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Research paper

Bioavailability of probucol from lipid and surfactant based formulations in minipigs: Influence of droplet size and dietary state

Flemming Seier Nielsen, Kamilla Buchberg Petersen [1], Anette Müllertz *

Department of Pharmaceutics, The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark

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Abstract

The influence of droplet size on the absorption from lipid and surfactant based formulations was evaluated from two self-emulsifying formulations, a surfactant solution, and an oil solution. The self-emulsifying formulations was a self-emulsifying (SEDDS) and a self-nanoemulsifying (SNEDDS) formulation containing equal lipid and surfactant load, but exhibiting a large difference (approx 100 times) in the mean particle diameter of the resultant emulsion. The formulations were evaluated in a bioavailability study in fasted and fed Göttingen minipigs using probucol as model drug. In order to determine the bioavailability, an oil-in-water emulsion was included as i.v. formulation. The fasted group was fasted overnight and offered the first daily meal approx 4 h after treatment. The fed group was offered the first daily meal (50% energy from fat) 30 min prior to treatment. In the fasted group the SNEDDS exhibited a slightly faster absorption and higher bioavailability than the SEDDS, though non-significant. Furthermore, the bioavailability from the surfactant solution and the oil solution were slightly lower compared to the SNEDDS, indicating that both small particle size and digestibility are important in ensuring optimal bioavailability. Comparing the absorption in fasted and fed minipigs showed that probucol exhibited no significant food effect, when formulated in a lipid and surfactant based formulations.

Keywords: Self-emulsifying drug delivery system (SEDDS); Bioavailability; Oral absorption; Food interactions; Surfactants; Emulsion/nanoemulsion

1. Introduction

The use of lipid and surfactant based formulations is one of several approaches found to be efficient in improving the bioavailability of lipophilic drug compounds. Numerous factors are influencing absorption from lipid and surfactant based formulations: Rate of dispersion, degree of emulsification, particle size and precipitation of drug from the formulation upon dispersion [1,2]. Effect of particle size has been investigated by several authors; however the majority of the studies employed formulations which differed both in the content and the type of surfactant and lipid phase [3–6]. This limits a thorough conclusion of the impact of the particle size on the drug absorption.

The underlying mechanisms governing absorption from lipid and surfactant based formulations are not yet fully understood; however, release of compound is believed to take place by partitioning from the intact vehicle, also referred to as interfacial transfer, and by degradation of the vehicle driving the compound out. There is still controversy as to whether lipid and surfactant based formulations must be degraded to ensure optimal absorption of the loaded compound [7,8]. Recent studies by de Smidt et al. suggest that while digestion is important for crude oil formulations of medium chain triglyceride, it is only of minor influence for sub-micron emulsions of medium chain triglycerides [9]. A number of typically employed excipients are susceptible to enzymatic degradation in the GI tract.

* Corresponding author. Department of Pharmaceutics and Analytical Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen O, Denmark. Tel.: +45 35306440; fax: +45 35306030.
E-mail address: amu@farma.ku.dk (A. Müllertz).
[1] Present address: H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark.

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Excipients susceptible to degradation include natural di- and triglycerides as well as some commonly used surfactants [10,11]. Level of solubilization in the digested formulation has been shown to correlate with bioavailability [12].

Some surfactants typically used in pharmaceutical formulations, including Cremophor EL, have been shown to be substrates or non-competitive inhibitors of the P-glycoprotein (PGP) efflux transporters [13]. It has therefore been suggested that some of the bioavailability enhancing properties of lipid and surfactant based formulations of drugs which are PGP substrates, could be attributed to this mechanism [14].

The influence of food on the bioavailability of lipophilic compounds from a solid formulation has been well documented [15]. The impact of concurrent food administration on the bioavailability from lipid and surfactant based formulations has been the subject of far fewer investigations.

In the present study, four lipid and surfactant based formulations and a powder formulation of the model drug, probucol, were tested orally together with an i.v. formulation. Probucol is a highly lipophilic, non-ionisable compound with a calculated *C*log *P* of 11 (ChemDraw Ultra, version 8.0.3), a low water solubility of 2–5 ng/mL [16]. The lipid and surfactant based formulations all contained probucol in solution, which did not precipitate upon dispersion when tested *in vitro*. Two of the lipid and surfactant based formulations tested were self-emulsifying formulations: a self-emulsifying drug delivery system (SEDDS) and a self-nanoemulsifying drug delivery system (SNEDDS), previously characterized [17]. The SEDDS and SNEDDS contained equal lipid and surfactant loads but exhibited a large difference (approx 100 times) in the mean particle diameter of the resultant emulsions. Two other lipid and surfactant based formulations were tested: a surfactant solution, which dispersed into a micellar solution, and an oil solution for which the dispersion relies solely on the dispersion capacity and digestion in the small intestine. A powder formulation composed of micronized probucol dispersed with lactose was included as an example of a conventional solid formulation.

The purpose of the present study was to (a) investigate the influence of droplet size of a self-emulsifying formulation on drug absorption, (b) compare the absorption from self-emulsifying formulations with a surfactant solution, a lipid solution and a powder formulation and (c) investigate the impact of a concurrent high fat meal on the absorption, (d) investigate if probucol if a PGP substrate.

2. Materials and methods

2.1. Materials

Cremophor RH40® was from BASF – BASIS kemi A/S, Denmark and Maisine 35-1® was from Gattefossé s.a., France. Sesame oil was from Apoteket AB, Sweden and absolute ethanol was from Kemetyl AB, Sweden. Lecithin (Lipoid S-75) was kindly provided by Lipoid, Germany. Lactose monohydrate (Ph.Eur.) and glycerol (Ph.Eur.) were from Unikem, Denmark. Hanks balanced salt solution (HBSS) was from Invitrogen, Denmark. Packard Ultima Gold Scintilation fluid and [^{14}C]mannitol with a specific activity of 51.50 mCi/mmol were from Sigma–Aldrich, Denmark. [3H]Etoposide with a specific activity of 654 mCi/mmol was obtained from Moravek Biochemicals Inc. Water was freshly prepared using a Milli-Q water purification system from Millipore, Denmark.

All other chemicals used were of analytical grade and were used as received.

2.2. Lipid and surfactant based formulations

Formulation vehicles were prepared by weighing appropriate amounts (Table 1) of ethanol and pre-heated (50 °C) Cremophor RH40, Maisine 35-1 and Sesame oil into a glass vial and mixed. Final formulations were prepared one day before dosing through addition of probucol. Prior to administration, the final formulation was weighed into size 00 gelatine capsules (Capsugel, Belgium) according to the weight of the animal.

Equilibrium solubility of probucol in the lipid and surfactant based formulations was determined using HPLC as previously described [17]. Precipitation was evaluated after dispersing the probucol load pre-concentrate loaded in 0.7% saline. Precipitation of drug from the emulsions was evaluated by visual inspection after 24 h.

Dispersion time of the lipid and surfactant based formulations from gelatine capsules was evaluated using a USP dissolution apparatus 2, containing USP simulated gastric fluid without pepsin at 100 rpm as previously described [17].

The mean particle size (calculated on % mass using regularization histogram) of the resultant emulsions (1%) in saline (0.7%) for the SNEDDS and Cremophor formulation were determined using a DynaPro running Dynamics software version 5.26.38 (ProteinSolutions, UK). The mean particle size (mean volume diameter, $D[4,3]$) for the SEDDS dispersed in saline (0.7%) was determined using a Malvern Mastersizer S equipped with a 300RF lens and a Small Volume Sample Preparation Unit in conjunction with Mastersizer software version 2.19 (Malvern Instruments, UK).

2.3. Powder formulation

A powder formulation was prepared by sieving micronized probucol and lactose monohydrate through a size 300 sieve followed by thorough mixing of equal amounts of each. Mean particle size (mean volume diameter, $D[4,3]$) of the probucol powder after sieving was determined to be 18.5 ± 0.6 μm using a Malvern Mastersizer S equipped with a 300F lens and an MSX Dry Powder Feeder in conjunction with Mastersizer software version 2.19 (Malvern Instruments, UK).

Table 1
Description and characterization of lipid and surfactant based oral formulations

	Cremophor solution	SNEDDS	SEDDS	Oil solution
Dose (mg/kg)				
Cr RH40	109.3	36.4	36.4	0
M:O	0	72.8	72.8	109.3
EtOH	12.1	12.1	27.3	12.1
Probucol	10.0	10.0	10.0	10.0
% loading	73.2%	69.9%	ND	80.0%
Droplet size	14.5 ± 0.3 nm	45.0 ± 3.4 nm	4.58 ± 0.84 μm	NA
Dispersion time	<10 min	<3.5 min	<1.5 min	NA

Cr RH40, Cremophor RH40; M:O, Maisine 35-1:Sesame oil 1:1, w/w; EtOH, Absolute ethanol. Particle size of the resultant emulsions (1%) was determined in saline. Dispersion time from gelatine capsules was evaluated using a USP dissolution apparatus 2, containing USP simulated gastric fluid without pepsin at 100 rpm.

2.4. O/W emulsion for intravenous administration

The formulation for intravenous administration contained 0.5% w/w probucol solubilized in a lecithin stabilized o/w emulsion containing 20% w/w sesame oil, 2% w/w lecithin (Lipoid S-75), 2.5% w/w glycerol and 75% w/w water was freshly prepared 1 day before each dosing day, as previously described by Karpf and co-workers [18].

2.5. Bioavailability study

All *in vivo* experimental procedures were performed at Scantox A/S (Lille Skensved, Denmark). All procedures were approved and performed in accordance with Danish National Legislation. Twelve male Göttingen minipigs (Ellegaard Göttingen minipig A/S, Denmark) were divided into two groups: a fasted group and a fed group. The study was conducted as two parallel six-way cross-over studies with 1 week washout between dosing. The minipigs in the fasted and fed groups weighed 14.1 ± 0.8 kg and 13.8 ± 1.3 kg on the first day of dosing and 17.2 ± 0.9 kg and 16.7 ± 1.3 kg on the last day of dosing, respectively. A minipig diet (SMP MOD, Special Diets Services, UK) containing approx 200 g per animal per meal was offered twice daily. The diet consisted of 2.13% crude oil, 13.03% crude protein and 14.52% crude fibre and had a total digestible energy content of 11.43 kJ/g.

The fasted group was fasted overnight and offered the first daily meal approx 4 h after dosing. On the day of dosing, the fed group was offered its first daily meal 30 min prior to treatment. This meal consisted of a standard minipig diet enriched with 20 g of olive oil per 80 g diet and lead to a high fat diet with approx 50% of the caloric content coming from fat, as recommended for clinical food effect studies [19]. All other meals prepared for the fed group were similar to the ones prepared for the fasted group.

The oral formulations (10 mg/kg) were administered in gelatine capsules with the aid of a biting block and approx 10 mL of tap water was administered subsequently. The i.v. administration (2.5 mg/kg) was given as a constant infusion over 10–15 min using an infusion pump. During the infusion, the minipigs were sedated using a mixture of Zoletil 50 Vet. (Virbac, France), Rompun Vet. (Bayer, Germany), Ketaminol Vet. (Veterinaria AG, Switzerland) and Methadon DAK (Nycomed DAK, Denmark).

After oral administration blood samples were taken predose, at 45 min and at 1.5, 2.25, 3, 4, 5, 6, 8, 12, 24 and 48 h post-dose. The sampling schedule following the i.v. administration was pre-dose, at 15 and 30 min as well as 1, 2, 3, 4, 6, 8, 12, 24 and 48 h post-dose. Blood samples were collected from the jugular vein or the bijugular trunk into tubes containing heparin. Plasma was separated by centrifugation and stored at −20 °C until analysis.

2.6. Analytical methods

Plasma samples were prepared and analyzed by HPLC using a modified method previously described by Nourooz-Zadeh et al. [20]. Probucol was analyzed by injecting a 50 μL sample on a 150 × 4.6 mm Phenomenex Luna® C18(2) (5 μm) column fitted with a 4 × 3.0 mm Phenomenex Securityguard™ C18 guard column and detected at 242 nm. The HPLC system consisted of a Hewlet Packard HPLC 1100 series with a diode array detector and also equipped with a column oven. The mobile phase consisted of an acetonitrile:water mixture (96:4, v/v). Probucol was eluted isocratically with a typical elution time of approx 4 min using a flow rate of 1.5 mL/min at a column temperature of 60 °C.

Samples were prepared by initially transferring 100 μL aliquots of plasma to 15 mL glass tubes followed by adding 800 μL ethanol and then vortexing for 30 s. Subsequent to this, 1.70 mL of iso-octane and 1000 μL of water were added to each tube with 30 s of sample vortexing following each addition. Samples were centrifuged at 4500 rpm for 5 min after which a 1.50 mL aliquot of the upper layer (iso-octane) was transferred to a glass centrifuge tube using a glass pipette. The samples were evaporated to dryness under a stream of nitrogen at 35 °C and residues were reconstituted with 100 μL of acetonitrile and analysed on HPLC.

Calibration curves for probucol in plasma were made each day by spiking blank minipig plasma with two independent sets of reference solutions containing probucol diluted in absolute ethanol but otherwise prepared as the plasma samples. This provided a calibration curve covering the range from 0.1 to 10 μg probucol per mL plasma. Concentrations were calculated using Microsoft® Excel (Microsoft Corporation). Correlation coefficients (r^2) for the calibration curves were calculated to be >0.993 and recovery studies showed an extraction efficiency of 85.8–92.3%. All reported plasma concentrations were mean values from two independent analyses with a difference of less than 10%.

2.7. Pharmacokinetic and statistical data analysis

Non-compartmental pharmacokinetic parameters were derived using WinNonlin™ version 3.3. (Pharsight Corporation, USA). Within each dosing group, T_{max} values were compared using Kruskal–Wallis one-way analysis of variance on ranks. In case of significance ($p < 0.05$), differences between formulations were allocated by multiple comparison using the Wilcoxon signed rank test. Comparisons of T_{max} values from the fasted and fed state study were tested using the Mann–Whitney rank sum test. Also, values for C_{max}, AUC and $t_{1/2}$ within each dosing group were compared using one-way analysis of variance. In cases of significance ($p < 0.05$), differences between formulations were allocated by multiple comparison using the paired t-test (Student–Newman–Keuls method). The C_{max} (fasted state study) failed the test for normality and was therefore compared using Kruskal–Wallis one-way analysis of variance on ranks with differences between formulations allocated by multiple comparisons using the Wilcoxon signed rank test. Comparisons of C_{max} from the fasted and fed state studies were tested using the Mann–Whitney rank sum test. Comparisons of $t_{1/2}$ and bioavailability from the fasted and fed state study were tested using the unpaired t-test (Student–Newman–Keuls method). The bioavailability of each treatment was, as the study was conducted as a cross-over study, calculated as an average of the individual bioavailabilities and not using pooled data. All statistical calculations were performed using SigmaStat version 3.1 (Systat Software Inc., USA).

2.8. Caco-2 experimental procedures

Caco-2 cells were obtained from the American Type Culture Collection. The Caco-2 cells were cultured and transport experiments were performed between day 24 and 28 after seeding as previously described by Nielsen et al. [21]. Apical (A) to basolateral (B) and B to A fluxes of etoposide was measured in HEPES buffer and in the presence of 50 μM probucol on both donor and receiver side. Probucol was dissolved in DMSO and diluted with HEPES buffer to a final concentration of 0.1% v/v DMSO. The concentration of etoposide on the donor side was 100 μM spiked with labelled etoposide to a final concentration of 120 μM. Samples of 20 μL were taken from the donor solution at $t = 0$ and 210 min and 150 μL samples were taken from the receiver solution and replaced with fresh buffer ($t = 30$, 60, 90, 120, 150 min). Samples were transferred to scintillation vials, where 2 mL of scintillation fluid was added to each vial and the radioactivity was measured in a liquid scintillation analyzer.

Steady state flux was obtained after 60 min and calculated as the mean of fluxes ($n = 3$) by linear regression in the time interval 60–150 min. The integrity of the Caco-2 cell monolayers was evaluated by concurrent [^{14}C]mannitol transport studies. The apparent permeability of mannitol was unaffected by the presence of probucol when values for permeability was 0.0012 ± 0.0001 cm/h in the absence and 0.0013 ± 0.0004 cm/h in the presence of probucol.

3. Results and discussion

Lipid and surfactant based systems have been extensively evaluated in attempts to improve bioavailability of poorly water soluble compounds. In spite of this, only a limited number of studies have focused on the influence of the particle/droplet size of comparable formulations on the absorption and bioavailability.

In order to elucidate this, two self-emulsifying formulations were selected from a system composed of Cremophor RH40, Maisine 35-1:sesame oil (1:1, w/w) and ethanol. As proper controls a Cremophor RH40 micellar solution and a Maisine:sesame oil (1:1, w/w) solution were used. Performance of the lipid and surfactant based formulations were compared to the performance of a conventional solid formulation exemplified by a powder formulation composed of micronized probucol dispersed with lactose. In order to determine the bioavailability from the formulations, an oil-in-water emulsion was included as i.v. formulation. An o/w emulsion of probucol was chosen, due to the low solubility of probucol in water and low incorporation into cyclodextrin solution.

Probucol was selected as model compound to represent Class II compounds according to the Biopharmaceutical Classification System and because of its high solubility in lipid and surfactant based formulations ensuring a high dosing potential.

Probucol has been reported to undergo lymphatic transport [22]. Lymphatic transport is a post-absorption event and the fraction transported through the mesenteric lymphatic are not subjected to hepatic first pass metabolism. However, the contribution to the total exposure due to lymphatic transport is not expected to be profound, as probucol is a low clearance compound (0.6 ml/min/kg, calculated from i.v. data, data not shown).

It is well-known that the intake of food concurrent to lipophilic drug compounds can improve absorption greatly [15]. The impact of food on absorption from lipid and surfactant based formulations has yet to be investigated further. Consequently all formulations were tested in both

fasted minipigs and minipigs fed a high fat meal just prior to administration.

3.1. Lipid and surfactant based formulations

The two self-emulsifying formulations, SNEDDS and SEDDS, contained equal amounts of lipids and surfactants, but differed in EtOH content. The SNEDDS formulation was characterized in a recently publication by Nielsen et al. [17]. The SNEDDS was found to disperse into a nanoemulsion with a monomodal particle size distribution with a mean particle diameter of 45.0 ± 3.4 nm in saline [17]. The SNEDDS was evaluated following dispersion in media simulating fasted and fed state, FaSSIF and FeSSIF, respectively, and the particle size of the resultant emulsion was similar to when disperser in saline [17].

The influence of dilution on the particle size was investigated in saline in concentrations of SNEDDS vehicle ranging from 0.1% to 10% v/v and the particle was found to decrease slightly (NS, Student's *t*-test) from 45.9 ± 1.6 to 38.3 ± 0.9 nm.

In contrast, the SEDDS dispersed into a polydisperse emulsion with a mean particle diameter of 4.85 ± 0.84 μm in saline (Table 1). The particle size of the SEDDS was measured using a laser diffraction analysis. The technique and the employed equipment require that the concentration of the formulation is adjusted to a proper concentration range (signal count rate range) in order to give reliable results. As a result of this it was not possible to investigate particle size in a large concentration range. However, the SEEDS vehicle show quite robust dispersion characteristics in different concentrations ranges (0.1–10%, v/v) as well as in FaSSIF and FeSSIF based on dispersion time and visual appearance.

The EtOH content of the SNEDDS and SEDDS is expected to redistribute into the water phase upon dispersion and is not expected to impact drug absorption in the given amounts [23].

The *in vivo* performance of the two self-emulsifying formulations were compared with a surfactant solution, dispersing into a micellar system upon dilution in water (mean particle diameter of 14.5 ± 0.3 nm in saline), and an oil solution whose dispersion relies only on the dispersion capacity and digestion in the small intestine (Table 1).

The loading of probucol was selected in order to obtain formulations, which were non-saturated with probucol, and would ensure solubilization capacity after dispersion. The solubility of probucol in the pre-concentrates was found to be largely independent of vehicle composition and ranged from 95.1 mg/g in the oil formulation to 108.9 mg/g in the SNEDDS. The probucol dose in the formulations was based on a load equivalent to 80% of the solubility in the formulation with the lowest solubility. The actual percent loading of probucol with respect to the equilibrium solubility in the formulations is shown in Table 1.

The resultant emulsions formed after dispersion of the pre-concentrates (SEDDS) potentially possess a drug solubilization capacity that differs from the pre-concentrate. Precipitation and particle size was evaluated after dispersion (1%) and probucol did not precipitate after dispersion and the particle size distribution did not change within the tested time frame (24 h) for all lipid and surfactant based formulations.

3.2. Fasted state study

The mean plasma concentration–time profiles after oral administration to fasted minipigs are depicted in Fig. 1. Administration of the powder formulation led to very low plasma concentrations until after the minipigs were fed, approx 4 h post-dosing, when plasma concentrations increased slightly. The 4 h lag-time in absorption suggests that absorption from the powder formulation was related to feeding. The data also suggest that the powder formulation was incompletely emptied from the stomach after administration in the fasted state, and the remaining part was emptied and to some extent dissolved and absorbed after feeding. It has previously been shown that stomach emptying in pigs is bimodal and incomplete for up to 24 h [24,25]. The median T_{max} for the powder formulation was 24 h ranging from 5 to 48 h. This large variation in T_{max} values may in part be related to the low absorption (2.5% BA) following administration of the powder formulation and lack of a distinct C_{max}. However, the total transit time of pellets in domestic pigs has previously been reported to be between 24 and 48 h which implies the possibility of a T_{max} within this time range [26].

In contrast this phenomenon was not observed for the lipid and surfactant based formulations where the absorption occurred with a short lag time (Fig. 1). Fig. 1 indicates that the two self-emulsifying formulations (SEDDS and SNEDDS) exhibit slightly faster absorption compared to the Cremophor solution and the oil formulation.

All lipid and surfactant based formulations investigated in this study improved the AUC_{0-48h} of probucol significantly compared with the powder formulation in fasted minipigs (Table 2). The lipid and surfactant based formulations also exhibited significantly higher C_{max} than the powder formulation as well as faster T_{max}, though not significantly. No significant differences in AUC_{0-48h} were found in-between individual lipid and surfactant based formulations. In the fasted minipigs, the SNEDDS formulation exhibited the highest bioavailability of 8.9%. The Cremophor solution exhibited slightly higher bioavailabilities compared to the SEDDS formulation and the oil solution with bioavailabilities of 7.7%, 7.1% and 6.9%, respectively.

A trend was noted whereby the SEDDS was found to exhibit a slightly lower C_{max} and a slightly lower bioavailability (7.1% BA) compared with the SNEDDS (8.9% BA).

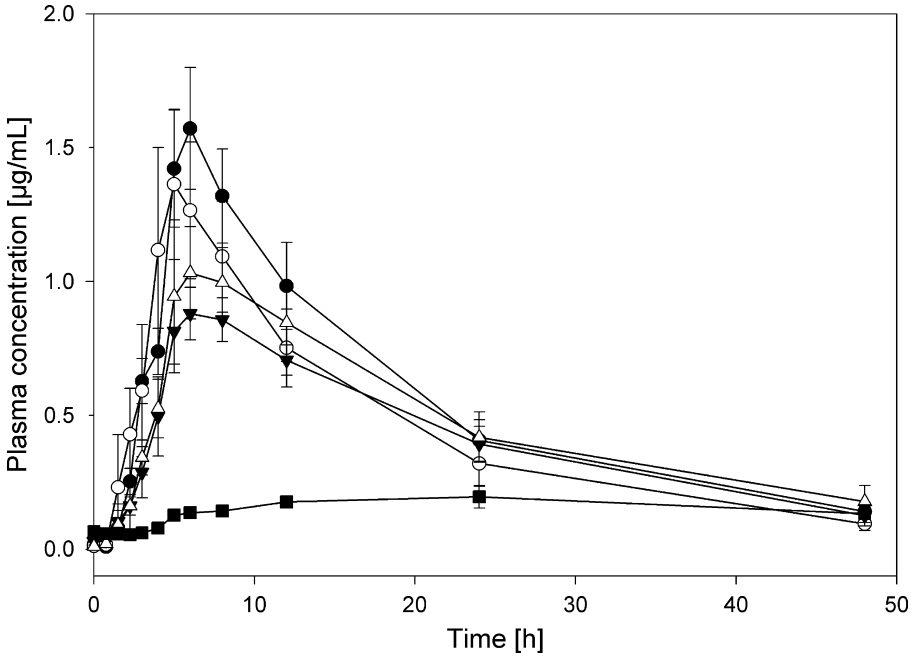

Fig. 1. Fasted state study. Mean plasma concentration versus time profiles (mean ± SE, $n = 6$) for probucol following oral administration (10 mg/kg) to minipigs fasted overnight and fed 4 h after administration. Probucol was administered in gelatine capsules containing either SNEDDS (●), SEDDS (○), Oil solution (▼), Cremophor solution (Δ) or Powder formulation (■).

Table 2
Pharmacokinetic parameters for probucol obtained from non-compartmental analysis

	C_{max} (µg mL^{-1})		T_{max} (h)		$T_{½}$ (h)		AUC_{0-48h} (h µg mL^{-1})		BA (%)	
Fasted state study										
SNEDDS	1.84	±0.53[a,b,1]	5.0	[5.0–6.0][3]	13.4	±2.3	26.2	±8.8[e]	8.9	±2.8
SEDDS	1.58	±0.87[a]	5.5	[3.0–8.0]	11.4	±2.0	21.6	±10.2[e]	7.1	±2.9
Oil solution	0.98	±0.23[a,b]	7.0	[5.0–8.0][4]	15.3	±7.8	19.9	±4.1[e]	6.9	±2.0
Cremophor solution	1.31	±0.60[a]	6.0	[5.0–12]	16.1	±5.6	22.9	±9.4[e]	7.7	±2.9
Powder formulation	0.24	±0.05[2]	24.0	[5.0–48]			7.5	±2.9	2.5	±0.9[5]
IV infusion					19.3	±3.8	74.4	±12.2		
Fed state study										
SNEDDS	1.11	±0.35[c,1]	8.0	[8.0–24][3]	21.2	±8.8	34.4	±13.5	9.0	±2.6
SEDDS	1.31	±0.41[d]	8.0	[4.0–24]	15.4	±5.7	35.5	±15.8	9.1	±2.6
Oil solution	0.72	±0.09[d]	12.0	[12.0–24][4]	16.3	±9.5	21.5	±4.4	5.7	±0.4
Cremophor solution	0.82	±0.39	8.0	[5.0–12]	21.6	±13.2	19.9	±5.7	5.3	±1.7
Powder formulation	0.69	±0.28[c,2]	24.0	[5.0–48]			21.6	±7.8	5.8	±2.4[5]
IV infusion					19.8	±4.3	95.5	±23.2		

Non-compartmental analysis of plasma profiles obtained from fasted state study ($n = 6$) and fed state study ($n = 5$). Mean ± SD or median and range in brackets.

Probucol was administered i.v. as an infusion (2.5 mg/kg) or orally in gelatine capsule containing either SNEDDS, SEDDS, Oil solution, Cremophor solution or Powder formulation (10 mg/kg).

[a] Statistically significant different C_{max} when compared with the powder formulation in the fasted state study ($p < 0.013$).
[b] Statistically significant difference between C_{max} from SNEDDS and oil formulation in the fasted state study ($p = 0.031$).
[c] Statistically significant difference between C_{max} from SNEDDS and powder formulation in the fed state study ($p = 0.012$).
[d] Statistically significant difference between C_{max} from SEDDS and oil formulation in the fed state study ($p = 0.021$).
[e] Statistically significant AUC when compared with the powder formulation in the fasted state study ($p < 0.011$).
[1] Statistically significant C_{max} from SNEDDS when tested in the fasted and in the fed state study ($p = 0.030$).
[2] Statistically significant C_{max} from powder formulation when tested in the fasted and in the fed state study ($p = 0.017$).
[3] Statistically significant C_{max} from SNEDDS when tested in the fasted and in the fed state study ($p = 0.004$).
[4] Statistically significant C_{max} from oil formulation when tested in the fasted and in the fed state study ($p = 0.004$).
[5] Statistically significant bioavailability from powder formulation when tested in the fasted and in the fed state study ($p = 0.009$).

The administered SNEDDS and SEDDS formulations only differ in the amount of ethanol and the particle size of resultant emulsions (Table 1).

The slightly higher bioavailability from SNEDDS compared with SEDDS indicates that smaller particle size and, hence, a larger surface area only promoted the absorption

of probucol to a minor extent. A number of studies have reported higher bioavailability with decreased particle size when comparing self-emulsifying formulations [3–6]. However, the formulations used in these studies contained either different surfactant and lipid phases or different ratios between the surfactant and the lipid phase. Since it is also well documented that formulations with matching particle size, but different lipid phases may exhibit different bioavailability [12,27,28], no clear cut conclusions can be drawn at this time with regard to the effect of droplet size on oral absorption [12,27,28].

The Cremophor solution dispersed into a micellar system with a very small particle size. Contrary to expectations that smaller particle size increases absorption, this did not lead to any improvement in C_{max} and bioavailability. The Cremophor solution was found to exhibit slightly lower C_{max} compared to SEDDS and SNEDDS whereas the bioavailability (7.7%) was comparable to SEEDS (7.1%) and slightly lower than from the SNEDDS (8.9%). Bioavailability of paclitaxel reportedly decreased when administered orally together with Cremophor EL and this was explained by the entrapment of paclitaxel within Cremophor micelles [29,30]. The slightly lower bioavailability from the Cremophor solution compared with the SNEDDS may be related to the fact that Cremophor RH40 is only degraded to a minor extent in the GI tract. As a result, the Cremophor may entrap probucol and prevent it from being released and subsequently absorbed. Limited Cremophor degradation was found when tested *in vitro* by means of a lipolysis model (data not shown).

The oil formulation exhibited the lowest bioavailability (6.9%) of the lipid and surfactant based formulations tested, and had a C_{max} value significantly lower than that of the SNEDDS. The oil formulation was dependent on emulsification and subsequent digestion in the gastrointestinal tract in order to release the compound, which seems to be a limiting factor in the absorption of probucol and explains the lower bioavailability found.

3.3. Fed state study

One minipig in the fed state group exhibited markedly higher AUC_{0-48h} (2- to 6-fold) from three of the five oral administrations as well as an i.v. plasma profile with multiple peaks and a higher AUC_{0-48h} compared with the other minipigs in the group. This minipig was considered an outlier and data from this animal was excluded from the entire data set.

Plasma profiles following oral administration to fed minipigs are depicted in Fig. 2. Fig. 2 indicates that the two self-emulsifying formulations (SEDDS and SNEDDS) exhibit slightly faster absorption compared to the Cremophor solution and noticeable faster than the oil and powder formulations.

No significant differences were seen between the determined AUC_{0-48h} in the minipigs fed a high fat meal and there were no significant differences between the determined T_{max} values. The C_{max} after administration of SNEDDS was found to be significantly higher than that of the powder formulation and the C_{max} after administration of SEDDS was significantly higher than that of the oil solution.

The SEDDS and SNEDDS formulations exhibited the highest bioavailabilities: 9.1% and 9.0%, respectively, and exhibited the highest C_{max} values. Absorption from two marketed self-emulsifying formulations of Cyclosporine A, Neoral® and Sandimmune® in healthy volunteers fed a high fat breakfast showed that Neoral® exhibited a higher bioavailability than Sandimmune® [31]. Neoral® and Sandimmune® self-emulsify into a micro-emulsion and a crude emulsion. However, their different lipid and surfactant types, makes it impossible to compare the findings directly to those of the present study [32].

The Cremophor solution exhibited faster absorption, higher C_{max} and faster Tmax but a slightly lower bioavailability (5.3%) compared to the oil solution (5.7%) and powder formulation (5.8%). This indicates that having a highly dispersed system improves absorption rate but is not enough to ensure optimal bioavailability.

A high bioavailability from the powder formulation was expected as the high fat meal consisted of a considerable amount of olive oil (approx 40 g). The powder formulation exhibited a bioavailability of 5.7% which was similar to the bioavailability obtained from the oil solution (5.7%).

3.4. Comparison of fasted and fed state study

Food was found to increase magnitude and variability of the T_{max} for the lipid and surfactant based formulations whereas C_{max} decreases. The Increased magnitude and variability of the T_{max} could be due to delayed and variable gastric emptying in the fed state. However, the variability of the C_{max} and AUC was comparable in the fasted and the fed state study. As expected, food was found to have no impact on the determined apparent terminal half-life of probucol. Moreover, when formulated in a powder formulation, probucol exhibited a significant food effect (BA up 2.3-fold, C_{max} up 2.9-fold and T_{max} unchanged). The SNEDDS exhibited comparable bioavailability of 8.9% and 9.0% when tested in fed and fasted minipigs, respectively. The oil and Cremophor solutions had decreased bioavailabilities in the fed minipigs group compared with the fasted group whereas the SEDDS exhibited an increase. However, overall, there were no significant differences in the bioavailabilities obtained from lipid and surfactant based formulations following comparison of data from the fasted and fed state study.

The SNEDDS formulation and the oil solution were comparable with regard to bioavailability and AUC in the fasted and the fed state study. The SNEDDS formulation did, however, exhibit a significantly lower C_{max} in the fed state study whereas both the SNEDDS formulation and the oil solution exhibited significantly prolonged T_{max} in the fed state study. Previous studies with Cyclosporine A

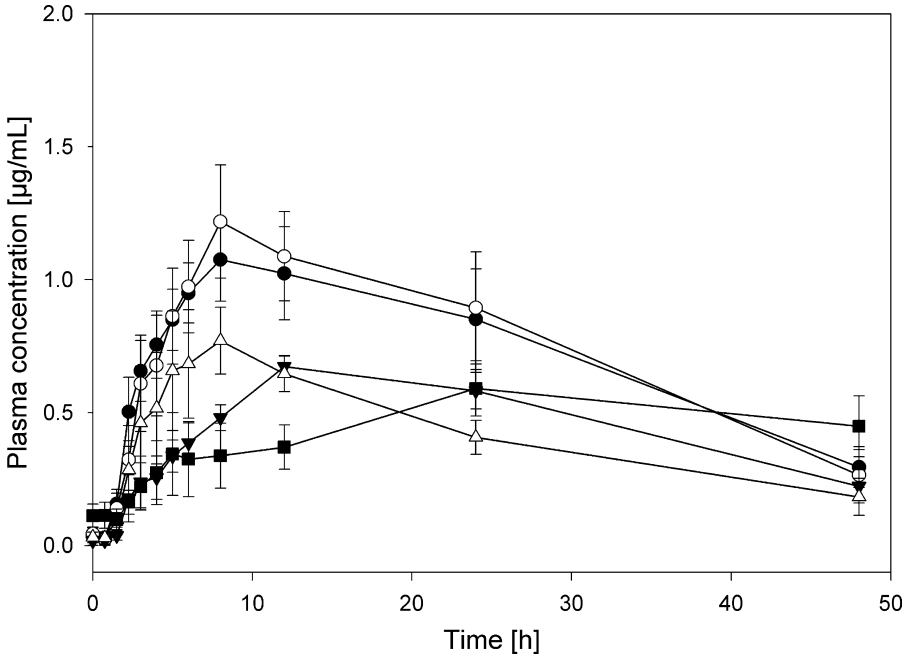

Fig. 2. Fed state study. Mean plasma concentration versus time profiles (mean ± SE, $n = 5$) for probucol following oral administration (10 mg/kg) to minipigs fed a high fat meal 30 min prior to administration. Probucol was administered in gelatine capsules containing either SNEDDS (●), SEDDS (○), Oil solution (▼), Cremophor solution (Δ) or Powder formulation (■).

from marketed formulations showed that neither Neoral® nor Sandimmune® were bioequivalent in healthy volunteers who fasted or were fed a fat rich meal [33]. Kawakami and co-workers have reported that the bioavailability of Nitrendipine exhibited a pronounced food effect when administered as a suspension in non-fasted rats as compared to fasted rats. In addition, the food effect on bioavailability was diminished by an oil solution (150% bioavailability compared to fasted rats) and three surfactant formulations (84–125% bioavailability compared to fasted rats) [34].

3.5. P-glycoprotein efflux transporter

The SEDDS, SNEDDS and Cremophor formulations contain Cremophor RH40 and resulted in slightly higher bioavailability compared to oil solution. Cremophor RH40 is very similar to Cremophor EL, which has been shown to have an affinity for the P-glycoprotein (PGP) [35] and has increased digoxin (PGP substrate) transport over the intestinal mucosa in the rat everted gut sac model [13]. Recently, Cremophor EL has been shown to be a non-competitive inhibitor of the known PGP substrate rhodamine123 in a diffusion chamber system using rat intestinal mucosa [36].

The possibility of probucol being a PGP substrate, and hence the possibility that Cremophor RH40 would enhance bioavailability, was investigated indirectly by measuring the flux of etoposide due to the lack of sensitivity of the analytical method. Etoposide is a known PGP substrate and has been used previously in the Caco-2 cell model to identify PGP substrates [37]. Etoposide flux in the A to B direction was 14.7 ± 0.5 pmol cm^{-2} min^{-1} while flux in the B to A direction was 57.2 ± 0.5 pmol cm^{-2} min^{-1}. With 50 μM probucol, the etoposide flux was found to be 13.7 ± 0.3 pmol cm^{-2} min^{-1} and 57.1 ± 2.3 pmol cm^{-2} min^{-1} in the A to B and B to A direction, respectively. Intracellular etoposide accumulation was found to be unaffected by the presence of probucol (data not shown).

On this basis, it seems that probucol does not act as a substrate for the PGP transporter at concentrations up to 50 μM, concentration magnitudes higher than the intrinsic water solubility. Sugimoto et al. showed that a 50 μM concentration of probucol had an effect on both the A to B and B to A flux of Cyclosporine A. However, it was concluded that the effect was not mediated by PGP [38]. It can therefore be concluded that enhancement of probucol bioavailability for Cremophor containing formulations tested, were not related to any PGP inhibitory effects of Cremophor.

4. Conclusion

In the present study, two self-emulsifying formulations differing only in the amount of ethanol and particle size of the resultant emulsion were compared with an oil solution, a Cremophor solution and a powder formulation. In fasted minipigs, the SNEDDS exhibited a slightly faster absorption and a higher bioavailability of probucol than the SEDDS, though non-significant. The bioavailability from the surfactant solution and the oil solution were slightly lower (non-significant) compared to the SNEDDS, indicating that both dispersion and digestibility are impor-

tant factors when ensuring optimal bioavailability from lipid and surfactant based formulations. Comparing the absorption in fasted and fed minipigs showed that probucol, when formulated in a powder formulation, exhibited a significant food effect. None of the tested lipid and surfactant based formulations, with probucol in solution, exhibited significantly different bioavailability when tested in fasted and fed minipigs. The difference in bioavailability of lipophilic, poorly soluble compounds found in fed compared to fasted state can be circumvented by using a lipid or surfactant based formulation.

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References

[1] C.J. Porter, W.N. Charman, In vitro assessment of oral lipid based formulations, Adv. Drug Deliv. Rev. 50 Suppl. 1 (2001) S127–S147.
[2] C.W. Pouton, Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems, Eur. J. Pharm. Sci. 11 Suppl. 2 (2000) S93–S98.
[3] J. Drewe, R. Meier, J. Vonderscher, D. Kiss, U. Posanski, T. Kissel, K. Gyr, Enhancement of the oral absorption of cyclosporin in man, Br. J. Clin. Pharmacol. 34 (1992) 60–64.
[4] J.M. Kovarik, E.A. Mueller, J.B. van Bree, W. Tetzloff, K. Kutz, Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation, J. Pharm. Sci. 83 (1994) 444–446.
[5] Z.G. Gao, H.G. Choi, H.J. Shin, K.M. Park, S.J. Lim, K.J. Hwang, C.K. Kim, Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A, Int. J. Pharm. 161 (1998) 75–86.
[6] J.M. Odeberg, P. Kaufmann, K.G. Kroon, P. Hoglund, Lipid drug delivery and rational formulation design for lipophilic drugs with low oral bioavailability, applied to cyclosporine, Eur. J. Pharm. Sci. 20 (2003) 375–382.
[7] W.N. Charman, Lipids, lipophilic drugs, and oral drug delivery – some emerging concepts, J. Pharm. Sci. 89 (2000) 967–978.
[8] T. Gershanik, S. Benita, Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur. J. Pharm. Biopharm. 50 (2000) 179–188.
[9] P.C. de Smidt, M.A. Campanero, I.F. Troconiz, Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification versus digestibility, Int. J. Pharm. 270 (2004) 109–118.
[10] M. Khossravi, Y.H. Kao, R.J. Mrsny, T.D. Sweeney, Analysis methods of polysorbate 20: a new method to assess the stability of polysorbate 20 and established methods that may overlook degraded polysorbate 20, Pharm. Res. 19 (2002) 634–639.
[11] F. Seeballuck, E. Lawless, M.B. Ashford, C.M. O'Driscoll, Stimulation of triglyceride-rich lipoprotein secretion by polysorbate 80: in vitro and in vivo correlation using Caco-2 cells and a cannulated rat intestinal lymphatic model, Pharm. Res. 21 (2004) 2320–2326.
[12] C.J. Porter, A.M. Kaukonen, B.J. Boyd, G.A. Edwards, W.N. Charman, Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation, Pharm. Res. 21 (2004) 1405–1412.
[13] G. Cornaire, J. Woodley, P. Hermann, A. Cloarec, C. Arellano, G. Houin, Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo, Int. J. Pharm. 278 (2004) 119–131.
[14] R.N. Gursoy, S. Benita, Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs, Biomed. Pharmacother. 58 (2004) 173–182.
[15] L.E. Schmidt, K. Dalhoff, Food–drug interactions, Drugs 62 (2002) 1481–1502.
[16] N. Yagi, Y. Terashima, H. Kenmotsu, H. Sekikawa, M. Takada, Dissolution behavior of probucol from solid dispersion systems of probucol-polyvinylpyrrolidone, Chem. Pharm. Bull. (Tokyo) 44 (1996) 241–244.
[17] F.S. Nielsen, E. Gibault, H. Ljusberg-Wahren, L. Arleth, J.S. Pedersen, A. Mullertz, Characterization of prototype self-nanoemulsifying formulations of lipophilic compounds, J. Pharm. Sci. 96 (2007) 876–892.
[18] D.M. Karpf, R. Holm, H.G. Kristensen, A. Mullertz, Influence of the type of surfactant and the degree of dispersion on the lymphatic transport of halofantrine in conscious rats, Pharm. Res. 21 (2004) 1413–1418.
[19] Center for Drug Evaluation and Research (CDER), Guidance for Industry, Food–Effect Bioavailability and Fed Bioequivalence Studies, 2002.
[20] J. Nourooz-Zadeh, N.K. Gopaul, L.A. Forster, G.A. Ferns, E.E. Anggard, Measurement of plasma probucol levels by high-performance liquid chromatography, J. Chromatogr. B. Biomed. Appl. 654 (1994) 55–60.
[21] C.U. Nielsen, J. Amstrup, B. Steffansen, S. Frokjaer, B. Brodin, Epidermal growth factor inhibits glycylsarcosine transport and hPepT1 expression in a human intestinal cell line, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (2001) G191–G199.
[22] K.J. Palin, C.G. Wilson, The effect of different oils on the absorption of probucol in the rat, J. Pharm. Pharmacol. 36 (1984) 641–643.
[23] L. de Campo, A. Yaghmur, N. Garti, M.E. Leser, B. Folmer, O. Glatter, Five-component food-grade microemulsions: structural characterization by SANS, J. Colloid Interface Sci. 274 (2004) 251–267.
[24] E. Kokue, M. Shimoda, K. Sakurada, J. Wada, Pharmacokinetics of oral sulfa drugs and gastric emptying in the pig, J. Pharmacobiodyn. 11 (1988) 549–554.
[25] R.F. Witkamp, M. Monshouwer, Pharmacokinetics in vivo and in vitro in swine, Scand. J. Lab. Anim. Sci. 25 (1998) 45–56.
[26] S.S. Davis, L. Illum, M. Hinchcliffe, Gastrointestinal transit of dosage forms in the pig, J. Pharm. Pharmacol. 53 (2001) 33–39.
[27] S.M. Khoo, A.J. Humberstone, C.J.H. Porter, G.A. Edwards, W.N. Charman, Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine, Int. J. Pharm. 167 (1998) 155–164.
[28] R. Holm, C.J. Porter, G.A. Edwards, A. Mullertz, H.G. Kristensen, W.N. Charman, Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides, Eur. J. Pharm. Sci. 20 (2003) 91–97.
[29] M.M. Malingre, J.H. Schellens, O. van Tellingen, M. Ouwehand, H.A. Bardelmeijer, H. Rosing, F.J. Koopman, M.E. Schot, W.W. Bokkel Huinink, J.H. Beijnen, The co-solvent Cremophor EL limits

absorption of orally administered paclitaxel in cancer patients, Br. J. Cancer 85 (2001) 1472–1477.
[30] H.A. Bardelmeijer, M. Ouwehand, M.M. Malingre, J.H. Schellens, J.H. Beijnen, O. van Tellingen, Entrapment by Cremophor EL decreases the absorption of paclitaxel from the gut, Cancer Chemother. Pharmacol. 49 (2002) 119–125.
[31] J. Gonzalez-Llaven, J.A. Palma-Aguirre, R. Garcia-Arreola, V. Brizuela, J. Nava-Rangel, S. Moran-Lira, J. Vela-Ojeda, G. Castaneda-Hernandez, F.J. Flores-Murrieta, Comparative bioavailability evaluation of two cyclosporine oral formulations in healthy Mexican volunteers, Arch. Med. Res. 30 (1999) 315–319.
[32] B.A. Klyashchitsky, A.J. Owen, Drug delivery systems for cyclosporine: achievements and complications, J. Drug Target 5 (1998) 443–458.
[33] E.A. Mueller, J.M. Kovarik, J.B. van Bree, J. Grevel, P.W. Lucker, K. Kutz, Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation, Pharm. Res. 11 (1994) 151–155.
[34] K. Kawakami, T. Yoshikawa, T. Hayashi, Y. Nishihara, K. Masuda, Microemulsion formulation for enhanced absorption of poorly soluble drugs. II. In vivo study, J. Control. Release 81 (2002) 75–82.
[35] D. Wagner, H. Spahn-Langguth, A. Hanafy, A. Koggel, P. Langguth, Intestinal drug efflux: formulation and food effects, Adv. Drug Deliv. Rev. 50 Suppl. 1 (2001) S13–S31.
[36] Y. Shono, H. Nishihara, Y. Matsuda, S. Furukawa, N. Okada, T. Fujita, A. Yamamoto, Modulation of intestinal P-glycoprotein function by cremophor EL and other surfactants by an in vitro diffusion chamber method using the isolated rat intestinal membranes, J. Pharm. Sci. 93 (2004) 877–885.
[37] V.D. Makhey, A. Guo, D.A. Norris, P. Hu, J. Yan, P.J. Sinko, Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells, Pharm. Res. 15 (1998) 1160–1167.
[38] K. Sugimoto, T. Sudoh, S. Tsuruoka, Y. Yamamoto, S. Maezono, Y. Watanabe, A. Fujimura, Effect of probucol on the oral bioavailability of cyclosporine A, Eur. J. Pharm. Sci. 22 (2004) 71–77.