Improving the High Variable Bioavailability of Griseofulvin by SEDDS

Adi Issam Arida,*,[a] Moawia Mohammed Al-Tabakha,[b] and Hantash Abdel Jalil Hamoury[a]

[a] *Faculty of Pharmacy, Philadelphia University; P.O. Box 1, Postal Code 19392, Jordan: and* [b] *Faculty of Pharmacy and Health Sciences, Ajman University of Science and Technology Network; P.O. Box 2202, Al-Fujairah, U.A.E.*

To enhance the dissolution and oral absorption of poorly water-soluble griseofulvin (GF), self-emulsifying drug delivery system (SEDDS) composed of oil, surfactant and cosurfactant for oral administration of griseofulvin was formulated, and its physicochemical properties and pharmacokinetic parameters were evaluated. The solubility of griseofulvin was further improved by the addition of hydrochloric acid. Droplet size of griseofulvin emulsion was kept constant both in simulated gastric fluid without pepsin and simulated intestinal fluid throughout 12 weeks incubation period. Griseofulvin in the SEDDS rapidly dissolved in different dissolution media. This was not the case for the commercial GRIS-PEG® tablets. In different fed diet groups, $AUC_{0 \to 24\,h}$, Cp_{max}, and T_{max} of griseofulvin after oral administration of SEDDS in rats were comparable to those after oral dose of GRIS-PEG® tablet. Although, in fed lipidic diet group, the mean AUC and Cp_{max} after oral administration of GRIS-PEG® in rats were 1.28 and 1.15 fold higher, respectively, compared with those of SEDDS, these have not shown to be significantly different. These results demonstrate that the SEDDS of griseofulvin composed of Capmul® GMO-50, Poloxamer and Myvacet 9-45 greatly enhanced the dissolution of griseofulvin (without ultramicronisation). However, food intake effect on the bioavailability of griseofulvin has remained. Thus, this system may provide a useful dosage form for oral water-insoluble drugs which have problems in their dissolution.

Key words   griseofulvin; self-emulsifying drug delivery system; bioavailability; dissolution

Self-emulsifying drug delivery systems (SEDDS) are mixtures of oils and surfactants, ideally isotropic, and sometimes containing cosolvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation.[1] Recently, SEDDS have been formulated using medium chain triglyceride oils and nonionic surfactants, the latter being less toxic. Upon peroral administration, these systems form fine emulsions (or microemulsions) in gastro-intestinal tract (GIT) with mild agitation provided by gastric mobility.[2,3] Potential advantages of these systems include enhanced oral bioavailability enabling reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug(s) toward specific absorption window in GIT, and protection of drug(s) from the hostile environment in gut.[4—6]

The process of self-emulsification proceeds through formation of liquid crystals (LC) and gel phases, the properties of which significantly affect the formation of droplets and interface available for partitioning of drug.[5—9] Many workers claim various rational applications of SEDDS for delivering and targeting lipophilic drugs (*e.g.*, WIN 54954,[1] N-4472,[10] idebenone,[11] coenzyme Q10,[12] vitamin E,[13] halofantrine,[14] and cyclosporin A.[15] However, very few reports are available of SEDDS of water insoluble or poorly soluble hydrophobic compounds. Therefore the concept of using griseofulvin in SEDDS was considered for the present study, where the drug is present in solution form.

Griseofulvin is an antifungal agent first isolated from a *Penicillium* spp. in 1939. It is effective after oral ingestion and reaches the skin and hair. It is deposited primarily in keratin precursor cells. Ingestion with a heavy meal and reduction in particle size enhances the absorption of griseofulvin.[16] Griseofulvin systematic (IUPAC) name is (2*S*,6′*R*)-7-chloro-2′,4,6-trimethoxy-6′-methyl-3*H*,4′*H*-spiro[1-benzofuran-2,1′-cyclohex[2]ene]-3,4′-dione (Fig. 1), and its formula is $C_{17}H_{17}ClO_6$ with molecular weight 352.766 g/mol. The

compound is insoluble in water (8.64 mg/l). It has a logP/hydrophobicity 2.15 (partition system octanol/water), and its pK_a/isoelectric point is not available.[17] Griseofulvin inhibits fungal mitosis by disrupting the mitotic spindle through interaction with polymerized microtubules.[18]

Griseofulvin is administered orally. Its microcrystalline and ultramicrocrystalline forms are available as tablets. The microcrystalline form also comes in a pediatric suspension form. The typical dose of microcrystalline form is 500—1000 mg/d. Ultramicrocrystalline form is administered at doses of 330—990 mg/d.[19] Since the bioavailability of poorly water-soluble drugs can be influenced by interactions with food or by the physicochemical conditions in the gastrointestinal (GI) tract,[20] oral preparation of griseofulvin is commonly prescribed to be administered according to a fixed dosing schedule. The oral bioavailability of griseofulvin is highly variable (25 to 70%).[16]

The Biopharmaceutics Classification System[21] classifies drugs into four categories, depending on their solubility and permeability characteristics. According to this scheme, griseofulvin belongs to Class II drugs and their solubility is too low to be consistent with complete absorption. Six *et al.*[22] stated that correlation of *in vivo* results with dissolution tests is likely to be best for Class II drugs, because in this case the dissolution rate is the primary limiting aspect to absorption.
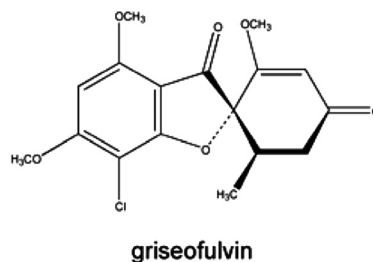

Fig. 1.   The Molecular Structure of Griseofulvin

∗ To whom correspondence should be addressed.   e-mail: arida@go.com.jo

As the dissolution step is the rate limiting step to absorption of griseofulvin, the circumstances of GI tract, such as post-prandial, preprandial states, affect extensively drug absorption.

Griseofulvin absorption from the gastrointestinal tract varies considerably among individuals, mainly because of insolubility of the drug in aqueous media of the upper GI tract; duodenum. The bioavailability of the microsize griseofulvin is variable; ranging from 25 to 70% of an oral dose, while that of ultramicrosize griseofulvin is almost completely absorbed. Ultramicrosize or microsize refers to the size of the drug (griseofulvin) crystals or particles. The peak serum level for griseofulvin (0.5—2 μg/ml) needs approximately 4 h following administration of a single dose of 250 mg of ultramicrosize griseofulvin, or 500 mg of microsize griseofulvin, with biological half life ($t_{1/2}$) 9—24 h.[23,24] Levels may be increased by giving the drug with a high-fat diet. GI absorption of the ultramicrosize products is about 1.5 times that of the microsize products; there is no evidence that this causes any difference in the safety and effectiveness of the drug compared with the microsize form. The ultramicrosize of crystals of griseofulvin (GRIS-PEG) are smaller particles and more completely absorbed into the body than the conventional microsize crystals, with more reaching the blood stream. Because of this 1/3 less ultramicrosize (than microsize) is needed to get the same effect and results.[19,25,26]

The dissolution rate of poorly water-soluble drugs often becomes a rate-limiting step in their absorption from the GI tract.[27,28] Various solubilization methods have been used to increase the drug solubility and dissolution properties, including the use of surfactants, water-soluble carriers, polymeric conjugates, particle size reduction, suitable polymorph, anhydrous or organic solvate forms and solid dispersions.[29—33]

However, hydrophobic drugs can be dissolved in SEDDS allowing them to be encapsulated as unit dosage form for peroral administration. When such a formulation is released into the lumen of the gut, it disperses to form a fine emulsion, so that the drug remains in solution in the gut, avoiding the dissolution step which frequently limits the rate of absorption of hydrophobic drugs from the crystalline state.[5] The commercial success of the SEDDS formulation Sandimmune Neoral® (cyclosporin), as well as the novel self-emulsifying formulations such as Norvir® (ritonavir) and Fortovase® (saquinavir), has raised the interest in such promising emulsion-based drug delivery system.[34] Hong *et al.*[35] formulated a self-emulsifying formula of itraconazole that enhanced its bioavailability and at the same time the formula was not affected by food intake.

Here, in this work, and after the protocol of Hong *et al.*,[35] a trial to develop a similar new self-emulsifying formula for griseofulvin (without micronization) in which there will be a formula with enhanced bioavailability, that is not affected by food, and has enhanced dissolution, and solubility properties has been performed. The physicochemical properties of SEDDS and pharmacokinetic parameters were evaluated in comparison to the ultramicrocrystalline 125 mg GRIS-PEG® tablets (Pedinol Pharmacal Inc.); in addition, the relative bioavailability was investigated according to different food intake states.

Experimental

Materials Griseofulvin was obtained from Sigma-Aldrich (St. Quentin Fallavier, France), Commercial GRIS-PEG® tablets (griseofulvin ultramicrosize), USP 125 mg, were obtained from Pedinol Pharmacal Inc. (NY, U.S.A.). Castor oil was obtained from Sigma Chemical Co. (St. Louise, MO, U.S.A.), Lauroglycol® FCC (propylene glycol laurate) was obtained from Gattefosse (Seoul, Korea), Miglyol 812 (caprylic/capric triglyceride, triglycerides of the fractionated vegetable fatty acids C8 and C10) was obtained from Sasol Corp. (Werk Witten, Germany), Myvacet 9-45 (acetylated monoglycerides) was obtained from Eastman Chemical Products Inc. (Kingsport, Tenn, U.S.A.), Acconon® CA-40 (PEG-40 castor oil), Acconon CC-6, EP (macrogol 6 glycerol caprylocaprate), Acconon MC8-2, EP/NF (polyoxyethylene[8]) caprylic/capric glycerides), Capmul® GMO-50, EP/NF (glycerol mono-oleate), Caprol® PGE, 860 (polyglycerol-10 mono-dioleate) were obtained from ABITEC Corporation, (Columbus, Ohio, U.S.A.). Carbitol (diglycol monoethyl ether), Labrafac® hydrophile (a mixture of C_8/C_{10} ethoxylated glycerides, HLB 1), Lubrafil® M 1944 CS (composed largely of triglycerides based on oleic and linoleic acid (C_{18}) and pegylated derivatives, HLB 4), was obtained from Gattefosse (Gennevilliers, France). Poloxamer (polyethylene-polypropylene glycol, a hydrophilic non-ionic surfactant), Sodium dodecylsulfate (sodium lauryl sulfate, an ionic surfactant), Tween 80 (polyoxyethelene 20 sorbitan monooleate), Lecithin (a lipid material composed of choline and inositol), Sodium taurocholate (conjugation product of cholic acid with taurine and the principal constituent of the bile of carnivorous animals) were purchased from Merck Ltd. (Whitehouse Station, NJ, U.S.A.). Methanol of HPLC quality grade was purchased from Baker (Deventer, Holland). Water was purified by a Milli-Q system (Millipore, Bedford, MA, U.S.A.). Normal and lipidic food (AIN-76A purified rodent diet with 8.0% sodium chloride powder containing: casein, DL-methionine, cornstarch, sucrose, cellulose, corn oil, salt mix #200000, vitamin mix #300050, choline bitartrate, sodium chloride) were obtained from Jungang Lab. Animal Co (Seoul, Korea) and Dyets® Inc. (Pennsylvania, U.S.A.), respectively.

Methods. Preparation of the SEDDS An excess amount of griseofulvin was added to various oils, surfactants and cosurfactants, and mixed by vortexing. The mixture was then kept at ambient temperature for 1 week to get to equilibrium. The equilibrated sample was centrifuged at 1000 rpm for 10 min to remove the undissolved griseofulvin. The supernatant was taken and diluted for quantification of griseofulvin by HPLC. High-performance liquid chromatography (HPLC) was performed on an analytical reverse column (Intersphere C18 5 μm, 250×4.6 mm, Interchim, Monthucon, France). Mobile phase (methanol/water 85 : 15 v/v) was delivered by a 305-Pump (Gilspn, France) at a constant flow rate of 0.5 ml/min. Samples were injected as pure methanolic solutions (10 mg/ml) in a Rheodyne valve (1-ml sample loop). Volumes of 500 μl were injected by a 500-μl syringe (Hamilton, Reno, NV, U.S.A.). Detection was performed at 254 nm using a 115-UV model detector (Gilson) coupled to a SE-120 model integrator (BBC Guerz Metrawatt). Each solvent (ethyl acetate, methylene chloride, hexane and methanol) was of Normapur quality (SDS, Peypin, France) and distilled prior to use. Water was purified by a Milli-Q system (Millipore, Bedford, MA, U.S.A.).

Effect of Variables on SEDDS In order to study the effect of hydrochloric acid on the solubility of griseofulvin, an excess amount of griseofulvin was added to surfactants with increasing the added volume of hydrochloric acid up to 20 μl. Moreover, the effect of surfactant–oil ratio on the solubility of griseofulvin was also investigated. An excess amount of griseofulvin was added to each composition of SEDDS at weight ratios of surfactant mixture (S_{mix}) to oil ranging from 2 : 1 to 6 : 1. After equilibration for 1 week at ambient temperature, the equilibrated samples were centrifuged and the dissolved amount of griseofulvin was determined by HPLC as described above.

The homogeneous mixtures of chosen emulsifiers in varying ratios were blended with the oil in different weight ratios. Griseofulvin was dispersed into the mixture of oil- and surfactants (emulsifers) with constant stirring and kept at 50—60 °C for 10 min to obtain a good blend of oil–S_{mix} mixture at a liquid state. Premicroemulsion concentrate (500 μl) containing griseofulvin, prepared as described in Table 1, was added to 200 ml of simulated gastric fluid without pepsin (SGF, pH 1.2) or simulated intestinal fluid (SIF, pH 6.8), and then incubated for 2 h at 37 °C. The particle size of microemulsion was determined as function of time using a laser particle analyzer (Malvern 2600HSD laser diffraction particle sizer, Malvern Instruments, Worcester, U.K.).

***In-Vitro* Tests** The dissolution test was performed according to the USP 24 in a USP paddle dissolution apparatus (Hanson Research Corporation,

Table 1. SEDDS Formulations under Test

Formulation	#1	#2	#3	#4	#5	#6
S_{mix} (surfactant : cosurfactant)	2 : 1	2 : 1	2 : 1	4 : 1	4 : 1	4 : 1
S_{mix} : oil	6 : 1	4 : 1	2 : 1	6 : 1	4 : 1	2 : 1
Capmul® GMO-50 (mg)	570	534	444	686	640	534
Poloxamer (mg)	285	267	222	169	161	132
Myvacet 9-45 (mg)	145	199	334	145	199	334
35% HCl (mg)	23.6					
Total (mg)	1023.6					

Chats Worth, California, U.S.A.). Each preparation of SEDDS of griseoful-vin equivalent to 125 mg of griseofulvin, and GRIS-PEG® tablets was put into a sinker. This sinker was loaded with 1000 ml water with sodium dode-cylsulfate (5.4 mg/ml) at 37 ± 0.5 °C with paddle speed of 75 rpm. Each sample was withdrawn at 5, 10, 20, 30, 40, 50, 60, and 120 min with replacement by an equal volume of temperature-equilibrated media, and centrifuged at 1000 rpm for 10 min. After appropriate dilution with solution of methanol and water, the concentration of griseofulvin was determined by HPLC.

***In-Vivo* Tests** Male Sprague–Dawley rats weighing 300 ± 20 g were used. All experiments were performed according to the International guidelines of experimental animal care; "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). The animals were divided into three groups; the first group of rats was fasted for 12 h before drug administration, the second group was continuously fed with normal diet for 12 h before drug administration, and the third group was continuously fed with li-pidic diet for 12 h before drug administration. The femoral artery was cannulated with 23-gauge polyethylene cannula for blood samples withdrawals, after anesthesia with diethylether. The cannula was flushed with 0.3 ml of heparin (50 IU) saline solution to prevent blood clotting. After rats recovered from the anesthesia, GRIS-PEG® tablets and SEDDS (125 mg of griseoful-vin in 1023.6 mg mixture) equivalent to 10 mg/kg of griseofulvin, were administered orally to rats using oral sonde. Each preparation was dispersed into 500 μl of distilled water by vortexing for 20 s immediately prior to dosing. Each blood sample (250 μl) was withdrawn at designated time intervals and frozen at -20 °C until analysis. The concentration of griseofulvin in rat plasma was determined by HPLC; R51012 solution (*cis*-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]-methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methyl-butyl)-3*H*-1,2,4-triazol-3-one) as an internal standard was added into the plasma. Then the plasma samples were extracted by vortexing and centrifuging. The residue of the organic layer was reconstituted in the mobile phase. Finally, after vortexing another time, the sample was used for HPLC as above.

The area under the drug concentration–time curve from zero to infinity ($AUC_{0 \to 24h}$) was calculated using the trapezoidal rule. The maximal plasma concentration of drug (Cp_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma data. The data from different formulations were compared for statistical significance by ANOVA.

Results and Discussion

Determination of Solvents The surfactants used in SEDDS formulations are known to improve the bioavailability by various mechanisms.[12,36] Moreover, the addition of co-surfactants has been shown to increase the extent of the microemulsion region in lecithin/triglyceride systems.[37] Myvacet 9-45 led to the highest solubility of griseofulvin (6.78 mg/g) among the oils studied (Table 2). On the other hand, Capmul® GMO-50 and Poloxamer showed the maximum solubility of griseofulvin as 7.21 and 5.60 mg/g, respectively (Tables 1, 2). From these results, Myvacet 9-45, Capmul® GMO-50 and Poloxamer were chosen as oil and surfactants for preparing a microemulsion system of griseofulvin for further work in this study.

The commercial griseofulvin oral formulation, GRIS-PEG® tablets, USP, contains 125 mg griseofulvin in one tablet. In this work, the solubility of excess raw griseofulvin

Table 2. Solubility of Griseofulvin in Various Oils and Surfactants ($n=3$)

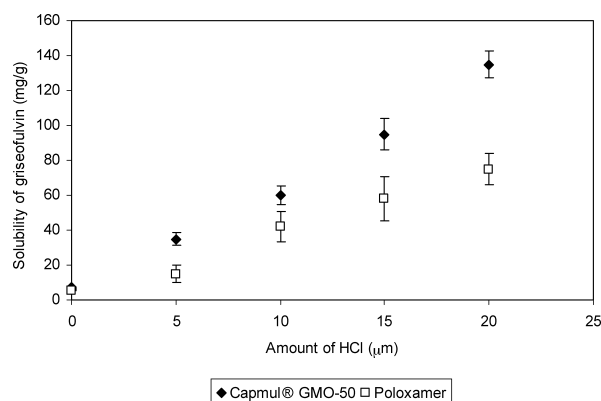|	Solubility (mg/g) $\pm$ S.D.
Surfactants	
Acconon® CA-40	4.99 ± 0.62
Acconon CC-6, EP	5.12 ± 0.23
Acconon MC8-2, EP/NF	4.75 ± 0.49
Capmul® GMO-50	7.21 ± 0.44
Caprol® PGE, 860	4.49 ± 0.34
Poloxamer	5.60 ± 0.52
Carbitol	4.32 ± 0.42
Tween 80	1.18 ± 0.05
Oils	
Castor oil	1.85 ± 0.52
Lauroglycol® FCC	2.78 ± 0.34
Miglyol 812	0.12 ± 0.09
Myvacet 9-45	6.78 ± 0.87
Labrafac® hydrophile	0.96 ± 0.32
Lubrafil® M 1944 CS	0.22 ± 0.12

Fig. 2. Effect of Hydrochloric Acid on the Solubility of Griseofulvin in Surfactants

Data are expressed as mean $\pm$ S.D. ($n=3$).

in oils and surfactants was less than 8 mg/g as shown in Table 2. To enhance the solubility of griseofulvin, 35% hydrochloric acid was added. The solubility of griseofulvin in each 1 g of Capmul® GMO-50 and Poloxamer increased proportionately with added amount of 35% hydrochloric acid ranging from 0 to 20 μl (Fig. 2). Since griseofulvin is protonated in acidic condition,[38] the solubility of griseofulvin increased in proportion to the amount of added hydrochloric acid. When added hydrochloric acid amount was 20 μl, the solubility of griseofulvin increased 19 folds in Capmul® GMO-50, and 13 folds in Poloxamer compared with the solubility of griseofulvin without hydrochloric acid (Fig. 2). Because Capmul® GMO-50 showed the higher solubility of griseofulvin than Poloxamer, Capmul® GMO-50 was chosen as a surfactant and Poloxamer as a cosurfactant. To solubilize at least 125 mg of griseofulvin per 1 g of SEDDS, 20 μl of hydrochloric acid was chosen.

A key aspect of this work was this remarkable increase in griseofulvin solubility in the excipients in the presence of HCl. Corvis *et al.*[39] studied the interactions of griseofulvin with lipid membranes trying to elucidate the mechanisms of GF-membrane interactions using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dilauroyl-*sn*-glycero-3-phos-phocholine (DLPC), and 1,2-myristoyl-*sn*-glycero-3-phos-phoethanolamine (DMPE) monolayers spread at the air/water

interface. They proposed that nonpolar interactions are by and large responsible for GF retention in the monolayers.

Moreover, a look at the structure of griseofulvin (Fig. 1), the authors can see that the chemical structure possesses three major functional groups, which might have a direct effect on the oil/water distribution ratio, absorption, and the dissolution characteristics of the drug. Two of these functional groups are carbonyls; one of these is a highly reactive $\alpha\beta$-unsaturated ketone ring, and the other one is a simple carbonyl moiety. The chemical structure of the compound contains also an ether oxygen ring.

In the process of protonation with 35% HCl, it seems to the authors that the acid, by itself, does not increase or enhance the solubility of the drug (by any complexation mechanism, for example), but rather it may reduce the polarity of the functional groups *via* protonation process which play a great part in directing the balance of oil/water behavior towards increasing the hydrophobic character of the drug as well as it might decrease the rate of the metabolism and excretion processes of the drug.

As the ratio of S_{mix} to Myvacet 9-45 increases, the solubility of griseofulvin was moderately increased from 139 to 167 mg/g (Fig. 3). However, the solubility of griseofulvin showed no significance between different ratios of Capmul® GMO-50 to Poloxamer. At the S_{mix} ratios of 2:1 and 4:1 (Capmul® GMO-50 to Poloxamer), the solubility of griseofulvin was almost 155 mg/g over the ratio 4:1 of S_{mix} to oil. These values were sufficient to solubilize 125 mg griseofulvin per 1 g of SEDDS.

Determination of Particle Size Particle size distribution is one of the most important characteristics of emulsion for the evaluation of its stability,[1] and also *in vivo* fate of emulsion.[40]

Droplet size of griseofulvin microemulsion was decreased with reducing the oil content in SEDDS. When S_{mix}: oil ratio was 2:1, the bigger particle formed in comparison with ratios 4:1 and 6:1 of S_{mix}: oil. Generally, at 2:1 ratio of surfactant and cosurfactant, smaller particle was formed than 4:1 ratio of S_{mix}. The smallest volumetric median diameter of microemulsion (272 nm) was obtained at the 6:1 ratio of S_{mix} to oil. Since griseofulvin is stable in acidic condition, all SEDDS formulations formed smaller particle in simulated gastric fluid (SGF) than in simulated intestinal fluid (SIF) (Fig. 4). Hence, the ratios of 6:1 (S_{mix} to oil) and 2:1 (surfactant to cosurfactant) were used for further studies.

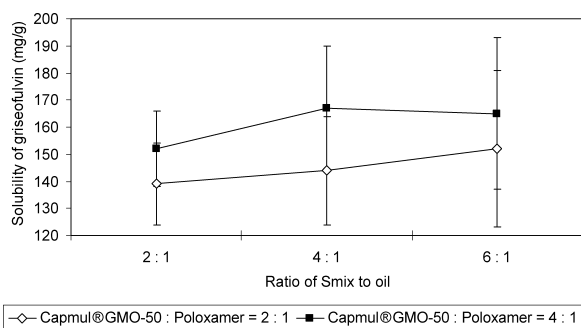Griseofulvin-loaded SEDDS was stable at room tempera-ture for 12 weeks as shown in Fig. 5. Mean particle size was constant both in simulated gastric and intestinal fluid. Moreover, the content of griseofulvin was maintained in the range of 90—115% and showed no significant difference.

Dissolution Test The dissolution profiles of griseofulvin from GRIS-PEG® 125 mg tablets and SEDDS were different according to the fluid pH and the usage of surfactant (SDS) (Fig. 6). While GRIS-PEG® tablet was dissolved in 1000 ml of water containing 5.4 mg/ml sodium dodecylsulfate (SDS) (Fig. 6a), and in acidic condition, SGF (Fig. 6b), the tablet dissolution in SIF at pH 6.8 (Fig. 6d) was lower than the SGF conditions. However, dissolution was improved with increasing amount of surfactant in SIF (Fig. 6c). On the other hand, the dissolution of griseofulvin from SEDDS was not influenced by pH and surfactant added, and these results were statistically not significant. Griseofulvin from SEDDS was completely and rapidly dispersed regardless of the fluid condition.

The results of griseofulvin dissolution from the commercial GRIS-PEG tablets *in vitro* were found to be different in the four media ($p=0.04$). ANOVA was employed in the statistical analysis, considering the percentage dissolved at 60 min as after this time there was no significant increase in the concentration. Also at 60 min there was an overlap in the error bars of the data. Therefore, Tukey HSD was used to further analyze the data. This *post hoc* test (or multiple comparison test) can be used to determine the significant differ-
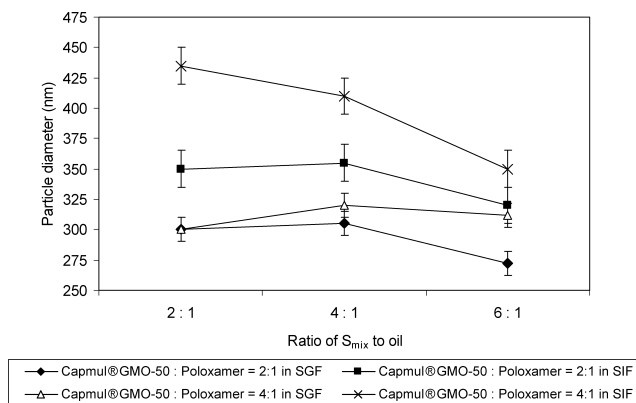
Fig. 4. Particle Diameter of Griseofulvin in Various Ratio of S_{mix} to Myvacet 9-45 When S_{mix} Is Variable

Data are expressed as mean±S.D. ($n=3$).

Fig. 3. Solubility of Griseofulvin in Various Ratio of S_{mix} to Myvacet 9-45 When S_{mix} Is Variable
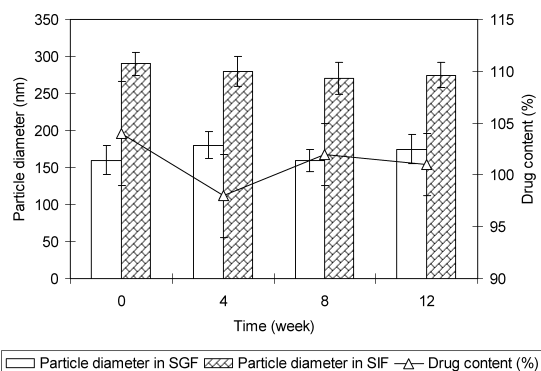
Data are expressed as mean±S.D. ($n=3$).

Fig. 5. Physical and Chemical Stability of SEDDS

Data are expressed as mean±S.D. ($n=3$).

ences between group means in an analysis of variance setting. By applying the Tukey HSD test ($p<0.05$), only the drug dissolved in 1000 ml water with 5.4 mg/ml SDS (87%) showed significant difference when compared to the dissolution in SIF (41%) at 60 min. ANOVA analysis on the other hand did not show significant difference when considering the dissolution of SEDD formulation in the different dissolution media at 60 min. When comparing GRIS-PEG tablets with the SEDD formulation in each of the media using t-test at 60 min, the only difference was found in the dissolution medium containing water and SDS ($p=0.04$). For SEDD formulation the average was 86% in that dissolution medium. The results also showed almost complete and faster dissolu-

tion of the SEDD formulation during the 60 min in all of the dissolution media when compared to GRIS-PEG tablet results. SEDD formulation also showed less variable dissolution characteristic in the different media.

Pharmacokinetic Studies An *in vivo* absorption study was undertaken to determine whether or not the enhanced solubility and *in vitro* dissolution of griseofulvin in a SEDDS could increase the GI absorption of drug after oral administration regardless of dietary conditions. Table 3 shows the pharmacokinetic variables measured in this study which were area under the concentration-*versus*-time curve (*AUC*), maximum plasma concentration (Cp_{max}), and time to Cp_{max} (T_{max}). *AUC* was calculated by using the trapezoidal rule, and Cp_{max} and T_{max} were calculated by direct observation. The *AUC* of griseofulvin *versus* time was calculated based on a period that extends to complete drug elimination (24 h).

Using ANOVA two factor analysis, it was found that *AUC* and Cp_{max} of both GRIS-PEG® and SEDD formulation were affected by fasting or food intake whether normal or lipidic ($p=0.000023$). There has been an increase in the *AUC* from 5.41 to 18.28 µg·h/ml in the case of GRIS-PEG® tablets and an increase from 5.09 to 14.28 µg·h/ml in the case of SEDD
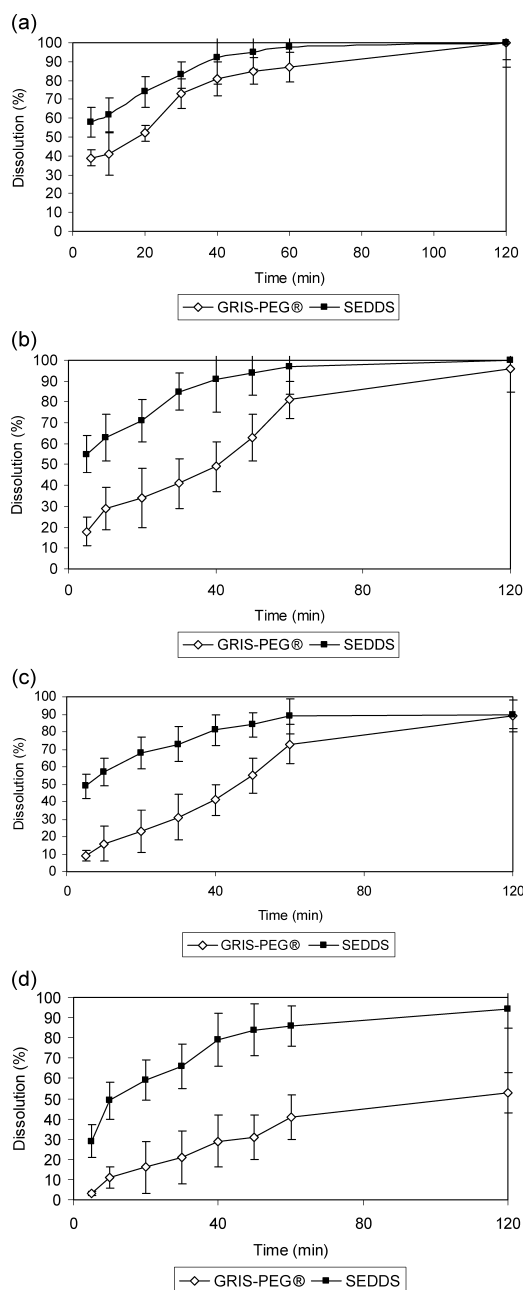

Fig. 6. Dissolution Profiles of Griseofulvin from GRIS-PEG® Tablets and SEDDS in: Water+5.4 mg/ml SDS (a), SGF+5.4 mg/ml SDS (b), SIF+5.4 mg/ml SDS (c), and in SIF (d)

SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SDS, sodium dodecylsulfate. Data are expressed as mean±S.D. ($n=3$).
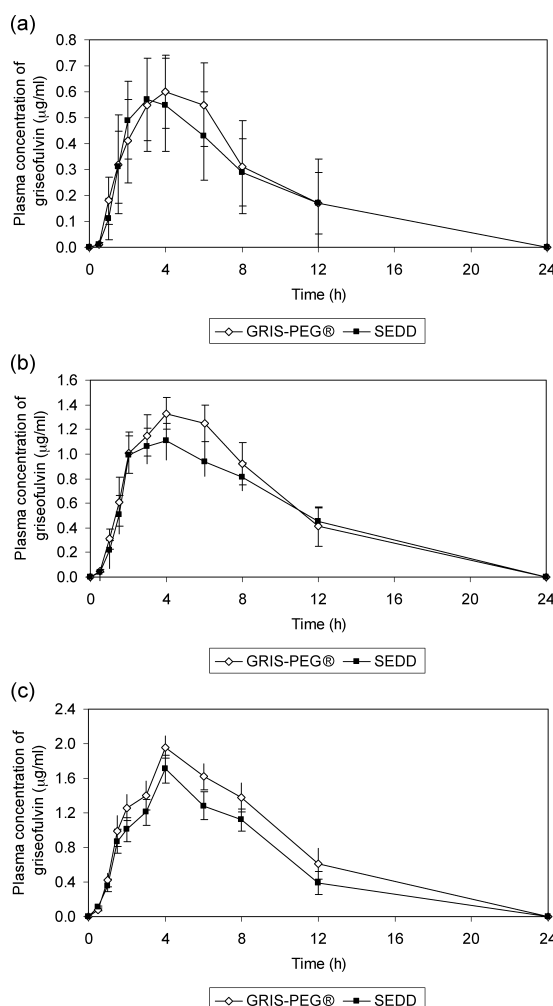

Fig. 7. Plasma Concentration–Time Profile of Griseofulvin in Rats after Oral Administration of GRIS-PEG® Tablet or SEDDS of Griseofulvin at a Dose Equivalent to 10 mg of Griseofulvin/kg of Body Weight

Before the treatment, the animals were fasted overnight (F) (a), received normal diet continuously (N) (b), or lipidic diet (L) (c). Data are expressed as mean±S.D. ($n=5$).

Table 3. Pharmacokinetic Parameters after Oral Administration of Griseofulvin Formulations to Rats ($n=5$)

Formulation	Parameter	Feeding condition		
		No food	Normal food	Lipidic food
GRIS-PEG® tablet	$AUC_{0\to24h}$ (μg h/ml)	5.41±3.85	12.92±5.72	18.28±5.73
	T_{max} (h)	4.30±1.86	5.20±1.10	4.00±0.00
	Cp_{max} (μg/ml)	0.91±0.16	1.39±0.27	1.96±0.29
SEDDS	$AUC_{0\to24h}$ (μg h/ml)	5.09±2.66	11.76±1.55	14.28±4.34
	T_{max} (h)	4.60±1.64	4.20±1.10	4.00±0.00
	Cp_{max} (μg/ml)	0.94±0.12	1.31±0.16	1.71±0.35

formulation when lipidic food was introduced in comparison to fasting subjects. This together with similar increases in Cp_{max} indicates a greater extent of griseofulvin absorption with food. Cp_{max} has increased from 0.91 to 1.96 μg/ml in the case of GRIS-PEG® tablets when lipidic food was introduced with the drug intake. In comparison, there was similar increase with SEDD formulation as Cp_{max} increased from 0.94 to 1.71 μg/ml; almost doubling. The analyses did not show significant differences between the AUCs or the Cp_{max} of both formulations. The time to reach maximum concentration was not affected by griseofulvin formulation or food intake and was averaged around 4.4 h. Although it was not significant, T_{max} was earlier in fed state (Fig. 7, Table 3). In particular, lipidic dietary group showed earlier T_{max} than normal diet group. The T_{max} of SEDDS was also earlier in fed state. It can be concluded that this new formula of griseofulvin formulated as SEDD has comparable results to the ultramicrosized coomercial GRIS-PEG® formula, and that this SEDDS formula is rapidly dissolved and reaches its therapeutic levels.

Bile salts play an important role in the solubilization of lipid digested products and poorly water-soluble drugs. Typical concentrations of bile salts in the fasting intestine are 4—6 mM compared with postprandial concentrations of 10—20 mM.[41] However, rats have no gall bladder and always secret bile salt whether there is food intake or not, and the pulsatile response to food which occurs in dogs and human is absent.[35,42] Therefore, in fasting state, GRIS-PEG® tablet could be dissolved by bile salt without interference of food, and also by the naturally excreted acid in the stomach. However, in fed lipidic diet state, bile salt improved the dissolvation of lipid in the food and which by itself (the lipid) also improves the absorption of the drug from GRIS-PEG® tablet, consequently the absorption of griseofulvin of GRIS-PEG® tablet after lipidic food was the highest among all other groups. Meanwhile, normal food contains little lipid and gastric residence time will be more in comparison with fasting state therefore drug absorption also increased here.

From the pharmacokinetic study in rats, the absorption of GRIS-PEG® tablet shows differences between different diet groups. These results suggested that the absorption of griseofulvin in rats after administration of GRIS-PEG® tablet would be improved by the help of food type as well as bile salt. The absorption of griseofulvin in SEDDS was also affected by food intake.

In the fed state, the absorption of hydrophobic drug can be enhanced compared with that in the fasting state. Fatty meals provide the greatest effects on GI physiology, and the systemic drug availability is maximally affected. First, fatty meals can increase gastric residence time thereby increasing the time available for solubilization of insoluble drug. Second, fatty meals may enhance the solubilization of drugs by lipids contained in the meal or by increasing the amount of bile salts released in the intestine. This would agree with the finding of Hong *et al*.[35]

Conclusion

Griseofulvin is a representative poorly water-soluble drug that is used as antifungal. The marketed formulation GRIS-PEG® 125 mg tablet which contains the ultramicrosize griseofulvin showed little differences between post and pre-prandial state in human. Self-emulsifying formulation developed in this study, shows similar absorption to GRIS-PEG® after oral administration in respect of dietary conditions. Since SEDDS rapidly forms fine particles sized *ca.* 250—450 nm, this shows good self emulsification, and in other words we can say that this SEDDS has assisted the subsequent true dissolution process, *i.e.* partition out of the dispersion into free solution, and hence the absorption was improved through this system. There was no need of bile salts for SEDDS that formed microemulsion in the stomach, because the formulation was sufficiently solubilized by itself. From these results, SEDDS might be a useful system to improve the bioavailability of griseofulvin without micronisation that shows similar absorption and prone to similar food intake effects like the ultramicrosized GRIS-PEG® 125 mg tablets. This technique may be applied to similar drugs such as colchicine which is used for the management of gout, and also to drugs such as cytarabine and dacarbazine which are used for cancer.

References

1) Charman S. A., Charman W. N., Rogge M. C., Wilson T. D., Dutko F. J., Pouton C. W., *Pharm. Res.*, **9**, 87—93 (1992).
2) Pouton C. W., *Int. J. Pharm.*, **27**, 335—348 (1985).
3) Shah N. H., Carvajal M. T., Patel C. I., Infeld N. H., Malick A. W., *Int. J. Pharm.*, **106**, 15—23 (1994).
4) Pouton C. W., Charman W. N., *Adv. Drug Deliv. Rev.*, **25**, 1—2 (1997).
5) Pouton C. W., *Adv. Drug Deliv. Rev.*, **25**, 47—58 (1997).
6) Kim C. K., Cho Y. J., Gao Z. G., *J. Controlled Release*, **70**, 149—155 (2001).
7) Constantinides P. P., *Pharm. Res.*, **12**, 1561—1572 (1995).
8) Craig D. Q. M., Barker S. A., Banning D., Booth S. W., *Int. J. Pharm.*, **114**, 103—110 (1995).
9) Porter C. J., Charman W. N., *Adv. Drug Deliv. Rev.*, **50** (Suppl. 1), S127—S147 (2001).
10) Itoh K., Tozuka Y., Oguchi T., Yamamoto K., *Int. J. Pharm.*, **238**, 153—160 (2002).
11) Kim H. J., Yoon K. A., Hahn M., Park E. S., Chi S. C., *Drug Dev. Ind. Pharm.*, **26**, 523—529 (2000).
12) Kommuru T. R., Gurley B., Khan M. A., Reddy I. K., *Int. J. Pharm.*, **212**, 233—246 (2001).
13) Julianto T., Yuen K. H., Noor A. M., *Int. J. Pharm.*, **200**, 53—57 (2000).
14) Khoo S. H., Humberstone A. J., Porter C. J. H., Edwards G. A., Charman W. N., *Int. J. Pharm.*, **167**, 155—164 (1998).
15) Gao Z. G., Choi H. G., Shin H. J., *Int. J. Pharm.*, **161**, 75—86 (1998).
16) Lin C., Magat J., Chang R., McGlotten J., Symchowicz S.. *J. Pharma-

col. Exp. Ther., **187**, 415—422 (1973).
17) Drug Bank Griseofulvin (APRD01004) http://redpoll.pharmacy. ualberta.ca/ drugbank/cgi-bin/getCard.cgi? CARD=APRD01004.txt (accessed 2 January 2007).
18) Gull K., Trinci A. P. J., *Nature* (London), **244**, 292—294 (1973).
19) Korting H. C., Schäfer-Korting M., Zienicke H., Georgii A., Ollert M. W., *Antimicrob. Agents Chemother.*, **37**, 2064—2068 (1993).
20) Dongowski G., Fritzsch B., Giessler J., Härtl A., Kuhlmann O., Neubert R. H. H., *Eur. J. Pharm. Biopharm.*, **60**, 147—151 (2005).
21) Amidon G. L., Lennernas H., Shah V. P., Crison J. R., *Pharm. Res.*, **12**, 413—420 (1995).
22) Six K., Verreck G., Peeters J., Brewster M., Van Den Mooter G., *J. Pharm. Sci.*, **93**, 124—131 (2004).
23) Grifulvin V (Ortho), "PDR Physicians desk reference," 43rd ed., Medical Economics Company, Oradell, NJ, 1989, p. 1515.
24) Fulvicin-U/F (Schering), "PDR Physicians desk reference," 43rd ed., Medical Economics Company, Oradell, NJ, 1989, p. 1923.
25) Lin C., Lim J., DiGiore C., Gural R., Symchowicz S., *J. Int. Med. Res.*, **10**, 274—277 (1982).
26) Straughn A. B., Meyer M. C., Raghow G., Rotenberg K., *J. Pharmacokinet. Biopharm.*, **8**, 347—362 (1980).
27) Maeda T., Takenaka H., Yamahira Y., Noguchi T., *J. Pharm. Sci.*, **68**, 1286—1289 (1979).
28) Chiba Y., Kohri N., Iseki K., Miyazaki K., *Chem. Pharm. Bull.*, **39**, 2158—2160 (1991).
29) Kislalioglu M. S., Khan M. A., Blount C., Goettsch R. W., Bolton S., *J. Pharm. Sci.*, **80**, 799—804 (1991).
30) Ho H. O., Su H. L., Tsai T., Sheu M. T., *Int. J. Pharm.*, **139**, 223—229 (1996).
31) Moyano J. R., Arias M. J., Gines J. M., Rabasco A. M., *Drug Dev. Ind. Pharm.*, **23**, 379—385 (1997).
32) Okonogi S., Oguchi T., Yonemochi E., Puttipipatkhachorn S., Yamamoto K., *Int. J. Pharm.*, **156**, 175—180 (1997).
33) Yoo S. D., Lee S. H., Kang E., Jun H., Jung J. Y., Park J. W., Lee K. H., *Drug Dev. Ind. Pharm.*, **26**, 27—34 (2000).
34) Gursoy R. N., Benita S., *Biomed. Pharmacother.*, **58**, 173—182 (2004).
35) Hong J. Y., Kim J. K., Song Y. K., Park J. S., Kim C. K., *J. Controlled Release*, **110**, 332—338 (2006).
36) Garcia-Celma M. J., *Indust. Appl. Microemulsions*, **66**, 123—145 (1997).
37) Trotta M., Pattarino F., Grosa G., *Int. J. Pharm.*, **174**, 253—259 (1998).
38) Mehdizadeh M., Grant D. J., *J. Pharm. Sci.*, **73**, 1195—1203 (1984).
39) Corvis Y., Barzyk W., Brezesinski G., Mrabet N., Badis M., Hecht S., Rogalska E., *Langmuir*, **22**, 7701—7711 (2006).
40) Tarr B. D., Yalkowsky S. H., *Pharm. Res.*, **6**, 40—43 (1989).
41) Humberstone A. J., Charman W. N., *Adv. Drug Deliv. Rev.*, **25**, 103—127 (1997).
42) O'Driscoll C. M., *Eur. J. Pharm. Sci.*, **15**, 405—415 (2002).