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# Preparation and evaluation of solid supersaturable self-nanoemulsifying drug delivery system of candesartan cilexetil

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**Abstract**

Solubility problem of many effective pharmaceutical molecules is still one of the major challenges in the formulation of these molecules. Candesartan cilexetil (CC) is angiotensin II receptor antagonist and has very low water solubility and as a result of that low and variable bioavailability was produced. Supersaturable solid self- emulsifying drug delivery system (S-SSEDDS) showed a promising result in overcome solubility problem of many drug molecules with significant improvement in in-vivo bioavailability of drug molecules. CC was prepared as S-SSEDDS by using novel combination of two surfactants (tween 80 and cremophore EL) and tetraglycol as cosurfactant, in addition to use of triacetin as oil and solidify using different adsorbents (Avicel PH101, Avicel PH102, Aerosil and dibasic calcium phosphate), after that a suitable precipitation inhibitor was used (HPMC K100). Different tests were performed to confirm the stability of the final product which includes; measurement of micrometric properties of the resultant powder, in-vitro drug release, SPM, FTIR, X-ray powder diffraction, DSC and in-vivo plasma level measurement. The results suggest that preparation of CC. as S-SSEDDS is a promising technique for oral delivery of CC. in order to improve its bioavailability.

**Key words:** Candesartan cilexetil, Supersaturable, in-vivo evaluation, solid self-emulsification.

## INTRODUCTION:

Oral rout for drug delivery represent more than 70% of total dosage forms utilized by humans, and this can be related to its convenient and acceptability as a mean for the administration of drug molecules to patients since it associates with a high rate of patient compliance in one hand and economic and flexible dosage design in others. [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 to reach the 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 the biological membrane, termed as permeability. This classification system is called the Biopharmaceutical Classification System (BCS). (3)

Drug molecules that belong to class II have a 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 are crucial to overcome this major obstacle and improve the fraction that will reach the systemic circulation. (4)

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

SEDDS is a type of lipid-based drug delivery system, and it is isotropic mixture consists of oil, surfactant and co-surfactant or co-solvent that can 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 the action of G.I.T. peristalsis along with aqueous media is sufficient for emulsification process to complete. In addition, to enhancing drug solubilization, drug release and absorption also improve since; the drug was already dissolved and, upon emulsification, it will produce a very fine particle with a large surface area. (7)

Solid SEDDS (SSEDDS) provide an attractive alternative that can overcome most of SEDDS limitations still preserving all the advantages of it. Converting SEDDS into powder dosage form, by various solidification techniques, and then to one of solid oral dosage form, say; tablet, capsule, or pellets, will impart the merits of solid dosage forms (for example, improving patient acceptance, better stability, reduction of production cost and reproducibility) to the SEDDS. (8)

Drug precipitation is a process in which a drug solute precipitates in vivo when the solubilization capacity of the formulation for the drug has decreased. Several reasons attributed to this phenomenon and from these reasons are sharp pH change, dilution of the formulation with body fluids or digestion of solubilizing excipients in formulations. (9, 10) This precipitation results in a decrease the available amount of drug in-vivo that are ready for absorption and hence, affect the bioavailability and efficacy. (11)

Supersaturable SSEDDS are designed to minimize precipitation of drug from SSEDDS in the gastrointestinal tract.

Supersaturable SSEDDS are thermodynamically stable formulations which contain a reduced amount of surfactant and a polymeric precipitation inhibitor. Precipitation inhibitors prevent precipitation of drug by generating and maintaining the supersaturated state in vivo following dilution with water.

Polymeric precipitation inhibitors employed in supersaturable SSEDDS formulation are mainly water-soluble cellulosic polymers like HPMC, PVP, Methyl

cellulose, HPMC phthalate, sodium CMC which can sustain the supersaturated state by preventing the precipitation of drug. (12)

Candesartan cilexetil (CC) is a selective, reversible, competitive angiotensin II receptor-1 antagonist, and it was used for the management of hypertension, heart failure, and myocardial infarction. It was also used in patients with impaired left ventricular systolic function, either when ACE inhibitors were not tolerated, or in addition to ACE inhibitors. (13, 14)

CC is a prodrug, and also it's a racemic mixture containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group. (15)

After oral administration, CC subjects to 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's a colorless to off-white crystals or powder, with a melting point range of (160-175 °C), and it's 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 a molecular weight of 610.66 Dalton. (16, 17).

## 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, was 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.

Preparation of CC SSEDDS

After selection of proper SEDDS consist of (triacetin as oil, tween 80 and cremophore EL as surfactants and tetraglycol as cosurfactant) in previous work, solid self-nanoemulsifying drug delivery system (SSEDDS) powders of CC, (table 1), were prepared by mixing (0.1 ml) amount of one of five of the best liquid SEDDS formulas, with different type of the adsorbent mixtures. Adsorbent mixtures used were: avicel 101 plus aerosil 200, avicel 101 plus dibasic calcium phosphate anhydrous, avicel 102 plus aerosil 200 and avicel 102 plus dibasic calcium phosphate anhydrous. The adsorbent mixture was added to each formula and then was mixed by using mortar and pestle for 10 min. After that, the prepared mixture was dried in an oven at 40°C for a period of 48 hr. After drying, a quantity of this system (250 mg) equivalent to 8 mg of CC was taken and filled into hard gelatin capsule size 0. (18)

Table (1): Compositions of CC SSEDDS

| *Solid Formula code* | *Avicel 101 (mg)* | *Avicel 102 (mg)* | *Aerosil 200 (mg)* | *Dibasic calcium phosphate anhydrous (mg)* |
|---|---|---|---|---|
| SF-1 | 150 | | 5 | |
| SF-2 | 150 | | | 5 |
| SF-3 | | 150 | 5 | |
| SF-4 | | 150 | | 5 |
| SF-5 | 150 | | 5 | |
| SF-6 | 150 | | | 5 |
| SF-7 | | 150 | 5 | |
| SF-8 | | 150 | | 5 |
| SF-9 | 150 | | 5 | |
| SF-10 | 150 | | | 5 |
| SF-11 | | 150 | 5 | |
| SF-12 | | 150 | | 5 |
| SF-13 | 150 | | 5 | |
| SF-14 | 150 | | | 5 |
| SF-15 | | 150 | 5 | |
| SF-16 | | 150 | | 5 |
| SF-17 | 150 | | 5 | |
| SF-18 | 150 | | | 5 |
| SF-19 | | 150 | 5 | |
| SF-20 | | 150 | | 5 |

**Evaluation of prepared SSEDDS of CC.**

**Angle of Repose measurement**

The angle of repose is an angle between the sides of cone and horizontal surface after pouring powder through a funnel onto a horizontal surface". (19) The angle is an indicator of the cohesiveness of the powder, since the point at which the interparticle attraction exceeds the gravitational pull on a particle is expressed by it. Particle size, shape and moisture content are among the most important factors that may affect the angle of ribose. (20)

Formation of a cone with facile edges assigned a low angle of repose corresponding to a free-flowing powder; whereas a cone with steeper edges assigned a poor-flowing capability with a high angle of repose. The value obtained was calculated using (Equation 1) and compared with ranges mentioned in the table (2). (21)

$\tan \theta = h/r$…….. (Equation 1)

Where: h= height of powder cone; and r =radius of powder cone.

Table (2): The Angle of Repose of the Resultant Powder (19)

| *Angle of repose (degrees)* | *Type of flow* |
|---|---|
| 25-30 | Excellent |
| 31-35 | Good |
| 36-40 | Fair |
| 41-45 | Passable |
| 46-55 | Poor |
| 56-65 | Very poor |
| Over 66 | Very, very poor |

2.3.7.2. Measurement of Poured Density and Bulk Density
A quantity of 2g of SSEDDS poured into 10 ml measuring graduated cylinder. Initial volume recorded, and the cylinder was allowed to fall under its weight from a height of 2.5 centimeters into a surface at 2 seconds intervals. When there is no further change in volume of the powder obtained then the tapping process stopped. Both densities were calculated using (Equation 2) and (Equation 3). (22)

Poured density (BD) = (Weight of powder blend)/ ( Poured volume of powder blend ) ..... (Equation 2)
Tapped density (TD) = (weight of powder blend )/(Tapped volume of powder blend)..... (Equation 3)
2.3.7.3.Measurement of Hausner's Ratio and Carr's Index
Hausner's ratio and Carr's index (compressibility index) are two terms give a useful measure of powder flowability and calculated using (Equation 4) and (Equation 5), respectively which were illustrated below. Hausner's ratio is related to interparticulate friction and varies from about 1.2 for free-flowing powders to 1.6 for cohesive powders (table 3). Carr's index is a direct measure of the potential powder arch or bridge strength and stability. It is classified into ranges as listed in table 13. (20, 21, 23)
Hausner's ratio= (Tapped bulk density)/(Poured bulk density) … (Equation 4)
Carr's index= (100×(Tapped bulk density-Poured bulk density))/(Tapped bulk density)…... (Equation 5)

Table (3): Carr's Index and Hausner's Ratio for Supersaturable SSEDDS.

| Carr's index | Hausner's ratio | Type of flow |
|---|---|---|
| **1-10** | 1.00-1.11 | Excellent |
| **11-15** | 1.12-1.18 | Good |
| **16-20** | 1.19-1.25 | Fair |
| **21-25** | 1.26-1.34 | Passable |
| **26-31** | 1.35-1.45 | Poor |
| **32-37** | 1.46-1.59 | Very poor |
| **>38** | > 1.60 | Very, very poor |

- **Determination of Drug Content**
  Drug content was measured in each formula using UV/Vis spectrophotometer. Taking about 250 mg of SSEDDS which contains 8 mg of drug was dissolved in 100 ml of ethanol and sonicated for 15 min to ensure complete mixing. From this solution, 1ml was taken and diluted to 25 ml with ethanol, filtered through 0.45 µm membrane and absorbance was taken in UV/Vis spectrophotometer at 255nm against a blank and drug content was determined. (24)
- **In vitro Drug Release Study of CC SSEDDS**
  The in vitro release of SSEDDS filled in hard gelatin capsule performed in 900 ml of 0.5% Tween 20 in 0.1 N HCl, and the temperature maintained at 37˚ C with paddle operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals of 5, 10, 15, 20, 30, and 40 min with replenish it with fresh dissolution media.
  Aliquot was analyzed after filtration through Whatman filter paper (No.41), spectrophotometrically at 255 nm. (25)
- **In vitro Drug Release Kinetics Study of CC SSEDDS**
  To study the kinetics and mechanism of CC release from various SSEDDS formulations, data obtained from in vitro drug release study was plotted in various mathematical models including: zero order, first order, Higuchi's model, Korsmeyer's model, and Hixson Crowell's model. (26, 27)

**Selection of Optimum CC. SSEDDS**
The choice of the best CC SSEDDS formula was achieved based on the results gained from the evaluation tests including: angle of repose, Hausner's ratio, Carr's index, drug content and in vitro drug release study.

**Preparation of Supersaturable SSEDDS of CC**.
Supersaturable SSEDDS (S-SSEDDS) of CC prepared by using two different hydrophilic polymers with different concentration as shown in table (4).
The method of preparation was involved mixing of precipitation inhibitor (HPMC and/or PVP K30) with selected SSEDDS for 15 minutes by using mortar and pestle. (19)

Table (4): Composition of Supersaturable SSEDDS.

| Formula Code | *HPMC K100* | *PVP K30* |
|---|---|---|
| **SSF-1** | 6.75 mg (2.5% (w/w)) | |
| **SSF-2** | 12.5 mg (5%(w/w)) | |
| **SSF-3** | | 6.75 mg (2.5%(w/w)) |
| **SSF-4** | | 12.5 mg (5%(w/w)) |
| **SSF-5** | 6.75mg (2.5 (w/w)) | 6.75mg (2.5 (w/w)) |

**In-vitro precipitation test**
One hard gelatin capsule contains approximately 262 mg of S-SSEDDS formulations added to 200 mL of 0.1N HCl previously placed in a dissolution vessel of USP Dissolution apparatus II (Paddle). The temperature kept at 37◦C, and the rotation rate adjusted at 100 rpm. Solution samples, each of 3 mL were withdrawn from the test medium without volume replenishment at 5, 10, 15, 20, 25, 30, 45, 60, 90 and 120 min. Withdrawn samples were filtered through a 0.22 µm filter syringe and analyzed for CC content by measuring their UV absorbance at 255 nm. (28)

**Optimum CC SSEDDS Evaluations**
*Morphology Examination*
Scanning probe microscope (SPM) was used for this purpose. The principle of this instrument is relay on the interaction between a sharp tip and a surface to obtain the image. (29)
*Fourier Transform Infrared Spectroscopy*
This test performed using FT-IR instrument at the range of 4000 cm-1 to 500 cm-1 to detect drug–excipients interaction. FT-IR performed for the pure drug (CC), SF-17

of SSF-2 (optimum formula) to check if there is any incompatibility between drug and the whole system.(30)

*Differential Scanning Calorimetry*

The differential scanning calorimetry (DCS) is a thermal analysis performed to study the thermotropic properties and physical transformation of various substances and to check for any interaction between drug and excipients. The DSC technique performed for the pure drug (CC), SF-17 and SSF-2 (optimum formula). The procedure includes taking about 5 mg of each sample, sealed in an aluminum pan and heated at a heating rate of 10 °C/min with a temperature range of 40°C to 300°C in DSC instrument. (31, 32)

*X-ray Powder Diffractometry*

Structural pattern of drug, polymorphic changes, drug-excipients interaction are important factors, which may affect the drug dissolution and bioavailability, therefore X-ray powder diffraction measurement was overlooked on four samples include: pure CC powder, physical mixture of optimum formula, SF-17 and SSF-2 using a diffractometer covering a range of 0–50° (2θ) using the copper (Cu)-target X-ray tube and xenon (Xe)-filled detector and relying on the fact that this radiation has a strong penetrating power in materials with a rate of absorption depending on the density of the material. (33, 34)

**Pharmacokinetic study (35)**

.Drug administration

Fifteen male (2.3–2.9 kg) healthy adult albino rabbits divided into three groups. Each group consisting of five rabbits each. Each rabbit was housed individually under environmentally controlled conditions (25±2 °C, 12 h light/dark cycle). Prior to each experiment, the rabbits were starved for 24 h and were allowed free access to tap water. Suspension of Atacand® tablet, SF-17 and SSF-2 were orally administered into each.

Sampling procedures

Blood samples (2 mL) were collected at 0.0, 0.25, 0.5, 0.75, 1.5, 2, 4, 6, 8 and 12 h of drug administration from a marginal ear vein into EDTA tubes and placed on ice, protected from light. Samples stored at -20°C until assayed.

Preparation of plasma samples

Liquid–liquid extraction technique was employed for all the plasma samples (the method validated regarding selectivity, specificity, linearity, precision, and accuracy). An aliquot of 500 mL plasma mixed with 25 mL of internal standard (paracetamol, 10 mg/mL) was spiked with 3mL of HPLC grade acetonitrile by vortex mixing for 3 min. The mixture centrifuged at 10500 rpm for 10 min, the supernatant filtered through 0.45 mm filter (Millipore), and the organic supernatant evaporated to dryness at room temperature. The residue reconstituted with 200 mL of mobile phase, then the solution centrifuged at 15000 rpm for 10 min; finally, an aliquot of 20 mL of the solution injected into the HPLC equipped with a UV detector at a flow rate of 1 mL/min, and the run time was 10 min. The mobile phase comprised 60% acetonitrile: 40% methanol at pH 6.0 determined at UV wavelength of 255 nm.

## RESULT AND DISCUSSION

**Preparation of CC SSEDDS**

This result in the formulation of twenty formulas of white, fluffy, and free flowing powder and it symbolized as SF-1 to SF-20.

**Evaluation of prepared SSEDDS of CC.**

Powder flowing properties

Three micrometric properties measured for the prepared powder of SEDDS of CC, and the result shown in table (5). The result showed that the powders, in general, have either excellent or good micrometric property which indicated good flow and good compressibility.

Determination of drug content

Drug contents measured for all SSEDDS and the result listed in table (6), and these result showed that all of these formulations had drug content within the accepted range ( using USP as reference).

Table (5): Micrometric Properties of Solid SEDDS of CC. (mean ±SD, n=3)

| Formula no. | Angle of Ribose | Flow character | Hausner's ratio | Flow character | Carr's index | Flow character |
|---|---|---|---|---|---|---|
| **SF-1** | 26.19 ±1.50 | Excellent | 1.07±0.0084 | Excellent | 8.12 ±1.40 | Excellent |
| **SF-2** | 28.60 ±1.10 | Excellent | 1.11±0.003 | Excellent | 7.20 ±1.18 | Excellent |
| **SF-3** | 31.72 ±2.10 | Good | 1.121±0.004 | Good | 12.41 ±2.08 | Good |
| **SF-4** | 31.70 ±1.12 | Good | 1.14±0.001 | Good | 11.80 ±2.28 | Good |
| **SF-5** | 33.20 ±1.17 | Good | 1.24±0.021 | Good | 11.03 ±0.76 | Good |
| **SF-6** | 29.24 ±1.69 | Excellent | 1.11±0.0010 | Excellent | 8.04 ±1.02 | Excellent |
| **SF-7** | 28.90 ±1.90 | Excellent | 1.04±0.0031 | Excellent | 6.92 ±1.80 | Excellent |
| **SF-8** | 32.99 ±0.81 | Good | 1.152±0.003 | Good | 12.84 ±1.30 | Good |
| **SF-9** | 31.96 ±1.40 | Good | 1.141±0.0175 | Good | 14.09 ±1.20 | Good |
| **SF-10** | 26.56±1.88 | Excellent | 1.066±0.0054 | Excellent | 7.30±3.10 | Excellent |
| **SF-11** | 31.96 ±1.69 | Good | 1.394±0.0023 | Good | 12.43 ±2.84 | Good |
| **SF-12** | 26.05±1.20 | Excellent | 1.105±0.0005 | Excellent | 7.98 ±1.62 | Excellent |
| **SF-13** | 32.02 ±1.12 | Good | 1.331±0.0063 | Good | 13.05 ±1.90 | Good |
| **SF-14** | 26.56 ±2.10 | Excellent | 1.057±0.0032 | Excellent | 6.84±1.30 | Excellent |
| **SF-15** | 33.02 ±2.20 | Good | 1.148±0.0062 | Good | 12.80 ±2.11 | Good |
| **SF-16** | 26.56 ±1.57 | Excellent | 1.068±0.0041 | Excellent | 6.36 ±1.10 | Excellent |
| **SF-17** | 27.75 ±2.30 | Excellent | 1.012±0.0072 | Excellent | 7.47 ±1.04 | Excellent |
| **SF-18** | 26.66 ±1.17 | Excellent | 1.062±0.0033 | Excellent | 6.48 ±1.90 | Excellent |
| **SF-19** | 27.40 ±2.10 | Excellent | 1.071±0.0025 | Excellent | 8.60 ±1.18 | Excellent |
| **SF-20** | 26.56±1.43 | Excellent | 1.032±0.0031 | Excellent | 8.25±1.90 | Excellent |

Table (6): Drug Content of Solid-SEDDS of CC. (mean ±SD, n=3)

| Formula code | % drug content | Formula code | % drug content |
|---|---|---|---|
| **SF-1** | 98.32 ± 0.42 | SF-11 | 96.29± 0.93 |
| **SF-2** | 99.03 ± 0.21 | SF-12 | 96.99±1.08 |
| **SF-3** | 98.8±1.02 | SF-13 | 98±0.98 |
| **SF-4** | 97.11±0.88 | SF-14 | 99.2±0.79 |
| **SF-5** | 98.22±0.81 | SF-15 | 98.08±1.28 |
| **SF-6** | 96.71±1.2 | SF-16 | 99.02±0.99 |
| **SF-7** | 98.62±0.73 | SF-17 | 98.33±1.18 |
| **SF-8** | 98.89±0.89 | SF-18 | 98.2±0.83 |
| **SF-9** | 99.21±1.22 | SF-19 | 98.68±0.79 |
| **SF-10** | 97.41±1.42 | SF-20 | 98.07±1.14 |

Table (7): Release Kinetic of CC. SSEDDS Formulations

| Formula code | Zero-order | First-order | Higuchi | Hixon | Korsmeyer-peppas | n | Release mechanism |
|---|---|---|---|---|---|---|---|
| **SF-1** | 0.948 | 0.918 | 0.912 | 0.973 | 0.98 | 0.76 | anomalous |
| **SF-2** | 0.961 | 0.917 | 0.915 | 0.976 | 0.983 | 0.76 | anomalous |
| **SF-3** | 0.959 | 0.913 | 0.916 | 0.979 | 0.983 | 0.76 | anomalous |
| **SF-4** | 0.961 | 0.929 | 0.905 | 0.979 | 0.986 | 0.77 | anomalous |
| **SF-5** | 0.959 | 0.879 | 0.921 | 0.974 | 0.981 | 0.76 | anomalous |
| **SF-6** | 0.959 | 0.888 | 0.92 | 0.977 | 0.979 | 0.76 | anomalous |
| **SF-7** | 0.964 | 0.912 | 0.914 | 0.978 | 0.985 | 0.77 | anomalous |
| **SF-8** | 0.969 | 0.926 | 0.914 | 0.98 | 0.985 | 0.77 | anomalous |
| **SF-9** | 0.855 | 0.918 | 0.914 | 0.944 | 0.949 | 0.73 | anomalous |
| **SF-10** | 0.869 | 0.93 | 0.921 | 0.946 | 0.951 | 0.74 | anomalous |
| **SF-11** | 0.916 | 0.948 | 0.917 | 0.963 | 0.971 | 0.75 | anomalous |
| **SF-12** | 0.957 | 0.943 | 0.903 | 0.979 | 0.987 | 0.76 | anomalous |
| **SF-13** | 0.871 | 0.914 | 0.917 | 0.931 | 0.94 | 0.74 | anomalous |
| **SF-14** | 0.915 | 0.94 | 0.926 | 0.957 | 0.96 | 0.75 | anomalous |
| **SF-15** | 0.942 | 0.95 | 0.913 | 0.973 | 0.977 | 0.76 | anomalous |
| **SF-16** | 0.96 | 0.945 | 0.91 | 0.974 | 0.979 | 0.77 | anomalous |
| **SF-17** | 0.791 | 0.784 | 0.908 | 0.823 | 0.913 | 0.72 | anomalous |
| **SF-18** | 0.812 | 0.915 | 0.916 | 0.9 | 0.921 | 0.72 | anomalous |
| **SF-19** | 0.864 | 0.926 | 0.918 | 0.933 | 0.945 | 0.74 | anomalous |
| **SF-20** | 0.878 | 0.926 | 0.92 | 0.939 | 0.947 | 0.74 | anomalous |

**In-vitro dissolution**

The result of the in-vitro release of SSEDDS illustrated in comparatively in figures (1 and 2).

SF-9, SF-10, SF-13, SF-18, SF-19, and SF-20 reached their maximum release after 30 min., while SF-17 reached its maximum release (99.88) after only 20 min.

Formulas that contained avicel 101 showed more efficient release rate comparing to avicel 102, and this could be related to the following reasons:

1- Avicel 101 has a lower particle size (about 40 % are less than 50µm) compare to avicel 102(particle size may reach to 200 µm), and small adsorbent particle size with its large surface area exposed and highly porous structure lead to a better soaking of the drug and more contact with dissolution medium, which also increases the drug dissolution rate. (36)
2- Physical properties of avicel 101 differ from that of avicel 102. Particles of avicel 102 have very rough surfaces, with many wrinkles, folds and are irregular in shape. (37)

Also, all formulations that contain aerosil 200 gave better release rate compare with that of dibasic calcium phosphate anhydrous and this because the smaller particle size of aerosol 200 (15nm) compare to that of dibasic calcium phosphate anhydrous (94 µm). This means that the surface area that will expose to dissolution media is much higher in the case of aerosol 200 than when we use dibasic calcium phosphate anhydrous.

Result also showed that formulations that contain a mixture of surfactants had better release profile than formulations that contain only one surfactant, and this may be related to a better coating of carrier around the drug molecules in case of surfactants mixture which leads to improving solubility and dissolution rate of the drug. (38)

**In-vitro release kinetic of SSEDDS**

The result of release kinetic of all CC. SSEDDS formulations illustrated in table (7), 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 indicates that the release mechanism is anomalous and the release is ruled by both diffusion of the drug and dissolution/ erosion.

**Selection of optimum CC. SSEDDS.**

Based on the results of flow properties and in-vitro dissolution, SF-17 was selected as best formula which contain triacetin 20% (w/w), cremophore EL 45%(w/w), tween 80 15%(w/w), avicel 101 150mg and aerosil 200 5mg, since it showed excellent flowing properties as indicated by the angle of repose (27.75) , Carr's index

(7.47), Hausner's ratio (1.012), optimum drug content (98.33) and fast in-vitro cumulative drug release (99.88 in 20 min), and that is why it was selected to test the best supersaturation inhibitors among two hydrophilic polymers HPMC K100 and PVP K30.

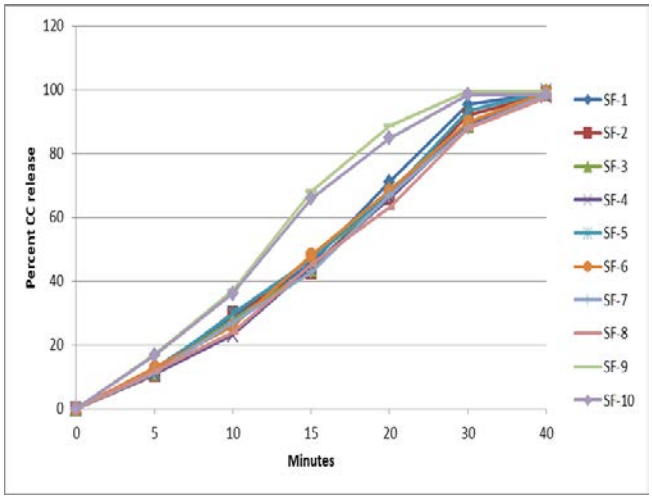


Figure (1): A comparative dissolution profile of CC. of SF-1 to SF-10 in 900 ml of 0.1 N HCl (pH 1.2) with 0.5% tween 20 at 37 °C.

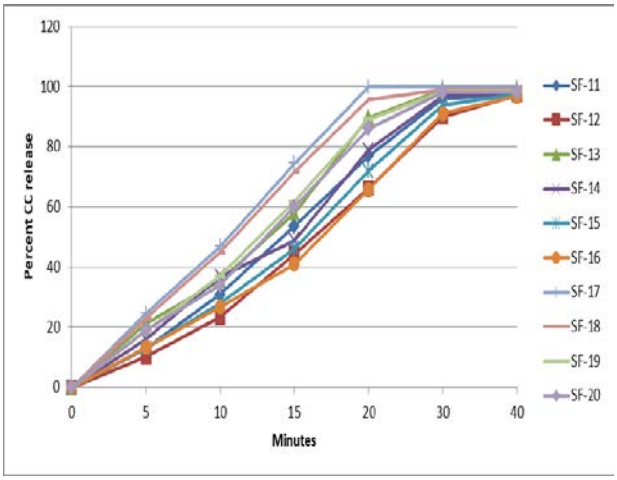


Figure (2): A comparative dissolution profile of CC. of SF-11 to SF-20 in 900 ml of 0.1 N HCl (pH 1.2) with 0.5% tween 20 at 37 °C.

**Preparation of Supersaturable SSEDDS of CC.**

Two hydrophilic polymers were used to prepare the supersaturable SSEDDS(S-SSEDDS), which are HPMC K100 and PVP K30, and they filled in capsule size 0 after mixing with SF-17.

**In-vitro precipitation**

In order to evaluate the drug concentration sustained in the supersaturated state and the degree of supersaturation as a function of time, an in-vitro test was performed. The total volume of the medium chosen was 200 mL, based on physiological considerations of the total volume of the residual stomach fluid in order to yield a non-sink condition for CC. The non-sink condition and inevitable precipitation of CC., related to the high degree of supersaturation, resulted in the complicated state of the drug in the medium: free drug, solubilized molecules partitioned into the dispersion, and precipitated solid particles. The distribution of CC among these states was dynamic and changed rapidly over time. CC concentration determined was a measure of the total concentration of drug present in various states rather than a measure of the free drug concentration in the test medium. (39)

Upon mixing with the selected media, the SSEDDS formulation initially appeared as a nanoemulsion with a colorless to bluish reflection. However, the solution developed cloudiness after 10 min, and about 20 min, solid precipitates of CC observed, which suggested that the medium was in a supersaturated state. As shown in Figures (8).

The results show that both hydrophilic polymers (HPMC K100 and PVP K30) had good precipitation inhibitory properties, but using HPMC K100 in 5% (w/w) was significantly ($p< 0.05$) retard the precipitation of CC.

The ability to generate a supersaturated state with HPMC may be due to the formation of a widely spaced cellulosic-polymer network that is created by the HPMC chains in water, since solutions of HPMC consist of 'cellulosic bundles resulting in a tenuous network of swollen clusters with hydrophobic substituents surrounded by sheaths of structured water HPMC polymer chain that inhibit nucleation, as well as crystal growth inhibited by adsorption of the HPMC molecules onto the surface of the nuclei, or onto the surface of crystals. (40)

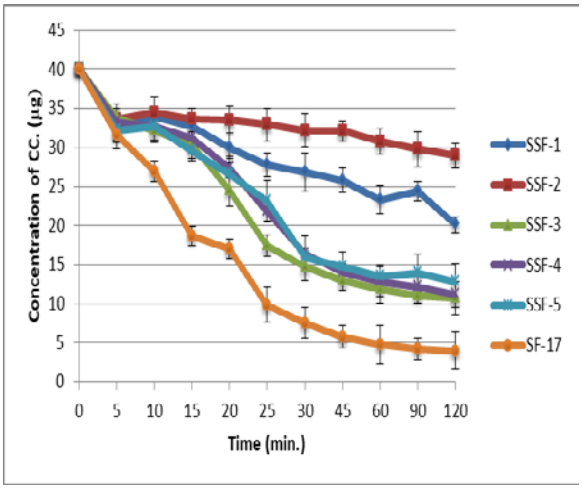


Figure (3): Effect of HPMC K100 and/or PVP K30 on the precipitation of the optimum formula. (mean ±SD, n=3)

**Morphology Examination**

The result that obtained from SPM of SSF-2 illustrated in figure (4), and it showed a group of spheres with little interparticulate contact and generally good properties. SPM analysis confirmed the nanometric droplet diameter of formulated S-SSEDDS of SSF--2 with an average droplet diameter of (77.45 nm). The particle size difference between this technology and that obtained by particle size analyzer can be attributed to the agglomeration of particles placed on a glass slide of SPM instrument, beside that the small volume of sample loaded also could be a reason since it made SPM unable to get a precise statistical determination of particle size distribution (PSD). (41)

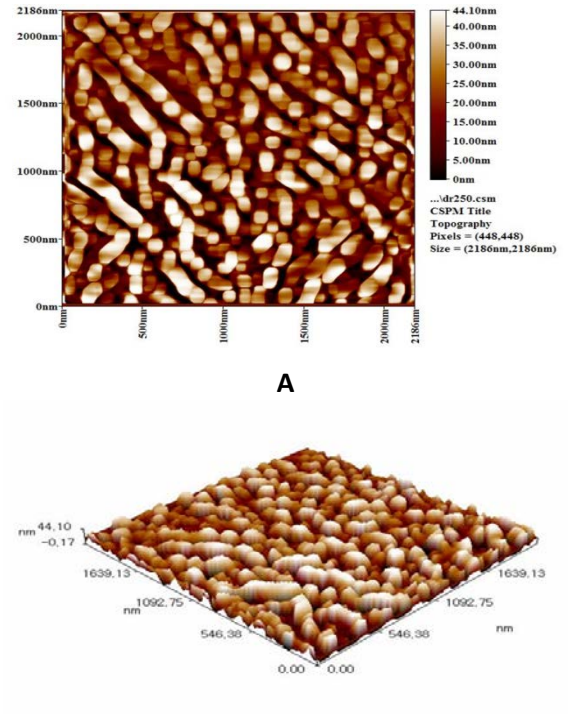


Figure (4): Scanning probe microscopic image of SSF-2 in: A) Two dimensional image (scope of image: 2488 nm, 2521 nm), and B) Three dimensional section. (Magnification power=106 X)

**Fourier Transform Infrared Spectroscopy**

The Fourier transform infrared spectroscopy (FT-IR) spectra of pure CC powder showed characteristic peaks which are: 2940.91 cm-1 due to aromatic (-C-H) stretching, 2861.85 cm-1 for (O-H) stretching, 1752.98 cm-1 and 1714.41 cm-1 for ester (−C=O) stretching vibration, 1276.65 cm-1 and 1313.29 due to (-C-O) stretching of the carbonyl group of aromatic ester and 746.32 due to (-O-) substitution. All obtained peaks were within an acceptable range of pure CC (42)

Results of FT-IR spectrum for SSF-2 (optimum formula) showed all the characteristic major peaks of CC which are: 1644.98 cm-1 corresponding for ester (−C=O) carbonyl stretching, a peak at 1743.33 cm−1 corresponding for ester (−C=O) carbonyl stretching and another peak at 1230.36 cm−1 corresponding to (-C-O) stretching in the aromatic ester. Accordingly, it suggests the lack of drug-carrier interaction. (43)

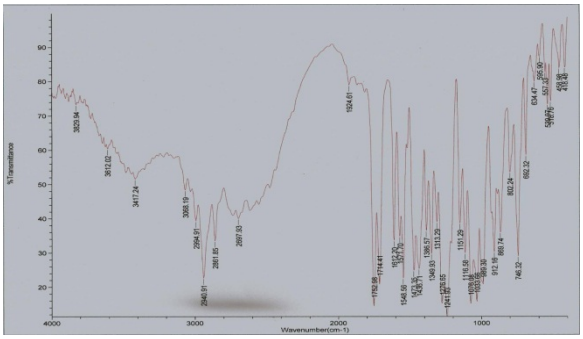

Figure (5): FTIR of CC. powder.

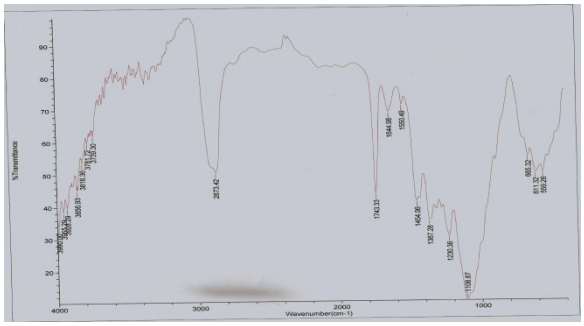

Figure (6): FTIR of SF-17.

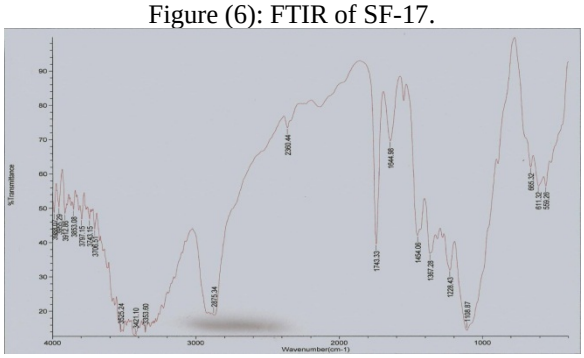

Figure (7): FTIR of SSF-2.

**Differential Scanning Calorimetry**

Pure CC showed a characteristic endothermic peak at (173.46 °C) which indicating its melting point and confirms the reported one.

No representative peak of CC was observed for both SF-17 and SSF-2 formulations, indicates that the drug was present in an amorphous form or in a molecularly dissolved state in triacetin oil core as was shown in figures (8-10). (44)

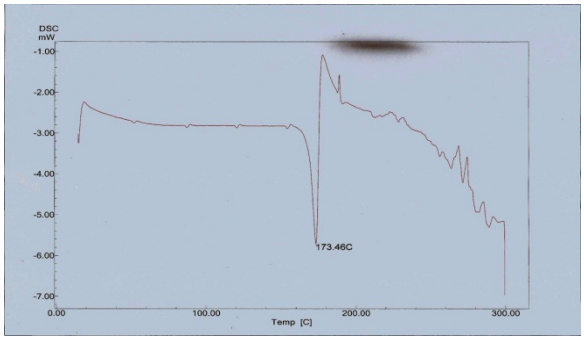


Figure (8): DSC of pure CC. powder.

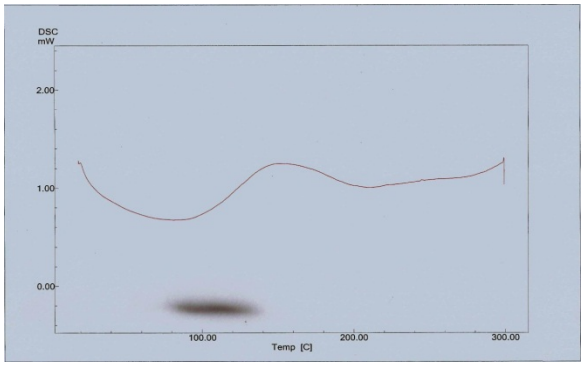


Figure (9): DSC of SF-17.

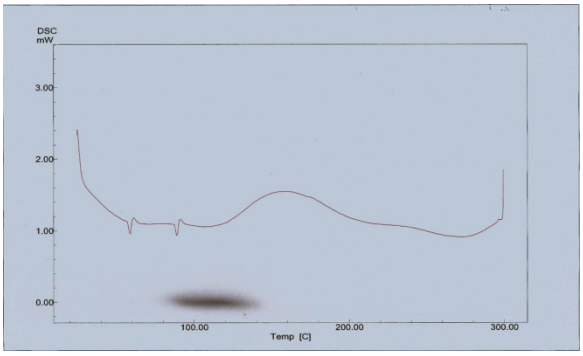


Figure (10): DSC of SSF-2.

**X-ray Powder Diffractometery**

The X-ray diffractograms of pure CC, a blank physical mixture of the optimum formula (SSF-2), SF-17 and SSF-2 are shown in Figures (11, 12, 13, and 14) respectively. The X-ray powder diffraction (XRPD) pattern of pure CC powder revealed that the drug was clearly in a crystalline state as it showed sharp, distinct peaks notably at 2θ diffraction angles of 20.400, 22.1558°, 23.3538°, 25.0807°, 27.6891°, and 29.2056° similar to the record values. (45)

The results also showed an absence of obvious peaks representing crystals of CC in optimum formula (SSF-2), indicating that the drug was in an amorphous or disordered crystalline phase in the oily inner core. (44)

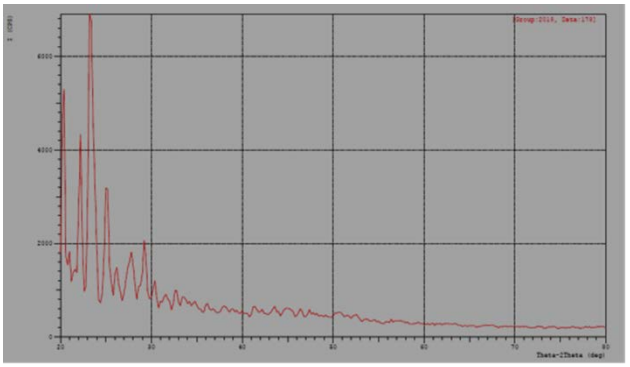

Figure (11): X-ray powder diffraction for pure CC. powder.

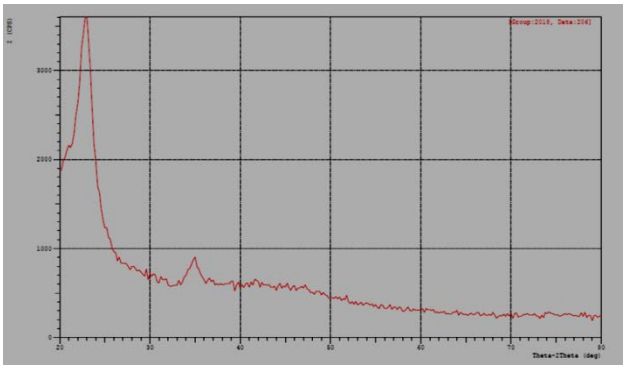

Figure (12): X-ray powder diffraction for physical mixture of SSF-2.

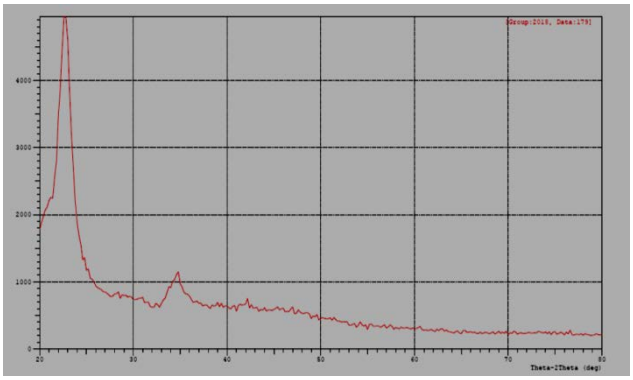

Figure (13): X- ray powder diffraction of SF-17.

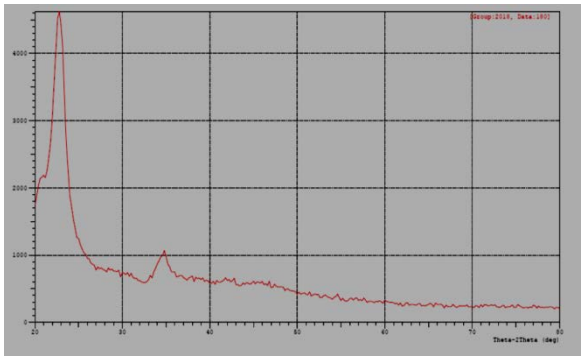

Figure (14): X-ray powder diffraction of SSF-2.

**Pharmacokinetic study**

In vivo studies in rabbits were conducted to evaluate whether there is a change in bioavailability occur and study the effect of supersaturation on the optimum formula.

The plasma concentrations of CC. were determined by HPLC method to evaluate the pharmacokinetic behavior of SSF-2, SF-17, and control (marketed CC product(Atacand®)). The plasma concentration– time profiles of CC after single-dose oral administration of each formula was presented in Figure (15) and the corresponding pharmacokinetic parameters were summarized in Table (8).

The experimental results showed a significant ($p<0.001$) difference occurred between the pharmacokinetic profiles of SSF-2 in comparison to that of control.

Taken together, at each time point, the plasma concentration of CC from SSF-2 was higher than those measured for the control and SF-17.

The peak concentrations ($C_{max}$) of SSF-2, SF-17, and control founded to be133.37 ±3.8, 52.31±4.5, and 24.97± 4.1 µg/mL, respectively, which was significantly higher ($p<0.05$) when compared to both SF-17 and control.

However, there was no change in the time to reach peak concentration ($T_{max}$) for both SSF-2 and SF-17 (2 hr), but significantly changed ($p<0.05$) compare with that of control (4 hr.), indicating that CC could be absorbed more rapidly after its prodrug, if it formulated as supersaturable-SSEDDS.

Areas under the concentration–time curves (AUC) for SSF-2 (200.42 mg h/mL) were enhanced by about 2-fold over the area under the concentration–time profiles of both SF-17, and control (110.97 and 98.34 µg h/mL; $p<0.05$).

Several mechanisms either alone or in combination might have contributed for the increased bioavailability of CC

from S-SSEDDS formulations. They were: (i) the enormous effective surface area by virtue of the nanosize of the used formulation might have resulted in an increased rate of absorption, (ii) the small size of the S-SSEDDS permit to adhere to GI tract and also to enter the intervallic spaces thus increasing the residence time for increased bioavailability, (iii) the influence of surfactant on the preferential uptake of lipid particles by Peyer's patches also result in improved bioavailability of CC due to the avoidance of first pass metabolism. (46)

Also, a change in the elimination half-life was observed between SSF-2, SF-17, and control (4.74, 5.25 and 4.23 hr) respectively.

This revealed that the oral bioavailability of CC could be increased in a significant manner if it formulated as S-SSEDDS.

The therapeutic effect of CC. occurred at 4–6 h after the oral administration of CC. tablets. Hence, the more rapid absorption of CC. from formulations could be helpful in the treatment of hypertension or heart failure in clinical therapeutics. (47)

Table (8): Pharmacokinetic Data for SSF-2 after Single Dose Administration to Rabbit.

| Parameter | SSF-2 | SF-17 | Control |
|---|---|---|---|
| $T_{max}$ hr | 2 | 2 | 4 |
| $C_{max}$ µg/ml | 133.37 | 52.31 | 24.97 |
| $K_{elimination}$ hr-1 | -0.146 | -0.132 | -0.164 |
| $t_{1/2}$ hr | 4.75 | 5.25 | 4.23 |
| AUC µg/ml hr | 200.42 | 110.97 | 98.34 |

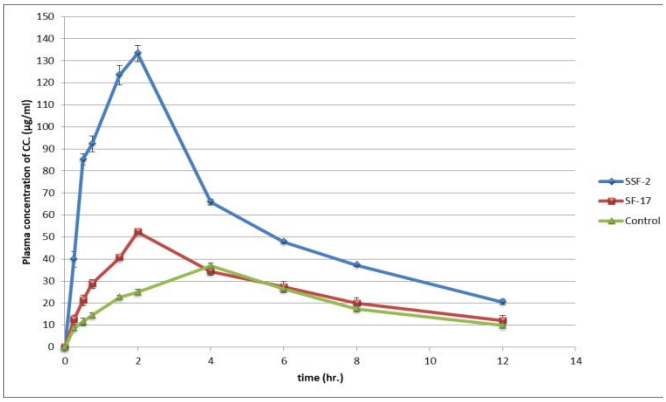


Figure (15): Plasma concentration–time profile for SF-17, SSF-2 and control after single dose administration to rabbit.

## CONCLUSION

The new formulations (S-SSEDDS) of CC are a promising technique for the formulation of CC. The oral delivery of water-insoluble drugs like CC may be possible by using S-SSEDDS approach, as it showed significant improvement in oral bioavailability. The results demonstrated that S-SSEDDS containing 20% w/w triacetin (oil), 15% w/w, Tween 80 + 45% cremophore EL (surfactant combination), 20% w/w tetraglycol (co-surfactant), Avicel PH101 150mg, Aerosil 5 mg and 10% HPMC K100 was successfully increase the in-vivo bioavailability of a poorly water-soluble drug (CC) when compared to SSEDDS and marketed form of the drug. Thus, this study confirms that the S-SSEDDS of CC can be used as a possible alternative drug delivery to traditional oral formulations of CC with improved solubility, drug release, and bioavailability.

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