Formulation of avanafil in a solid self-nanoemulsifying drug delivery system for enhanced oral delivery☆

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ABSTRACT

Avanafil was incorporated into solid self-nanoemulsifying systems with the aim of improving its oral bioavailability. Labrafal, Labrafac, and Miglyol 812 N were investigated as oils, Tween 80 and Cremophor EL as surfactants, and Transcutol HP as a co-surfactant. Nine formulations produced clear solutions of 13.89–38.09 nm globules after aqueous dilution. Adsorption of preconcentrate onto Aeroperl 300 Pharma at a 2:1 ratio had no effect on nanoemulsion particle size. Differential scanning calorimetry, X-ray diffraction, and scanning electron microscopy indicated that avanafil was molecularly dispersed within the solid nanosystems. A formulation containing 10% Labrafal, 60% Tween 80, and 30% Transcutol HP had the highest drug loading (44.48 mg/g) and an acceptable in vitro dissolution profile (96.42% within 30 min). This formulation was chemically and physically stable for 6 months under accelerated storage conditions and it produced a 3.2-fold increase in bioavailability in rabbits, as compared to conventional commercially available avanafil tablets (Spedra®).

☆ The authors declare no conflict of interest.

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1. Introduction

Avanafil is a phosphodiesterase type 5 inhibitor that was recently approved by the United States Food and Drug Administration (FDA) and the European Medicines Agency for the treatment of erectile dysfunction (European Medicines Agency, 2013; US Food and Drug Administration, 2012). This compound has a faster onset of action and fewer visual side effects than other inhibitors of this type (Alwaal et al., 2011). However, avanafil has poor aqueous solubility and relatively low oral bioavailability (38–41%) (Burke and Evans, 2012). There are many established techniques for improvement of oral bioavailability of water-insoluble drugs. These include nanocrystal formation (Chen et al., 2016), inclusion complexation (Ammar et al., 2006), formulation of solid dispersions (Mohammed et al., 2016) and lipid-based systems (Shehata et al., 2016). Formulation of poorly soluble drugs in nanoemulsion systems provides an efficient approach to improving their bioavailability (Parmar et al., 2011). Self-nanoemulsifying drug delivery systems (SNEDDS) are mixtures of lipids, surfactants, co-surfactants, and drugs that rapidly form an oil/water nanoemulsion on mild agitation in an aqueous medium (Singh et al., 2010). When administered orally, SNEDDS mix with gastrointestinal tract fluids during peristaltic movement; this results in the generation of <100-nm globules with an extremely large surface area that contain the water-insoluble drug, thus enhancing drug dissolution and bioavailability (Shakeel et al., 2013). Fahmy et al. (2015) formulated a liquid SNEDDS containing avanafil in an attempt to improve its oral bioavailability in rats. However, solid SNEDDS (S-SNEDDS) are considered superior because they combine the solubility and bioavailability enhancement properties of liquid SNEDDS with enhanced stability and patient compliance in addition to accurate dosing (Bari et al., 2015). S-SNEDDS can be prepared by spray-drying, freeze-drying, or by direct adsorption of nanoemulsion preconcentrates onto carriers (Date et al., 2010; Tang and Cheng, 2008). Several recently published studies investigated the formulation of different water-insoluble drugs in S-SNEDDS where the drugs were dispersed within the carrier in an amorphous form, which improved their oral bioavailability (Balakrishnan et al., 2009; Kang et al., 2012; Shanmugam et al., 2011). Nevertheless, the chemical and physical stability of the dispersed amorphous drug remains a concern. The present study aimed to optimize an amorphous dispersion of avanafil within S-SNEDDS. The prepared formulations were thoroughly evaluated in vitro and the stability and oral bioavailability of a selected formulation was investigated.
2. Material and methods

2.1. Materials

Avanafl was purchased from Arcadia Biotechnology (Shanghai, China). Methanol and high-performance liquid chromatography (HPLC) grade acetonitrile were purchased from Sigma–Aldrich. Absolute ethanol was purchased from Piocheme (Cairo, Egypt). Tween 80 (polyoxyethylene sorbitan monoooleate, hydrophilic–lipophilic balance [HLB] = 15, iodine value 19–24) and hydrochloric acid were purchased from El-Nasr Pharmaceuticals (Cairo, Egypt). Aeroperl®300 Pharma (oleoyl macrogolglycerides, HLB = 4, iodine value 79–89), Labrafac™ lipophile WL 1349 (medium chain triglycerides, HLB = 1, iodine value <2), and Transcutol®HP (diethylene glycol monoethyl ether, HLB = 4.2) were kindly gifted by Evonik (Essen, Germany). Labrafil M 1944 CS (polyethoxylated triglycerides, HLB = 13, iodine value 25–35) was purchased from Sigma-Aldrich (Taufkirchen, Germany). Miglyol 812 N (medium chain triglycerides, HLB = 5, iodine value <1) was kindly gifted by Cremer (Hamburg, Germany). Dapoxetine HCl and transparent hard gelatin capsule shells (size 0) were supplied by the Center of Applied Research and Advanced Studies, Faculty of Pharmacy, Cairo University.

2.2. Construction of pseudo-ternary phase diagrams

Three nanoemulsion preconcentrate systems were prepared, each using the 36 possible surfactant/co-surfactant/oil combinations shown in Fig. 1. These systems employed Labrafil/Tween 80/Transcutol HP, Labrafac/Tween 80/Transcutol HP, and Miglyol 812 N/Cremophor EL/Transcutol HP.

The surfactant/co-surfactant/oil mixtures were prepared and shaken with excess avanafl at 30 °C for 24 h (Tulabo, SW-20 C, USA) prior to filtering the solution through a 0.45-μm membrane filter to remove excess drug. An accurately weighed 0.5 g of the filtrate was diluted with 50 mL distilled water, and magnetically stirred at 10 g for 5 min (Wisd Wisestir MSH 20-D; Witteg, Germany). The diluted systems were visually inspected against a dark background and were classified as clear, translucent, or biphasic. The surface morphologies of avanafl were observed after re-inspection at 24 h post dilution. The selected S-SNEDDS were examined. Images were taken using a transmission electron microscope (JEM 2100; Jeol, Japan) (Villar et al., 2012).

2.3. Drug loading capacity

Excess avanafl was shaken with the selected preconcentrates and filtered, as described above. The filtrate (0.5 g) was diluted to 10 mL with 50% (v/v) alcohol. This dilution was repeated twice with 50% (v/v) alcohol at a ratio of 1:10. The drug solubility in the preconcentrate was measured spectrophotometrically at a wavelength of 311 nm (UV-1601 PC; Shimadzu, Japan). One-way analysis of variance (ANOVA) followed by post-hoc analysis was used to investigate whether there were significant differences between the drug loading capacity of clear formulations of each system.

2.4. Particle size analysis

2.4.1. Photon correlation spectroscopy

Clear and translucent formulations were selected for particle size analysis using dynamic light scattering. They were diluted with distilled water (1:100), magnetically stirred at 10 g for 5 min, filtered through a 0.45-μm membrane filter to remove any precipitated drug, and inspected at 24 h post dilution. Z-average values and the polydispersity indices (PDI) were determined in duplicate for each formulation at room temperature using a scattering angle of 173° to the incident beam (Zetasizer nano ZS; Malvern, UK). One-way ANOVA was used to investigate whether clear formulations of each system showed significantly different Z-averages.

2.4.2. Transmission electron microscopy

A sample drop of each nanoemulsion was applied to a collodion-coated 300 mesh copper grid at 24 h post dilution and left for 10 min to allow adherence of the particles to the collodion. After removal of excess dispersion using filter paper, a drop of 2% aqueous uranyl acetate was applied for 1 min. The sample was air dried and examined using a transmission electron microscope (JEM 2100; Jeol, Japan) (Villar et al., 2012).

2.5. Drug solubility in water

An accurately weighed 0.5 g of each selected formulation was loaded with excess drug and mechanically shaken for 24 h at room temperature. The supernatant was diluted with 50 mL distilled water, stirred for 5 min at 10 g, and filtered through a 0.45-μm membrane filter. The filtrate was diluted twice with 50% (v/v) alcohol at a ratio of 1:10, and the concentration of dissolved avanafl was determined using UV spectroscopy at a wavelength of 311. The results of these analyses were analyzed by one-way ANOVA, followed by a post-hoc analysis.

2.6. Preparation and characterization of S-SNEDDS

Avanafl was dissolved within the Labrafil, Labrafac, and Miglyol-based formulations at concentrations of 40 mg/g, 30 mg/g, and 20 mg/g, respectively. The drug-loaded preconcentrates were left to equilibrate for 24 h prior to adsorption on Aeroperl by direct trituration in a glass mortar at a preconcentrate:adsorbent ratio of 2:1 (Sanka et al., 2016; Soliman et al., 2012). The prepared solid preconcentrates were characterized by particle size analysis, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM).

2.6.1. Particle size analysis

The prepared solid preconcentrates were diluted, filtered, and examined using photon correlation spectroscopy, as described above. The Z-average of each solid preconcentrate was compared with the Z-average of its corresponding liquid nanoemulsion preconcentrate using a two-sided t-test.

2.6.2. DSC

DSC was used to compare the solid phase of the selected S-SNEDDS to those of avanafl, Aeroperl, and their physical mixture. Four milligrams of the solids were heated in an aluminum cell (DSC-50; Shimadzu, Japan) under nitrogen at a flow rate of 25 mL/min. The hold temperature was set at 250 °C and the temperature acceleration rate was 10 °C/min.

2.6.3. PXRD

The X-ray diffraction pattern of the selected S-SNEDDS was compared to those of avanafl, Aeroperl, and their physical mixture using an X-ray diffractometer equipped with Co Kα (2100E; Dianocorp, USA). The tube was operated at 45 kV, 9 mA.

2.6.4. SEM

The surface morphologies of avanafl, Aeroperl, their physical mixture, and the selected S-SNEDDS were examined. Images were taken at an excitation voltage of 20 kV using a Quanta FEG 250 scanning electron microscope (FEI, USA).
2.7. In vitro dissolution study

This experiment was performed in accordance with FDA guidelines for the dissolution of avanafil (US Food and Drug Administration, 2015). The selected S-SNEDDS was filled into size 0 hard gelatin capsules so that each contained 15 mg avanafil. Dissolution of avanafil from the prepared capsules was assessed at 37 °C, relative to that of the pure drug, using the USP Dissolution Tester, Apparatus II (DIS-6000; Copley, UK) with 900 mL simulated gastric fluid (without pepsin) as the dissolution medium at a rotation rate of 50 rpm. Aliquots of 5 mL were withdrawn at 5, 10, 15, 20, and 30 min and replaced with an equal volume of fresh dissolution medium. The samples were withdrawn through a sintered glass filter and analyzed for their avanafil content by measuring the absorbance at 306 nm, using 0.1 N HCl as a blank. Replicate batches were used for dissolution studies.

The percentage drug released at 5 min (Q5) and the area under the dissolution curve for 0–30 min (dissolution efficiency [D.E.(0–30)]) (Anderson et al., 1998; Sanka et al., 2014a) were calculated for pure avanafil and the prepared formulation and compared using a two-sided one sample t-test.

2.8. Effect of storage

The prepared capsules were stored at 40 ± 2 °C with 75 ± 5% relative humidity for 6 months in a constant climate chamber (KBF 115; Binder, Germany) and samples were withdrawn at 0, 1, 2, 3, 4, 5, and 6 months, in accordance with the International Conference on Harmonisation guidelines (World Health Organization, 2011). The withdrawn samples were visually inspected for any change in color, odor, or texture of the hard gelatin capsules. The contents of the withdrawn samples were dissolved in 10 mL acetonitrile. After filtration through a 0.45-μm membrane filter, the filtrate was diluted to 1:100 with acetonitrile and the drug content was determined by HPLC using acetonitrile:water (90:10) (v/v) as the mobile phase, at a flow rate of 1 mL/min (LC-10 AD isocratic pump; Shimadzu, Japan). The HPLC system included a reversed phase C18 column (3.9 mm × 300 mm, particle size 4 μm; Waters, USA) and a spectrofluorimetric detector at a wavelength of 238 nm (RF-551; Shimadzu, Japan).

The solid contents of the stored capsules were investigated using DSC and PXRD, as described above. The nanoemulsion droplet sizes of the stored capsules were determined and their dissolution was assessed as described previously. The cumulative percentage drug released, Q5, and D.E.(0–30) were calculated. Two sided t-tests were used to compare the Q5, D.E.(0–30), and Z-average of the stored capsules with those of the fresh capsules.

2.9. In vivo evaluation

This study compared avanafil bioavailability from formulation 2 with that of conventional Spedra® tablets (Berlin-Chemie; batch number 28003, 200-mg avanafil tablets). Eight rabbits (2.8 ± 0.2 kg) were randomly allocated to two groups. Each group received a single oral dose of the test formulation or Spedra®, separated by a washout period of 1 week, in a crossover design. Animals were fasted overnight for 12 h with free access to water before drug administration, and for up to 4 h post dosing. A dose of 5.5 mg avanafil/kg was given to each rabbit (corresponding to a 100-mg human dose) based on the body surface area normalization method (US Food and Drug Administration, 2005). This involved administration of 206 mg/kg of formulation 2 or 11 mg/kg of Spedra® tablets. Blood samples were withdrawn from the marginal ear vein at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. The study protocol adhered to the European community standards and the legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU) (European Union Law, 2010) and was approved by the Research Ethical Committee of the Faculty of Pharmacy.
Cairo University (approval number PI-1495). The samples were collected into heparinized tubes and then centrifuged at 1800g for 10 min (Nuve, NF 815, Ankara, Turkey). The clear plasma samples were then deep-frozen at −20 °C until analysis.

Analysis of samples was performed using an HPLC system with an isocratic pump, a spectrophotometric detector operated at 238 nm, and a reversed phase C18 column (3.9 mm × 300 mm, particle size 4 μm; Waters, USA). A 35:65 (v/v) mixture of acetonitrile and phosphate buffer (pH 4) was used as the mobile phase, at a flow rate of 2 mL/min. Dapoxetine HCl (500 mg/mL) was used as the internal standard. Samples were prepared for column injection by adding acetonitrile, vortex mixing for 30 s (Stuart, UK), and centrifuging for 10 min at 1800g, in order to separate plasma proteins (Sedgwick et al., 1991). The supernatant was evaporated under vacuum using a sample concentrator (Stuart, UK) and the residue was extracted with 1 mL n-hexane:ethyl acetate (10:90 v/v). The assay procedure was validated in terms of linearity and extraction recovery. Relative recoveries of 25–3000 ng/mL avanafil were evaluated by assaying the plasma standards as described above and comparing the peak area ratios with those obtained from direct injection of unprocessed reference solutions of the same concentrations.

The plasma concentration–time data were plotted and analyzed using the Kinetta 5 program, extravascular model. The following pharmacokinetic parameters were calculated: peak plasma concentration (Cmax, ng/mL), time to Cmax (Tmax, h), the area under the plasma concentration–time curve extrapolated to infinity (AUC(0–∞), ng·h/mL), and the ratio of Cmax/AUC(0–∞), h−1. Percentage relative bioavailability was also calculated as (AUC(0–∞) S-SNEDDS / AUC(0–∞) market product) × 100. The data were statistically analyzed by one-way ANOVA, and differences were considered significant at p < 0.05.

3. Results

3.1. Pseudo-ternary phase diagrams

Fig. 1 shows the pseudo-ternary phase diagrams for nanoemulsion preconcentrate systems containing excess avanafil. Visual inspection indicated that all of the clear and translucent formulations maintained their appearance at 24 h post dilution. All clear systems had an oil concentration of 10%, while higher levels of oil resulted in biphasic emulsions. The Miglyol 812 N/Cremophol EL/Transcutol HP system exhibited a larger clear zone than either the Labrafac/Tween 80/Transcutol HP or Labrafil/Tween 80/Transcutol HP systems. The clear combinations had total HLB values in the range of 7.80–11.44, whereas the total HLB values of the translucent combinations comprising Labrafac/Tween 80/Transcutol HP and Miglyol 812 N/Cremophol EL/Transcutol HP were 9.28 and 6.92, respectively (Table 1).

3.2. Drug loading capacity

The drug loading capacities of the selected preconcentrates ranged from 8.70 ± 0.03 mg/g to 48.41 ± 0.65 mg/g (Table 1). The solubility of avanafil in these preconcentrates decreased as the surfactant/co-surfactant ratio and the resulting HLB increased. This drug had greater solubility in Labrafil-based systems than in Labrafac or Miglyol-based systems.

One-way ANOVA showed that formulation 1 had a significantly higher drug loading capacity than formulation 2, with an F-value of 28.7 (p < 0.05). There was no significant difference between formulations 4 and 5. In contrast, there was a significant difference in the drug loading capacity of formulations 7, 8, 9, 10, and 11, with an F-value of 77.3. Post-hoc LSD analysis showed that formulation 7 exhibited a significantly higher drug loading capacity than any of the other formulations.

3.3. Particle size

Photon correlation spectroscopy showed that clear formulations had Z-average values below 100 nm (Table 1). The intensity size distribution curves of selected formulations are shown in Fig. 2A. Each of formulations 2, 5, 7, 8, 9, 10, and 11 showed a single intensity peak, with Z-average values ranging from 13.89 ± 0.31 to 25.67 ± 7.10 nm and a narrow PDI ranging from 0.06 to 0.20. On the other hand, formulations 1 and 4 had 2 intensity peaks, with the major one showing an average relative intensity of 97.75% and 97.30%, respectively. The translucent formulations, 3 and 6, had higher Z-averages (above 100 nm), in addition to wider PDI values (Table 1).

One-way ANOVA showed that formulation 2 had a significantly lower Z-average than formulation 1, with an F-value of 67.7 (p < 0.05). On the other hand, formulations 4 and 5 did not show significantly different Z-averages. The Z-averages of formulations 7, 8, 9, 10, and 11 did not differ significantly.

Comparing formulations 2, 4, and 7 by one-way ANOVA revealed a significant difference in Z-average, with an F-value of 154.2 (p < 0.05). Post-hoc analysis showed that both formulations 2 and 4 had significantly lower Z-averages than formulation 7.

Transmission electron microscopy (Fig. 2B) revealed regular spherical globules of formulation 2, with no signs of coalescence or drug precipitation at 24 h post dilution. The nanoemulsion droplets emerged as dark spots in bright surroundings. A surfactant/co-surfactant layer was clearly visible around each oil globule. Measurement of the diameter showed a globule size that was close to the previously determined Z-average.

3.4. Drug solubility in water

The drug-loaded formulations 2, 4, and 7 showed aqueous drug solubility values of 409.35 ± 4.25, 434.84 ± 4.25, and 392.35 ± 7.08 μg/mL, corresponding to respective improvements in drug solubility (as compared to pure avanafil) of 52.54-, 55.82-, and 50.36-fold. One-way ANOVA followed by post-hoc analysis showed that formulation 4 produced significantly higher aqueous drug solubility than formulations 2 and 7, with an F-value of 15.9 (p < 0.05).

3.5. Preparation and characterization of S-SNEDDS

3.5.1. Particle size

S-SNEDDS with formulations 2, 4, and 7 had Z-average values of 17.16 ± 1.38, 17.24 ± 0.75, and 23.78 ± 1.63 nm, respectively. Two sided t-tests identified no significant differences between the Z-average values of the solid and liquid nanoemulsions (p > 0.05).

3.5.2. DSC

Fig. 3A shows that the avanafil DSC thermogram had a sharp endothermic peak at 162 °C, corresponding to its melting point. The DSC thermogram of Aeroperl showed a broad endothermic peak at 75 °C. The DSC thermogram of the drug-Aeroperl physical mixture showed both of these peaks. On the contrary, the DSC thermogram of formulation 2 exhibited complete disappearance of the sharp endothermic peak of avanafil.

3.5.3. PXRD

Fig. 3B shows prominent and sharp peaks in the X-ray diffraction pattern of avanafil at 2θ in the range of 20–30°. Aeroperl showed no sharp X-ray diffraction peaks. Avanafil peaks were clearly distinguished in the X-ray diffraction pattern avanafil-Aeroperl physical mixture. However, PXRD analysis of formulation 2 revealed complete absence of the sharp peaks of avanafil.
3.5.4. SEM

Fig. 4 shows the rod-shaped crystals of avanalil, while Aeroperl appeared as completely spherical granules with a hollow interior. The image of the physical mixture showed both of these structures, without any sign of interaction. The drug crystals were absent from the image of formulation 2. Investigation of this formulation under higher magnification power revealed that the smooth surface of the Aeroperl granules was coated with the nanoemulsion preconcentrate.

3.6. In vitro dissolution study

Fig. 5 shows that capsules containing formulation 2 exhibited 96.42% in vitro dissolution in 0.1 N HCl within 30 min, representing a 3.18-fold improvement over pure avanalil. Pure avanalil and formulation 2 had Q5 values of $4.49 \pm 0.79\%$ and $61.77 \pm 1.21\%$, and D.E.$(0-30)$ values of $480.79 \pm 4.24\%$ and $2311.95 \pm 9.10\%$, respectively. One sample t-test showed that the S-SNEDDS exhibited significantly higher Q5 and D.E.$(0-30)$ than avanalil ($p < 0.05$).

3.7. Effect of storage

The stored capsules of formulation 2 showed no change in color, odor, or texture during the study period. The avanalil content of the stored capsules decreased to 96.91% of the initial content after 6 months of storage at accelerated storage conditions (Table 2). The DSC thermograms and X-ray diffraction patterns of the contents of stored capsules were similar to those of the fresh formulation, as shown in Fig. 2. The Z-average of the stored capsule contents are shown in Table 2. Analysis by two-sided, one sample, t-test did not identify any significant

Table 1
Compositions and evaluation of the formulations selected from different phase diagrams.

<table>
<thead>
<tr>
<th>Formulation number</th>
<th>Self-emulsifying mixture composition (% w/w)</th>
<th>Visual appearance</th>
<th>HLB$^a$</th>
<th>Drug loading capacity (mg/g) $^c$</th>
<th>Z-average (nm) $^c$</th>
<th>PDI$^b$</th>
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<td>Oil</td>
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<td>Co-surfactant</td>
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<td>(± SD, n = 2)</td>
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$^a$ HLB, hydrophilic-lipophilic balance.

$^b$ PDI, polydispersity index.

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Fig. 2. Particle size analysis of nanoemulsions: (A) intensity size distribution curves of selected formulations, (B) transmission electron micrograph of formulation 2.
differences between the Z-average of the fresh formulation and those of the stored capsules during the study period ($p > 0.05$). There was no significant change in the Q$_2$ and D.E.($t_{0.30}$) values of the capsule contents during the first month of storage, although both values had increased significantly after 2 months.

3.8. In vivo evaluation

HPLC analysis of blank rabbit plasma spiked with avanafil and dapoxetine HCl showed that these peaks were sufficiently separated and readily distinguishable, eluting at 7.04 and 12.63 min, respectively. The assay procedure showed a linear relationship between the peak area ratio of avanafil and its concentration within the tested range, with a correlation of determination ($r^2$) of 0.9974 and an intercept that was not significantly different from zero at a 5% significance level. The mean extraction recovery was 100.30%.

The plasma concentration-time curves following administration of both formulation 2 and the Spedra$^\text{TM}$ tablets are shown in Fig. 6. The mean values of C$_{\text{max}}$ for avanafil were 3293.87 ± 541.62 ng/mL and 944.07 ± 54.65 ng/mL following oral administration of formulation 2 and Spedra$^\text{TM}$ tablets, respectively. Both preparations had the same $T_{\text{max}}$ (1 h). The mean AUC$_{(0-\infty)}$ values of formulation 2 and Spedra$^\text{TM}$ tablets were 8694.63 ± 603.40 ng·h/mL and 2693.72 ± 329.77 ng·h/mL, respectively. These results indicated that formulation 2 produced a 3.2-fold increase in bioavailability, as compared to Spedra$^\text{TM}$ tablets. The mean values of C$_{\text{max}}$/AUC$_{\text{c}}$ were 0.38 ± 0.03 h$^{-1}$ and 0.36 ± 0.05 h$^{-1}$ for formulation 2 and Spedra$^\text{TM}$, respectively.

One-way ANOVA showed that administration of formulation 2 produced significantly higher C$_{\text{max}}$ and AUC$_{(0-\infty)}$ values than Spedra$^\text{TM}$ tablets ($p < 0.05$, with F-values of 130.4 and 533.1, respectively). The C$_{\text{max}}$/AUC$_{\text{c}}$ values did not differ significantly between these preparations.

4. Discussion

Formulation of avanafil in S-SNEDDS improved its oral bioavailability. The high AUC$_{(0-\infty)}$ obtained after oral administration of formulation 2 indicated a significant improvement in the extent of drug absorption. However, the C$_{\text{max}}$/AUC$_{(0-\infty)}$ Values indicated that this nanoemulsion formulation did not influence the rate of drug absorption (Endrenyi et al., 1991; Lacey et al., 1994). This improvement in oral bioavailability was produced by incorporating avanafil into a lipid-based system.

![Fig. 3. Solid state characterization of avanafil, Aeroperl, their physical mixture, fresh and 6-month stored formulation 2: (A) differential scanning calorimetric thermograms, (B) X-ray diffraction patterns.](image-url)
selected as surfactants in this study due to their high HLB and their chemical structures, which enhance self-nanoemulsification (Date and Nagarsenker, 2007; Rao and Shao, 2008; Zhao et al., 2010). Moreover, the Labrafil/Tween 80/Transcutol HP system was previously reported by Shakeel et al. (2013) to be capable of forming ultra-fine SNEDDS. The wider nanoemulsion zone of the Miglyol 812 N/Cremophor EL/Transcutol HP system may be attributed to the highly branched chemical structure of Cremophor EL rather than Tween 80; this enhanced the flexibility of the oil/water interface and promoted further globule size reduction, as reported by Rao and Shao (2008). Moreover, the identified range of HLB values indicated that it was not the only determinant of nanoemulsion formation and that the chemical structures of both the surfactant and the co-surfactant played a role in producing globules with sizes in the nanometer range which was also reported by Rao and Shao (2008) and Wang et al. (2009). The significantly lower Z-average of formulation 2 as compared to formulation 1, may have resulted from its higher surfactant/co-surfactant ratio. This coincides with the findings of both Kang et al. (2012) and Yoo et al. (2010), who stated that increasing the surfactant concentration up to 60–75% reduced the droplet size. On the other hand, the failure of nanoemulsion formation in formulations 3 and 6 could reflect their relatively lower surfactant/co-surfactant ratios.

Based on Z-average values and drug loading capacities, formulations 2, 4, and 7 were selected as representatives of the 3 systems and were used to prepare S-SNEDDS. Aeroperl was used as a solid adsorbent for these nanoemulsion preconcentrates because of its high adsorption power and large surface area (Mahmoud et al., 2009; Shrivastava et al., 2009). The actual amounts of loaded avanafil within the solid preconcentrates were 3.4–11.2% lower than the predetermined drug loading capacities, to avoid any possible drug precipitation due to temperature fluctuation during storage. Adsorption of the nanoemulsion preconcentrates on Aeroperl did not affect the globule size of the nanoemulsion. Formulation 4 exhibited significantly higher aqueous drug solubility than formulation 2, despite the non-significant difference between their Z-averages, indicating that Labrafac oil improved aqueous drug solubility more effectively than Labrafil. Nevertheless, formulation 2 was selected for subsequent in vitro dissolution, stability, and bioequivalence studies due to its high drug-loading capacity (44.48 mg/g).

The increase in percentage drug released at 5 min and the dissolution efficiency during the storage period may be attributed to the absorption of moisture by the capsules, resulting in enhanced powder
Table 2

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Drug content (%) (±SD, n = 2)</th>
<th>Z-average (nm) (±SD, n = 2)</th>
<th>Q_d (%) (±SD, n = 2)</th>
<th>DE (%)(±SD, n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
<td>17.16 ± 1.38</td>
<td>61.37 ± 0.86</td>
<td>2311.95 ± 6.43</td>
</tr>
<tr>
<td>1</td>
<td>99.38 ± 0.12</td>
<td>14.90 ± 0.23</td>
<td>53.77 ± 1.95</td>
<td>2299.90 ± 9.75</td>
</tr>
<tr>
<td>2</td>
<td>99.37 ± 0.31</td>
<td>15.22 ± 0.03</td>
<td>72.87 ± 1.21</td>
<td>2590.01 ± 2.16</td>
</tr>
<tr>
<td>3</td>
<td>99.14 ± 0.20</td>
<td>14.75 ± 0.20</td>
<td>69.57 ± 0.87</td>
<td>2447.55 ± 12.12</td>
</tr>
<tr>
<td>4</td>
<td>98.91 ± 0.15</td>
<td>15.30 ± 0.20</td>
<td>89.00 ± 0.52</td>
<td>2663.90 ± 16.05</td>
</tr>
<tr>
<td>5</td>
<td>97.94 ± 0.34</td>
<td>14.76 ± 0.17</td>
<td>73.93 ± 1.23</td>
<td>2513.57 ± 16.21</td>
</tr>
<tr>
<td>6</td>
<td>96.91 ± 0.37</td>
<td>14.90 ± 0.12</td>
<td>79.01 ± 0.35</td>
<td>2611.24 ± 7.45</td>
</tr>
</tbody>
</table>

\( Q_d \) percentage drug released at 5 min.

\( DE \) dissolution efficiency within 30 min.

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**Fig. 6.** Plasma concentration time profiles of avanafil after oral administration of formulation 2 and Spedra® tablets to eight rabbits.

dissolution and drug release. Although this effect is not likely to occur under normal storage conditions, further studies will be required to confirm this.

We did not calculate the similarity factor (\( f_2 \)) of the in vitro dissolution profiles in the present study because the prepared S-SNEDDS capsules exhibited 87.85% in vitro drug release within the first 15 min, indicating that drug bioavailability from the S-SNEDDS was not limited by dissolution (Stevens et al., 2015; US Food and Drug Administration, 1997).

The nanoemulsion preconcentrate formulation 2, consisting of 10% Labrafac, 60% Tween 80, and 30% Transcutol HP, had an avanafil loading capacity of 44.48 mg/g and produced a 5.2-fold increase in aqueous drug solubility. After adsorption on Aeropel, the S-SNEDDS was chemically and physically stable at accelerated storage conditions and produced a 3.2-fold increase in bioavailability in rabbits, as compared to Spedra®. This easily prepared formulation could provide a promising solid dosage form that is acceptable to patients and enhances drug bioavailability.

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**References**


