Preparation and in-vitro evaluation of cilostazol self-emulsifying drug delivery system

Ali N. Wannas, Nidhal K. Maraie*

**Department of Pharmaceutics / College of Pharmacy/ Mustansiriyah University*

DOI: https://doi.org/10.32947/ajps.20.01.0436

Article Info:

Received 29 July 2019
Accepted 28 Nov 2019
Published 1 Mar 2020

Corresponding Author email:
pham.dr.nidhal.khazaal@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0001-5628-1479

Abstract:

This work reported a first liquid self-nanoemulsifying drug delivery system (SEDD) of cilostazol using oleic acid as oil phase, tween 80 as surfactant, and transcutol as co-surfactant. Cilostazol is a poor water-soluble phosphodiesterase III inhibitor, which has antiplatelet and vasodilator effect used to relief intermittent claudication symptoms. Cilostazol solubility was determined in various oils, surfactants and co-surfactants and phase diagram was constructed at different oil: surfactant: co-surfactant ratios to determine the existence of nano-emulsion region. The in-vitro dissolution profile showed an optimized cilostazol SEDD formula (LT1) containing oleic acid (10%) as oil, tween 80 (45%) as surfactant, and transcutol (45%) as co-surfactant in comparison with the commercial conventionally Tablets. The LT1 formula was thermodynamically sTable, with a zeta potential of -30.48 mV and droplet size 154 nm. The LT1 capsule showed a superior dissolution profile (100%) when compared to the commercial Tablet (64%) of cilostazol.
The objective of the present study is to formulate cilostazol as an oral liquid SEDD with better solubility and drug release to overcome a variable bioavailability of the commercial Tablet in which a high-fat meal increases absorption to approximately 90%.

Key words: Self-emulsifying drug delivery system, nano-emulsion, and cilostazol.

التحضير والتقييم المختبري لنظام ايصال دواء السيلوستازول بشكل سائل ذاتي الاستحلاب

علي نعمه وناس، نضال خزعل مرعي

الجامعة المستنصرية/كلية الصيدلة/ فرع الصيدلانيات

الخلاصة:

هذه هي المرة الأولى التي يتم فيها تحضير نظام إيصال الدواء المستحلب الذاتي النانوي (SEDD) من سيلوستازول باستخدام زيت الأوليك اسد ، و توين 80 كمضاد للشد السطحي و ترانسكيوتول كمساعد مضاد للشد السطحي. سيلوستازول هو مركب قليل الذوبان بالماء مثبط فسفوديستريز III والذي له تأثير مضاد للصفائح الدموية ومضخم للأوعية يستخدم لتخفيف أعراض العرج المتقطع. تم تحديد قابلية ذوبان سيلوستازول في مختلف الزيوت ، مضادات الشد السطحي، ومساعدات مضادات الشد السطحي، وتم تعيين المستحلب النانوي باستخدام مخطط ثلاثي الحاله بنسب مختلفه من الزيت و مضادات الشد السطحي ، ومساعدات مضادات الشد السطحي تمت مقارنة قابليه تحرر الدواء مختبريا بين الصيفه (SEDD)المثاليه LT1 واللتي تحتوي علي زيت اوليك اسد (10%) و توين 80 كمضاد للشد السطحي (45%) و ترانسكيوتول كمساعد مضاد للشد السطحي(45%) مع حب السيلوستازول المتوفر تجاربا. كانت الصيغة LT1 مقاومه للتخفيف ، مستقرة بالديناميكا الحرارية مع جهد زيتا من -30.48 مللي فولت وحجم القطرة 154 نانومتر. أظهر قابليه تحرر الدواء مختبريا تفوق (100%) LT1 تحرير دواء مقارنة ب (64%) تحرر دواء في حب السيلوستازول المتوفر تجاربا

كان الهدف من هذه الدراسة هو صياغة سيلوستازول كسائل SEDD عن طريق الفم مع أفضل ذوبان وتحرر الدواء للتغلب على التوافر البيولوجي المتغير للقرص التجاري الذي تزيد فيه وجبة الدهن المرتفع من الامتصاص ، مع زيادة بنسبة 90٪ تقريبًا.

الكلمات المفتاحية: نظام ايصال دواء المستحلب الذاتي النانوي، المستحلب النانوي، سيلوستازول.

Introduction

SEDD is pre-concentrate oil, co-surfactant, and surfactant mixture that used in the preparation of in-situ emulsion formation upon water dilution in the gastrointestinal tract. Its formation depends on the ease of water penetration into a gel, crystalline or liquid formed on the exterior surface of droplets. The size of produced emulsion droplets ranging from nano-meter to several microns, for this reason the SEDD can be classified into self-nano emulsifying (SNEDD) and self-micro emulsifying (SMEDD) drug delivery system [1].

The pre-concentrated formula of SEDDS commonly packed into soft or hard gelatin shell capsules that produce a clear or translucent emulsion after oral administration with the agitation. That is usually produced from normal gastrointestinal motility to form an emulsion inside the stomach that is sTable after dilution [2]. Self-emulsification will occur according to the thermodynamic theory, which suggests the entropy change for dispersion exceeds the free energy for increasing the surface area of dispersion [3]. The SEDD can improve drug bioavailability by different mechanisms such as a decrease in droplet size, maintaining the drug in a solubilized form, resulting in enhanced drug absorption from high concentration gradient hence improve drug penetration across the intestinal mucosal membrane [4].

Cilostazol is a quinolone derivative that inhibits cellular phosphodiesterase (more specific for phosphodiesterase III). It is a weakly basic compound with a pKa 11.8, and its molecular weight is 369.46 gram/mole (Dalton). Cilostazol is 6-[4-(1-cyclohexyl-1*H*-tetrazole-5-yl) butoxy]-3,4-dihydro-2(1*H*)-quinolinone. Cilostazol belongs to class II drugs according to the biopharmaceutics classification system (BSC) [5]. A high-fat meal can increase its absorption to approximately 25% in AUC and 90% in C max. It has an apparent elimination half-life of about 11-13 hours (CL/F; 0.18 L/h/kg), practically insoluble in water, 0.1N HCl pH 1.2 and 6.8 with pka 11.8 [6].

Only a few approaches have been reported for improving the solubility and hence bioavailability of cilostazol, such as solid dispersion using mixture of Eudragit L100 and Eudragit S100, complex inclusion using β-cyclodextrin, nano-emulsion. Self-emulsion approach differs from other approaches by the presence of solubilized form of cilostazol readily to be absorbed instead of other steps. In addition, the presence of surfactant and co-surfactant can further increase drug absorption and bioavailability in comparison to nano-emulsion. Self-emulsion has no water in the formula that reduces its size and allows capsule packaging [7, 8].

The aim of this study is to formulate cilostazol as an oral self-emulsifying capsule that may enhance the bioavailability, reduces dose size and side effects.

Materials and methods:

Materials:

Cilostazol cremophore, triacetin, olive oil, paraffin oil, Lemon oil, castor oil, peppermint oil, linoleic acid, and safflower oil were supplied from Hangzhou Hyper chemicals (China), oleic acid, methanol, and clove oil were obtained from G.C.C., (UK), PEG 400, propylene glycol, were obtained from Merck (Germany), transcutol was obtained from Gattefosse Corporation (USA) and Tween (20, 40 and 80) were supplied from CDH (India).

Methods:

Determination of saturated solubility of cilostazol

The saturated solubility of cilostazol is measured in various oils, surfactants, and co-surfactants. The oils used were (oleic acid, castor oil, olive oil, liquid paraffin, safflower oil, clove oil, linoleic oil, peppermint oil, lemon oil, and triacetin). The surfactants (tween 20, 40, 80, cremophore), and co-surfactants (PEG 400, propylene glycol, and transcutol).

The measurement of solubility was done by adding an excess amount of cilostazol powder to 5ml of each oil, surfactant, and co-surfactant in tightly closed small glass tubes. Then these tubes placed in a shaking water bath at (25 ± 0.5) ˚C for 72 hours after that the samples centrifuged at 3000 rpm for 20 min, then the supernatant layer for each example filtered by using filter membrane (0.45 µm). The samples diluted with suiTable solvent. The solubility is determined by using UV-visible spectrophotometer. The measurement was done in triplicate [9]

Construction of Pseudo-ternary Phase Diagrams

The aqueous titration method was used for the pseudo-ternary phase diagrams construction using the selected oil and emulsifier (surfactant and co-surfactant) from the solubility study (oleic acid as oil, tween 80 as a surfactant, propylene glycol or transcutol as co-surfactant). For each phase diagram construction, many Smix (1:1, 1:2, 1:3, 1:4 and 2:1, 3:1, 4:1) ratios of surfactant and co-surfactant were used, and oil to specific Smix ratio (ranging from 0.5:9.5 to 9.5:0.5) were prepared [10].

Preparation of liquid self-emulsifying drug delivery (SEDD)

Liquid self-emulsifying drug delivery (SEDD) formulations of cilostazol were prepared by mixing of cilostazol (50mg) with oleic acid oil, tween 80 and propylene glycol or transcutol (Smix) in a screw-capped glass vial using a vortex mixer and heated at a temperature (50 °C) for 30 min in a water bath to facilitate homogenization using oleic acid oil, tween 80 and propylene glycol or transcutol (Smix) in ratios (1:1, 1:2, 1:3, 1:4, 2:1, 3:1 and 4:1) and oil: Smix at 1:9, 2:8, 3:7 ratio[11]. The components of the (42) prepared formulas are presented in Tables (1and 2).

Table (1): Contents of the prepared liquid SEDD of cilostazol using propylene glycol as co-surfactant.

Formula no.	Oil:Smix ratio	Oleic acid %	Tween80 %	Propylene glycol%	Smix ratio
LP1	1:9	10%	45%	45%	1:1
LP2	1:9	10%	60%	30%	2:1
LP3	1:9	10%	67.50%	22.50%	3:1
LP4	1:9	10%	72%	18%	4:1
LP5	1:9	10%	30%	60%	1:2
LP6	1:9	10%	22.50%	67.50%	1:3
LP7	1:9	10%	18%	72%	1:4
LP8	2:8	20%	40%	40%	1:1
LP9	2:8	20%	53.40%	26.70%	2:1
LP10	2:8	20%	60%	20%	3:1
LP11	2:8	20%	64%	16%	4:1
LP12	2:8	20%	26.70%	53.40%	1:2
LP13	2:8	20%	20%	60%	1:3
LP14	2:8	20%	16%	64%	1:4
LP15	3:7	30%	35%	35%	1:1
LP16	3:7	30%	46.70%	23.30%	2:1
LP17	3:7	30%	52.50%	17.50%	3:1
LP18	3:7	30%	56%	14%	4:1
LP19	3:7	30%	23.30%	46.70%	1:2
LP20	3:7	30%	17.50%	52.50%	1:3
LP21	3:7	30%	14%	56%	1:4

Table (2): Contents of the prepared liquid SEDD of cilostazol using transcutol as co-surfactant

Formula no.	Oil:Smix ratio	Oleic acid %	Tween80 %	Transcutol %	Smix ratio
LT1	1:9	10%	45%	45%	1:1
LT2	1:9	10%	60%	30%	2:1
LT3	1:9	10%	67.50%	22.50%	3:1
LT4	1:9	10%	72%	18%	4:1
LT5	1:9	10%	30%	60%	1:2
LT6	1:9	10%	22.50%	67.50%	1:3
LT7	1:9	10%	18%	72%	1:4
LT8	2:8	20%	40%	40%	1:1
LT9	2:8	20%	53.40%	26.70%	2:1
LT10	2:8	20%	60%	20%	3:1
LT11	2:8	20%	64%	16%	4:1
LT12	2:8	20%	26.70%	53.40%	1:2
LT13	2:8	20%	20%	60%	1:3
LT14	2:8	20%	16%	64%	1:4
LT15	3:7	30%	35%	35%	1:1
LT16	3:7	30%	46.70%	23.30%	2:1
LT17	3:7	30%	52.50%	17.50%	3:1
LT18	3:7	30%	56%	14%	4:1
LT19	3:7	30%	23.30%	46.70%	1:2
LT20	3:7	30%	17.50%	52.50%	1:3
LT21	3:7	30%	14%	56%	1:4

Evaluation of prepared cilostazol SEDD

Self-emulsification time:

The time required to complete self-emulsification for all prepared liquid SEDDS of cilostazol (LP1-LP21 and LT1-LT21) was determined using the USP type II dissolution apparatus. Where 1 ml of each formula was added to 500 ml of 0.1N HCl at 37°C with gentle stirring (at 50 rpm) until a transparent homogenous phase was observed visually. One minute is considered as the optimum time for the formation of transparent SEDD because longer time will have milky appearance [12]. According to the reported grade as shown in Table (3).

Table 3 SEDD Visual Observation Grades [13].

Grade	Nanoemulsion formation time	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

Turbidity measurement:

The turbidity of the resultant self-emulsion in the self-emulsification time study was measured by taking twenty milliliters from each formula LP1-LP5 and LT1-LT6 placed in a glass cell and the turbidity recorded by turbidimeter using 0.1 N HCl solution as control [14].

Light transmittance:

The transmittance of light through the prepared self-emulsion was measured for each formula (LP1-LP5 and LT1-LT6) using UV- visible spectrophotometer at 650nm and 0.1 N HCl solution as a blank [15].

Droplet size distribution and polydispersity index measurement

Particle size distribution and polydispersity index (PDI) measurements were performed for SEDD formulations (LP1-LP5 and LT1-LT6) using particle size analyzer ABT-9000 nanolaser (Brookhaven, USA). The lower the value of (PDI) the better uniformity of droplet size within the formulation [16].

Dilution test

One milliliter of each prepared liquid SEDD formulas (LP1-LP5 and LT1-LT6) was diluted to 100 ml, and 1 ml of each formula was diluted to 1000 ml with 0.1N HCl, phosphate buffer (6.8) or water (each dissolution medium separately). The diluted product was stored overnight and observed for any signs of drug precipitation or phase separation [17].

Determination of drug content

Drug content was measured for the best formulas (LP1-LP5 and LT1-LT6) using UV spectrophotometer (Shimadzu 1800, Japan). 1 ml of the prepared SEDDS formulas was added to 100 ml of methanol and vortexed for 5 minutes for complete mixing. A volume of 0.1ml was withdrawn from the solution and diluted to 20 ml with methanol, filtered through a 0.45 µm filter medium, and the absorbance was measured using a UV spectrophotometer at 257nm [18].

Thermodynamic tests

Centrifugation test

The SEDDS formulations (LP1-LP5 and LT1-LT6) were centrifuged for 20 min at 3000rpm and checked for phase separation,

creaming, or cracking [19]. The formulations that succeeded and passed the centrifugation test will be examined by another thermodynamic test

Heating/Cooling Cycles Test

Heating and cooling test were done by keeping the formulations (LP1-LP5 and LT1-LT6) at 40˚C for 48 hours, then refrigerated at 4˚C for another 48 hours. This is performed in triplicate. This test is used to indicate the racking effect on formulations stability [20].

Freezing/ Thawing Cycles Test

The formulations (LP1-LP5 and LT1-LT6) were frozen at (-20˚C) overnight followed by keeping them at 25˚C to melt for further testing of the thermodynamic stability for the prepared liquid SEDD formulas [21].

In-vitro drug release study of cilostazol SEDDS

The *in-vitro* release of liquid SEDDS (LP1-LP5 and LT1-LT6) was performed in 900 ml of 0.3% SLS in 0.1N HCl, and the temperature was maintained at 37˚ C using USP dissolution apparatus II (Erweka DT720 GmbH, Germany) rotated at 75 rpm. At predetermined intervals of 5, 10, 20, 30, and 60 min, an aliquot of 5 ml was withdrawn and replenished by a fresh new dissolution media to maintain sink condition [22]. The aliquot was analyzed after filtration through a filter membrane (0.45µm), spectrophotometrically at 257 nm [23].

Zeta potential measurement

Liquid SEDD formulations (LP1 and LT1) were analyzed by zeta potential analyzer instrument (nano brook zeta plus, USA). Particles with +30mv or -30mv were considered to be sTable [24].

Results and discussion:

Determination of cilostazol saturated solubility

The proper selection of appropriate SEDD components that showed the highest solubility for cilostazol is so critical to achieving a sTable system. The solubility of cilostazol in various oils can be arranged as follow: liquid paraffin < olive oil < peppermint oil < lemon oil < castor oil < linoleic oil < triacetin < safflower oil < clove oil < oleic acid; therefore, the oleic acid is chosen as the oil phase of SEDD since it showed the highest solubility for cilostazol than other formulas as reported before [25].

The solubility of cilostazol in surfactant can be arranged as follow: tween 40 < cremophore < tween 20 < tween 80. In addition, cilostazol solubility tested in different co-surfactants that are important for elasticity of interfacial film and lowering interfacial tension to zero or less than zero. The results of cilostazol solubility in different co-surfactant are PEG 400 < transcutol < propylene glycol.

Pseudo-ternary Phase Diagram Construction

Figure (1 and 2) showed the cilostazol saturated solubility in different oils, surfactants and co-surfactants, oleic acid was used as oils phase, tween 80 as surfactant and propylene glycol or transcutol as co-surfactants (components of the pseudo-ternary phase triangle).

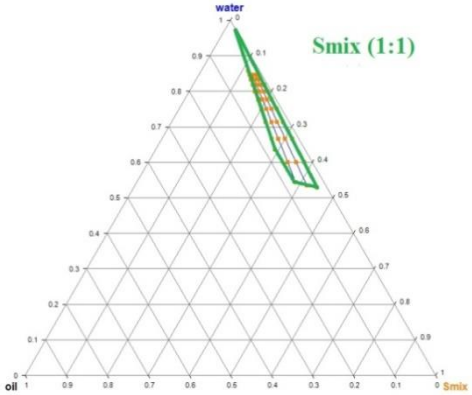
When the co-surfactant (propylene glycol or transcutol) concentration increased (1:2, 1:3 and 1:4) the nano-emulsion area decreased as compared to 1:1 ratio, This could be attributed to the reduction in the surfactant (tween 80) concentration from 50% in 1:1 ratio to 20% in 1:4 ratio. This will confirm the fact that the surfactant by their chemical structure can be adsorbed on the surface of oil droplet provide the essential barrier for the formation of o/w emulsion while the co-surfactant has an auxiliary role by imparting flexibility to

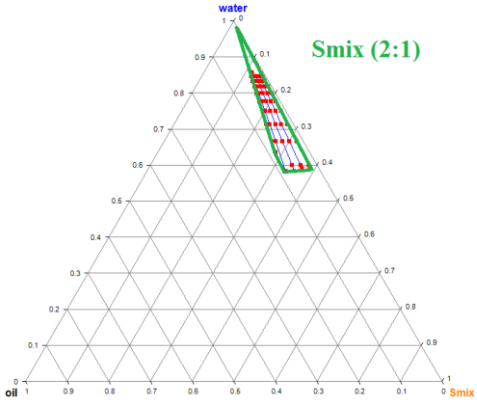
the formed barrier film provide further reduction in the interfacial tension [26].

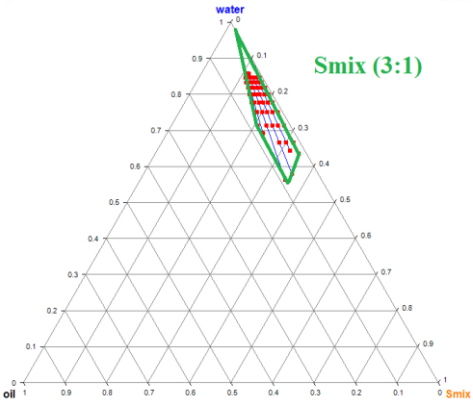
On the other hand, the increase in tween 80 concentration in the Smix (2:1, 3:1 and 4:1) had little or even a negative effect on the nano-emulsion area due to the low flexiblelity in the interfacial film that might be produced with the lowering in the co-surfactant concentration (from 50% in 1:1 ratio to 20% in 4:1 ratio) that may destabilize the nanoemulsion [27].

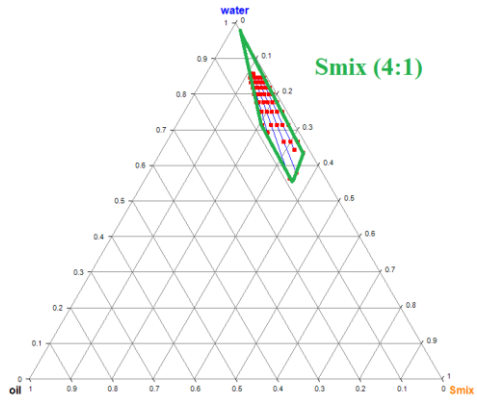
The results furthermore showed that there was no difference in the nano-emulsion area between propylene glycol and transcutol as co-surfactant, indicating the capability of both in producing flexibility.

The best o/w nano-emulsion area was obtained with low oil: Smix ratio (1:9, 2:8, 3:7) because it gave better stability and lower droplet size, this result agreed with the reported data [28]. Therefore, these oils: Smix ratios were selected for further studies









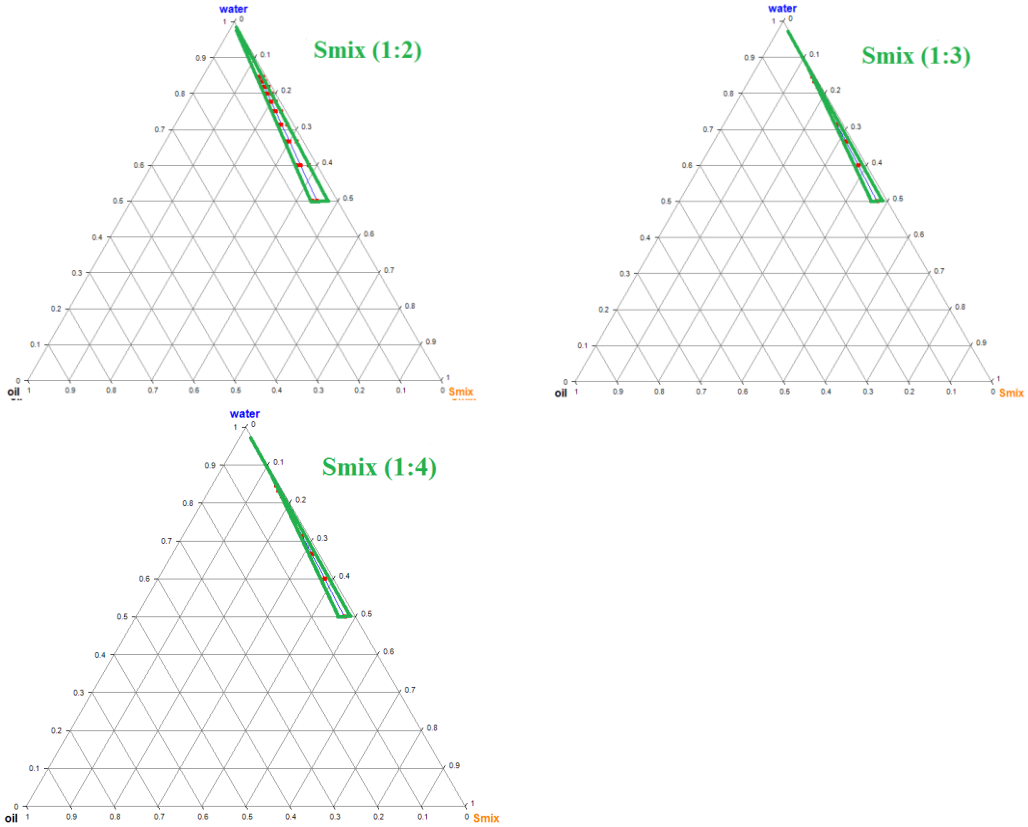
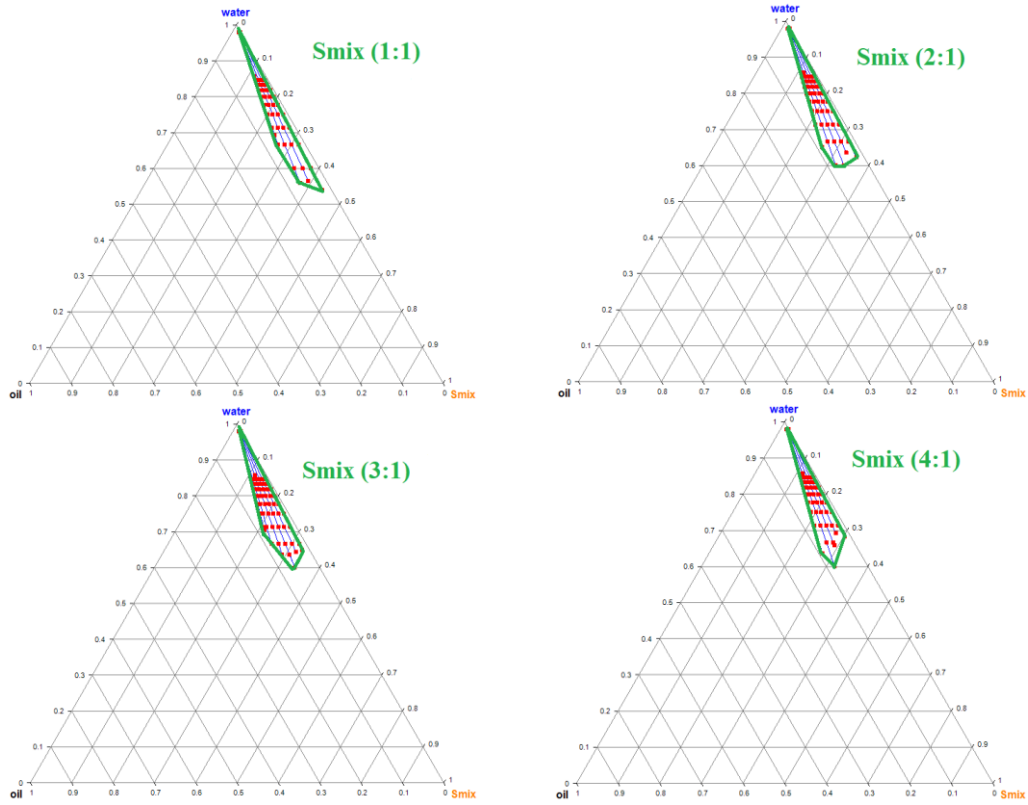


Figure (1): Pseudo-ternary phase diagram of oleic acid, Smix (Tween80: propylene glycol), and deionized water.



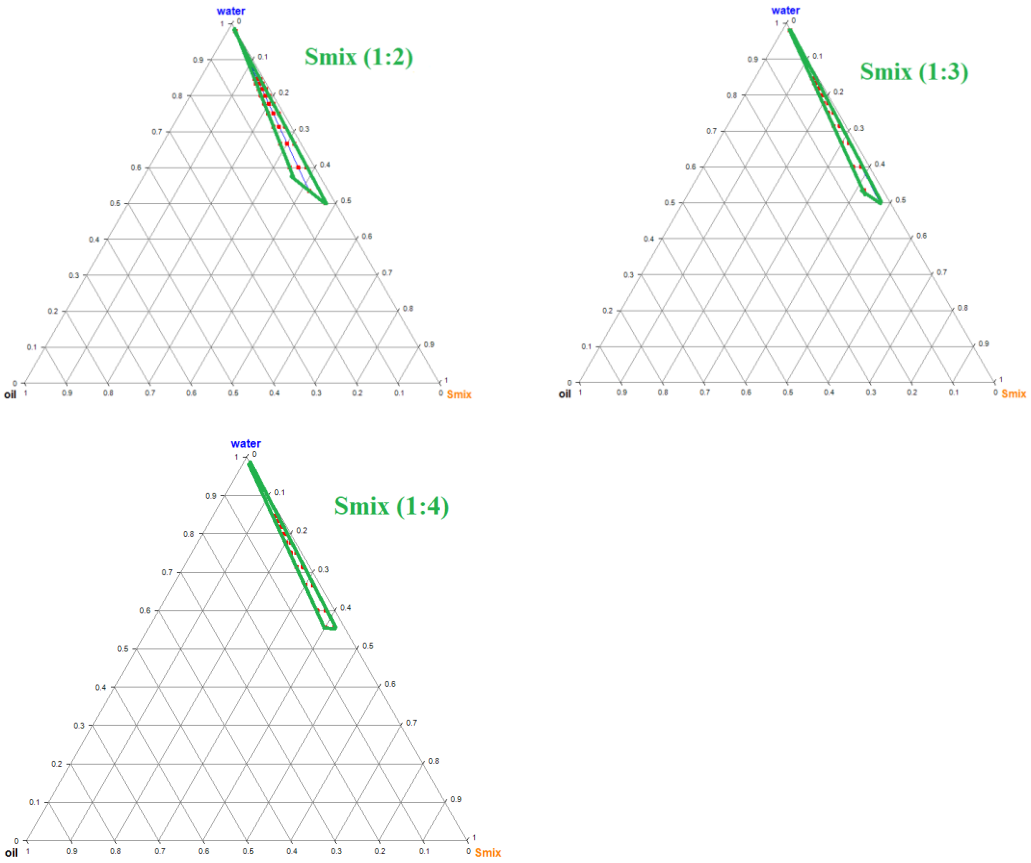


Figure (2): Pseudo-ternary phase diagram of oleic acid, Smix (Tween80: transcutol), and deionized water.

Evaluation of prepared cilostazol SEDD

Self-emulsification time:

One minute is considered as the optimum time for the formation of transparent nano-emulsion, and so eleven formulas from the total forty-two prepared SEDD formulas gave clear nano-emulsion within one minute (class A). Table (4) shows the emulsification time of the formulas and their visual appearance grading [29].

Table (4): SEDD self-emulsification time and visual observation classification

SEDD formula	emulsifying time/ class	SEDD formula	emulsifying time/ class	SEDD formula	emulsifying time/ class
LP1	8 sec/A	LP15	60 sec/B	LT8	10sec/B
LP2	10 sec/A	LP16	65 sec/C	LT9	12sec/B
LP3	20 sec/A	LP17	75 sec/C	LT10	15sec/B
LP4	25 sec/A	LP18	75 sec/C	LT11	18sec/B
LP5	4 sec/A	LP19	>120 sec/D	LT12	25sec/B
LP6	3 sec/B	LP20	45 sec/C	LT13	15sec/B
LP7	2 sec/C	LP21	60 sec/C	LT14	35sec/C
LP8	20 sec/B	LT1	8 sec/A	LT15	40sec/C
LP9	25 sec/B	LT2	8 sec/A	LT16	45sec/B
LP10	55 sec/B	LT3	9 sec/A	LT17	50sec/C
LP11	60 sec/B	LT4	13sec/A	LT18	55sec/B
LP12	15 sec/B	LT5	5 sec/A	LT19	>120 sec/D
LP13	30 sec/C	LT6	5 sec/A	LT20	>120 sec/D
LP14	35 sec/C	LT7	5 sec/B	LT21	>120 sec/D

It was noticed that increase in oil to Smix ratio caused a slowing down in the emulsification process and increased droplet size (appeared as dull or ash emulsion) in addition to increasing surfactant (tween 80) to co-surfactant (propylene glycol or transcutol) ratio. This can resulte in reduction in the flexibility of interfacial film that would cause slow down emulsification process and hence increase droplet size (turbid appearance). This occurs by interfacial disruption phenomenon that can triggered by increasing the water penetration into the droplets that will eject the oil into the aqueous phase. While increasing co-surfactant to surfactant ratio could increase droplet size due to the reduction in the amount of surfactant which is required for the formation of the interfacial film around dispersed droplets [30].

Light transmittance and Turbidity measurement:

Based on the self-emulsification test, the best eleven SEDD formulas (Lp1-Lp5 and LT1-LT6) were characterized by light transmittance and turbidity measurement. The smaller the droplet size resulted in small turbidity value and high transparency (weak light scatters). It can be seen in the Table (5) and Figure (3) the results are consistent with the previous visual observation for nano-emulsion (SEDD visual observation classification), indicating good self-emulsification with good miscibility and small droplet size [31].

Table (5): Light transmittance and Turbidity measurement for the selected SEDD formulas

SEDD formula	Light transmittance	Turbidity (NTU)	SEDD formula	Light transmittance	Turbidity
LP1	97.7	4.16	LT2	98.4	3.7
LP2	97.3	5.5	LT3	98	4.81
LP3	95.5	5.68	LT4	97.9	6.76
LP4	95.2	5.74	LT5	97.2	7.64
LP5	94.6	6.36	LT6	96.7	7.91
LT1	99.6	3.22			

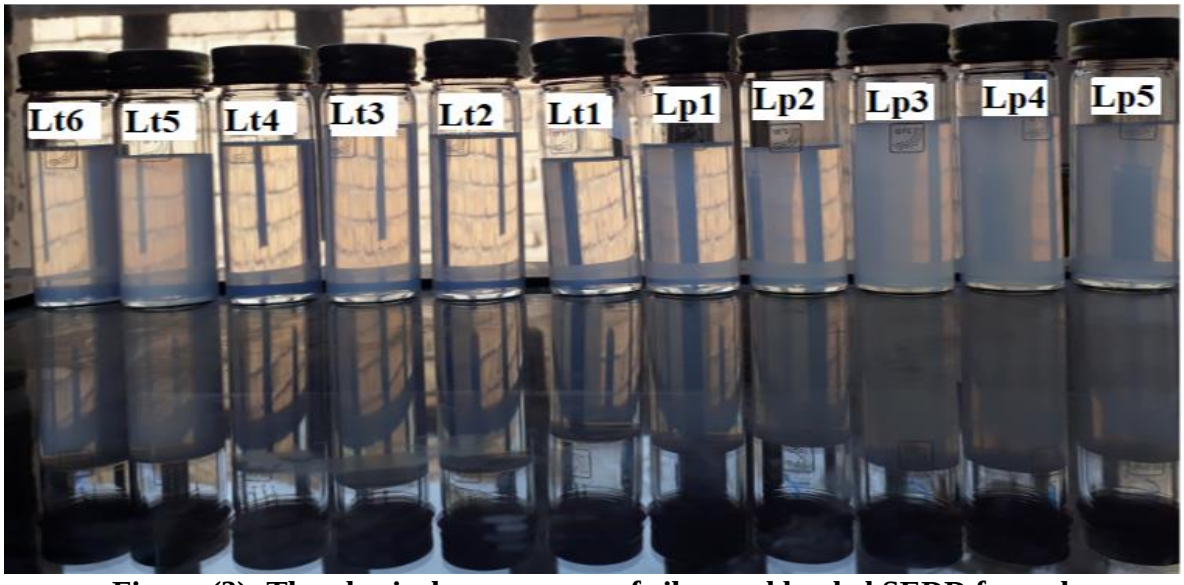


Figure (3): The physical appearance of cilostazol loaded SEDD formulas.

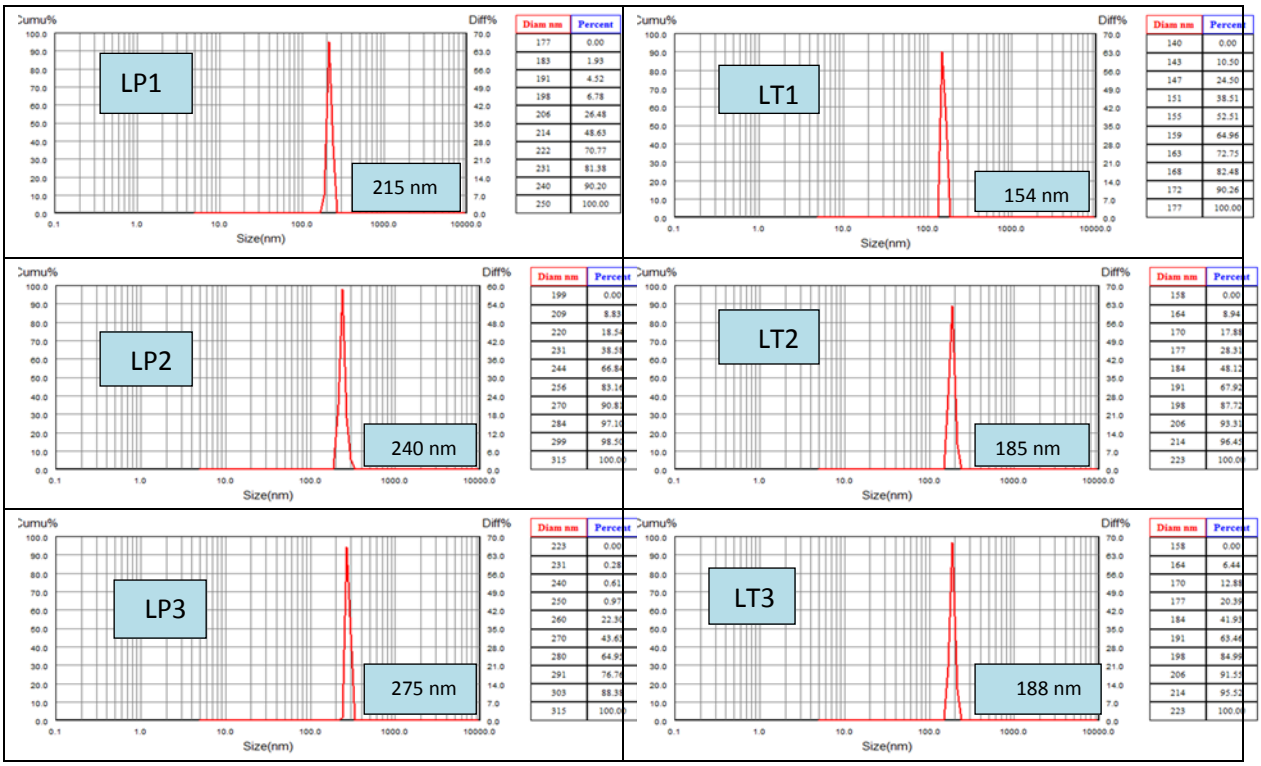
Droplet size distribution and poly-dispersity index measurement

The best eleven SEDD formulas (Lp1-Lp5 and LT1-LT6) were also characterized by measuring the droplet size and poly-dispersity index (PDI) as shown in Table (6) and Figure (4).

Table (6): Droplet size distribution and polydispersity index measurement (PDI) for the selected SEDD formulas

SEDD formula	Droplet size	PDI	SEDD formula	Droplet size	PDI
LP1	215	0.014	LT2	185	0.014
LP2	240	0.014	LT3	188	0.015
LP3	275	0.015	LT4	215	0.017
LP4	315	0.016	LT5	288	0.099
LP5	410	0.159	LT6	425	0.277
LT1	154	0.011			

It was noticed that changing the type of co-surfactant from propylene glycol (Lp1-Lp5) to transcutol (LT1-LT6) affected the droplet size of the resulted emulsion due to the difference in the viscosity between propylene glycol and transcutol (42 and 4.8 m.pas, respectively) [32]. The lower viscosity of transcutol in comparison to propylene glycol decreases the emulsification time and improves the flexibility of barrier film, which is important in the self-emulsification process ($P<0.05$) [33, 34].



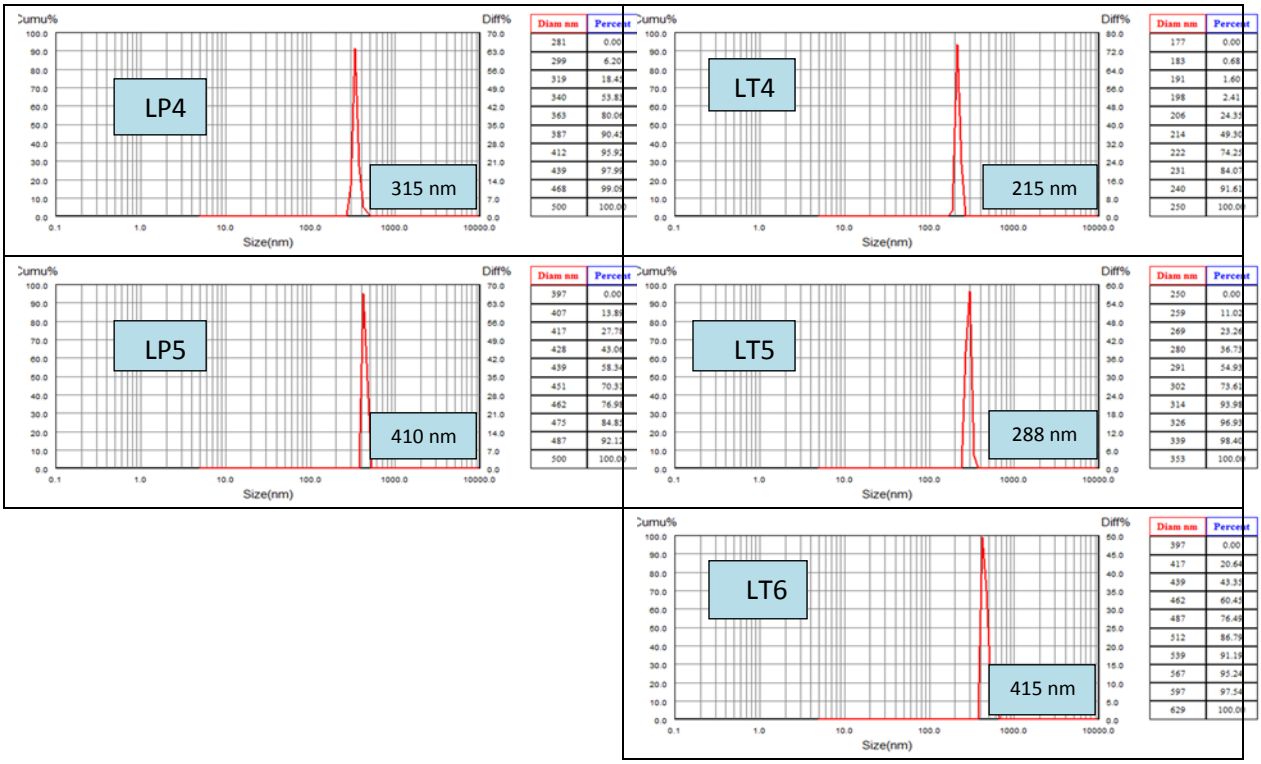


Figure (4): Droplet size distribution of the selected SEDD formulas (Lp1-Lp5 and LT1-LT6).

Dilution test

All of the selected SEDD formulas were robust to dilution (no signs of drug precipitation or phase separation) after 24 hours storage and this indication for the production of s Table o/w nano-emulsion [35].

Determination of drug content

Drug content was determined for the selected SEDD formulas (Lp1-Lp5 and LT1-LT6), and the high drug content observed due to the high drug solubility in the surfactant, co-surfactant, and oil together with the proper choice surfactant/co-surfactant/oil combination [36], as shown in Table (7).

Table (7): Drug content of the selected SEDD formulas

SEDD formula	% of drug content	SEDD formula	% of drug content
LP1	99.56	LT2	98.43
LP2	99.6	LT3	98.85
LP3	98.24	LT4	98.95
LP4	98.91	LT5	99.04
LP5	98.78	LT6	97.07
LT1	99.55		

Thermodynamic study

The thermodynamic study was designed to identify and exclude the unsTable system (formula). As shown in Table (8) all the selected SEDD formulas had successfully passed the test without phase separation, creaming, cracking or drug precipitation under the extreme condition of the test [37]. Indication the efficiency of the method to obtain sTable emulsion.

Table (8): Thermodynamic study for the selected SEDD formulas

SEDD formula	Centrifugation test	Heating-cooling cycles test	Freeze-thawing cycles test
LP1	pass	Pass	Pass
LP2	pass	Pass	Pass
LP3	pass	Pass	Pass
LP4	pass	Pass	Pass
LP5	pass	Pass	Pass
LP6	pass	Pass	Pass
LP7	pass	Pass	Pass
LP8	pass	Pass	Pass
LP9	pass	Pass	Pass
LP10	pass	Pass	Pass
LP11	pass	Pass	Pass

In-vitro drug release study of cilostazol SEDDS

The release profiles for cilostazol loaded SEDD formulas (LP1-LP5 and LT1-LT6) are shown in Figure (5 and 6). All the prepared formulas showed an initial burst effect within the first 10 min, and the release continued for 60 min. Formula Lp1 and Lp2 showed higher release than Lp3 and Lp4 (containing less % of co-surfactant) because there might be a reduction in the flexibility of the interfacial barrier film due to the reduction in the amount of co-surfactant leading to increase in the droplet size as well as decrease in the drug release [38]. While Lp5 showed lower drug release than Lp1 and Lp2 due to the reduction in the amount of surfactant (tween 80), which is required for the formation of the interfacial barrier layer [39]. The same results obtained for formulas LT1-LT6 (containing transcutol as co-surfactant) and LT1 showed a 100% release after 60 minutes. Both LP1 and LT1 formulas were selected for further characterization.

When drug release was compared between optimum liquid SEDD formula of cilostazol (LT1) and cilostazol commercial Tablet; the LT1 showed a significant ($p < 0.05$) higher release than the commercial Tablet as shown in Figure (7), and this is related to the ability of LT1 to form spontaneous nano-emulsion.

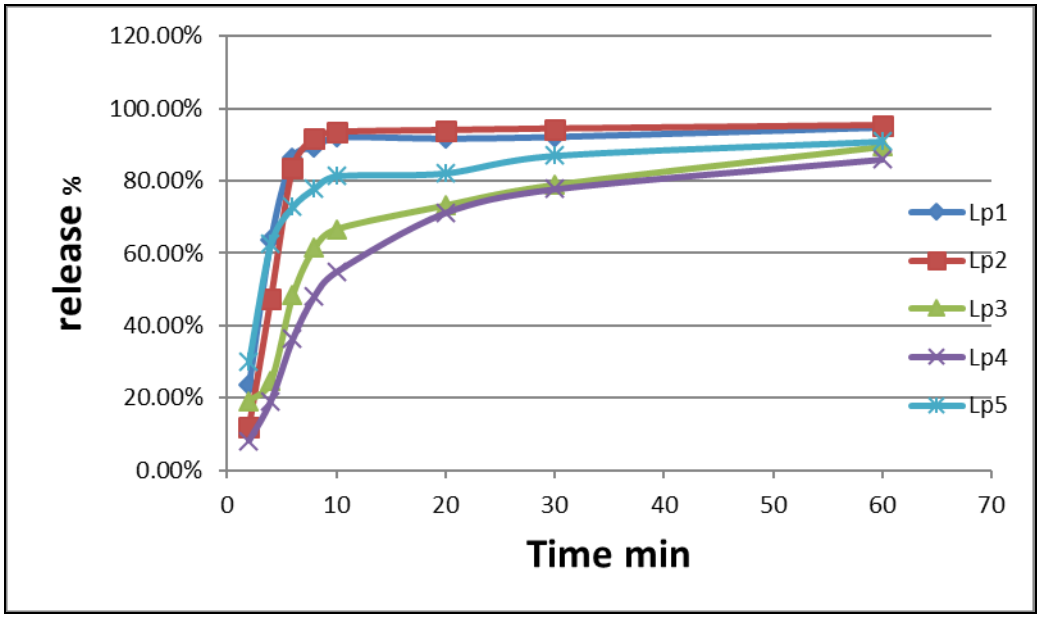


Figure (5): In-vitro release of cilostazol from the prepared liquid SEDD formulas (Lp1-Lp5) containing propylene glycol as co-surfactant, in 0.1N HCl at 37˚C (n=3).

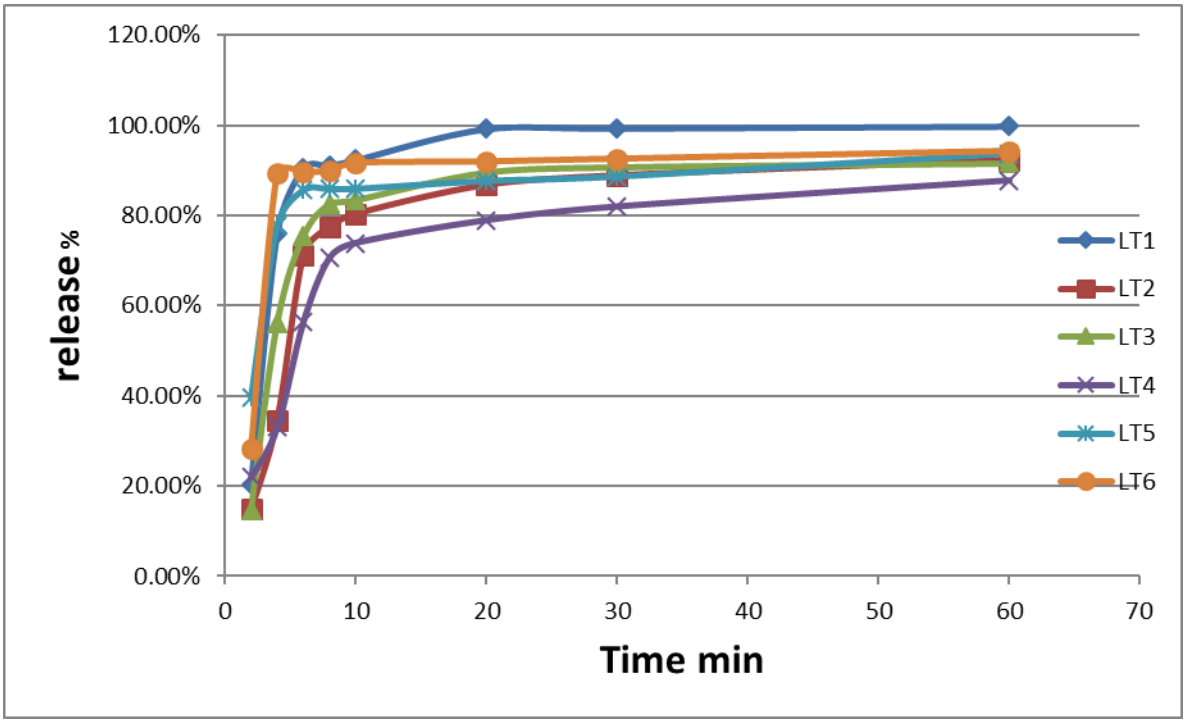


Figure (6): In-vitro release of cilostazol from the prepared liquid SEDD formulas (LT1-LT6) containing transcutol as co-surfactant, in 0.1N HCl at 37˚C (n=3).

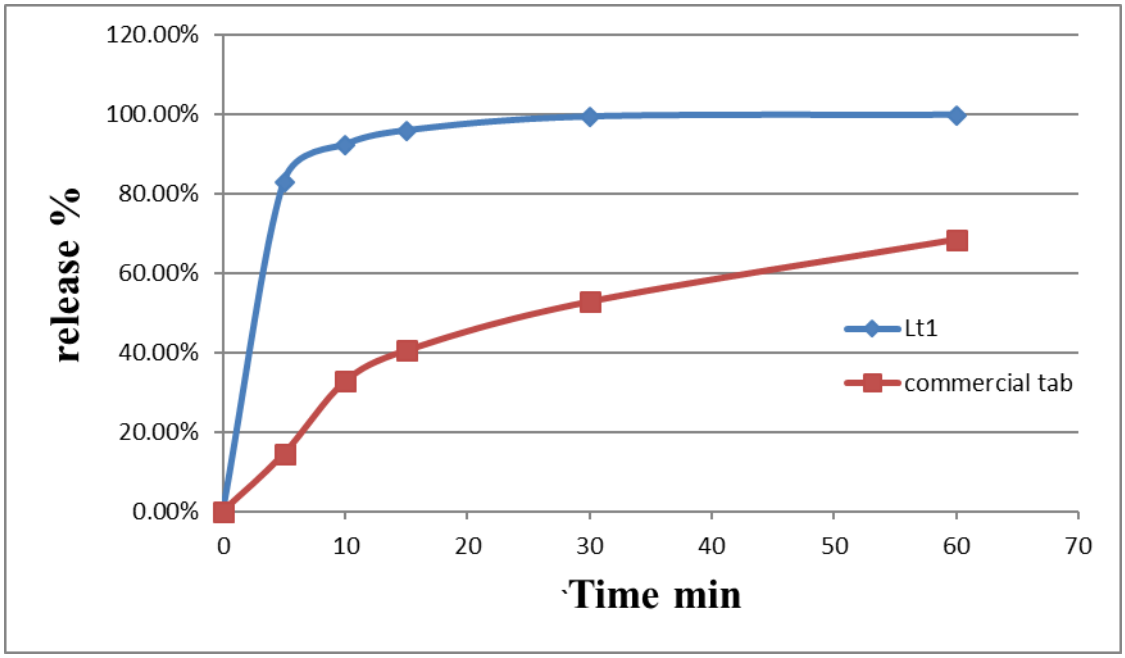


Figure (7): Comparative in-vitro release profile of cilostazol from the selected liquid SEDD formula (LT1) and commercial Tablet in 0.1N HCl at 37˚C (n=3).

Zeta potential measurement

As shown in Figure (8), the zeta potential is considered as an important indicator of the stability of the nanoparticle in the dispersion medium. According to the thumb rule, when the value of zeta potential in the range (-5 mV to +5) mV indicates fast aggregation, value in the range (-20 mV to +20) mV provides short-term stability. Values of zeta potential in the range (≤ -30mV) to (≥+30mV) indicated that there were good stability and values in the range (≤ -60mV) to (≥ +60mV) which indicates that there was excellent stability in the formulation [40].

In the case of tween 80 (non-ionic surfactant with large molecular weight) which acts by steric stabilization. The value of zeta potential of 20mV or lower provides an efficient stabilization [41]. The measured zeta potential for the liquid SEDD formulas Lp1 and Lt1 was -20.83 and -30.48 mV, respectively.

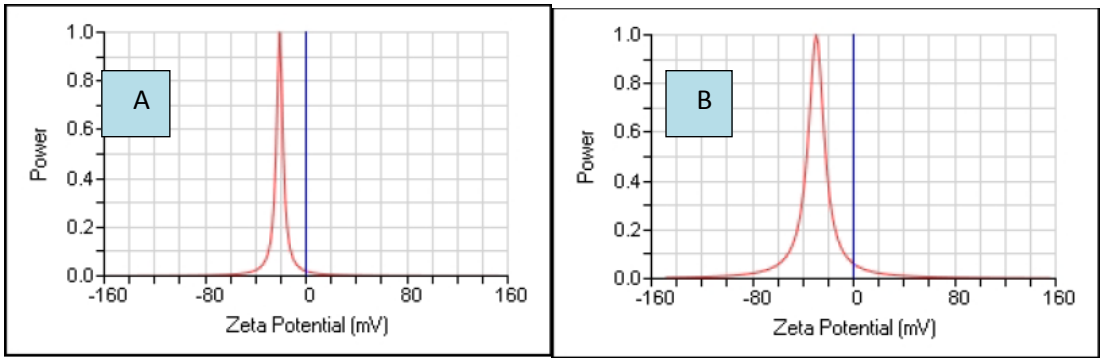


Figure (8): Zeta potential values of cilostazol SEDD nanoemulsions of A Lp1, B LT1.

Conclusion:

For the first time an oral spontaneous dosage form for cilostazol was successfully done which can form in-situ nano-emulsion in the stomach to give sTable self-emulsifying drug delivery system that might improve drug absorption and bioavailability. This will help pharmaceutical company in reducing dose size that may reduce drug side effects of the selected drug.

Acknowledgment

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad-Iraq for its support to the present work.

References:

1- Wang L., Dong J., Chen J., Eastoe J., Li X. Design and optimization of a new self-nanoemulsifying drug delivery system. Journal of colloid and interface science. 2009;330(2):443-8.
2- Tatyana G., Simon B. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. European journal of pharmaceutics and biopharmaceutics. 2000;50:179-88.
3- Gursoy R. N., Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomedicine & Pharmacotherapy. 2004;58(3):173-82.
4- Hauss D. J. Oral lipid-based formulations. Advanced drug delivery reviews 2007;59(7):667-76.
5- Ibrahim F., Sharaf El Din M., Heba Abd El Aziz. Selective methods for cilostazol assay in presence of its oxidative degradation product and co formulated telmisartan application to Tablet formulation. Journal of chromatography & separation techniques. 2016;7(5).
6- Steven L. Bramer, William P. Forbes, Suresh M. Cilostazol pharmacokinetics after single and multiple oral doses in healthy males and patients with intermittent claudication resulting from peripheral arterial disease. Clinical pharmacokinetic. 1999;37(2):1-11.
7- Park J. H., Choi H. K. Enhancement of solubility and dissolution of cilostazol by solid dispersion technique. Arch Pharm Res. 2015;38(7):1336-44.
8- Patel S. G., Rajput S. J. Enhancement of oral bioavailability of cilostazol by forming its inclusion complexes. AAPS PharmSciTech. 2009;10(2):660-9.
9- Sheela A., Yadav D. S., Sushilkumar P. Influence of components of nanoemulsion system for transdermal drug delivery of nimodipine. Asian journal of pharmaceutical and clinical research. 2012;5(3):209-14.
10- Pathak R., Dash R. P., Misra M., Nivsarkar M. Role of mucoadhesive polymers in enhancing delivery of nimodipine microemulsion to brain via intranasal route. Acta Pharm Sin B. 2014;4(2):151-60.
11- Panner S. R, Kulkarni P.K, Mudit D. Preparation and evaluation of self-nanoemulsifying formulation of Efavirenz. Indian journal of pharmaceutical education and research. 2013;47(1):47-54.
12- Meena A. K., Sharma K., Kandaswamy M., Rajagopal S., Mullang R. Formulation development of an albendazole self-emulsifying drug delivery system (SEDDS) with enhanced systemic exposure. Acta Pharm. 2012;62(4):563-80.
13- Khasia H. V, Khasia V. D. A Review on Self Emulsifying Drug Delivery System. International journal of pharmaceutical and chemical sciences. 2012;1(1):353-9.
14- Rahn-Chique K., Puertas A. M., Romero-Cano M. S., Rojas C., Urbina-Villalba G. Nanoemulsion stability: experimental evaluation of the flocculation rate from turbidity measurements. Advances in colloid and interface science 2012;178:1-20.

15- Sharma S., Sahni J. K., Ali J., Baboota S. Effect of high-pressure homogenization on formulation of TPGS loaded nanoemulsion of rutin - pharmacodynamic and antioxidant studies. Drug delivery. 2015;22(4):541-51.
16- Inas F. Abd-AlRazaq, Firas A. Rahi, Mohammed S. Al-lami. Preparation and Characterization of Nimesulide Nanoparticles For Dissolution Improvement. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2018:46-60.
17- Gupta A.K., Mishra D.K., Mahajan S.C. Preparation and in-vitro evaluation of self emulsifying drug delivery system of antihypertensive drug valsartan. International journal of pharmacy and life sciences. 2011;2(3):633-9.
18- Cheng G., Hu R., Ye L., Wang B., Y. G, al. e. Preparation and in vitro/in vivo evaluation of puerarin solid self-microemulsifying drug delivery system by spherical crystallization technique. American association of pharmaceutical scientists pharmSciTech. 2016;17(6):1336-46.
19- Estanqueiro M., Conceição J., Amaral M. Helena, Santos D., Silva J. Baptista. Characterization and stability studies of emulsion systems containing pumice. Brazilian journal of pharmaceutical sciences. 2014;50(2):361-9.
20- Al-Sabagh A.M., Khalil S. A., Abdelrahman A., Nasser N. M., Noor Eldin M.R., et al. Investigation of oil and emulsion stability of locally prepared metalworking fluids. Industrial lubrication and tribology. 2012;64(6):346-58.
21- Hussein AA, Safa Fakher Mekhilef. Novel combination for self-nanoemulsifying drug delivery system of candesartan cilexetil. Iraqi journal of pharmaceutical sciences (P-ISSN: 1683 - 3597 , E-ISSN : 2521 - 3512). 2018:123-34.
22- Obitte N., Ugorji L., Ezichi L., Ogbodo S., Onyishi V., et al. ibuprofen self-emulsifying drug delivery system. world journal of pharmacy and pharmaceutical sciences. 2014;4(2):887-99.
23- Kale A. A., Patravale V. B. Design and evaluation of self-emulsifying drug delivery systems (SEDDS) of nimodipine. American association of pharmaceutical scientists pharmSciTech. 2008;9(1):191-6.
24- Esraa G., Alaa A. Abdulrasool, Jafar J. Al-Tamimi, Nawal A. Nebivolol hydrochloride loaded nanostructured lipid carriers as transdermal delivery system: part 1: Preparation, characterization and in vitro evaluation. Al Mustansiriyah journal of pharmaceutical sciences. 2016;16(2):1-14.
25- Jeevana J. B., Sreelakshmi K. Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. Journal of young pharmacists 2011;3(1):4-8.
26- Azeem A., Rizwan M., Ahmad F. J., Iqbal Z., Khar R. K., et al. Nanoemulsion components screening and selection: a technical note. AAPS PharmSciTech. 2009;10(1):69-76.
27- Yuan Y., Li S. M., Mo F. K., Zhong D. F. Investigation of microemulsion system for transdermal delivery of meloxicam. International journal of pharmaceutics 2006;321(1-2):117-23.
28- Alliod O., Valour Jean-Pierre, Urbaniak S., Fessi H., Dupin D., et al. Preparation of oil-in-water nanoemulsions at large-scale using premix membrane emulsification and Shirasu Porous Glass (SPG) membranes. Colloids and surfaces a: physicochemical and engineering aspects. 2018;557:76-84.
29- Prajapati S. T., Joshi H. A., Patel C. N. Preparation and characterization of self-microemulsifying drug delivery system of olmesartan medoxomil for

bioavailability improvement. J Pharm (Cairo). 2013;2013:728425.
30- Pouton CW. Formulation of self-emulsifying drug delivery systems. Advanced Drug Delivery Reviews. 1997;25:47-58.
31- Avachat A. M., Patel V. G. Self nanoemulsifying drug delivery system of stabilized ellagic acid-phospholipid complex with improved dissolution and permeability. Saudi Pharm J. 2015;23(3):276-89.
32- Enriqueta R. Lo´ pez, Luis L., Marı´a J. P. Comun˜ as, Josefa G., Josefa F.´ ndez. Liquid density measurements of diethylene glycol monoalkyl ethers as a function of temperature and pressure. Journal of chemical & engineering data. 2004;49:376-9.
33- Nikolakakis I, Panagopoulou A, Salis A, Malamataris S. Relationships between the properties of self-emulsifying pellets and of the emulsions used as massing liquids for their preparation. AAPS Pharm SciTech. 2015;16(1):129-39.
34- Eid A. M., El-Enshasy H. A., Aziz R., Elmarzugi N. A. The preparation and evaluation of self-nanoemulsifying systems containing Swietenia oil and an examination of its anti-inflammatory effects. Internatinal journal of nanomedicine. 2014;9:4685-95.
35- Beg S., Jena S. S., Patra Ch. N., Rizwan M., Swain S., et al. Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids Surf B Biointerfaces. 2013;101:414-23.
36- Onyirioha N. N., Barminas J., Osemeahon S. Formulation and characterization of self emulsifying drug delivery system of simvastatin using maringa oleifera and banished oil extract for potential pharmaceutical application. Journal of applied chemistry. 2018;11(5):36-42.
37- Kshitija K., Swati M. self-emulsifying drug delivery system: a review International journal of pharmaceutical sciences and research. 2013;4(12):International Journal of Pharmaceutical Sciences and Research.
38- Vajjha R. S., Das D. K., Godwin A.Chukwu. An experimental determination of the viscosity of propylene glycol/water based nanofluids and development of new correlations. Journal of fluids engineering. 2015;137(8).
39- Shailesh V. Biradar , Ravindra S. Dhumal , Anant P. Rheological investigation of self-emulsification process: effect of co-surfactant. Journal of pharmacy and pharmaceutical sciences. 2009;12(2):164-74.
40- Honary S., Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 2). Tropical journal of pharmaceutical research. 2013;12(2).
41- Hoeller S., Sperger A., Valenta C. Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation. nternational journal of pharmaceutics. 2009;370(1-2):181-6.