Original Article

Preparation and *in vitro* evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) containing clotrimazole

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ABSTRACT: This study sought to formulate and evaluate a self-nanoemulsified drug delivery system (SNEDDS) for clotrimazole (CT), a poorly water-soluble antimycotic drug, used in vaginal delivery. SNEDDS was developed to increase the CT dissolution rate, solubility, and ultimately bioavailability. The solubility of CT in various oils, surfactants, and co-surfactants was determined. Based on solubility studies, oil phase (oleic acid without or with coconut oil), surfactant (Tween 20), and co-surfactants (PEG 200 and *n*-butanol) were selected and grouped in two combinations for phase studies. Pseudo-ternary phase diagrams were used to evaluate the area of self-nanoemulsification. Essential properties of the prepared systems with regard to emulsion droplet size and turbidity value were determined. In order to investigate the potential for interaction between any of the SNEDDS ingredients used, FTIR spectroscopy was performed. *In vitro* release studies were performed with SNEDDS formulations in capsules, and the plain drug served as a control. The droplet size of the nanoemulsion was greatly affected by the ratio of the surfactant and co-surfactant. Based on the results with regard to droplet size, turbidity values, and complete drug release after 3 h, three optimized formulations were selected; each contained oleic acid/coconut oil/Tween 20/PEG 200/*n*-butanol in ratios of 10:0:60:15:15 (%, w/w), 7.5:2.5:53.5:13.3:13.3 (%, w/w), and 6.7:3.3:60:10:10 (%, w/w), respectively. Results suggested that the prepared SNEDDS formulations produced acceptable properties in terms of immediate drug release and could increase the bioavailability of CT.

Keywords: Clotrimazole, self-nanoemulsion (SNEDD)

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1. Introduction

Clotrimazole (CT), a lipophilic imidazole derivative with antimycotic action, is widely and effectively employed locally for the treatment of vulvovaginal candidiasis. It is formulated in creams, foams, tablets, gels, irrigations, and pessaries. Unfortunately, oral use of CT is unacceptable due to its severe side effects, so topical administration of CT is recommended. However, its use is limited because of its very low water solubility, resulting in the need for it to be incorporated into a suitable vehicle (*1*). Microemulsion-based formulations offer rapid dispersion and an enhanced drug absorption profile. Microemulsions are thermodynamically stable, isotropically clear dispersions of water, oil, and surfactants with the potential to serve as drug-delivery vehicles (*2*,*3*). Microemulsions appear to have the ability to deliver larger amounts of topically applied agents into the mucosa than do traditional lotions and creams because they provide a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization (*4*). Nanoemulsions or mini-emulsions are transparent or translucent oil-in-water (o/w) or water-in-oil (w/o) droplets with a mean droplet diameter in the range of 100-600 nm. They are also known as submicron emulsions and, unlike thermodynamically stable microemulsions, nanoemulsions are kinetically stable with great stability in suspension due to their small droplet size (*5*). Furthermore, self-nanoemulsion drug delivery systems (SNEDDS) have been reported to result in more reproducible plasma concentration profiles and oral bioavailability of pharmaceuticals (*6*). The aim of the present study was to prepare a CT SNEDDS to enhance the solubility of CT and consequently its absorption profile.

2. Materials and Methods

2.1. *Materials*

CT was generously provided by Alexandria Pharmaceuticals and Chemical Industries Co. (Alexandria, Egypt). Miglyol 812 (medium chain triglyceride oil from coconut oil), α-tocopherol acetate

(vitamin E acetate), Tween® 60 (polyoxyethylene 20 sorbitan monostearate), Arlacel 83 (sorbitan sesquioleate), and Labrafil M 1944 (a polyoxyethylated kernel oil) were generously provided by GlaxoSmithKline (Cairo, Egypt). Oleic acid, citric acid, sodium hydroxide, conc. hydrochloric acid, polyethylene glycol 4000 (PEG 4000), methanol, 1-octanol, and *n*-butanol were from ADWIC (Cairo, Egypt). Castor oil, sesame oil, palm oil, coconut oil, olive oil, corn oil, and linseed oil were from Lab Chemicals Trading (Cairo, Egypt). Sweet almond oil was from Escoda & Nicolau, S.A. (Spain). Sorbitol and Tween® 80 (polyoxyethylene 20 sorbitan monooleate) were from ADCO (Alexandria, Egypt). PEG 200 (polyethylene glycol 200), Span® 80 (sorbitan monooleate), and Tween® 20 (polyoxyethylene 20 sorbitan monolaurate) were from Sigma-Aldrich (St. Louis, MO, USA). PEG 400 (polyethylene glycol 400) and PEG 600 (polyethylene glycol 600) were from Winlab (Middlesex, UK). Isopropyl myristate, propylene glycol (PG), Span® 20 (sorbitan monolaurate), and Tween® 40 (polyoxyethylene 20 sorbitan monopalmitate) were from Fluka (Buchs, Switzerland).

2.2. *Solubility studies*

Solubility of CT in various oils, surfactants, and co-surfactants was determined (*7-9*). Two grams of each of the selected vehicles were added to each cap vial containing an excess of CT. After the vial was sealed, the mixture was heated at 40°C in a water bath to facilitate solubilization using a sonicator (Ultrasonic model SS101H, Sonix IV, Huntington Beach, CA, USA). Mixtures were shaken with a shaker (shaking water bath, Weiss-Gallenkamp, Loughborough, UK) at 25°C for 48 h. Each vial was centrifuged using a centrifuge (Nuve, NF 815, Ankara, Turkey) at 3,000 rpm for 5 min and excess insoluble CT was discarded by filtration using hydrophilic polyvinylidene fluoride (PVDF) Acrodisc LC membrane filter discs (0.2 μm). The clear filtrate was diluted with methanol and was measured spectrophotometrically using a spectrophotometer (Model 6105 UV/V, Jenway Ltd., Essex, UK) at 261 nm.

2.3. *Apparent partition coefficient studies*

The previous saturation of equal volumes of the citrate buffer (pH 4.5) and 1-octanol was accomplished by shaking both in a shaker for 3 h, and the two phases were left to separate overnight. A known concentration of CT was added to the separated 1-octanol phase with gentle shaking until the CT dissolved. The 1-octanol phase containing the dissolved drug was mixed with the citrate buffer (pH 4.5) phase. The mixture was then agitated for 6 h at room temperature, and the two phases were then separated again after centrifugation. The drug concentration in the citrate buffer (pH 4.5) phase was determined spectrophotometrically at 263 nm after suitable dilution.

2.4. *Construction of phase diagrams*

Based on previous solubility studies (*10,11*), an oil phase (oleic acid without or with coconut oil), surfactant (Tween® 20), and co-surfactants (PEG 200 and *n*-butanol) were selected and grouped in two combinations for phase studies (Table 1). Surfactant and co-surfactants were mixed (Smix) in different weight ratios (2:1:1, 4:1:1, and 6:1:1, respectively). These Smix ratios were chosen to reflect increasing concentrations of surfactant with respect to co-surfactants. For each phase diagram, the oil phase (consisting of oleic acid alone or in combination with coconut oil in ratios of 2:1, 3:1, and 4:1) and the specific Smix ratio were mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.33, 1:2, 1:1.5, 1:1, 1:0.66, 1:0.43, 1:0.25, and 1:0.11) were produced to study phase diagrams. Pseudo-ternary phase diagrams were developed using aqueous titration with a magnetic stirrer (Thermolyne, Dubuque, IA, USA). Slow titration with the aqueous phase was performed for each combination of oil and Smix separately. The amount of the aqueous phase added was varied to produce a water concentration in a range of 5% to 95% of total weight in increments of around 5%.

2.5. *Preparation of SNEDDS*

Based on the pseudo-ternary phase diagrams observed, homogenous mixtures of Tween 20 (surfactant), PEG 200, and *n*-butanol (co-surfactants) in varying ratios were blended with oleic acid with or without coconut oil (groups I and II) in different weight ratios using a magnetic stirrer.

2.6. *Emulsion droplet size analysis*

The morphology and size of the emulsion were studied (*6,12-17*) using a transmission electron microscope (TEM, JEM 1010, JEOL, Tokyo, Japan) capable of point-to-point resolution. The combination of bright field imaging at increasing magnification and diffraction modes was used to reveal the form and size of the emulsion. A drop of CT emulsion was placed on a carbon-coated copper grid, stained with 2% uranyl acetate aqueous solution, and examined using the TEM.

2.7. *Turbidity measurement*

Each formulation (1.6 g) was diluted with citrate buffer (pH 4.5) to 400 mL and gently mixed. The resultant emulsions were evaluated for their turbidity. The turbidity

Table 1. Composition of SNEDDS formulations

Formula	O:Smix	Omix ratio	Smix ratio	Ingredients (%, w/w)				
				Oleic acid	Coconut oil	Tween 20	PEG 200	*n*-butanol
F1	1:9	–	2:1:1	10	–	45	22.5	22.5
F2	1:8	–	2:1:1	10	–	40	20	20
F3	1:7	–	2:1:1	10	–	35	17.5	17.5
F4	1:6	–	2:1:1	10	–	30	15	15
F5	1:9	–	4:1:1	10	–	60	15	15
F6	1:8	–	4:1:1	10	–	53.3	13.3	13.3
F7	1:7	–	4:1:1	10	–	46.7	11.7	11.7
F8	1:6	–	4:1:1	10	–	40	10	10
F9	1:5	–	4:1:1	10	–	33.3	8.3	8.3
F10	1:3.5	–	4:1:1	10	–	23.3	5.8	5.8
F11	1:8	4:1	4:1:1	8	2	53.3	13.3	13.3
F12	1:7	4:1	4:1:1	8	2	46.7	11.7	11.7
F13	1:6	4:1	4:1:1	8	2	40	10	10
F14	1:8	3:1	4:1:1	7.5	2.5	53.3	13.3	13.3
F15	1:9	–	6:1:1	10	–	67.5	11.25	11.25
F16	1:8	–	6:1:1	10	–	60	10	10
F17	1:7	–	6:1:1	10	–	52.5	8.75	8.75
F18	1:6	–	6:1:1	10	–	45	7.5	7.5
F19	1:5	–	6:1:1	10	–	37.5	6.25	6.25
F20	2:8	–	6:1:1	10	–	30	5	5
F21	1:3.5	–	6:1:1	10	–	26.25	4.375	4.375
F22	1:9	4:1	6:1:1	8	2	67.5	11.25	11.25
F23	1:8	4:1	6:1:1	8	2	60	10	10
F24	1:7	4:1	6:1:1	8	2	52.5	8.75	8.75
F25	1:6	4:1	6:1:1	8	2	45	7.5	7.5
F26	1:5	4:1	6:1:1	8	2	37.5	6.25	6.25
F27	1:8	3:1	6:1:1	7.5	2.5	60	10	10
F28	1:7	3:1	6:1:1	7.5	2.5	52.5	8.75	8.75
F29	1:6	3:1	6:1:1	7.5	2.5	45	7.5	7.5
F30	1:5	3:1	6:1:1	7.5	2.5	37.5	6.25	6.25
F31	1:8	2:1	6:1:1	6.7	3.3	60	10	10
F32	1:7	2:1	6:1:1	6.7	3.3	52.5	8.75	8.75

of the resulting emulsions given in nephelometric turbidity units (NTU) was measured using a turbidity meter (TRB 550, WTW, Weilheim, Germany). Turbidity measurements were performed on 15 mL of the emulsion stored in amber screw-capped vials.

2.8. *Fourier transform infrared (FTIR) spectroscopy*

In order to investigate the potential interaction between any of the SNEDDS ingredients used, FTIR spectroscopy was performed using a FTIR spectroscope (FT/IR-5300, JASCO, Tokyo, Japan) fitted with a single cuvette or a single bounce diamond at 45° that internally reflected incident light, providing a sampling area 1 mm in diameter with a sampling depth of several microns. Samples analyzed were CT powder, oleic acid, coconut oil, Tween 20, PEG 200, *n*-butanol, a physical mixture of CT powder, oleic acid, Tween 20, PEG 200, and *n*-butanol at a ratio 1:1:1:1:1, and a physical mixture of CT, oleic acid, coconut oil, Tween 20, PEG 200, and *n*-butanol at a ratio 1:1:1:1:1:1. A small amount of the sample was directly scanned for absorbance over a range from 4,000 to 400 wave numbers (cm^{-1}).

2.9. *In vitro release test*

Release studies were performed with SNEDDS formulations in capsules, and the plain drug served as a control. The *in vitro* release test was performed in a dissolution apparatus I (Dissolution Test Apparatus, USP standard, DA-6D, Bombay, India). Each CT-SNEDDS formulation equivalent to 100 mg of CT was placed in two hard gelatin capsules (*2,18-20*) (size 00) while ensuring that the capsule was completely intact. The same SNEDDS formulation of the same weight but free of CT was placed in two hard gelatin capsules and subjected to dissolution to serve as a blank. These capsules were placed in a basket and rotated at 100 rpm using 400 mL citrate buffer, simulating vaginal

pH (pH 4.5) (*21*) with a temperature maintained at 37 ± 0.5°C. The samples (4 mL each) were removed at specified time intervals, namely, 15, 30, 45, 60, 90, 120, 180, and 240 min (*22*). The withdrawn samples were filtered using PVDF Acrodisc LC membrane filter discs (0.2 μm) and the drug content was determined spectrophotometrically at the predetermined λ_{max} against a blank of the same SNEDDS formulation but free of CT. An equal volume of citrate buffer (pH 4.5) was added to the release medium to maintain constant dissolution volume. The experiments were done in triplicate. The release data were kinetically analyzed using different kinetic models (Zero-order, First-order, and Higuchi diffusion models) to determine the mechanism of CT release from the different SNEDDS.

3. Results and Discussion

3.1. *Screening of oils and surfactants*

Development of microemulsion systems for poorly water-soluble drugs is crucial. The volume of the formulation should be kept to a minimum to deliver the therapeutic dose in an encapsulated form. Components selected for the formulation should have the ability to solubilize the drug at a high level in order to obtain a concentrated form of microemulsion (*23*).

The solubility of CT in various vehicles is shown in Figure 1. The best results in terms of the highest drug solubility were obtained using oleic acid followed by coconut oil (139 and 43.7 mg/mL, respectively). In contrast, Tween 20, PEG 200, and *n*-butanol had a maximum solubility of CT of 47.2, 73.8, and 183 mg/mL. Based on these results, oleic acid (*20,24,25*) and coconut oil (*26*) were chosen as the oil phase, Tween 20 as the surfactant, PEG 200 and *n*-butanol (*27*) as co-surfactants.

An important criterion for the selection of surfactants is that the hydrophilic lipophilic balance (HLB) value required to form an o/w nanoemulsion be greater than

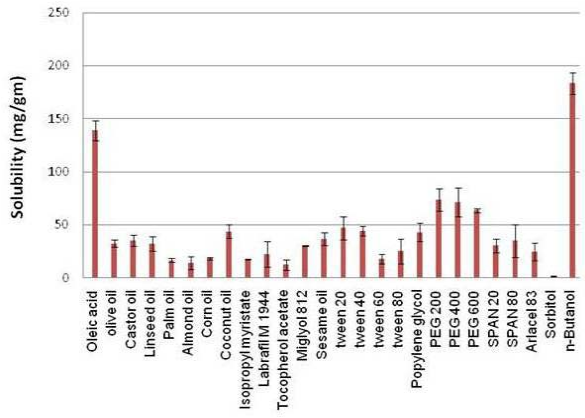

Figure 1. Solubility of CT in different oils, surfactants, and co-surfactants.

10 (*3*). In the present study, Tween 20, a nonionic surfactant, was selected because it has a HLB of 16.7.

Transient negative interfacial tension and a fluid interfacial film are rarely achieved with the use of a single surfactant, usually necessitating the addition of a co-surfactant. The presence of co-surfactants decreases the bending stress of the interface and allows an interfacial film with sufficient flexibility to assume different curvatures required to form a nanoemulsion over a wide range of compositions (*6*) and it also adjusts the HLB value of the formulation by making the polar solvent less hydrophilic. Due to the low water solubility of CT and rigidity of the oily surface, a quantity of alcohol (*n*-butanol) was added to dissolve the drug and increase the curvature of the oil layer. Alcohol incorporated into the nanoemulsion system not only reduces the interfacial tension between the oil phase and the aqueous phase but also makes the lipophilic drug soluble in the system (*28*). Thus, the co-surfactants selected for the study were PEG 200 and *n*-butanol.

3.2. *Apparent partition coefficient*

With a calculated octanol-citrate buffer, pH 4.5 [the pH in the vagina (*21*)], the partition coefficient (log P) value for CT was 2.33. This high value suggested good solubility of CT in lipophilic solvents.

3.3. *Pseudo-ternary phase diagram*

Pseudo-ternary phase diagrams were constructed in the absence of CT (*29*) to identify self nano-emulsifying regions and to select suitable concentrations of oil, surfactant, and co-surfactants for the SNEDDS formulation.

SNEDDS form fine o/w emulsions with only gentle agitation upon introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as providing a mechanical barrier to coalescence. A visual test is used to measure the apparent spontaneity of emulsion formation (*8*).

Oleic acid and coconut oil (oil), Tween 20 (surfactant), and PEG 200 and *n*-butanol (co-surfactants) were put in two groups to study the phase diagrams in details. Pseudo-ternary phase diagrams were created separately for each group, as shown in Figure 2, so that o/w nanoemulsion regions could be identified. In both groups, increasing the ratio of surfactant/co-surfactants (Tween 20/PEG 200/*n*-butanol) from 2:1:1 to 6:1:1 in SNEDDS formulations was found to increase the spontaneity of the self-emulsification region. Therefore, a much higher concentration of surfactant led to a much higher self-emulsifying region in phase diagrams.

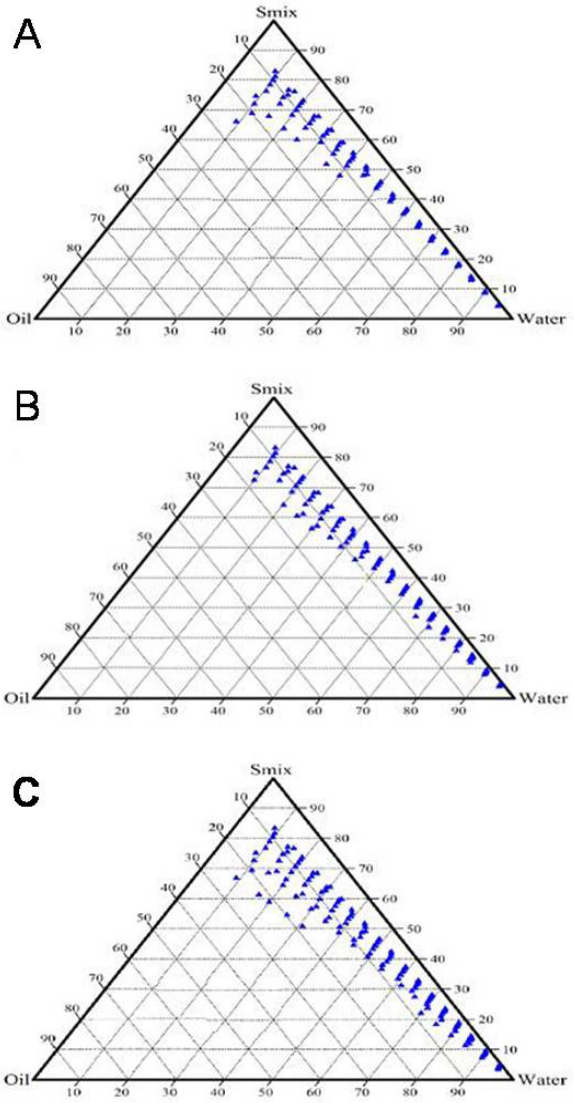

Figure 2. Pseudo-ternary phase diagrams of the o/w nanoemulsion region of certain CT systems at different Smix ratios. Smix ratios (Tween 20/PEG 200/*n*-butanol) were 2:1:1 **(A)**, 4:1:1 **(B)**, and 6:1:1 **(C)**. Closed triangles indicate points with o/w nanoemulsion.

These results agree with those of Derle *et al*. (*28*) who designed topical microemulsions of nimesulide, a poorly water-soluble nonsteroidal anti-inflammatory drug, using olive oil as the oil phase and Tween 80/iso-octanol as surfactant/co-surfactant.

3.4. *Droplet size and turbidity analysis*

TEM analysis revealed that the emulsion droplet was almost spherical in shape (Figure 3). The droplet size of the diluted SNEDDS formulations was evaluated by TEM as described elsewhere (*10,12,14*). Nanoemulsions are characterized in the nanometer size range. Therefore, droplet size analysis was performed to see whether the resultant emulsions were indeed nanoemulsions. All of the formulations prepared were found to be in the nanometer size range except F7, F10,

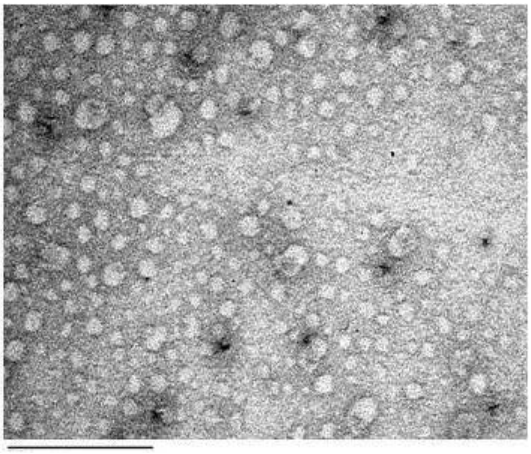

Figure 3. Typical TEM photographs of clotrimazole-containing microemulsion droplets of formulation F14. Bar, 500 nm. Original magnification, ×50,000.

F18, F19, and F26, which were in the micrometer size range (1,500-2,000 nm). The formulations F5-F9, F12, F14, F15, F20, and F31 had a size of < 200 nm, while the formulations F4, F13, F16, F21-25, F27-30, and F32 had a size of 200-500 nm and the formulations F1-3, F11, and F17 had a size of 500-700 nm. The measured turbidity of the formulations is summarized in Table 2. As is apparent, the formulations with a high turbidity (> 1,000) had a droplet size diameter of more than 1.5 µm, indicating a significant correlation between the droplet size and turbidity (in an ANOVA test, the correlation coefficient $r = 0.737$ and the two-tailed p value < 0.001, so the correlation is considered extremely significant).

3.5. *FTIR spectroscopy*

FTIR spectra are mainly used to determine if there is any interaction between the drug and any of the excipients used. The existence of an interaction is detected by the disappearance of an important functional group of the drug. CT compatibility with the ingredients of SNEDDS formulations was tested using FTIR, as shown in Figure 4. The FTIR spectrum of CT was characterized by bands at 1,585.63, 1,487.25, and 1,305.93 cm^{-1} (benzene ring stretching); 904.7, 823.68, and 744.59 cm^{-1} (C-H stretching); 3,169.33 and 3,042.02 cm^{-1} (aromatic C-H stretching); 1,084.09 cm^{-1} (chlorobenzene), and 1,203.69 cm^{-1} (C-N stretching).

After careful inspection of the spectra of the physical mixture of CT with the ingredients of SNEDDS formulations, the –C-N group, benzene ring, and –C-H stretching were found to be affected by the presence of these ingredients, as evidenced by the slightly higher absorption although the activity of the whole compound as well as the activity of characteristic groups of CT were unaffected. This finding confirms that CT did not interact with any of the ingredients of SNEDDS formulations.

Table 2. Mean droplet sizes, turbidity values, and cumulative release of clotrimazole from different SNEDDS formulations

Formulation	Mean droplet size (nm)	Turbidity value (NTU)	Cumulative% release after 240 min
F1	646	893	38.5
F2	723	518	32.6
F3	572	699	43.1
F4	434	344	55.2
F5	81	313	100
F6	144	296	51.8
F7	1,965	> 1,000	13.9
F8	183	816	93.9
F9	191	695	92.7
F10	1,532	> 1,000	32.5
F11	513	263	43.6
F12	179	515	94.4
F13	486	635	46.6
F14	98	88	100
F15	113	551	74.8
F16	492	157	48.7
F17	680	828	38.0
F18	1,714	> 1,000	30.8
F19	1,901	> 1,000	16.7
F20	118	88	29.7
F21	473	458	48.9
F22	303	353	77.7
F23	324	226	73.1
F24	402	276	65.0
F25	454	288	49.3
F26	1,850	> 1,000	20.2
F27	275	671	82.0
F28	404	301	63.2
F29	433	371	51.6
F30	333	438	75.8
F31	168	327	100
F32	345	83	71.0

3.6. *In vitro release study*

Release studies were performed with SNEDDS formulations in capsules as well as with the plain drug. When the release of CT from these formulations was evaluated in citrate buffer (pH 4.5), the percentage release of CT after 240 min from SNEDDS formulations was significantly greater than that of plain CT (13.5%) (Table 2). Complete drug release (100%) was obtained with F5 and F14 after 180 min and with F31 after 240 min (Figure 5). According to correlation coefficient (*r*), the *in vitro* release data suggested diffusion release kinetics except for F5, which displayed first-order release kinetics. The values of n for all of these formulations were ≤ 0.5, indicating Fickian (case I) transport (*20*), except for F31, which had an n that fell between 0.5 and 1, *i.e.*, non-Fickian (anomalous) transport (*30*,*31*).

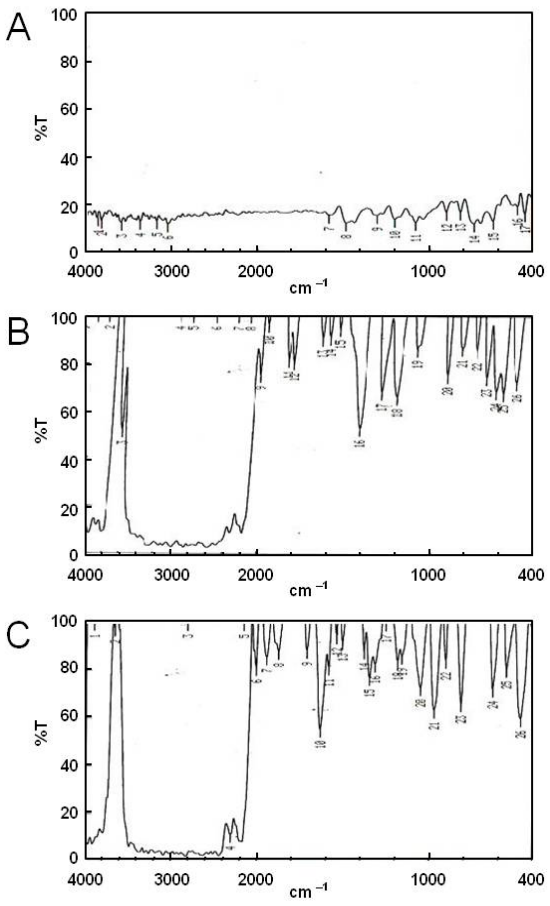

Figure 4. FTIR spectra. (A) clotrimazole; **(B)** physical mixtures of clotrimazole, oleic acid, Tween® 20, PEG 200, and *n*-butanol in a 1:1:1:1:1 ratio; **(C)** physical mixtures of clotrimazole, oleic acid, coconut oil, Tween® 20, PEG 200, and *n*-butanol in a 1:1:1:1:1:1 ratio.

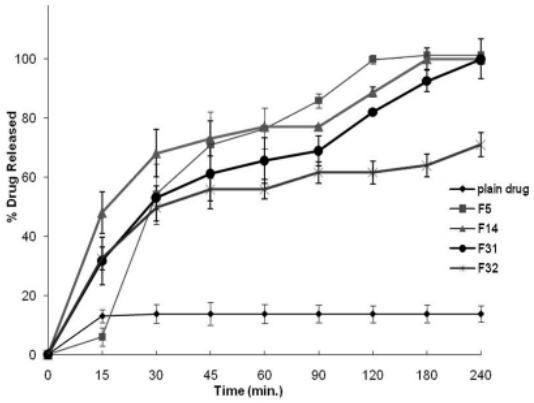

Figure 5. *In vitro* release of clotrimazole from SNEDDS formulations F5, F14, F31, and F32 and plain drug.

4. Conclusion

Results suggested that the prepared self-nanoemulsified formulations of CT produced acceptable properties in terms of droplet size, turbidity values, and immediate drug release that could increase the bioavailability profile of CT.

References

1. Ning MY, Guo YZ, Pan HZ, Yu HM, Gu ZW. Preparation and evaluation of proliposomes containing clotrimazole. Chem Pharm Bull (Tokyo). 2005; 53:620-624.
2. Date AA, Nagarsenker MS. Design and evaluation of self-nanoemulsified drug delivery systems (SNEDDS) for cefpodoxime proxetil. Int J Pharm. 2007; 329:166-172.
3. Constantinides PP. Lipid microemulsions for improving drug dissolution and oral absorption: Physical and biopharmaceutical aspects. Pharm Res. 1995; 12:1561-1572.
4. D'Cruz OJ, Yiv SH, Uckun FM. GM-144, a novel lipophilic vaginal contraceptive gel-microemulsion. AAPS PharmSciTech. 2001; 2:E5.
5. Constantinides PP, Chaubal MV, Shorr R. Advances in lipid nanodispersions for parentral drug delivery and targeting. Adv Drug Deliv Rev. 2008; 60:757-767.
6. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm. 2007; 66:227-243.
7. Limayem Blouza I, Charcosset C, Sfar S, Fessi H. Preparation and characterization of spironolactone-loaded nanocapsules for pediatric use. Int J Pharm. 2006; 325:124-131.
8. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, Lee HB, Cho SH. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm. 2004; 274:65-73.
9. D'Cruz OJ, Uckun FM. Influence of long-term stability conditions on microbicidal nucleoside prodrug (WHI-07)-loaded gel-microemulsion. AAPS PharmSciTech. 2006; 7:73.
10. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, Khar RK, Ali M. Formulation development and optimization using technique: A technical note. AAPS PharmSciTech. 2007; 8:Article 28.
11. Sinko PJ (ed.). Martin's Physical Pharmacy and Pharmaceutical Sciences. 5th ed., Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2006; pp. 54-55.
12. Xi J, Chang Q, Chan CK, Meng ZY, Wang GN, Sun JB, Wang YT, Tong HH, Zheng Y. Formulation development and bioavailability evaluation of a self-nanoemulsified drug delivery system of oleanolic acid. AAPS PharmSciTech. 2009; 10:172-182.
13. Kelmann RG, Kuminek G, Teixeira HF, Koester LS. Carbamazepine parentral nanoemulsions prepared by spontaneous emulsification process. Int J Pharm. 2007; 342:231-239.
14. Kuo F, Subramanian B, Kotyla T, Wilson TA, Yoganathan S, Nicolosi RJ. Nanoemulsions of an anti-oxidant synergy formulation containing gamma tocopherol have enhanced bioavailability and anti-inflammatory properties. Int J Pharm. 2008; 363:206-213.
15. Singh KK, Vingkar SK. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. Int J Pharm. 2008; 347:136-143.
16. Vyas TK, Shahiwala A, Amiji MM. Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. Int J Pharm. 2008; 347:93-101.
17. Wan T, Hu ZW, Ma XL, Yao J, Lu K. Synthesis of silane monomer-modified styrene-acrylate microemulsion coatings by photopolymerization. Prog Org Coat. 2008; 62:219-225.
18. Patil P, Paradkar A. Porous polystyrene beads as carriers for self-emulsifying system containing loratadine. AAPS PharmSciTech. 2006; 7:E28.
19. Zidan AS, Sammour OA, Hammad MA, Megrab NA, Habib MJ, Khan MA. Quality by design: Understanding the formulation variables of a cyclosporin A self-nanoemulsified drug delivery systems by Box-Behnken design and desirability function. Int J Pharm. 2007; 332:55-63.
20. Mehta SK, Kaur G, Bhasin KK. Analysis of tween based microemulsion in the presence of TB drug rifampicin. Colloids Surf B Biointerfaces. 2007; 60:95-104.
21. Pavelić Ž, Škalko-Basnet N, Filipović-Grčić J, Martinac A, Jalšenjak I. Development and *in vitro* evaluation of a liposomal vaginal delivery system for acyclovir. J Control Release. 2005; 106:34-43.
22. Dash AK, Cudworth GC. Evaluation of an acetic acid ester of monoglyceride as a suppository base with unique properties. AAPS PharmSciTech. 2001; 2:E13.
23. Subramanian N, Ray S, Ghosal SK, Bhadra R, Moulik SP. Formulation design of self-microemulsifying drug delivery systems for improved oral bioavailability of celecoxib. Biol Pharm Bull. 2004; 27:1993-1999.
24. Borgia SL, Regehly M, Sivaramakrishnan R, Mehnert W, Korting HC, Danker K, Röder B, Kramer KD, Schäfer-Korting M. Lipid nanoparticales for skin penetration enhancement-correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. J Control Release. 2005; 110:151-163.
25. El-Laithy HM. Preparation and physicochemical characterization of dioctyl sodium sulfosuccinate (aerosol OT) microemulsion for oral drug delivery. AAPS PharmSciTech. 2003; 4:E11.
26. Fang JY, Hung CF, Hua SC, Hwang TL. Acoustically active perfluorocarbon nanoemulsions as drug delivery carriers for camptothecin: Drug release and cytotoxicity against cancer cells. 2009; 49:39-46.
27. Vandamme TF. Microemulsion as ocular drug delivery systems: Recent developments and future challenges. Prog Retin Eye Res. Ultrasonics. 2002; 21:15-34.
28. Derle DV, Sagar BSH, Pimpale S. Microemulsion as a vehicle for transdermal permeation of nimesulide. Indian J Pharm Sci. 2006; 68:622-625.
29. Parikh DK, Ghosh TK. Feasibility of transdermal delivery of fluoxetine. AAPS PharmSciTech. 2005; 6: E144-E149.
30. Attama AA, Nzekwe IT, Nnamani PO, Adikwu MU, Onugu CO. The use of solid self-emulsifying systems in the delivery of diclofenac. Int J Pharm. 2003; 262:23-28.
31. Swarnalatha S, Selvi PK, Ganesh Kumar A, Sekaran G. Nanoemulsion drug delivery by ketene based polyester synthesized using electron rich carbon/silica composite surface. Colloids Surf B Biointerfaces. 2008; 65:292-299.

(Received May 9, 2010; Revised June 4, 2010; Re-revised July 28, 2010; Accepted August 1, 2010)