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Research paper

Correlation between digestion of the lipid phase of smedds and release of the anti-HIV drug UC 781 and the anti-mycotic drug enilconazole from smedds

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Abstract

The present studies were conducted primarily to compare the drug release process of the anti-HIV drug UC781 from three different smedds to the smedds digestion profile. The influence of every formulation component on the digestion process, measured as the release of fatty acids, was determined. In addition, the release of the antimycotic drug enilconazole from a smedds was investigated as well in order to study the influence of the type of incorporated drug on oil digestion. Simulsol 1292, Tween 80, Cremophor RH40, ethanol and both drugs reduced the fatty acid release. C8, C10 and C12 fatty acids, originating from oil hydrolysis, were able to reverse the inhibitory effect of phospholipids present in the release medium. Similarly Cremophor RH40 lost its inhibitory capacities in combination with Captex 200P hydrolysis. In addition, UC781 did not decrease fatty acid release in combination with a Captex 200P–Tween 80–ethanol mixture.

The release of UC781 from smedds significantly increased compared to the dissolution of the pure drug. The drug release profiles were characterized by rapid and complete release followed by precipitation. In order to detect possible correlations between drug release and oil digestion, release results were compared to those of vehicle digestion experiments. Contrary to what one would assume, a higher extent of fatty acid liberation did not enhance drug release. In other words, drug release does not seem to be driven by the extent of lipid digestion.

Keywords: Smedds; Drug release; Oil digestion; Fatty acid liberation

1. Introduction

One of the major hurdles that the pharmaceutical industry has to face today is to enhance the bioavailability of poorly water soluble drug candidates. Drugs showing low aqueous dissolution rates/solubility but sufficient permeability are classified as Class II drugs in the Biopharmaceutical Classification System [1]. Release of drug from the dosage form will be the limiting step in the absorption process. Therefore enhancing drug solubility or increasing the surface area available for dissolution, e.g. by the use of salts, by micronisation or the formulation of solid dispersions, can improve bioavailability of these compounds. In case of sufficiently lipophilic drugs ($\log P > 4$) there has been a growing interest in the exploration of lipid-based delivery systems [2]. At least in part, this interest is due to the successful development of lipid-based formulations of cyclosporine A (originally marketed as "Sandimmune®" and now as the improved product "Neoral Sandimmun®") and two HIV protease inhibitors, ritonavir (Norvir®) and saquinavir (Fortovase®) [3–5]. By administering a compound in a dissolved state, one reduces the energy associated with a solid–liquid transition. Moreover, the presence of lipids in the GI tract will increase drug solubilisation and thus dissolution via a number of potential mechanisms such as an increased secretion of bile salts and endogenous

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biliary lipids together with an intercalation of administered lipids into bile salt structures, directly or after digestion and a reduced gastric transit time, resulting in an increased dissolution time and changes to the physical and biochemical barrier function of the intestinal tract [6–8]. Various lipid digestion products and surfactants show permeability enhancing properties or alternate the activity of intestinal efflux transporters.

Lipid formulations include lipid solutions, emulsions, microemulsions, self-emulsifying drug delivery systems (sedds) or self-microemulsifying drug delivery systems (smedds) [9–11,8]. A smedds is a mixture of an oil, a surfactant and sometimes a co-surfactant or co-solvent, that spontaneously forms a transparent (or at least translucent), isotropic and thermodynamically stable microemulsion upon dilution with water [12–15]. The interfacial tension at the oil–water interface is significantly reduced and the interfacial layer is kept highly flexible and fluid, allowing the formation of very small droplets (<200 nm), thereby increasing drug dissolution. These conditions are usually met by a careful and precise choice of the components and their respective proportions. Moreover smedds formulations are known to reduce inter- and intra-individual variations in bioavailability.

Not only can the oil and surfactant phase increase drug solubility, in theory also digestion products can enhance solubilisation as they can change the dissolution medium and be incorporated into o/w interfaces, resulting in a decrease of the oil droplet size or swelling of bile salts micelles already present [7,16]. Because this in turn affects drug dissolution, one would assume high correlations between both processes. However the exact influence of the different digestion processes on drug release has not yet been demonstrated.

This study focuses mainly on the release characteristics of the anti-HIV drug UC781 from various smedds in relation with the lipid digestion process. In addition, the influence of every smedds component on oil lipolysis is analysed. To investigate the influence of the incorporated drug on oil digestion, also enilconazole was incorporated into a smedds. UC781 is a second-generation NNRTI. It was found to be highly potent and a selective inhibitor of HIV-1 replication compared to first-generation NNRTIs [17,18]. Despite the potential of this compound against HIV-1 infected individuals, the development of an oral dosage form has been a problem as a result of the extremely poor solubility of UC781 in water. Enilconazole is an external antimycotic substance with a broad-spectrum activity [19,20]. It also suffers from poor aqueous solubility, but as for UC781, its lipophilicity allows it to be incorporated in a smedds formulation.

In a first part, the role of the oil phase, surfactant, ethanol UC781 and enilconazole in the oil digestion process will be analysed. Second, the assumed correlations between drug release characteristics and oil lipolysis are investigated.

2. Materials and methods

2.1. Materials

Captex 200P or propylene glycol mono- and dicaprylate and mono-and dicaprate was kindly supplied by Abitec Corp. (Janesville, WI, USA). Simulsol 1292 (polyoxyl 25 hydrogenated castor oil) was a generous gift from Seppic (Paris, France). Cremophor RH40 or polyoxyl 40 hydrogenated castor oil was purchased from BASF (Ludwigshafen, Germany) and Tween 80 (polyoxyethylene-20-sorbitan mono oleate) from VWR Int. (Leuven, Belgium). Imwitor 642 (Glyceryl hexanoate) was kindly provided by Sasol (Witten, Germany). Sodium taurodeoxycholate 99% (NaTDC), L-α-lecithin type X-E, Trisma-maleate and porcine pancreatin (activity 8× USP specifications) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ethanol p.a. and $CaCl_2{\cdot}2H_2O$ were purchased from Vel n.v. (Leuven, Belgium). NaCl and NaOH were from BDH (Poole, England). $(NH_4)_2HPO_4$ was obtained from Merck Eurolab (Leuven, Belgium). Enilconazole was kindly supplied by Janssen Pharmaceutica (Beerse, Belgium), UC781 was supplied by the Rega Institute (Leuven, Belgium).

2.2. Methods

2.2.1. Smedds preparation

Three different smedds with varying ratios of oil, surfactant and ethanol were prepared. Their composition is shown in Table 1. UC 781 was incorporated in each smedds formulation, while the properties of enilconazole were investigated in only one smedds. The amount of drug incorporated into the smedds was max. 25% of its solubility in the formulation. The drug was incorporated by mixing it during 12 h with the freshly prepared smedds vehicle using a tumbler mixer. For each measurement smedds were freshly prepared.

2.2.2. Drug solubility studies

An excess amount of drug was added to a test tube containing 1 ml of each smedds. After sealing, the mixture was left in a tumbler at 25 °C to facilitate dissolution. After 7 days, each test tube was centrifuged at 1600*g* for 30 min followed by filtration using 0.45 μm pore size filters (Machery-Nagel, Düren, Germany). Samples were diluted with acetonitrile and the concentration of each drug was quantified by HPLC (see Section 2.2.6).

Table 1
Smedds components: type and concentrations (expressed in % w/w)

	Oil	Surfactant	Co-solvent
Smedds 1	Imwitor 642: 24%	Simulsol 1292: 16%	Ethanol: 60%
Smedds 2	Captex 200P: 19%	Tween 80: 56%	Ethanol: 25%
Smedds 3	Captex 200P: 17%	Cremophor RH40: 69%	Ethanol: 14%

2.2.3. Preparation of drug release medium

Experiments were performed in the release medium simulating fasted state intestinal conditions as is described in Table 2. The pH was adjusted to 7.5 using NaOH.

The lecithin/NaTDC mixed micellar solutions were prepared by dissolving lecithin in chloroform followed by solvent evaporation with a rotavapor (Buchi, Flawil, Switzerland), leaving a thin film of lecithin on the walls of the flask. Specified amounts of NaTDC and a solution containing trisma-maleate, NaCl and $CaCl_2{\cdot}2H_2O$ (pH 7.5) were subsequently added and the suspension was stirred for 12 h. Prior to any experiments, the pH was checked again and adjusted if necessary.

2.2.4. In vitro digestion

In vitro lipolysis experiments were conducted in triplicate in accordance with a previously described in vitro lipid digestion model [21–23], using a pH-Stat automatic titration unit (718 STAT Titrino, Metrohm AG, Herisau, Switzerland). All experiments were conducted at 37 °C in a stirred and thermostatted glass vessel containing the release medium. Lipase extract is added to the vessel containing release medium and various samples, depending on the experiment, leading to oil digestion and fatty acid release, thereby lowering the pH. The pH-stat subsequently titrates and reports a measured volume of NaOH to maintain the initial pH and enzyme activity. The release medium has an inherently low buffering capacity, thereby ensuring detection of the pH drop caused by the fatty acid release after oil digestion. The pH of the reaction medium (pH 7.5) is slightly elevated compared to *in vivo* intestinal conditions, to ensure full ionisation of the fatty acids produced.

Pancreatin extract was prepared by adding 1 g of porcine pancreatin (containing pancreatic lipase and colipase) to 5 ml of salt solution (50 mM trisma-maleate, 150 mM NaCl, and 5 mM $CaCl_2{\cdot}2H_2O$). The suspension was stirred for 15 min followed by centrifugation at 1600*g* and 5 °C. The supernatant was collected and stored on ice. Pancreatin extracts were freshly prepared for each experiment.

2.2.4.1. Tributyrin experiments. The lipase activities were determined in triplicate using 6 ml of glyceryl tributyrate in 10 ml of release medium at pH 7.5 and expressed in terms of ml fatty acids released per min. Two millilitre of surfactant or ethanol was added and emulsified for 10 min prior to enzyme addition. The experiments were initiated by addition of 1 ml of pancreatin extract (containing 1796 tributyrin units or TBU, where 1 TBU is the amount of enzyme that can liberate 1 μmole of titratable fatty acid from tributyrin per min). 1 M NaOH was used as titrant.

2.2.4.2. Fatty acid experiments. To measure the influence of hexanoic, octanoic, decanoic or dodecanoic fatty acids on oil digestion, 0.1 ml of caproic, capric, caprylic or lauric acid was added to 25 ml release buffer also containing 0.5 ml Tween 80. The pH was adjusted to 7.5 using NaOH 1 M and 0.1 M. The experiments were initiated by addition of 2.5 ml of pancreatin extract after 60 s. NaOH 0.5 M was used as titrant. Experiments were carried out in triplicate.

2.2.4.3. Smedds experiments. The release of fatty acids from the oil phases, also in combination with a surfactant, a surfactant–ethanol or a surfactant–ethanol–drug mixture was measured in triplicate after the addition of pancreatin extract (1 ml extract per 10 ml release buffer) and was allowed to continue for 2 h. The oil phases, oil–surfactant, oil–surfactant–ethanol mixtures or smedds, were added and emulsified 10 min prior to enzyme addition. A specific amount of smedds was added in order to achieve smedds concentrations of 0.5% m/v. Fatty acids produced by sample digestion were titrated with 0.2 M NaOH or 0.1 M NaOH in order to maintain the pH at 7.5. In blank digestion experiments, where no sample was added to the release buffer, 0.01 and 0.05 M NaOH were used for fatty acid titration.

2.2.5. Drug release tests

Drug release studies were performed using a dissolution bath SR8 (Analis, Namur, Belgium) in 400 ml of release medium (as described in Section 2.2.3), at 37 °C and a paddle speed of 100 rpm. To start the experiment, 40 ml of pancreatin extract and 2 ml of smedds were added to the reaction vessel. During the release studies, samples of 8 ml were collected after 1, 5, 10, 20, 30, 60, 90, 120, 180 and 240 min using a syringe and were replaced with the same volume of release medium. A lipolysis inhibitor (4-bromophenylboronic acid 0.5 M in methanol, 9 μl per ml release medium) was added to the samples immediately after collection to stop further digestion. The samples were subsequently ultracentrifuged (334,000*g*, 37 °C, 30 min) to separate the pellet phase from the digested solution and the latter was subsequently filtered using 0.45 μm pore size membrane filters. All samples were analysed with HPLC. Experiments were performed in triplicate.

2.2.6. HPLC

The HPLC analysis was performed using a Merck Hitachi pump L-7100, an autosampler L-7200 and a UV–vis detector L-7420. The column was a Chromolith Performance RP-18e 100 × 4.6 mm (Merck KgaA, Darmstadt, Germany). A mobile phase of acetonitrile: water (55:45 v/v) was used in case of drug release tests while acetonitrile: $(NH_4)_2HPO_4$ (55:45) was used for solubility testing. Both

Table 2
Release medium

Ingredients	Concentration (mM)
Trisma-maleate	50
$CaCl_2{\cdot}2H_2O$	5
NaCl	150
Lecithin	1.25
Sodium taurodeoxycholate	5

mobile phases were used at a flow rate of 1.0 ml/min. 10 μl sample volume was injected onto the column, except for analysing samples of enilconazole, where the injection volume was increased to 50 μl. Each sample was analysed twice, while standards were measured three times. The standards for UC781measurements were prepared in methanol and detection of all UC781 samples was performed at a wavelength of 297 nm, whereas the standards for dissolution measurements of enilconazole were prepared in dissolution buffer and absorption of all enilconazole samples was analysed at 270 nm.

3. Results and discussion

Based on preliminary experiments (droplet size measurements on a large range of diluted oil–surfactant–ethanol combinations by multi-angle Dynamic Light Scattering), three smedds were selected based on the droplet diameters in diluted microemulsions, which should be below 200 nm. Smedds 1 consists of Imwitor 642–Simulsol 1292–ethanol, smedds 2 of Captex 200P–Tween 80–ethanol and smedds 3 contains a Captex 200P–Cremophor RH40–ethanol mixture. Their quantitative composition is given in Table 1.

To estimate the influence of drug properties, two model drugs, UC781 and enilconazole, were incorporated in these formulations. Both drugs suffer from low aqueous solubility, limiting their use in oral dosage forms. UC781 was incorporated in all three formulations, while enilconazole was only used to compare the influence of the drug on digestion in a Captex 200P–Cremophor RH40–ethanol smedds. As previously stated, by using smedds, drug release rates can be enhanced as a result of the higher solubilisation capacity and increased surface area from which dissolution can take place. In theory, digestion products can also increase drug solubility and because this in turn affects drug dissolution, one would assume high correlations between both.

Oil digestion can be monitored indirectly *in vitro* by titration of fatty acids in a pH-Stat method. To initiate lipolysis, the lipid formulation and lipase/colipase enzyme are added to the digestion media, leading to the production of fatty acids and transient lowering of the pH of the media. The reaction medium simulates the intestinal lumen in a fasted state and is composed in accordance with previous work [21–24]. The pH-stat subsequently titrates the fatty acids with NaOH and reports a measured volume of base to reinstall the initial pH.

3.1. The role of components of the smedds on digestion

In order to analyse lipolysis, the oil phase, an oil–surfactant mixture, an oil–surfactant–ethanol mixture or a smedds formulation was added to the digestion medium 10 min prior to the addition of the enzyme extract. As the oil phase was hydrolysed, fatty acids were produced thereby lowering the pH and initiating titration with NaOH until a pH of 7.5 is reinstalled.

Blank experiments including only digestion of release medium were performed to take into account the influence of digestible components present in this medium. Hydrolysis of these components is however partly inhibited by the presence of phospholipids in the release medium [25,26]. Experiments showed that the digestible components release only 6.68×10^{-3} mmol fatty acids per ml of release medium. This does not necessarily represent the full extent of digestible components (e.g. cholesterol, triglycerides and other impurities present in lecithin and sodium-taurodeoxycholate) present in the release medium as one of its components, more precisely phospholipids, can interfere with the lipolysis of digestible components of the medium and partly inhibit lipase activity [26–29].

As previously stated, the fatty acids that are formed after digestion can potentially increase the solubility of the drug. Considering the average molar weight of Captex 200P and Imwitor 642 (supplier information), one can estimate from pH Stat results described in Table 3 that Captex 200P, a propyleneglycol derivative, and Imwitor 642, a combination of mono-, di- and triglycerides, are both completely digested. In case of Imwitor 642 hydrolysis first yields fatty acids and monoglycerides that could undergo isomerisation and be further digested to fatty acids and glycerol. Captex 200P is digested into fatty acids and propyleneglycol. Both lipids are however not only fully digested, but also enhance the enzyme activity as even more fatty acids were liberated from the release medium than measured in blank experiments; 0.1 mmol Captex 200P caused an extra release of 0.292 mmol fatty acids from 25 ml release medium while 0.3 mmol Imwitor 642 in 25 ml release medium leads to an extra release of 0.421 mmol fatty acids. As mentioned above, lipase–colipase activity can be strongly inhibited by the presence of lecithin. Fatty acids can reverse this inhibition by changing interfacial properties and reactivate the enzyme as colipase binds preferentially and avidly to interfaces containing fatty acids when phospholipids are present [26,28,29]. This implies that the enzyme laterally concentrates substrates and products of lipolysis in its vicinity [27]. The effect of fatty acids on the activity of pancreatic lipase–colipase is related to both the saturation and the acyl chain lengths.

Captex 200P predominantly results in caprylic acid while also caproic, capric and lauric acids are present. In a next set of experiments, the effect of these free fatty acids was analysed in release medium containing Tween 80 as a solubiliser. Free fatty acids were added and neutralized followed by enzyme addition. It is clear from Fig. 1 that lipase activity is stimulated after addition of octanoic, decanoic and dodecanoic acids. Upon digestion of Captex 200P,

Table 3
Fatty acid liberation from the oil phases, expressed as mmol fatty acids released per ml oil ($n = 3$)

Imwitor 642 mmol FA/ml oil	Captex 200P mmol FA/ml oil
9.016	12.434

these fatty acids are present on the interface therefore being responsible for the previously discussed increased hydrolysation of release medium. Exogenous hexanoic acid, a lipolysis product from Imwitor 642, did not show any improved fatty acid release. This confirms data described by [25]. Other Imwitor 642 lipolysis products, e.g. octanoic, decanoic and lauric acids will also in this case reactivate the lipase complex. The extent of stimulation is however not as pronounced as for Captex 200P as these fatty acids are less present in Imwitor 642.

Not only the oil, but also the surfactant and ethanol can have a significant influence on the digestion process, either directly by solubilisation or interaction with the enzyme, or else indirectly by protecting the oil phase.

The interaction with the lipase complex was analysed by enzyme activity measurements, using tributyrin as the oil phase and expressing the activity as ml titrants per min. Table 4 shows that all three surfactants can partially inhibit the lipolysis process and this concurs with other reports [7,21]. The surfactant, situated mostly on the o/w interface, which is the site of action of the lipase complex, solubilises the oil phase thereby making it less accessible for pancreatic lipase. In addition, surfactants increase the amount of pure micelles to which colipase binds thus reducing oil–water interfacial pancreatic lipase concentrations [26,30]. In addition, some surfactants are able to bind to the active site of the enzyme, leading to a decrease of lipase activity by competitive inhibition [31]. If one takes into account the molecular weights, the activity results also seem to be in line with a phenomenon previously described in the literature, more precisely that higher HLB values (in the range of 12 to 17) result in an increased inhibition of the fatty acid liberation [21,32].

The activity of the enzyme is also reduced in combination with ethanol most probably because of the changed reaction medium.

Table 4
Activity of pancreatic lipase/colipase in combination with different surfactants or ethanol, expressed as mmol fatty acids released per ml enzyme per minute ($n = 3$)

	mmol/ml
Tributyrin	1.7963
Tributyrin + Simulsol 1292	0.5201
Tributyrin + Tween 80	0.3112
Tributyrin + Cremophor RH40	0.2760
Tributyrin + Ethanol	1.2498

PH Stat experiments on the oil–surfactant, oil–surfactant–ethanol and oil–surfactant–ethanol–drug samples, reported in Table 5, confirm some of these findings but also add some new perspectives.

Replacement of the oil still results in an inhibitory effect of Simulsol 1292 and Tween 80. In combination with tributyrin, also Cremophor RH40 caused an inhibition of oil digestion. However, in combination with Captex 200P this surfactant did not show any inhibition of fatty acid release. Cremophor RH40 is a hydrophilic surfactant, and should therefore in general inhibit lipid digestion. It has however been shown that this inhibition can be reversed by the inclusion of a lipophilic co-surfactant e.g. a blend of long chain mono- and diglycerides [21]. These results seem to agree with this phenomenon (in this case the combination with mono- and di-propyleneglycol derivatives reverses the inhibition). Moreover, not only is the inhibition completely reversed, a Captex 200P–Cremophor RH40 combination seems to increase lipase activity even more than the oil itself. The origin of this phenomenon is actually unknown, although this could be the result of an interaction of Captex 200P with Cremophor RH40 that causes a change in orientation and location of the surfactant at the oil–water interface of the droplet, enhancing hydrolysis. However, this could also be the result of an interaction

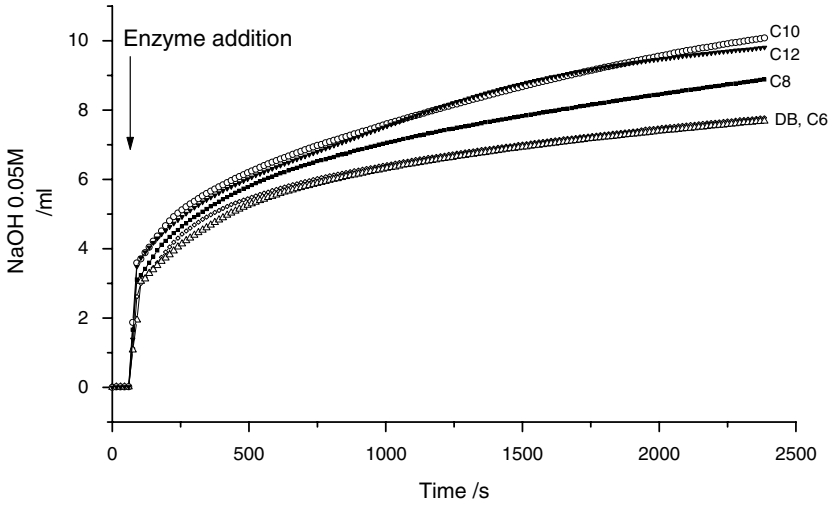

Fig. 1. Fatty acid release as measured by pH stat after addition of 0,1 ml hexanoic acid (C6, Δ), octanoic (C8, ■), decanoic (C10, ○) or dodecanoic acid (C12, ▼) to 25 ml release buffer also containing 0.5 ml Tween 80. Blank experiments (DB), containing release buffer and Tween 80, are represented by (◇).

Table 5
Interference of the smedds components with oil digestion, expressed as mmol fatty acids per ml surfactant or cosolvent or as mmol fatty acids per mg drug

Smedds	Surfactant	Cosolvent	Drugs	
Imwitor 642–Simulsol1292–ethanol	Simulsol 1292	Ethanol	UC781	
	-2.431 ± 0.88	-0.2692 ± 0.012	-0.2716 ± 0.034	
Captex 200P–Tween 80–ethanol	Tween 80	Ethanol	UC781	
	-4.312 ± 0.23	-2.944 ± 0.023	$+0.7525 \pm 0.013$	
Captex 200P–Cremophor RH40–ethanol	Cremophor RH40	Ethanol	UC781	Enilconazole
	$+1.7205 \pm 0.055$	-8.917 ± 0.88	-0.3646 ± 0.027	-0.3213 ± 0.0025

A negative number means that the amount of fatty acids is inhibited to be released by adding 1 ml or 1 mg component, a positive number means that the release of fatty acids is increased by the addition of 1 ml surfactant or 1 mg drug ($n = 3$).

of Cremophor RH40 and Captex 200P digestion products, which, in line with the previously discussed influence of fatty acids on the inhibitory effect of phospholipids, changes interfacial properties and improves the binding of the enzyme and its cofactor to the substrate surface [26,28].

Ethanol still partially inhibits lipolysis in all three formulations. Cremophor RH40 in the presence of ethanol inhibits lipid digestion possibly because the cosolvent reduces the interaction of Cremophor RH40 with Captex 200P or its reaction products. A Captex 200P–Tween 80 combination already revealed total inhibition of the fatty acid liberation of the oil phase and partial inhibition of the fatty acid liberation of the digestion phase. However also in this case, ethanol further increased the inhibition.

Finally, both drugs also influence the lipolysis process as they can interact with the apolar fraction of the surfactant inside the microemulsion droplet and can be incorporated in the interface, thereby changing the availability of the oil phase for digestion. This causes the fatty acid liberation to be partially inhibited by UC781 in Captex 200P–Cremophor RH40–ethanol formulations while the drug decreased the inhibitory effects of Tween 80 and ethanol in a Captex 200P–Tween 80–ethanol smedds.

The effect of enilconazole was investigated in only one smedds: Captex 200P–Cremophor RH40–ethanol. It also partially inhibits lipolysis but to a slightly smaller extent than UC781. The reason for this is not clear at this point. Differences in lipophilicity ($c\log P_{\mathrm{UC781}} = 4.43 + -0.65$; $c\log P_{\mathrm{enilconazole}} = 3.82 + -0.41$) (www.acdlabs.com), which can cause different orientations in the oil droplet, can interfere with oil availability for the enzyme, while for instance interactions of the drug with the oil phase and lipophilic surfactant tails can also influence the extent of lipolysis considerably.

3.2. Drug release and smedds digestion

As previously stated, one could assume that fatty acid liberation would increase drug release since they can be incorporated at the interface of the oil droplet. It has been proven that the presence of digestion products such as monoglycerides and fatty acids, incorporated in bile salt micelles, increases the solubility of poorly water soluble drugs and therefore can enhance their absorption [6,11,33]. The incorporation of both products will reduce the interfacial tension, decrease the droplet size and cause swelling of mixed micelles already present. Therefore the solubilisation capacity and the surface area available for drug release, two factors important in drug release, are considerably increased.

For both digestion and release experiments, the amount of drug that was incorporated into the smedds was max. 25% of the drug solubility, also ensuring similar drug doses for each drug. Drug doses and solubilities in the smedds formulations are reported in Table 6.

Fig. 2 depicts the release of pure UC781 as well as UC781 formulated in a Captex200P–Cremophor RH40–ethanol smedds while fatty acid liberation of the formulation is described in Table 7. Due to its hydrophobic nature

Table 6
Solubility of UC781 and enilconazole and their loading concentrations expressed in g/l, in the smedds formulations ($n = 3$)

	UC781		Enilconazole	
	Solubility g/l	Added amount g/l	Solubility g/l	Added amount g/l
Smedds 1	25.15 ± 1.52	5.78	ND	ND
Smedds 2	65.17 ± 0.71	5.81	ND	ND
Smedds 3	82.80 ± 2.14	5.21	594.24 ± 27.04	7.075

ND, not determined.

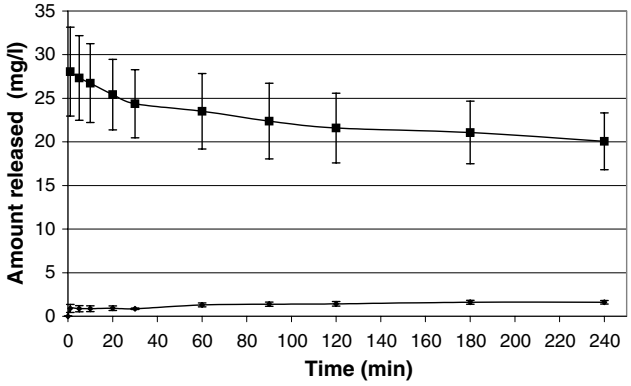

Fig. 2. Release of UC 781 from a Captex 200P–Cremophor RH40–ethanol formulation in simulated intestinal fluid (represented by ■). (▲) Represents the dissolution of pure drug in the same medium. Error bars indicate the standard deviation ($n = 3$).

only 6% of pure UC781 is dissolved after 4 h in the release medium. In a first stage of the release experiments, release rates from smedds are high since the presence of the surfactant, oil and a small amount of reaction products improves drug solubilisation. However, in time, digestion will proceed, breaking down the oil phase while the surfactant and fatty acids will spread out into the release medium, causing the drug to precipitate. Drug release still amounts up to 80–85% after 2 h, which is significantly higher than the dissolution of pure drug. The time to reach equilibrium values seems to correlate to the homogenisation of the medium, which is visible as the disappearance of an oily layer on top of the dissolution vessel, and probably also to the stabilisation of the fatty acid release. However one has to be careful in comparing kinetics of release and digestion experiments since hydrodynamics in both tests (i.e. release experiments and lipid digestion tests) were not identical as a consequence of differences in dissolution volumes and paddle equipments. Paddle speeds have an important influence on fatty acid liberation since a lower paddle speed will enable the oil phase to stay longer at the surface, which means that less surface area is available for oil digestion by lipase enzymes. This is shown in Fig. 3 where a decreased paddle speed shows slower fatty acid liberation. However doubling the paddle speed did not influence the release rate, which already points to an important issue that is opposite of what we assumed earlier and that we will discuss later on in this article, more precisely that the extent of fatty acid liberation does not seem to be the major driving force for drug release.

In order to investigate a possible influence of the drug on the release profile from a smedds formulation, we studied the release of enilconazole from the Captex 200P–Cremophor RH40–ethanol formulation. The results are shown in Fig. 4. Because of its hydrophobicity, solubility of pure enilconazole in water (pH 7.6) is limited to 0.018 g/100 ml. The release profile shows some variability in the initial phase of the experiment. It initially reaches values of 35–60%, but in contrast to the UC781 samples, drug release still increases during the first half hour until values of 70–80% are obtained. Subsequently the release profile decreases until equilibrium values of around

Table 7
Release of fatty acids and drugs ($n = 3$)

	Fatty acid liberation (mmol/ml)	Release of the drug in dissolution tests after 4 h (%)
Smedds 1 incorporating UC781	3.737 ± 0,20	45–50%
Smedds 2 incorporating UC781	7.323 ± 0,09	60–80%
Smedds 3 incorporating UC781	2.730 ± 0,18	80–85%
Smedds 3 incorporating enilconazole	2.615 ± 0,02	40–50%

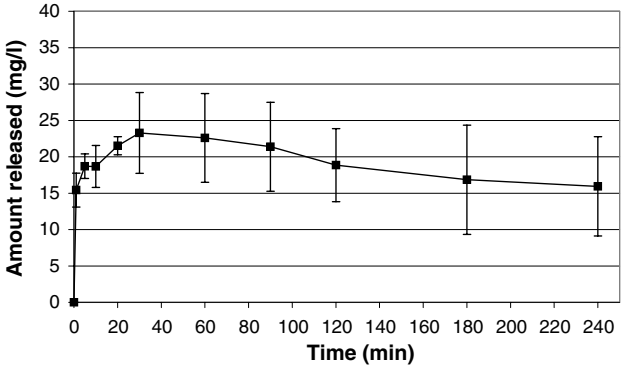

Fig. 4. Release profile of enilconazole from a Captex 200P–Cremophor RH40–ethanol formulation in simulated intestinal fluid.

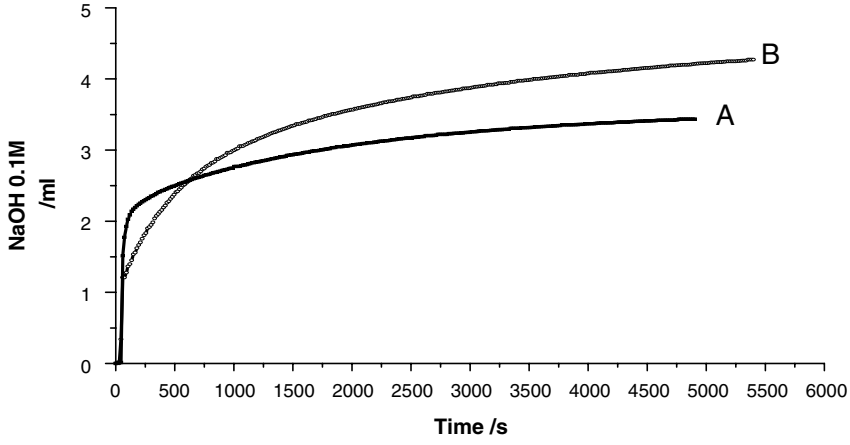

Fig. 3. Fatty acid release as measured by pH stat at two different paddle speeds. Profile A was achieved using a higher speed, while the lower paddle speed experiment is described by profile B. Error bars indicate the standard deviation ($n = 3$).

40–50% are reached, which is less than the obtained release profiles of UC781 in the same formulation.

Release of UC781 from a Captex 200P–Tween 80–ethanol smedds is shown in Fig. 5. Release of about 60% was obtained, which is 10 times more than in the absence of the smedds. The release is reduced in time as the oil is digested and the fatty acids are diluted. However, in this case there seems to be a slower initial increase in release rate. This is most probably caused by the voluminous oxyethylene chains of Tween 80 that are situated on the aqueous site of the interface and that sterically hinder the lipase from reaching the oil–water interface. If fatty acid liberation increases drug release, we expect the oil digestion to be less pronounced compared to the previously discussed Captex 200P–Cremophor RH40–ethanol formulation. Table 7 shows both fatty acid releases. Remarkably, the Captex 200P–Tween 80–ethanol smedds releases more fatty acids, again pointing out to the fact that oil digestion may not play the important role in drug release as one would assume.

Fig. 6 describes the release of UC 781 formulated in an Imwitor 642–Simulsols 1292–ethanol smedds. After only 1 min drug release increased from 60% to 85%. As drug release proceeded, the release curve initially showed some variability but after 2 h an equilibrium release value of 45% was reached, which is still considerably higher than that of pure UC781. Also in this case drug precipitation was observed. A comparison of the equilibrium drug release values and fatty acid liberations (Table 7) to the previously described smedds again confirms that the extent of oil digestion does not have an important influence on drug release. Drug release is minimal in case of an Imwitor 642–Simulsol 1292–ethanol formulation, while the fatty acid release is higher than in case of a Captex 200P–Cremophor RH40–ethanol smedds.

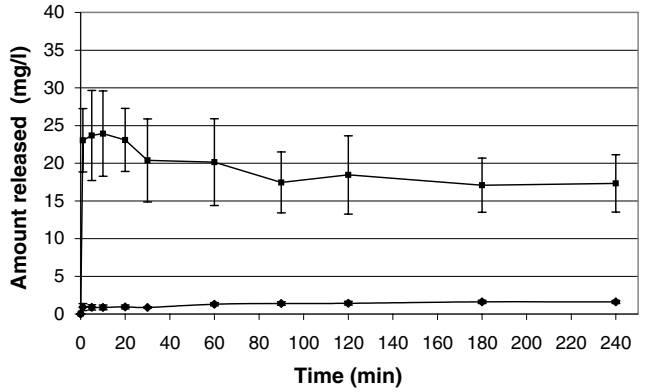

Fig. 5. Release experiments of UC 781 in a Captex 200P–Tween 80–ethanol formulation in simulated intestinal fluid (represented by ■). (▲) Describes the dissolution of pure drug in the same medium. Error bars indicate the standard deviation ($n = 3$).

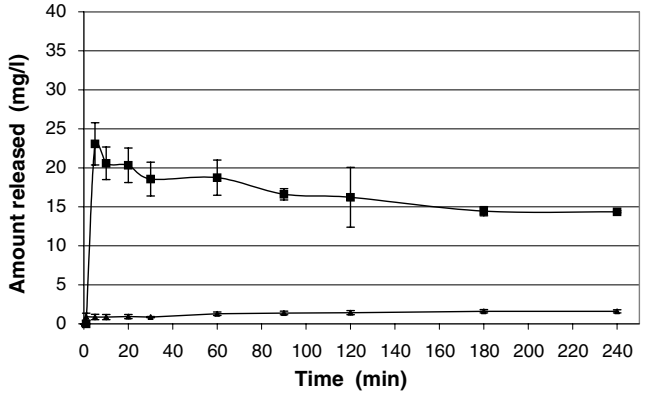

Fig. 6. Release of UC 781 incorporated in a Imwitor 642–Simulsol 1292–ethanol smedds formulation in simulated intestinal fluid (represented by ■). (▲) Represents the dissolution of pure drug in the same medium. Error bars indicate the standard deviation ($n = 3$).

Basically, a positive influence of fatty acid liberation on drug release would mean that a Captex 200P–Cremophor RH40–ethanol–UC781 formulation, that shows optimal drug releases, would also show the highest fatty acid liberation, i.e. more liberation than the Captex 200P–Tween 80–ethanol–UC781, which in turn would release more fatty acids than an Imwitor 642–Simulsol 1292–ethanol formulation. However, the results obtained in pH-stat experiments do not correlate with these assumptions as one cannot link the amount of fatty acid liberation with the equilibrium values of drug release (Table 7).

This points out that fatty acid liberation does not have the important influence on drug dissolution as often suggested or assumed. Other factors as e.g. droplet size, surface tension or the presence of liquid crystals during dilution should also be accounted for and their influence analysed.

4. Conclusion

The present studies show release profiles of UC781 incorporated in three different smedds and of enilconazole in one of the smedds formulations in combination with digestion experiments of the dosage forms. In addition, the influence of every component of the smedds on the digestion process, measured as release of fatty acids, was determined.

Lipase activities were partially inhibited by all three surfactants and were also limited by both drugs and ethanol. Surfactants, located at the oil droplet surface, can interfere with the attachment of the lipase complex to the oil–water interface while ethanol decreases enzyme activity by changing the digestion medium. The drugs solubilised in the oil droplets can interact with the surfactant chains and/or reduce the availability of the oil phase at the interface, thereby also interfering with the digestion process. The inhibitory effect of Cremophor RH40 however is reversed in combination with mono- and di-propyleneglycol-derivatives, probably because of a change in interfacial properties caused by the presence of Captex 200P digestion products at the o/w surface.

Compared to the results of pure drug, significant improvement of the release of UC781 has been demonstrated in case of each smedds formulation even showing up to 14 times the release of pure drug. Drug release started off very high but decreased in time due to drug precipitation. Equilibrium values were reached in 2 h.

Drug release does not seem to be driven by the digestion of the oil phase, as the results obtained with the release tests in these studies do not correlate with those obtained in pH-stat experiments. This means that, contrary to what is assumed in the literature, investigation of the extent of smedds digestion does not seem to predict drug dissolution behavior.

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References

[1] G.L. Amidon, H. Lennernas, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (1995) 413–420.
[2] A.J. Humberstone, W.N. Charman, Lipid-based vehicles for the oral delivery of poorly water soluble drug, Adv. Drug Del. Rev. 25 (1997) 103–128.
[3] C.T. Uede, M. Lemaire, G. Gsell, P. Misslin, K. Nussbaumer, Apparent dose-dependent oral absorption of cyclosporine A in rats, Biopharm. Drug Dispos. 5 (1984) 141–151.
[4] J. Grevel, E. Nuesch, E. Abisch, K. Kutz, Pharmacokinetics of oral cyclosporine A (Sandimmun) in healthy subjects, Eur. J. Clin. Pharmacol. 31 (1986) 211–216.
[5] J. Vanderscher, A. Meinzer, Rationale for the development of Sandimmune Neoral, Transplant. Proc. 26 (1994) 2925–2927.
[6] O. Hernell, J.E. Staggers, M.C. Carey, Physical–chemical behaviour of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase behaviour and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings, Biochemistry 29 (1990) 2041–2056.
[7] C.J.H. Porter, W.N. Charman, In vitro assessment of oral lipid based formulations, Adv. Drug Delivery Rev. 50 (2001) S127–S147.
[8] R.D. Vetter, M.C. Carey, J.S. Patton, Coassimilation of dietary fat and benzopyrene in the small intestine: an absorption model using killifish, J. Lipid Res. 26 (1985) 428–434.
[9] B.K. Kang, J.S. Lee, S.K. Chon, S.Y. Jeong, S.H. Yuk, G. Khang, H.B. Lee, S.H. Cho, Development of self-micro-emulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs, Int. J. Pham. 274 (2004) 65–73.
[10] C.W. Pouton, Effects of the inclusion of a model drug on the performance of self-emulsifying formulations, J. Pharm. Pharmacol. 36 (1985) 51P.
[11] 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 (2000) S93–S98.
[12] S.A. Charman, W.N. Charman, M.C. Rogge, T.D. Wilson, F.J. Dutko, C.W. Pouton, Self-emulsifying systems: formulation and biological evaluation of an investigative lipophilic compound, Pharm. Res. 9 (1992) 87–94.
[13] P. Constantinides, Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects, Pharm. Res. 12 (1995) 1561–1572.
[14] R.W. Greiner, D.F. Evans, Spontaneous formation of a water-continuous emulsion from water-in-oil microemulsion, Langmuir 6 (1990) 1793–1796.
[15] N.H. Shah, M.T. Carvajal, C.I. Patel, M.H. Infeld, A.W. Melick, Self-emulsifying drug delivery systems with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs, Int. J. Pharm. 106 (1994) 15–23.
[16] T. Gershanik, S. Benita, Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur. J. Pharm. Biopharm. 50 (2000) 179–188.
[17] J. Balzarini, W.G. Brouwer, D.c. Dao, E.M. Osika, E. De Clercq, Identification of novel thiocarboxanilide derivatives that supress a variety of drug-resistant mutant human deficiency virus type 1 strains at a potency similar to that for wild-type virus, Antimicrob. Agents Chemother. 40 (1996) 1454–1466.
[18] J. Balzarini, E. De Clercq, The thiocarboxanilides UC-10 and UC-781 have an additive inhibitory effect against human immunodeficiency virus type 1 reverse transcriptase and replication in cell culture when combined with other antiretroviral drugs, Antiviral Chem. Chemother. 8 (1996) 197–204.
[19] K. Hnilica, L. Medleau, Evaluation of topically applied enilconazole for the treatment of dermatophytosis in a Persian cattery, Vet. Derm. 13 (2002) 23–28.
[20] K. Moriello, D. Deboer, L. Volk, A. Sparkes, A. Robinson, Development of an *in vitro*, isolated, infected sore testing model for disinfectant testing of *Microsporum canis* isolates, Vet. Derm. 15 (2004) 175–180.
[21] K.J. MacGregor, J.K. Embleton, J.E. Lacy, A. Perry, L.J. Solomon, H. Seager, C.W. Pouton, Influence of lipolysis on drug absorption from gastro-intestinal tract, Adv. Drug Delivery Rev. 25 (1997) 33–46.
[22] L. Sek, C.J.H. Porter, W.N. Charman, Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with in situ densitometric analysis, J. Pharm. Biomed. Anal. 25 (2001) 651–661.
[23] L. Sek, C.J.H. Porter, A.M. Kaukonen, W.N. Charman, Evaluation of the in-vitro digestion profiles of long and medium chain triglycerides and the phase behaviour of their lipolytic products, J. Pharm. Pharmacol. 59 (2002) 29–41.
[24] A.M. Kaukonen, B.J. Boyd, C.J.H. Porter, W.N. Charman, Drug solubilization behaviour during *in vitro* digestion of simple triglyceride lipid solution formulations, Pharm. Res. 21 (2004) 245–253.
[25] A. Larsson, C. Erlanson-Albertsson, Effect of phophatidylcholine and free fatty acids on the activity of pancreatic lipase–colipase, Biochim. Biophys. Acta 876 (1986) 543–550.
[26] J.K. Embleton, C.W. Pouton, Structure and function of gastrointestinal lipases, Adv. Drug Delivery Rev. 25 (1997) 15–32.
[27] M. Dahim, H. Brockman, How colipase-fatty acid interactions mediate adsorption of pancreatic lipase to interfaces, Biochemistry 37 (1998) 8369–8377.
[28] H.L. Brockman, Kinetic behavior of the pancreatic lipase–colipase–lipid system, Biochimie 82 (2000) 987–995.
[29] I.P. Sugar, N.K. Mizuno, M.M. Momsen, H.L. Brockman, Lipid lateral organization in fluid interfaces controls the rate of colipase association, Biophys. J. 81 (2001) 3387–3397.
[30] J.S. Patton, M.C. Carey, Watching fat digestion, Science 204 (1979) 145–148.
[31] J. Hermoso, D. Pignol, B. Kerfelec, I. Crenon, C. Chapus, J.C. Fontecillacamps, Lipase activation by non-ionic detergents – the crystal structure of the porcine lipase–colipase–tetraethylene glycol monooctyl ether complex, J. Biol. Chem. 271 (1996) 18007–18016.
[32] L.J. Solomon, J.K. Embleton, C.W. Pouton, Inhibition of lipolysis of medium-chain triglycerides by non-ionic surfactants, a structure/activity study, in: P. Couvreur, D. Duchene, I. Kallas (Eds.), Formulation of poorly available drugs for oral administration, Editions de Santé, Paris, 1996, pp. 437–438.
[33] J.E. Staggers, O. Hernell, R.J. Stafford, M.C. Carey, Physical-chemical behaviour of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behaviour and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings, Biochemistry 29 (1990) 2028–2040.