsifying bilayer tablets for pH-independent fast release of candesartan cilexetil. Journal of Pharmazie.2012; 67: 917-924.

52. AboulFotouh KH., Allam A., El-Badry M., and El-Sayed A., Development and in vitro/in vivo performance of self-nanoemulsifying drug delivery systems loaded with candesartan cilexetil. European Journal of Pharmaceutical Sciences. 2017; 109: 503–513.

53. Seth A, Katti DS. A one-step electrospray-based technique for modulating morphology and surface properties of poly (lactide-co-glycolide) microparticles using pluronics. Int J nanomedicine. 2012; 7:5129-5136.

54. Prabhakar K, Afzal SM, Surender G, and Kishan V. Tween 80 containing lipid nanoemulsions for delivery of indinavir to brain. Acta Pharmaceutica Sinica B. 2013; 3(5):345-353.

55. Raval C, Joshi N, Patel J, and Upadhyay UM. Enhanced oral bioavailability of olmesartan by using novel solid self emulsifying drug delivery system. Int J Advanced Pharmaceutics. 2012; 2(2):82-92.

56. Padmapreetha J, and Arulkumaran KS. Effect of kolliphor EL on dissolution rate of leflunomide liquisolid compacts. J Pharm Sci and Res. 2016; 8(7):586-593.

57. Sharma B, Sharma A, Arora S, Gupta S, and Bishnoi M. Formulation, optimization and evaluation of atorvastatin calcium loaded microemulsion. J Pharm and Drug Deliv Res. 2012; 1(3):1-7.