Journal of Oleo Science

doi : 10.5650/jos.ess13236
J. Oleo Sci. **63**, *(6) 567-576 (2014)*

Solubility and Dissolution Enhancement of Tadalafil Using Self-Nanoemulsifying Drug Delivery System

Mahmoud El-Badry[1, 2], Nazrul Haq[1], Gihan Fetih[2] and Faiyaz Shakeel[1]*

[1] *Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[2] *Department of Pharmaceutics, College of Pharmacy, Assiut University, Assiut, Egypt*

Abstract: The aim of this study was to develop and evaluate self-nanoemulsifying drug delivery system (SNEDDS) of tadalafil (TDL) in order to enhance its aqueous solubility and dissolution rate. TDL SNEDDS were developed by aqueous phase titration method via construction of pseudo-ternary phase diagrams. The formulations which passed thermodynamic stability and self-nanoemulsification tests were further characterized in terms of droplet size, viscosity, % transmittance and drug content. Selected SNEDDS and drug suspension were subjected to in vitro drug release studies via dialysis membrane in phosphate buffer (pH 6.8). In vitro drug release studies showed 96.6% release of TDL from optimized SNEDDS F5 as compared to only 12.4% from drug suspension after 24 h of study. The results of solubility studies showed 1434 folds enhancement in TDL solubility from optimized SNEDDS F5 as compared to its aqueous solubility. Overall, these results indicated that developed SNEDDS could be successfully used to enhance solubility and dissolution rate of poorly soluble drugs such as TDL.

Key words: tadalafil, self-nanoemulsifying drug delivery system, dissolution, solubility.

1 INTRODUCTION

Tadalafil (TDL) is a poorly water soluble drug which was approved recently for the management and treatment of male erectile dysfunction[1-3]. Poor aqueous solubility of TDL is also related with poor in vitro dissolution rate which in turn results in poor oral bioavailability of TDL[2, 4]. Therefore, it is of great interest to enhance aqueous solubility and oral bioavailability of TDL in order to facilitate drug development process[1-4]. Recently, nanotechnology-based drug delivery systems (DDS) such as microemulsions, nanoemulsions, self-nanoemulsifying drug delivery systems (SNEDDS), liposomes, transferosomes, niosomes, nanoparticles, nanocapsules, nanocrystals, polymeric micelles and dendrimers have been investigated extensively in literature for the enhancement of solubility, bioavailability and therapeutic efficacy of several poorly soluble drugs[5-10]. Nanotechnology-based systems offered several advantages over conventional DDS[5, 6]. For solubility and dissolution enhancement of poorly soluble drugs, nanoemulsions and SNEDDS have also been investigated extensively in literature[11-19]. These lipid based carriers offered several advantages such as thermodynamic stability, great solubilization capacity, enhanced dissolution/permeation, enhanced bioavailability and ease of preparation[15-18]. The solubility of TDL has been enhanced via various formulation approaches such as co-crystal approach, solid dispersion, transdermal formulation and cyclodextrins complexation in literature[1-4, 20, 21]. However, SNEDDS approach has not been investigated for solubility and dissolution enhancement of TDL so far. Therefore, the aim of present study was to develop and evaluate SNEDDS of TDL in order to enhance its solubility and in vitro dissolution which will in turn enhance oral bioavailability. The pharmaceutically acceptable and safe excipients were used in the development of TDL SNEDDS.

2 EXPERIMENTAL

2.1 Materials

TDL and ethanol were purchased from Luna Pharmaceuticals (Cairo, Egypt) and Sigma Aldrich (St. Louis, MO), respectively. Propylene glycol monocaprylate-type I (Capryol-PGMC), propylene glycol monocaprylate-type II (Capryol-90), propylene glycol monolaurate-type I (Lauroglycol-FCC), propylene glycol monolaurate-type II (Lauroglycol-90), oleoyl macrogol-6-glyceride (Labrafil-M1944CS), propylene glycol dicaprylocaprate (Labrafac-

*Correspondence to: Dr. Faiyaz Shakeel, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA)
E-mail: faiyazs@fastmail.fm
Accepted February 20, 2014 (received for review December 29, 2013)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

PG), caprylic/capric triglyceride (Labrafac-Lipophile-WL 1349) and diethylene glycol monoethyl ether (Transcutol-HP) were obtained as kind gift samples from Gattefossé (Cedex, France). Polyethylene glycol-400 (PEG-400), propylene glycol (PG) and octylphenol ethylene oxide condensate (Triton-X100) were purchased from Fluka Chemicals (Bucsh, Switzerland). Ready-to-use dialysis membranes (MWCO 12,000 g/mole) were purchased from Spectrum Medical Industries (Mumbai, India). All the materials (drug and chemicals) used in the study were of high purity (>99%).

2.2 Screening of components for TDL SNEDDS preparation

The selection of oil phase and cosurfactant were based on solubility of TDL in these components. The saturated solubility of TDL in different oils (Capryol-PGMC, Capryol-90, Lauroglycol-FCC, Lauroglycol-90, Labrafac-PG, Labrafac-WL 1349 and Labrafil-M1944CS) and different cosurfactants (Transcutol-HP, PEG-400, PG and ethanol) was determined by adding the excess amount of TDL in 2 ml of each selected oil or cosurfactant in 5 ml capacity vials. These mixtures were continuously mixed in an isothermal water shaker bath (Julabo, MA) maintained at 37±0.2℃ at 100 rpm for 72 h to reach equilibrium[16, 18]. After 72 h, each mixture was removed from the shaker and centrifuged at 5000 rpm for 20 min, filtered, diluted suitably with methanol and subjected for analysis of TDL content spectrophotometrically at 283 nm[22]. The criterion for selection of surfactant was based on the hydrophilic lipophilic balance (HLB) value and safety of surfactants.

2.3 Nanophasic map construction and optimization of TDL SNEDDS

Based on solubility profile of TDL, Capryol-90 and Transcutol-HP were selected as oil phase and cosurfactant, respectively. Based on higher HLB value, Triton-X100 (HLB 13.5) was selected as surfactant. Deionized water was selected as an aqueous phase as water is the frequently used aqueous phase in literature[16, 18]. Surfactant (Triton-X100) and cosurfactant (Transcutol-HP) were mixed in the mass ratios of 1:0, 1:2, 1:3, 1:1, 2:1, and 3:1 in order to obtain various phase diagrams. For construction of pseudo-ternary phase diagrams, oil phase (Capryol-90) and a specific ratio of surfactant to cosurfactant (S_{mix}) were mixed thoroughly in 1:9 to 9:1 mass ratios. These mixtures of oil and S_{mix} were then titrated with deionized water as reported in our previous articles[16, 18]. SNEDDS zones were marked on a pseudo-ternary phase diagram in case of each S_{mix}.

2.4 Formulation development

The maximum SNEDDS zones were observed in 1:1 mass ratio of Triton-X100 and Transcutol-HP, hence this ratio was finely selected for the preparation of TDL SNEDDS. From the pseudo-ternary phase diagram, different formulations were selected. Almost entire range of SNEDDS zones in phase diagram was taken into account and varied oil compositions (8, 12, 16 and 20% w/w) with minimum surfactant (15-20% w/w) and cosurfactant (15-20% w/w) concentration were selected. 5 mg of TDL was directly solubilized in prepared SNEDDS to obtain drug-loaded SNEDDS (Table 1).

2.5 Thermodynamic stability and self-nanoemulsification tests

In order to eliminate unstable formulations and to select stable one, the developed TDL SNEDDS (F1-F8) were subjected to various thermodynamic stability tests. These tests were performed in terms of centrifugation, heating & cooling cycles and freeze-pump-thaw cycles[16, 18]. TDL SNEDDS (F1-F8) were subjected to centrifugation at 5000 rpm for the period of 30 min. Prepared SNEDDS were further subjected to heating & cooling cycles (3 cycles) between 4 and 50℃ for the period of 48 h. TDL SNEDDS which were found to be stable at above two stress conditions were further subjected to freeze-pump-thaw cycles (3

Table 1 Composition of tadalafil SNEDDS (F1-F8).

Code*	Formulation composition (% w/w)				S_{mix} ratio
	Capryol-90	Triton-X100	Transcutol-HP	Water	
F1	8	15	15	62	1:1
F2	12	15	15	58	1:1
F3	16	15	15	54	1:1
F4	20	15	15	50	1:1
F5	8	20	20	52	1:1
F6	12	20	20	48	1:1
F7	16	20	20	44	1:1
F8	20	20	20	40	1:1

*Five mg of tadalafil was loaded in each SNEDDS formulation

cycles) between −21 (freeze) and +25℃ (thaw) for the period of 24 h.

However, self-nanoemulsification test was carried out to investigate any drug precipitation or phase separation upon dilution with deionized water. To perform this test, 1 ml of each TDL SNEDDS (F1-F8) was diluted 500 times with deionized water and the efficiency of each SNEDDS was evaluated visually using the following grading systems[16, 18, 23]:

Grade A: Rapidly forming clear/transparent emulsion system
Grade B: Rapidly forming bluish white emulsion system
Grade C: Milky emulsions (take more than 2 minutes to emulsify)
Grade D: Dull, grayish milky emulsions
Grade E: Emulsions with oil globules at the surface

2.6 Physicochemical characterization of TDL SNEDDS

Developed TDL SNEDDS were characterized for various physicochemical parameters such as droplet size distribution, polydispersity index (PI), viscosity, percentage of transmittance (% T) and drug content.

The droplet size distribution and PI of TDL SNEDDS (F1-F8) were measured using Malvern Particle Size Analyzer (Malvern Instruments Ltd., Holtsville, NY) at 25℃ with a scattering angle of 90° as reported previously[16]. The zeta potential (ZP) of TDL SNEDDS was also measured using Malvern Zetasizer using glass electrodes as reported previously[18].

However, the viscosity of TDL SNEDDS (F1-F8) was measured by Brookfield Viscometer (Middleboro, MA) as reported previously[24].

% T of TDL SNEDDS was measured spectrophotometerically (SPUV-19, Dingelstadt, Germany) at detection wavelength of 550 nm as reported previously[25].

For determination of drug content, 1ml of each formulation (F1-F8) was mixed with 10 ml methanol in a volumetric flask and shaken vigorously for 5 min. The drug contents were determined spectrophotometrically at 283 nm[22].

Transmission electron microscopy (TEM) (Tecnai TF20, Hillsboro, OR) analysis was also performed to evaluate the surface morphology of optimized SNEDDS F5. TEM analysis was performed at 200 KV as reported in literature[26].

2.7 In vitro drug release studies via dialysis membrane

Based on lower droplet sizes, lower PIs, lower viscosities, higher % T and higher drug contents, TDL SNEDDS F5-F8 were selected for in vitro drug release studies. These studies were performed to compare the release of TDL from different SNEDDS and TDL suspension, all having same amount of TDL (5 mg). Drug release studies were carried out in 500 ml of phosphate buffer (pH 6.8) (dissolution media) using United States Pharmacopoeia (USP) XXIV method at 100 rpm and 37 ± 0.2℃ [16]. One ml of each SNEDDS and TDL suspension were placed in ready-to-use dialysis bag. Three ml of samples were withdrawn at 0, 1, 2, 3, 6, 18 and 24 h and same amount of drug free phosphate buffer (pH 6.8) was replaced every time[11]. The samples were analyzed for TDL content spectrophotometrically at 283 nm[22].

2.8 Drug release kinetic

Various mathematical models such as zero order, first order, Higuchi, Hixon-Crowell and Korsemeyer-Peppas models have been developed for proposed mechanism of drug release from formulations[27−29]. The drug release data of TDL from SNEDDS and drug suspension was fitted into the following models:

$$\text{Zero order} \quad Q_t = Q_0 + K_0 t \tag{1}$$

$$\text{First order} \quad Log\ C = Log\ C_0 - \frac{K_1 t}{2.303} \tag{2}$$

$$\text{Higuchi} \quad Q_t = kt^{0.5} \tag{3}$$

$$\text{Hixon-Crowell cube root} \quad (W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}}) = k_h t \tag{4}$$

$$\text{Korsemeyer-Peppas} \quad \frac{Q_t}{Q_\infty} = k_p t^n \tag{5}$$

Where, Q_0, Q_t and Q_ω are the amounts of TDL released initially, at time t and at time ω, respectively. C_0 and C represent the amounts of drug initially and at time t, respectively. W_0 and W_t are the amounts of drug in formulations initially and at time t, respectively. K_0, k_1, k, k_h and k_p are zero order, first order, Higuchi, Hixon-Crowell and Korsemeyer-Peppas rate constants, respectively. The exponent n (diffusion coefficient) is used to characterize drug release mechanism.

2.9 Determination of TDL solubility in water and optimized SNEDDS F5

The saturated solubility of TDL in water and optimized SNEDDS F5 was determined spectrophotometrically at 283 nm[22]. The excess amount of TDL was taken in water and optimized SNEDDS F5 in glass vials in triplicate. The rest of the procedure was same as described under screening of components section.

2.10 Statistical analysis

Results were expressed as the mean ± standard deviation. The data of physicochemical parameters and drug release studies were statistically evaluated by one way analysis of variance (ANOVA) using Dunnett's test with the help of SPSS Version 11 software.

Table 2 Equilibrium solubility data of tadalafil in various oils, cosurfactants, optimized SNEDDS F5 and water at 37℃ (n = 3).

Sample matrices	Solubility ± SD (mg/ml)
Capryol-90	7.88 ± 0.34
Capryol-PGMC	5.94 ± 0.28
Lauroglycol-90	2.65 ± 0.09
Lauroglycol-FCC	2.44 ± 0.08
Labrafil-M1944CS	5.42 ± 0.18
Labrafac-PG	0.85 ± 0.02
Labrafac-WL1349	0.41 ± 0.01
Transcutol-HP	33.56 ± 2.09
PEG-400	23.76 ± 1.78
PG	3.66 ± 0.13
Ethanol	3.54 ± 0.10
SNEDDS (F5)	28.68 ± 1.94
Water	0.02 ± 0.00

Polyethylene glycol-400 (PEG-400), propylene glycol (PG), self-nanoemulsifying drug delivery system (SNEDDS)

3 RESULTS

3.1 Screening of components for SNEDDS preparation

The saturated solubility data of TDL in different oils and cosurfactants at 37℃ is listed in Table 2. Among different oils investigated, the highest solubility was observed in Capryol-90 (7.88 ± 0.34 mg/ml) followed by Capryol-PGMC (5.94 ± 0.28 mg/ml), Labrafil-M1944CS (5.42 ± 0.18 mg/ml), Lauroglycol-90, Lauroglycol-FCC, Labrafac-PG and Labrafac-WL1349 (Table 2). However, the highest solubility was observed in Transcutol-HP (33.56 ± 2.09 mg/ml) among different cosurfactants followed by PEG-400, PG and ethanol. Based on solubility data of TDL, Capryol-90 and Transcutol-HP were selected as oil phase and cosurfactant, respectively. However, Triton-X100 and deionized water were selected as surfactant and aqueous phase, respectively.

3.2 Nanophasic map construction and optimization of TDL SNEDDS

Pseudo-ternary phase diagrams were constructed separately for each S_{mix} in order to identify SNEDDS zones in the phase diagrams (Fig. 1). It was observed that when Triton-X100 was used alone (S_{mix} 1:0), the SNEDDS zones in phase diagrams were very low (Fig. 1A). The maximum

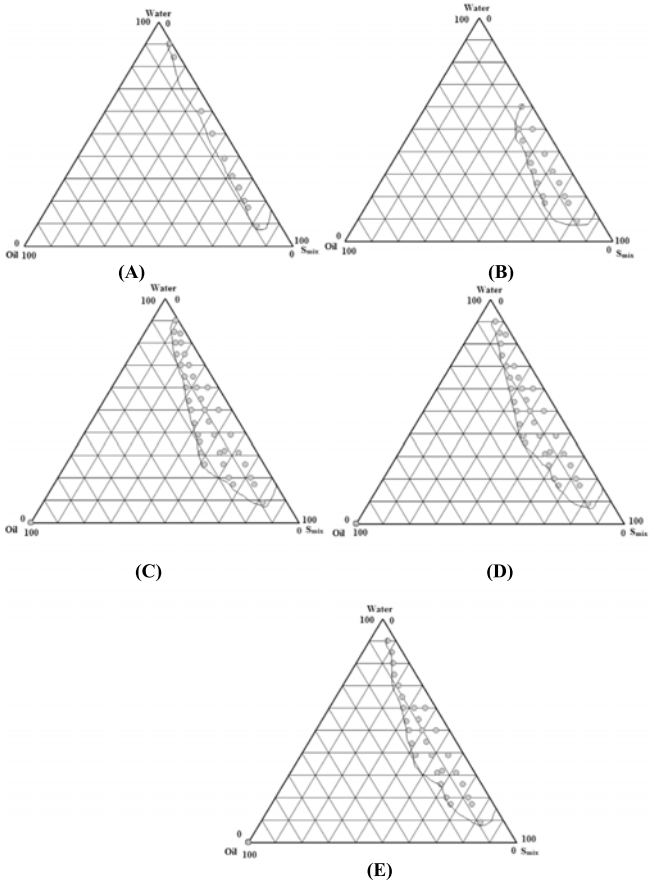

Fig. 1 Pseudo-ternary phase diagrams showing SNEDDS zones for oil phase (Capryol-90), aqueous phase (water), surfactant (Triton-X100) and cosurfactant (Transcutol-HP) at S_{mix} ratio of A. 1:0, B. 1:2, C. 1:1, D. 2:1 and E 3:1.

amount of Capryol-90 (oil phase) that was solubilized by this ratio was 9% w/w with very high amount of S_{mix} (82% w/w). When 1:2 mass ratio of Triton-X100 to Transcutol-HP (S_{mix}) was studied, the SNEDDS zones were found to be increased as compared 1:0 ratio (Fig. 1B). The maximum amount of Capryol-90 that was solubilized by this ratio was 17% w/w by incorporating 66% w/w of S_{mix}. When 1:1 mass ratio of Triton-X100 to Transcutol-HP was investigated, the SNEDDS zones were found to be increased rapidly as compared to 1:2 and 1:0 ratios (Fig. 1C). The maximum amount of Capryol-90 that was solubilized by this ratio (1:1) was found to be 22% w/w by utilizing 52% w/w of S_{mix}. However, when the mass ratios of 2:1 and 3:1 were studied, the SNEDDS zones were found to be decreased slightly as compared to 1:1 ratio (Fig. 1D and E).

3.3 Thermodynamic stability and self-nanoemulsification tests

Different thermodynamic stability tests viz. centrifugation, heating & cooling cycles and freeze-pump-thaw cycles were performed in order to eliminate unstable formulations and the results of these tests are listed in Table 3[16, 18, 23]. Only stable formulations were selected for further characterization and their compositions are listed in Table 1.

Thermodynamically stable SNEDDS (F1-F8) were further investigated for self-nanoemulsification test as it is mandatory for oral SNEDDS. This test was performed evaluate any phase separation or drug precipitation upon dilution with water[16, 23]. The results of these tests are listed in Table 3. It was observed that formulations F1-F4 passed this test with grade B. However, formulations F5-F8 passed this test with grade A.

3.4 Physicochemical characterization of TDL SNEDDS

The results of physicochemical characterization are listed in Table 4. The droplet size of SNEDDS (F1-F8) was observed in the range 64.7-211.8 nm. It was observed that when the concentration of S_{mix} was held constant at 30% w/w and the concentration of Capryol-90 (oil phase) was varied from 8 to 20% w/w (F1-F4), the droplet size was found to be increased significantly ($p < 0.05$). Similarly, when the concentration of S_{mix} was held constant at 40% w/w (F5-F8) and the concentration of Capryol-90 was varied from 8 to 20% w/w (F5-F8), the droplet size was found to be increased again (Table 4). The PI of TDL SNEDDS was observed in the range of 0.112-0.419 (Table 4). The ZP values of TDL SNEDDS (F1-F8) were observed in the range of -52.5 to -29.6 mV (Table 4). The lowest ZP was observed in F4 (-52.3 mV). However, the highest one was observed in F5 (-29.6 mV).

The viscosity of TDL SNEDDS (F1-F8) was observed in the range of 35.6-65.7 cp (Table 4). It was observed that when the concentration of S_{mix} was held constant at 30 (F1-F4) and 40% w/w (F5-F8) and the concentration of Capryol-90 (oil phase) was varied from 8 to 20% w/w, the viscosity was found to be increased significantly ($p < 0.05$) (Table 4). The% T of TDL SNEDDS (F1-F8) was observed in the range of 79.3-99.1% (Table 4). The% T of SNEDDS F5 was found to be highest (99.1 ± 0.3%) as compared to other formulations. However, the lowest one was observed in F4 (79.3 ± 0.1%). The% drug content of TDL SNEDDS (F1-F8) was observed in the range of 96.2-98.9% (Table 4). The highest and lowest drug content were observed in formulations F5 (99.2 (98.9 ± 0.4%) and F4 (96.2 ± 0.2%), respectively.

The purpose of TEM analysis was to evaluate the surface morphology and shape of droplets of optimized SNEDDS F5. Photomicrograph of optimized SNEDDS F5 was taken and interpreted for morphology. The size of all droplets was found to be less than 100 nm (Fig. 2).

Table 3 Results of thermodynamic stability and self-nanoemulsification efficiency tests of tadalafil SNEDDS (F1-F8) formulations.

Code	Self-nanoemulsification test grade	Thermodynamic stability tests		
		Cent.	H&T	FPT
F1	B	√	√	√
F2	B	√	√	√
F3	B	√	√	√
F4	B	√	√	√
F5	A	√	√	√
F6	A	√	√	√
F7	A	√	√	√
F8	A	√	√	√

√ (Passed the respective test), cent. (centrifugation), H&T (heating and cooling cycles), FPT (freeze-pump-thaw cycles)

Table 4 Physicochemical characterization of tadalafil SNEDDS in terms of droplet size, PI, viscosity, % transmittance and drug content.

Code	Characterization parameters					
	$\Delta_{dm}^{\#} \pm$ SD (nm)	p_i*	$\eta \pm$ SD (cp)	$\%T \pm SD$	*ZP*	*Drug content* (%) ± *SD*
F1	131.8 ± 6.6	0.318	42.8 ± 2.6	84.6 ± 0.1	− 42.1	98.1 ± 0.5
F2	163.4 ± 7.9	0.365	49.6 ± 3.0	82.3 ± 0.3	− 44.2	97.2 ± 0.4
F3	191.2 ± 8.6	0.412	56.3 ± 3.4	81.4 ± 0.2	− 46.5	97.0 ± 0.3
F4	211.8 ± 10.2	0.419	65.7 ± 4.1	79.3 ± 0.1	− 52.3	96.2 ± 0.2
F5	64.7 ± 3.7	0.112	35.6 ± 2.0	99.1 ± 0.3	− 29.6	98.9 ± 0.4
F6	76.2 ± 4.3	0.124	41.4 ± 2.4	98.2 ± 0.2	− 35.2	98.6 ± 0.3
F7	85.6 ± 5.1	0.158	47.8 ± 2.8	97.5 ± 0.1	− 37.4	98.0 ± 0.2
F8	96.8 ± 5.6	0.209	57.8 ± 3.7	96.4 ± 0.2	− 39.4	97.8 ± 0.1

#Mean droplet diameter (Δdm), *polydispersity index (p_i), viscosity mean (η); %T (% transmittance), zeta potential (ZP), standard deviation (SD)

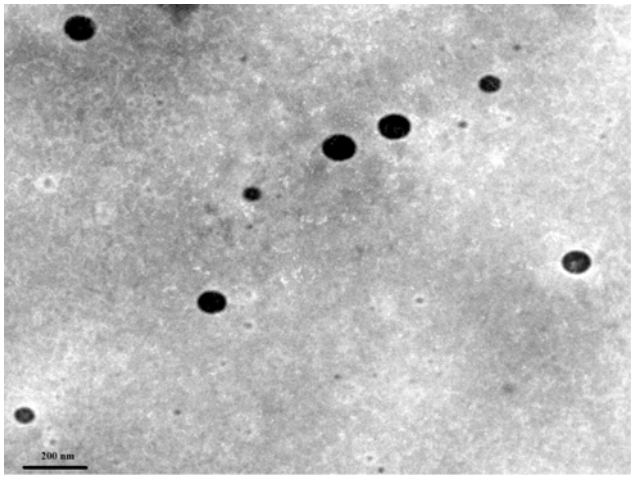

Fig. 2 Transmission electron microscopic (TEM) image of optimized SNEDDS (F5) showing non-spherical droplets.

3.5 In vitro drug release studies

The results of TDL release from SNEDDS and drug suspension are presented in Fig. 3. It was observed that initial release of TDL from all SNEDDS and drug suspension was rapid (immediate release profile). The release profile of TDL from all SNEDDS was significant as compared to drug suspension ($p < 0.05$). More than 60% of TDL was found to be released from all SNEDDS as compared to 8.2% from drug suspension after 6 h of study (Fig. 3). After 6 h, all SNEDDS and drug suspension showed slower release of TDL (sustained release profile). The highest drug release of TDL was observed with formulation F5 (Fig. 3). The% amount of TDL that was released from F5 after 24 h of study was found to be 96.6% as compared to 12.4% from drug suspension. Around 84% of TDL was released from F5 after 6 h of study. However, the lowest drug release profile was observed in drug suspension.

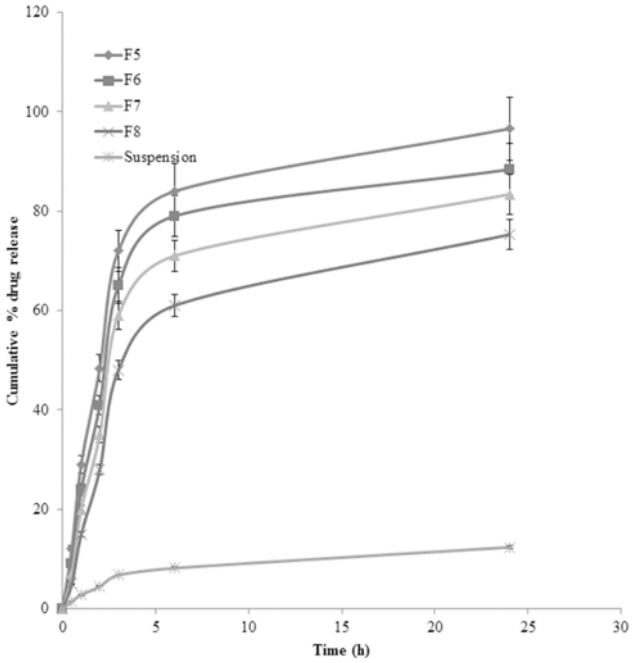

Fig. 3 Comparative in vitro drug release profile of tadalafil from TDL SNEDDS (F5-F8) and drug suspension in phosphate buffer (pH 6.8).

3.6 Drug release kinetic

Release kinetic parameters for different SNEDDS (F5-F8) and drug suspension were calculated using various mathematical models and results are listed in Table 5. The release from formulations F5, F6, F7 and drug suspension followed Korsemeyer-Peppas model with non-Fickian diffusion mechanism. However, the release from formulation F8 followed Korsemeyer-Peppas model with supercase II transport mechanism.

Table 5 Correlation coefficients and kinetic of drug release from SNEDDS (F5-F8) and drug suspension.

Formulation	Zero order		First order		Higuchi	Hixon-Crowell	Peppas	
	K_0	R^2	k_1	R^2	R^2	R^2	R^2	n
F5	14.05	0.866	2.051	0.951	0.941	0.923	0.942	0.788
F6	13.43	0.895	1.858	0.958	0.955	0.909	0.946	0.882
F7	12.96	0.896	1.636	0.936	0.949	0.916	0.943	0.944
F8	10.59	0.920	1.455	0.949	0.965	0.934	0.951	1.018
Suspension	1.345	0.886	1.028	0.888	0.958	0.884	0.970	0.705

Correlation coefficient (R^2), Zero order rate constant (K_0), first order rate constant (k_1), diffusion coefficient (n)

3.7 Determination of TDL solubility in water and optimized SNEDDS F5

The results of solubility studies of TDL in water and optimized SNEDDS F5 are listed in Table 2. The saturated solubility of TDL in water was observed as 0.02 mg/ml at 37℃. However, the saturated solubility of TDL in optimized SNEDDS F5 was found to be 28.68 mg/ml at 37℃ (Table 2), indicating TDL is present in its molecular state due to stable formation of SNEDDS[2]. Therefore, more than 25 mg of TDL could be loaded in 1 ml formulation of SNEDDS F5.

4 DISCUSSION

The solubility of drug (TDL) in oil phase is the most important step for screening of oils[10, 12]. However, several methods have been reported for screening of surfactants and cosurfactants including solubility[16, 18, 30–33]. In present study, the oils and cosurfactants were screened based on solubility of TDL in these components. However, the surfactant was selected based on its safety and higher HLB value because surfactants with higher HLB values favor the formation oil-in-water nanoemulsions. In present study, Triton-X100 was selected as surfactant because of its higher HLB value, safety, biodegradability and it has been investigated rarely as compared to other higher HLB surfactants such as Tween-80, Cremophor-EL and Labrasol[16–19]. With regard to pseudo-ternary phase diagrams, maximum SNEDDS zones were observed in 1:1 S_{mix} ratio of Triton-X100 to Transcutol-HP (Fig. 1C). Therefore, different formulations (F1-F8) with different concentrations of oil phase (8, 12, 16 and 20% w/w) and surfactant phase (30 and 40% w/w) were precisely selected from Fig. 1C. 5 mg of TDL was directly solubilized in each SNEDDS for further evaluation. The composition of these SNEDDS is listed in Table 1. Developed SNEDDS (F1-F8) were characterized in terms of droplet size, PI, viscosity, % T and drug content. The results of droplet size distribution indicated that the concentrations of Capryol-90 (oil phase) and S_{mix} have significance influence on droplet size of TDL SNEDDS ($p < 0.05$). Overall, the largest droplet size was observed in SNEDDS F4 (211.8 ± 10.2 nm) which was probably due to the presence of higher concentration of Capryol-90 (20% w/w). However, the lowest droplet size was observed in SNEDDS F5 (64.7 ± 3.7 nm) that was possible due to lower concentration of Capryol-90 (8% w/w) and higher concentration of S_{mix} (40% w/w). Generally, the PI of formulations F1-F4 was higher as compared to the PI of F5-F8, indicating more uniformity of droplets in F5-F8 as compared to F1-F4. The values of ZP in the magnitude of ± 30 mV characterizes the formation of stable formulation[18, 26]. In current study, the ZP of TDL SNEDDS F5-F8 was very close to 30 mV, indicating stable formation of F5-F8. However, the ZP of TDL SNEDDS F1-F4 was deviated from 30 mV, indicating metastable/unstable formation of F1-F4. The negative net charge of ZP for all TDL SNEDDS (F1-F8) was probably due to the presence of fatty acid esters in Capryoil-90 which was used as oil phase for TDL SNEDDS. The highest viscosity was observed in SNEDDS F4 (65.7 ± 4.1 cp) which was possible due to largest droplet size of F4. However, the lowest one was observed in SNEDDS F5 (35.6 ± 2.0 cp) which was due to the lowest droplet size of F5. The viscosity results were in good agreement with droplet size analysis and could be correlated with the different contribution of Capryol-90, Triton-X100 and Transcutol-HP used in stabilization of formulation compositions. TEM analysis indicated non-spherical shape of SNEDDS droplets which was probably due to the presence of Capryol-90 and Triton-X100 in the SNEDDS. The droplet size distribution of optimized SNEDDS F5 obtained by TEM analysis was in good agreement with droplet size distribution measured by Malvern Particle Size Analyzer. Based on self-nanoemulsification test with grade A, lower droplet sizes, lower viscosities, lower PIs, higher % T and higher drug contents, SNEDDS F5-F8 were selected for in vitro drug release studies. These studies were performed to compare the release profile of TDL trough dialysis membrane from selected SNEDDS (F5-F8) and drug suspension in order to optimize the formulation compositions. The results of drug release studies were in good agreement with the results of physicochemical characterization of SNEDDS. The highest drug release profile of TDL from F5 was probably due to its lowest droplet size, lowest viscosity, lowest PI, highest % T, highest drug content and presence of lower concentration

of Capryol-90. Two steps release profile of TDL from SNEDDS and drug suspension indicated diffusion controlled dissolution rate of TDL[18, 34]. Drug release kinetic studies showed that all formulations followed Korsemeyer-Peppas model. If the value of exponential n (diffusion coefficient) is equal to 0.5, it indicates Fickian diffusion mechanism. However, the value of n greater than 0.5 but less than 1.0 indicates non-Ficknian diffusion mechanism. On the other hand, the value of n greater than 1.0 indicates supercase II transport mechanism[27]. The value of n in formulations F5-F7 and drug suspension were observed in the range of 0.705-0.944, indicating non-Ficknian diffusion mechanism. However, the value of n for F8 was found to be 1.018, indicating supercase II transport mechanism. Based on highest drug release (96.6%), lowest droplet size (64.7 nm), lowest PI (0.112), lowest viscosity (35.6 cp), highest% T (99.1%), highest drug content (98.9%) and lower concentration of Capryol-90, formulation F5 was selected as an optimized formulation of TDL for further evaluation.

The poor aqueous solubility of drugs is the main cause of their formulation development which is directly related with dissolution rate and bioavailability of such drugs[18, 35]. Therefore, solubility enhancement of such drugs is of great significance in order to eliminate any barrier associated with formulation development. Hence, the solubility of poorly soluble drug TDL was enhanced by SNEDDS in present study. The enhancement in TDL solubility was observed as 1434 folds in optimized SNEDDS F5 as compared to its aqueous solubility (0.02 mg/ml). The enhanced solubility of TDL could be due to the molecular attractions between the molecules of TDL and the molecules of solvents such as Capryol-90, Triton-X100 and Transcutol-HP present in SNEDDS. The molecules of TDL could experience various kind of forces such as hydrophobic interactions, electrostatic forces and stereoscopic effects in solubilization process. TDL molecules have amide amine groups which could allow the dissolution of TDL molecules to breakdown the alignment of the sample matrices, which could further reduce the degree of order in the system and results in enhanced solubility of TDL. Therefore, the enhancement in TDL solubility in optimized SNEDDS F5 was possible due to the combined effects of Capryol-90, Triton-X100 and Transcutol-HP.

5 CONCLUSIONS

Based on lowest droplet size (64.7 nm), lowest PI (0.112), lowest viscosity (35.6 cp), highest% T (99.1%), highest drug content (98.9%), lower concentration of Capryol-90 (8% w/w), optimum surfactant (Triton-X100, 20% w/w) & cosurfactant concentrations (Transcutol-HP, 20% w/w) and highest drug release (96.6%), SNEDDS F5 has been optimized as an effective formulation of tadalafil. From these results, it was concluded that SNEDDS could be successfully used for solubility and dissolution enhancement of tadalafil. However, further pharmacodynamic and pharmacokinetic investigations are required to enhance oral bioavailability and therapeutic efficacy of tadalafil in comparison with marketed formulations. Pharmacodynamic and pharmacokinetic investigations are in process in the laboratory.

ACKNOWLEDGEMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding the work through the research group project no. RGP-VPP-299.

CONFLICT OF INTEREST

The authors report no declaration of interest. The authors alone are responsible for content and writing of the paper.

REFERENCES

1) Badr-Eldin, S. M.; Elkheshen, S. A.; Ghorab, M. M. Inclusion complexes of tadalafil with natural and chemically modified β-cyclodextrins. I: preparation and in-vitro evaluation. *Eur. J. Pharm. Biopharm.* **70**, 819-827 (2008).
2) Mehanna, M. M.; Motawwa, A. M.; Samaha, M. W. Tadalafil inclusion in microporous silica as effective dissolution enhancer: optimization of loading procedure and molecular state characterization. *J. Pharm. Sci.* **100**, 1805-1818 (2011).
3) Reddy, B. P.; Reddy, K. A.; Reddy, M. S. Validation and stability indicating RP-HPLC method for the determination of tadalafil API in pharmaceutical formulations. *Res. Pharm. Biotechnol.* **2**, 1-6 (2010).
4) El-Maghraby, G. M.; Alanazi, F. K.; Alsarra, I. A. Transdermal delivery of tadalafil. I. effect of vehicles on skin permeation. *Drug Develop. Ind. Pharm.* **35**, 329-336 (2009).
5) Shakeel, F.; Shafiq, S.; Haq, N.; Alanazi, F. K.; Alsarra, I. A. Nanoemulsions as potential vehicles for transdermal and dermal delivery of hydrophobic compounds: an overview. *Expert Opin. Drug Deliv.* **9**, 953-974 (2012).
6) Honda, M.; Asai, T.; Oku, N.; Araki, Y.; Tanaka, M.; Ebihara, N. Liposomes and nanotechnology in drug development: focus on ocular targets. *Int. J. Nanomed.* **8**, 495-504 (2013).

7) Andrade, F.; Rafael, D.; Videira, M.; Ferreira, D.; Sosnik, A.; Sarmento, B. Nanotechnology and pulmonary delivery to overcome resistance in infectious diseases. *Adv. Drug Deliv. Rev.* **65**, 1816-1827(2013).
8) Eldar-Boock, A.; Polyak, D.; Scomparin, A.; Satchi-Fainaro, R. Nano-sized polymers and liposomes designed to deliver combination therapy for cancer. *Curr. Opin. Biotechnol.* **24**, 682-689(2013).
9) Ariga, K.; Kawakami, K.; Hill, J. P. Emerging pressure-release materials for drug delivery. *Expert Opin. Drug Deliv.* **10**, 1465-1469(2013).
10) Izawa, H.; Kawakami, K.; Sumita, M.; Tateyama, Y.; Hill, J. P.; Ariga, K. β-Cyclodextrin-crosslinked alginate gel for patient-controlled drug delivery systems: regulation of host-guest interactions with mechanical stimuli. *J. Mater. Chem. B.* **1**, 2155-2161(2013).
11) Ghai, D.; Sinha, V. R. Nanoemulsions as self-emulsified drug delivery carriers for enhanced permeability of the poorly water-soluble selective β1-adrenoreceptor blocker talinolol. *Nanomed.* **8**, 618-626(2012).
12) Li, W.; Yi, S.; Wang, Z.; Chen, S.; Xin, S.; Xie, J.; Zhao, C. Self-nanoemulsifying drug delivery system of persimmon leaf extract: optimization and bioavailability studies. *Int. J. Pharm.* **420**, 161-171(2011).
13) Chhabra, G.; Chuttani, K.; Mishra, A. K.; Pathak, K. Design and development of nanoemulsion drug delivery system for amlodipine besilate for improvement of oral bioavailability. *Drug Develop. Ind. Pharm.* **37**, 907-916(2011).
14) Basalious, E. B.; Shawky, N.; Badr-Eldin, S. M. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine: I: development and optimization. *Int. J. Pharm.* **391**, 203-211 (2010).
15) Desai, N. S.; Nagarsenker, M. S. Design and evaluation of self-nanoemulsifying pellets of repaglinide. *AAPS Pharm. Sci. Tech.* **14**, 994-1003(2013).
16) Shafiq, S.; Shakeel, F.; Talegaonkar, S.; Ahmad, F. J.; Khar, R. K.; Ali, M. Design and development of oral oil in water ramipril nanoemulsion formulation: in vitro and in vivo assessment. *J. Biomed. Nanotechnol.* **3**, 28-44(2007).
17) Gong, Y.; Wu, Y.; Zheng, C.; Fan, L.; Xiong, F.; Zhu, J. An excellent delivery system for improving the oral bioavailability of natural vitamin E in rats. *AAPS Pharm. Sci. Tech.* **13**, 961-966(2012).
18) Shakeel, F.; Haq, N.; Elbadry, M.; Alanazi, F. K.; Alsarra, I. A. Ultra fine super self-nanoemulsifying drug delivery system(SNEDDS)enhanced solubility and dissolution of indomethacin. *J. Mol. Liq.* **180**, 89-94 (2013).
19) Xi, J.; Chang, Q.; Chan, C. K.; Meng, Z. Y.; Wang, G. N.; Sun, J. B.; Wang, Y. T.; Tong, H. H. Y.; Zheng, Y. Formulation development and bioavailability evaluation of a self-nanoemulsified drug delivery system of oleanolic acid. *AAPS Pharm. Sci. Tech.* **10**, 172-182(2009).
20) Vinesh, V.; Sevukarajan, M.; Rajalakshmi, R.; Chowdary, G. T.; Haritha, K. Enhancement of solubility of tadalafil by cocrystal approach. *Int. Res. J. Pharm.* **4**, 218-223(2013).
21) Vyas, V.; Sancheti, P.; Karekar, P.; Shah, M.; Pore, Y. Physicochemical characterization of solid dispersion systems of tadalafil with poloxamer 407. *Acta Pharm.* **59**, 453-461(2009).
22) Yunoos, M.; Sankar, D. G.; Kumar, B. P.; Hameed, S. UV spectrophotometric methos for the estimation of tadalafil in bulk and tablet dosage form. *E. J. Chem.* **7**, 833-836(2010).
23) Sing, S.; Pathak, K.; Bali, V. Product development studies on surface-adsorbed nanoemulsion of olmesartan medoxomil as a capsular dosage form. *AAPS Pharm. Sci. Tech.* **13**, 1212-1221(2012).
24) Shakeel, F.; Haq, N.; Alanazi, F. K.; Alsarra, I. A. Impact of various nonionic surfactants on self-nanoemulsification efficiency of two grades of Capryol (Capryol-90 and Capryol-PGMC). *J. Mol. Liq.* **182**, 57-63(2013).
25) Furlanetto, S.; Cirri, M.; Piepel, G.; Mennini, N.; Mura, P. Mixture experiment methods in the development and optimization of microemulsion formulations. *J. Pharm. Biomed. Anal.* **55**, 610-617(2011).
26) Anwer, M. K.; Jamil, S.; Ebnouf, E. O.; Shakeel, F. Enhanced antibacterial effects of clove oil by nanoemulsion. *J. Oleo Sci*. doi: 10.5650/jos.ess13214.
27) Costa, P.; Lobo, J. M. S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **15**, 123-133 (2001).
28) Dash, S.; Murthy, P. N.; Nath, L.; Chowdhury, P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol. Pharm.* **67**, 217-223(2010).
29) Nuchuchua, O.; Sakulku, U.; Uawongyart, N.; Puttipipatkhachorn, S.; Soottitantawat, A.; Ruktanonchai, U. In vitro characterization and mosquito(*Aedes aegypti*)repellent activity of essential-oils-loaded nanoemulsions. *AAPS Pharm. Sci. Tech.* **10**, 1234-1242 (2009).
30) Shakeel, F. Criterion for excipients screening in the development of nanoemulsion formulation of three anti-inflammatory drugs. *Pharm. Develop. Technol.* **15**, 131-138(2010).
31) Shakeel, F.; Ramadan, W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. *Coll. Surf. B.* **75**, 356-362(2010).
32) Azeem, A.; Rizwan, M.; Ahmad, F. J.; Iqbal, Z.; Khar, R. K.; Aqil, M.; Talegaonkar, S. Nanoemulsion components screening and selection: a technical note. *AAPS Pharm. Sci. Tech.* **10**, 69-76(2009).
33) Shinde, P. B. Components screening of miconazole ni-

trate nanoemulsion. *Asian J. Biomed. Pharm. Sci.* **3**, 33-40(2013).
34) Villar, A. M.; Naveros, B. C.; Campmany, A. C.; Trenchs, M. A.; Rocabert, C. B.; Bellowa, L. H. Design and optimization of self-nanoemulsifying drug delivery systems(SNEDDS)for enhanced dissolution of gemfibrozil. *Int. J. Pharm.* **431**, 161-175(2012).
35) Elbadry, M.; Fetih, G.; Fathy, M. Improvement of solubility and dissolution rate of indomethacin by solid dispersions in gelucire 50/13 and PEG 4000. *Saudi Pharm. J.* **17**, 217-225(2009).