Chase dosing of lipid formulations to enhance oral bioavailability of nilotinib in rats

Niklas J. Koehl[1], Rene Holm[2,3], Martin Kuentz[4], Brendan T. Griffin[1*]

[1]*School of Pharmacy, University College Cork, Cork, Ireland*

[2]*Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium*

[3]*Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark*

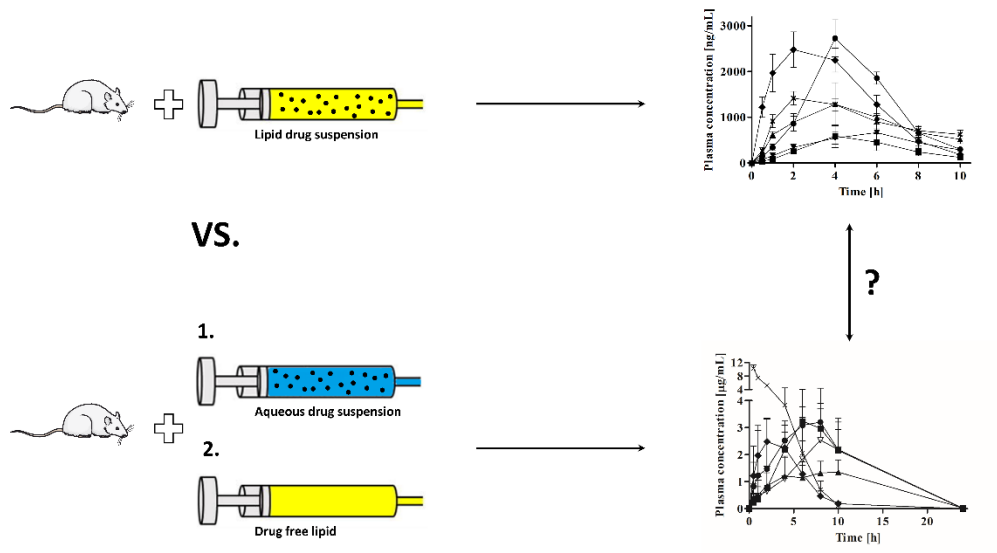
[4]*University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharma Technology, Muttenz, Switzerland*

[*] Corresponding author:

Brendan Griffin, University College Cork, School of Pharmacy, Cavanagh Building, College Road, Cork, Ireland

Phone: +353 (0) 21 490 1657, Fax: +353 (0) 21 490 1656

e-mail: Brendan.Griffin@ucc.ie



Graphical Abstract. The potential of bio-enabling lipid vehicles, administered via chase dosing and lipid suspensions, has been evaluated as an approach to enhance oral bioavailability of nilotinib.

Abstract

Purpose Lipid-based formulations (LBF) have shown oral bioavailability enhancement of lipophilic drugs, but not necessarily in the case of hydrophobic drugs. This study explored the potential of lipid vehicles to improve the bioavailability of the hydrophobic drug nilotinib comparing a chase dosing approach and lipid suspensions.

Methods Nilotinib *in vivo* bioavailability in rats was determined after administering an aqueous suspension chase dosed with blank olive oil, Captex 1000, Peceol or Capmul MCM, respectively. Absolute bioavailability was determined (relative to an intravenous formulation). Pharmacokinetic parameters were compared to lipid suspensions.

Results Compared to the lipid suspensions, the chase dosed lipids showed a 2- to 7-fold higher bioavailability. Both long chain chase dosed excipients also significantly increased the

bioavailability up to 2-fold compared to the aqueous suspension. Deconvolution of the pharmacokinetic data indicated that chase dosing of nilotinib resulted in prolonged absorption compared to the aqueous suspension.

Conclusion Chase dosed LBF enhanced the *in vivo* bioavailability of nilotinib. Long chain lipids showed superior performance compared to medium chain lipids. Chase dosing appeared to prolong the absorption phase of the drug. Therefore, chase dosing of LBF is favourable compared to lipid suspensions for 'brick dust' molecules such as nilotinib.

Keywords Chase dosing, Lipid suspension, Lipid based formulation, Brick dust molecule, poorly water-soluble drugs

Abbreviations: MC: medium chain; LC: long chain; MG: monoglyceride; TG: triglyceride; LBF: lipid-based formulation

1 Introduction

The oral bioavailability of many poorly water-soluble drugs is hindered by low solubility and slow dissolution in the gastrointestinal tract fluids. Lipid based formulations (LBF) are one option to improve the oral bioavailability of poorly water-soluble drugs and have shown numerous commercial success (1, 2). In particular, drugs in the Biopharmaceutical Classification System (BCS) class II and IV can benefit from usage of lipid excipients to enhance oral delivery. One of the primary biopharmaceutical advantages of LBFs is the ability to deliver the drug dissolved in lipid excipients and therefore avoiding the drug dissolution process. In addition, LBFs have been shown to increase drug solubilisation within the intestinal fluids, as well as intestinal permeability and promoting intestinal lymphatic uptake (3, 4). To date, the majority of successful examples of enhanced *in vivo* bioavailability via a LBF approach have been demonstrated for poorly water-soluble drugs with highly lipophilic characteristics (i.e. high log*P*). Such drugs tend to display high solubility in lipid excipients which supports high dose loading in LBFs. However, poor solubility in water is not always reflective of a good solubility in lipid vehicles. Solubility in lipid vehicles can be solid state limited due to a strong crystal lattice, i.e. so called 'brick dust' molecules. For example, it has been reported that poorly water-soluble drugs with a melting point > 150 °C display an insufficient solubility in glycerides (5), and hence lipid vehicles, which limits the aforementioned advantage in terms of bypassing drug dissolution via a LBF. However, for 'brick dust' drugs displaying both hydrophobic and high lipophilic properties it remains unclear whether the additional bio-enabling effects of lipid excipients can increase oral bioavailability. For such challenging compounds exhibiting low aqueous and lipid solubility the formulation as LBF is often disregarded. Recently, formulation strategies such as designing lipophilic salts or supersaturated LBFs have been proposed as an approach to increase the drug loading in a

LBF solution (6, 7). However, LBF suspensions (8, 9) and concomitant administration of drug and LBF excipients (chase dosing) (10, 11) have also shown promise in terms of enhancing the *in vivo* exposure. In the latter chase dosing, crystalline drug and the lipid excipient (or LBF) are administered immediately following each other. This can be done *ad hoc* via gavaging in rodents an aqueous suspension followed by the drug-free lipid vehicle or as a capsule-in-capsule approach in humans or larger animals. Lipid and aqueous suspensions are particularly useful in a pre-clinical setting, offering a balance in terms of ease of administration in rodent models, and also the potential of the lipid excipient to increase *in vivo* exposure which is a key consideration for example in the early stage of toxicological drug evaluation. This is particularly relevant, as large resource investment in formulation design and optimisation in pre-clinical development is risky given the high attrition rate of drug candidates.

Lipid suspensions have shown potential to enhance oral bioavailability for a number of poorly water-soluble drugs including danazol, fenofibrate and griseofulvin (8, 9, 12, 13). For example, in the case of danazol, a bioavailability increase of 4-9-fold was observed when dosed in a Labrafil M2125CS suspension compared to an aqueous suspension (8). However, in the case of 'brick dust' drugs like atovaquone and nilotinib, lipid excipients did not improve the bioavailability (14, 15). Atovaquone shows a positive food effect, high lipophilicity, high hydrophobicity and has a limited solubility in medium chain (MC) triglycerides (TG) of ~ 4 mg/mL. The bioavailability of an atovaquone MC TG suspension was similar to the bioavailability of an aqueous suspension in humans (15). In the case of nilotinib, which is highly lipophilic and hydrophobic with a MC TG solubility of ~ 0.05 mg/mL and a food effect of approximately 80 % (increase in AUC after a high fat meal), a similar bioavailability for both MC and long chain (LC) monoglyceride (MG) suspensions was observed. Interestingly, for nilotinib there was a significantly reduced bioavailability observed in case of both MC and

LC TG suspensions compared to an aqueous suspension (14). In the latter study, it was hypothesized that the main factor for the poor *in vivo* performance of the lipid suspensions was a kind of entrapment of the nilotinib drug crystal by a persistent lipid film due to a low dispersibility of the TGs. Such an entrapment may have hampered the drug interactions with the post digestive colloids in the gastrointestinal tract and hence drug solubilisation. Consequently, it was suggested that the separation of the drug and the lipid excipient would avoid such entrapment. The current study was hence designed to assess whether the concomitant administration of nilotinib as an aqueous suspension and a drug free LBF (chase dosing) would influence oral bioavailability relative to administration of either an aqueous or lipid-based drug suspension.

Lipid co-administration or chase dosing has been shown to successfully enhance the bioavailability compared to an aqueous suspension or even lipid solution across different preclinical species and in humans (10, 11, 16). Additionally, co-administration avoids any risk of formulation or active pharmaceutical ingredient instability. Larsen et al. demonstrated in rats that for danazol, halofantrine and cinnarizine a co-administered lipid resulted in a similar bioavailability compared to a lipid solution, which was higher than the bioavailability of an aqueous suspension (10). In humans the co-administration of the commercial cinnarizine tablets with a placebo-LBF was tested in the fasted and fed state (11). In the fasted state the co-administration showed a tendency to increase the bioavailability compared to the commercialized tablets. In the fed state a decreased food effect was observed based on the individual profiles (11). In a different study the co-administration of carvedilol was studied in dogs (16). Co-administering carvedilol with a drug-free-LBF showed a similar bioavailability compared to a LBF solution and a higher bioavailability compared to an aqueous suspension (16).

Given that most research on LBFs is focused on a relatively limited number of model poorly water-soluble drugs, there is a need to study other more recently licenced drugs, such as the tyrosine kinase inhibitor nilotinib. Nilotinib is considered a 'brick dust' molecule based on its high lipophilicity (logP 4.95) and high melting point of 236 ºC and hence displays low solubility in lipid excipients. The present study investigated the hypothesis that nilotinib would benefit from co-administration with lipids rather than its direct formulation as a lipid suspension. To compare the results of the previously published lipid suspensions to the present chase dosing approach, the previously tested lipid excipients olive oil (LC TG), Captex 1000 (MC TG), Peceol (LC MG) and Capmul MCM (MC MG) were also evaluated *in vivo*.

2 Materials and methods

2.1 Chemicals and materials

Nilotinib and sorafenib were purchased from Kemprotec Ltd. (UK). Olive oil (LC TG), highly refined and low acidity was obtained from Sigma-Aldrich (Ireland). Capmul MCM® (MC MG) and Captex 1000® (MC TG) were kindly donated by Abitec corporation (USA). A sample of Peceol® (LC MG) was kindly donated by Gattefossé (France). All other chemicals and solvents were of analytical or high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

2.2 Formulations for *in vivo* studies

For the lipid co-administration, an aqueous suspension with 20 mg/mL nilotinib was prepared by adding 20 mg nilotinib to 1 mL of the aqueous 0.5 % (w/v) methylcellulose solution and mixed thoroughly. To decrease any powder aggregates, the suspension was placed in an

ultrasonic bath for 5 sec and vortexed again afterwards. For the lipid suspensions the lipid excipients were melted as needed and 10 mg/mL nilotinib was added and stirred overnight. To ensure a homogeneous distribution of nilotinib, all suspensions were stirred prior to administration.

The intravenous formulation was prepared by adding 2.5 mL Cremophor EL to 7.5 mL water for injection. The pH was adjusted to 2.0 and 0.5 mg/mL nilotinib was added to the solution and stirred until dissolved.

2.3 *In vivo* study

The protocol used for the *in vivo* pharmacokinetic study was approved by the institutional animal ethics committee in accordance with Belgian law regulating experiments on animals and in compliance with EC directive 2010/63/EU and the NIH guidelines on animal welfare. Male Sprague-Dawley rats weighing 250-320 g on the day of the experiments were purchased from Charles River Laboratories Deutschland (Sulzfeld, Germany) and maintained on standard food and water ad libitum in the laboratory for at least 5 days before entering the experiment. Food was removed 16-20 h before dosing and water was available ad libitum at all times. For the oral study arms, parallel groups of animals were administered an aqueous suspension at a volume of 1 mL/kg by oral gavage with a nilotinib dose of 20 mg/kg immediately followed by oral administration of the drug free lipid excipient at a volume of 2 mL/kg. For the intravenous part, animals were slowly injected 5 mL/kg of the intravenous formulation (2.5 mg/kg nilotinib) via the tail vein. 200 µL blood samples were collected into plasma collection tubes containing dipotassium EDTA by individual tail vein puncture. Samples were taken at 0.5, 1, 2, 4, 6, 8, 10 and 24 h following oral dosing and at 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 h following intravenous

dosing. Plasma was harvested immediately by centrifugation for 10 min at 4 °C and 1,900 × *g* and stored at -20 °C until analysis. After the experiment the animals were euthanized.

2.4 Bioanalysis

The plasma concentrations of nilotinib were determined by reversed phase HPLC. The Agilent 1260 series HPLC system comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was controlled, and the data analysed with EZChrom Elite version 3.3.2. The employed method was described previously (14). In brief, a Zorbax Eclipse Plus-C18 column (5 µm, 4.6 mm x 150 mm) with a Zorbax Eclipse Plus-C18 guard column (5 µm, 4.6 mm x 12.5 mm) was used. The mobile phase consisted of water, methanol, acetonitrile and triethylamine (34:30:35:1 v/v) and was used at a flow rate of 0.9 mL/min. The sample and column temperature were set at 5 °C and 25 °C, respectively, and the detection wavelength was 267 nm. Nilotinib was extracted from the plasma samples by liquid-liquid extraction. To 50 µL of the plasma sample 66 µL of a methanol acetonitrile mixture (30:35 v/v), containing 1.25 µg/mL sorafenib as internal standard, was added. The mixture was mixed thoroughly and centrifuged at 22 °C, 11,500 x g for 9 min. 50 µL of the supernatant was injected to the HPLC system for analysis. The lower limit of detection (LOD) and lower limit of quantification (LOQ) in plasma by this method was 11 ng/mL and 37 ng/mL, respectively, determined using the standard error of y-intercept according to ICH Q2 (17). Linearity was confirmed between 37 ng/mL and 4.1 µg/mL.

2.5 Deconvolution

Concentration-time-profiles were deconvoluted to obtain information about the absorption process as a *in vivo* absorption function over time. This was done in Microsoft Excel by means

of system analysis and required no compartmental pharmacokinetic modelling or curve fitting (18, 19). The response observed after oral administration of nilotinib, i.e. the plasma concentration time profile, can be treated as the response function R(t). Additionally, a weighting function W(t) is needed, which can be obtained from intravenous or oral bolus administration. The correlation between both functions R(t) and W(t) depends on the input and can be described by the following integral (18, 19):

$$R(t) = \int_0^t I(\vartheta) W(t-\theta) d\theta$$

where R, I and W are the response, input and weighing function, respectively. In this study, the weighing and response functions were known from the oral and intravenous plasma concentration profiles. The input function combines the information about the release and absorption process and was sought. The integral is also known as the convolution integral and can be written as follows:

$$R(t) = I(t) * W(t)$$

where * is the convolution operator. The process to obtain I(t) is called deconvolution and was described by Langenbucher et al. as follows (18):

$$I(x_i) = \frac{[\frac{R(t)}{T} - \sum_{k-1}^{n} I(x_k) * W(x_{n-k+1})}{W(x_i)}$$

where T is the time interval, I (x_k) the average input rate and W(x_k) the weight between the times X_{k-1} and X_k. Considering the sample time intervals, W(T) can be calculated as follows:

$$W(T) = e^{(m*T+n)}$$

where T is the time interval (t_2-t_1), m the slope of the intravenous data and n the intercept of the intravenous data. With the information of W(T), I(t) can be calculated as follows (18, 19):

$$I(t) = \frac{R(t)}{W(T)}$$

where I(t) is the sought deconvolved input function. The amount of drug released and absorbed is the cumulative input curve, i.e. the sum of I(t) up to every time point. This amount can be converted to % by setting the sum of all I(t) equal to 100 % drug absorbed. In this study the total absorbed amount was set equal to 100 % as well as the absolute bioavailability (F_{abs}) in order to obtain information about the extent and kinetics of the absorption process at the same time. Therefore, the % drug released and absorbed was calculated as follows:

$$\%\ drug\ released\ and\ absorbed = \frac{F_{abs}\ or\ 100\ \%}{\sum_{t\ last}^{0} I(t)} * \sum_{t}^{0} I(t)$$

2.6 Data Analysis

The pharmacokinetic parameters were calculated using Microsoft Excel. The plasma concentration profiles were analysed by non-compartmental analysis and calculation of each area under the curve (AUC) was based on the linear trapezoidal rule.

The statistical analysis was performed using a one-way analysis of variance (one-way ANOVA) after using the Bartlett's test to check for equal variance. The pairwise comparison of the groups was done using Tukey's multiple comparison test. All statistical analyses were based on GraphPad Prism 5.

3 Results

3.1 Long chain lipid-based formulations increased bioavailability of nilotinib

Nilotinib was orally dosed as an aqueous suspension followed by oral administration of the pure lipid excipients olive oil (LC TG), Captex 1000 (MC TG), Peceol (LC MG) or Capmul MCM (MC MG). Additionally, an intravenous formulation was administered to allow absolute bioavailability to be determined. The mean plasma concentration versus time profiles (after

oral administration) are presented in Figure 1 together with the relative bioavailability (F_{rel}) compared to the aqueous suspension. The mean plasma versus time profile for the intravenous formulation are shown in Figure 2. Table 1 presents a summary of the pharmacokinetic parameters obtained for all dosed formulations.

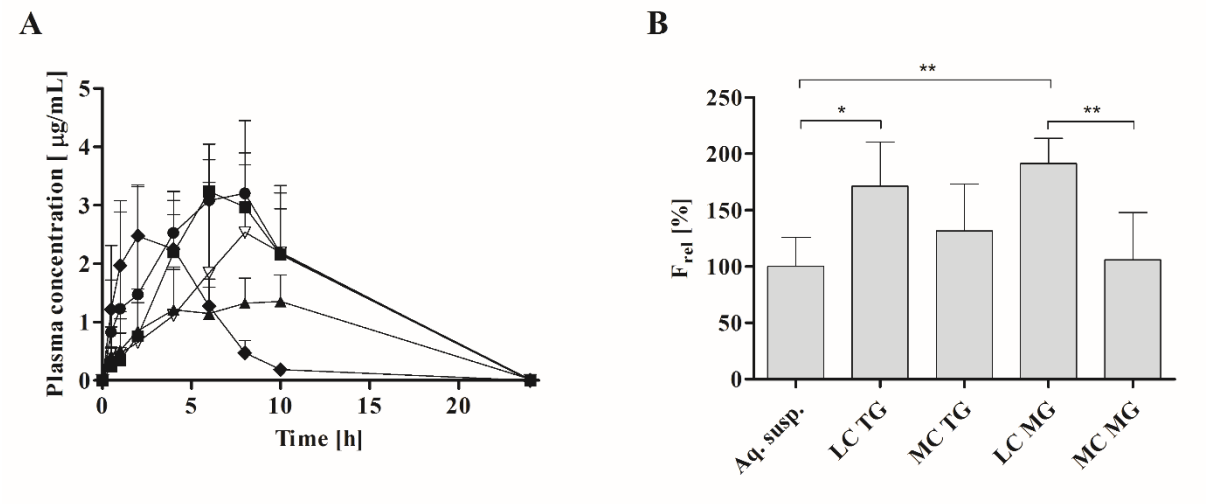


Fig. 1. A: Plasma concentration profiles as a function of time (mean ± SD, n = 5) for nilotinib aqueous suspension with a dose of 20 mg/kg co-administered with LC MG [Peceol] (●), LC TG [Olive oil] (■), MC MG [Capmul MCM] (▲) and MC TG [Captex 1000] (▽) in male Sprague-Dawley rats. Additionally, a nilotinib aqueous suspension (♦) with 20 mg/kg nilotinib in the same species previously reported (14) (mean ± SD, n = 5). B: Relative bioavailability of co-administered lipid excipients compared to the aqueous suspension previously published (14)

Co-administration of LC based LBFs resulted in a significant increase in nilotinib bioavailability relative to that obtained for an aqueous suspension alone ($p \leq 0.05$). The highest relative bioavailability was observed for the LC MG formulation (191 ± 22 %) followed by the LC TG vehicle (171 ± 39 %). Additionally, LC MG showed the highest c_{max} with

4.12 ± 0.67 µg/mL and the lowest variability for AUC and c_{max}. In the case of the MC formulations, a relative bioavailability of 131 ± 42 % for MC TG and 106 ± 42 % for MC MG was observed, which were not statistically different to the aqueous nilotinib suspension alone.

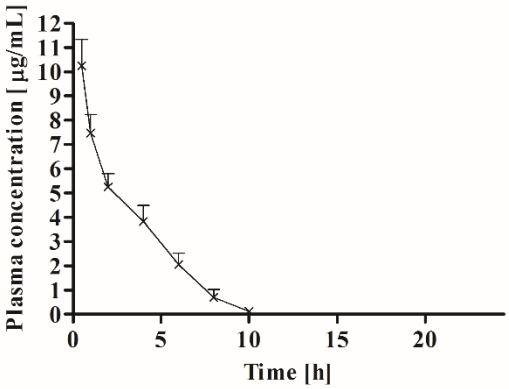


Fig. 2. Plasma concentration profiles as a function of time for an intravenous nilotinib formulation dosed at 2.5 mg/kg in male Sprague-Dawley rats (mean ± SD, n = 6).

Among the various lipid formulations evaluated, while there was a trend towards a lower bioavailability for the MC TG excipient, only the MC MG was significantly lower compared to LC MG ($p \leq 0.05$). The general ranking among the four formulations was LC MG ≥ LC TG ≥ MC TG ≥ MC MG. These results indicated that LC excipients performed better than MC excipients, which may be related to a long chain fatty acid mediated increase in bile salt release (20-22). Additionally, the results showed that *in vivo* exposure was not influenced by whether the LBF was administered as either a MG or a TG, when chase dosed.

Table I Pharmacokinetic parameters of an orally nilotinib aqueous suspension chase dosed with olive oil (LC TG), Captex 1000 (MC TG), Peceol (LC MG) and Capmul MCM (MC MG) and the corresponding lipid suspensions (14) as well as an aqueous suspension previously reported (14) (reanalysed with the intravenous data obtained in the present study). All oral parameters were obtained at a nilotinib dose of 20 mg/kg and n = 5. Pharmacokinetic parameters of an intravenous formulation were obtained with a nilotinib dose of 2.5 mg/kg and n = 6. All studies were performed in male Sprague-Dawley rats. MRT, MAT and t_{max} given as median (range), all other parameters as mean ± SD.

	Pharmacokinetic parameters					
	c_{max} [µg/mL]	t_{max} [h] (range)	AUC 0-inf. h [µg*h/mL]	MRT [h] (range)	MAT [h] (range)	F_{abs} [%]
Aq. susp. [a)]	2.65 ± 0.68	2 (2 - 4)	14.33 ± 3.71	4.14 (3.34 - 4.83)	2.49 (1.69 - 3.18)	12.90 ± 3.34
Lipid susp. Olive oil (LC TG) [a)]	0.61 ± 0.56	4 (2 - 6)	3.60 ±2.82	5.64 (4.42 - 6.42)	4.00 (2.78 - 4.77)	3.24 ± 2.54
Aq. susp., Olive oil (LC TG) (chase dosed)	3.48 ± 0.65	6 (4 – 8)	24.57 ± 5.65	7.87 (5.80 - 7.87)	6.22 (4.16 - 6.29)	22.11 ± 5.08
Lipid susp. Captex 1000 (MC TG) [a)]	0.77 ± 0.35	6 (4 - 10)	5.10 ± 2.35	6.18 (5.34 - 9.62)	4.54 (3.70 - 7.97)	4.58 ± 2.12
Aq. susp., Captex 1000 (MC TG) (chase dosed)	2.93 ± 1.09	8 (0.5 – 10)	18.86 ± 6.01	8.15 (5.47 - 8.72)	6.50 (3.82 - 7.07)	16.97 ± 5.41
Lipid susp. Peceol (LC MG) [a)]	2.80 ± 0.76	4 (4 - 6)	13.63 ± 2.70	5.29 (4.85 - 6.16)	3.64 (3.21 - 4.51)	12.26 ± 2.43

Aq. susp., Peceol (LC MG) (chase dosed)	4.12 ± 0.67	6 (2 - 8)	27.47 ± 3.22	7.25 (4.56 - 7.98)	5.60 (2.92 - 6.33)	24.72 ± 2.90
Lipid susp. Capmul MCM (MC MG) [a)]	1.32 ± 1.04	4 (1 - 6)	9.58 ± 5.50	5.78 (5.17 - 6.14)	4.13 (3.53 - 4.49)	8.62 ± 4.95
Aq. susp., Capmul MCM (MC MG) (chase dosed)	1.76 ± 0.47	8 (4 - 10)	15.18 ± 6.05	7.35 (5.49 - 8.24)	5.70 (3.85 - 6.59)	13.66 ± 5.44
intravenous	-	-	22.31 ± 4.50 [b)] 5.56 ± 1.19 [c)]	1.70 (1.19 - 2.39)	-	-

[a)] Data was previously reported (14) and re-analysed using intravenous data in the present study.

[b)] Dose [mg] corrected

[c)] Dose [mg/kg] corrected

3.2 Chase dosing leads to higher increases in bioavailability

The results of the chase dosing study conducted in the present work were compared to a previously published *in vivo* study in rats that investigated lipid suspensions for nilotinib (14). In both studies olive oil (LC TG), Captex 1000 (MC TG), Peceol (LC MG) and Capmul MCM (MC MG) were administered at the same dose and lipid volume. The previously reported aqueous and lipid nilotinib suspensions (14) were re-analysed using the intravenous data obtained in the present study. The absolute bioavailability (F_{abs}) is shown in Figure 3 and the pharmacokinetic parameters are summarised in Table 1.

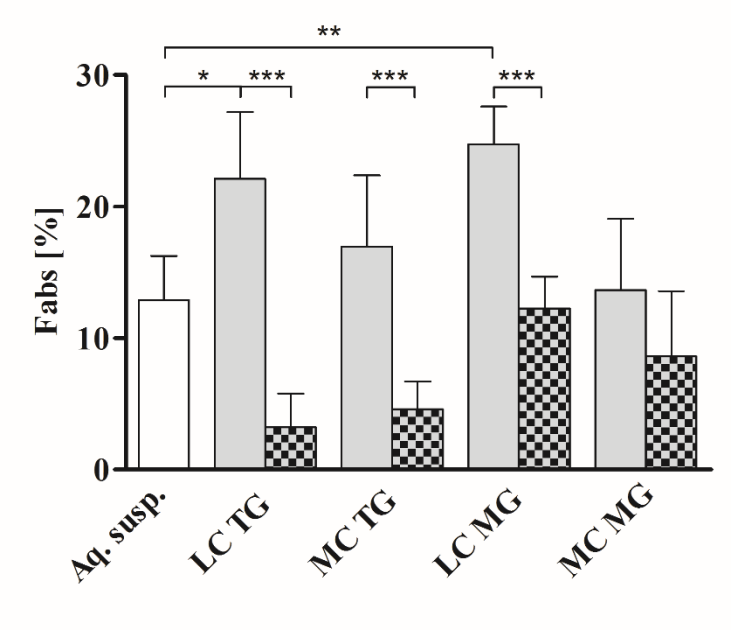


Fig. 3. *In vivo* absolute bioavailability of nilotinib co-administered with LC TG (olive oil), MC TG (Captex 1000), LC MG (Peceol) and MC MG (Capmul MCM) in male Sprague-Dawley rats (grey bars) in comparison to an aqueous suspension (white bar) and the four corresponding lipid suspensions (checked bars) in male Sprague-Dawley rats, which were reported previously. (14). All study legs are presented as mean ± SD, where n = 5.

In comparison to the lipid suspensions (14) the chase dosing approach of LC MG, LC TG and MC TG resulted in a statistically significant increase in the absolute bioavailability ($p \leq 0.05$). The largest increase was observed for LC TG, where the chase dosing increased the absolute bioavailability by approximately 22 %. This translates to a 6.8-fold increase in bioavailability, relative to the F_{abs} of 3 % for the LC TG suspension. For the MC TG and LC MG formulations, chase dosing increased bioavailability 3.7-fold and 2-fold, respectively, compared to the suspension approach. While MC MG displayed an increase of approximately 5 % (1.6-fold), the difference was not significant indicating that relative to this excipient, a lipid suspension approach already reached a reasonably high bioavailability. The rank order of bioavailability increases of the chase dosed formulations relative to the lipid suspensions (i.e. non chase dosed) was TG > MG (LC TG > MC TG > LC MG > MC MG). Therefore, the greatest impact of chase dosing was observed for the least dispersible formulations, when compared to the lipid suspensions. For more readily dispersible formulations, chase dosing offered a relatively lower increase. These findings supported the hypothesis from the previous study in that the lowest bioavailability was observed for the least dispersible formulations (14).

In the chase dosing study, the pharmacokinetic evaluation showed that the nilotinib absorption was prolonged. Relative to the lipid suspensions, there was a trend towards a prolonged time to reach the maximum plasma concentration (t_{max}), mean absorption time (MAT) and mean residence time (MRT). A consistent increase of 2 h in t_{max} from lipid suspensions to chase dosing was observed for LC TG, LC MG and MC TG. The same observation was made for MAT and MRT with an increase of approximately 2 h when the lipid excipient was chase dosed. For the MC MG excipient an increase of 4 h was observed for t_{max} and an increase of 2 h was observed for MRT, while MAT was only prolonged by 1 h. While these trends for prolonged absorption were not statistically significant, reflecting a high variability in rate of

absorption *in vivo*, the data suggested that the prolonged absorption may be a factor in explaining the improved bioavailability for chase dosing.

3.3 Deconvolution to explore differences in absorption

To further investigate the absorption from the tested formulations deconvolution was performed according to Langenbucher and Mysicka as described above (18). Using a numerical deconvolution technique, information about the *in vivo* drug absorption as a function of time was obtained. Deconvolution was calculated up to 8 h, which was the last plasma concentration within the limits of quantification of the bioanalysis. Normally, the cumulative integral for the last time point is set to 100 % drug absorbed (18, 19). Additionally, in this study, the cumulative integral for the last time point was set equal to the absolute bioavailability, as this approach provided insights on the extent and kinetics of the drug absorption process. The results of the deconvolution for the aqueous suspension, chase dosing and lipid suspensions of LC TG, LC MG, MC TG and MC MG, respectively, are shown in Figure 4. Additionally, Table 2 presents a summary of the time needed to absorb 25 % and 50 % of the maximum absorbed amount of nilotinib.

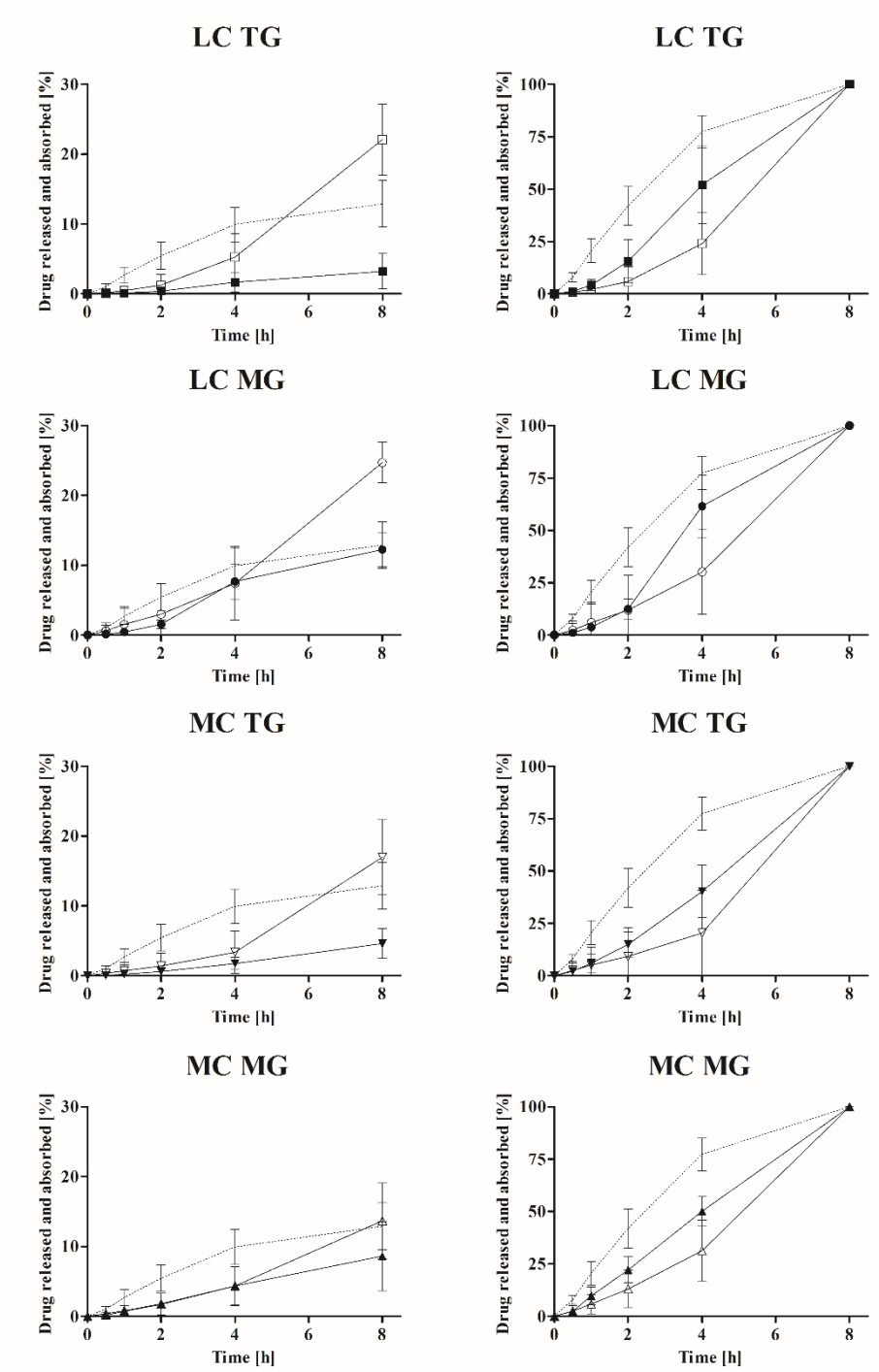


Fig. 4. Numerical deconvolution of the aqueous suspension (dotted line, no symbol), chase dosing (open symbol) and the lipid suspensions (filled symbol) for LC MG [Peceol] (●), LC TG [Olive oil] (■), MC MG [Capmul MCM] (▲) and MC TG [Captex 1000] (▼). On the left absolute bioavailability was set as maximum drug released and absorbed, on the right maximum drug absorption was set to 100 % (mean ± SD, n = 5).

All chase dosed excipients increased the absorption rate of nilotinib *in vivo* compared to the lipid suspensions between 4 and 8 h. The rate of absorption from the LC TG, MC TG and MC MG suspensions were linear from 0 – 8 h. While for LC MG suspension a linear rate of absorption was observed in the first 2 h after administration, the absorption rate increased between 2 and 4 h. In case of the chase dosed formulations, the absorption rate was lower compared to the lipid suspensions up to 4 h post-dosing. However, the rate of absorption increased between 4 and 8 h, which was in agreement with the observation of a longer MAT. This increased absorption rate was also evident in the absorbed extent of nilotinib, which distinctly increased 4 h post-dosing. Thus, the apparently slower initial absorption rate did not translate to an overall lower amount of nilotinib being absorbed. For example, in the case of LC MG the higher absorption rate for the lipid suspension between 2 and 4 h resulted in the same amount absorbed after 4 h compared to the chase dosing.

Table II Time to absorb 25 % and 50 % of the total fraction of absorbed drug for both lipid suspensions and chase dosed excipients with LC TG [olive oil], MC TG [Captex 1000], LC MG [Peceol] and MC MG [Capmul MCM] (mean ± SD, n = 5).

Time to absorb 25 % of the drug [h]		
	lipid suspension	chase dosed
LC TG	2.68 ± 0.78	3.97 ± 0.87
MC TG	2.92 ± 0.70	3.94 ± 1.46
LC MG	2.58 ± 0.39	3.49 ± 1.40
MC MG	2.20 ± 0.38	3.44 ± 1.00
Aq. susp.	1.24 ± 0.29	
Time to absorb 50 % of the drug [h]		
LC TG	3.99 ± 0.97	5.26 ± 0.71
MC TG	4.57 ± 0.69	5.29 ± 0.99
LC MG	3.28 ± 0.96	4.89 ± 1.22
MC MG	4.00 ± 0.47	4.97 ± 0.80
Aq. susp.	2.50 ± 0.43	

In contrast to the LBFs, where 50 % of drug was absorbed after 4 h, for the aqueous suspension the majority of drug was absorbed within the first 4 h. Within the first 4 h the aqueous suspension showed an absorption of approximately 10 % of the administered dose, which translates to approximately 77 % of the maximum absorbed amount. The remaining 3 % of the administered dose were absorbed between 4 and 8 h. In addition, the rate of absorption was linear and consistent throughout the absorption phase.

4 Discussion

LBFs have numerous biopharmaceutical benefits including the delivery of dissolved drug, a potential to promote lymphatic transport, improved intestinal permeability and most importantly an increased extent of *in vivo* solubilisation upon luminal dispersion and digestion of the formulation (3, 4). Many of the benefits of lipids in bio-enabling formulations are commonly demonstrated with drugs displaying high lipophilicity, i.e. 'grease ball' molecules, where the dose is soluble in the lipid vehicle to form a lipid solution. It has been reported that as a rule of thumb for a LBF approach a drug candidate should have a log*P* > 2 (23) and a melting point < 150 ºC (5). However, it is increasingly recognised that the hydrophobic characteristics of the drug merit consideration (24). For example, it has been reported that drugs with a melting point > 150 ºC may be less suited for formulation as lipid formulations, due to a high crystal lattice energy, which can lead to dose loading limitations in LBF (5). However, the merits of a LBF approach for drugs displaying both high hydrophobic and high lipophilic properties, may be theoretically limited due to the inability to dissolve drug in a reasonable lipid volume of the final dosage form. It remains unclear whether the additional biopharmaceutical benefits of lipid excipients for highly lipophilic drugs (e.g. *in vivo* solubilisation in post-digestive micellar fluids and/or increased permeability) may still lead to improved bioavailability. Therefore, for brick dust drugs, strategies focused on increasing dose loading in the lipid formulation have been reported such as supersaturated LBF (25, 26) or a lipophilic salt approach (6). Alternatively, the potential to administer a drug as a lipid suspension has been explored as a bio-enabling approach (8, 9, 14).

Nilotinib, a tyrosine kinase inhibitor used in the treatment of leukaemia that was licenced in 2007, shows a high lipophilicity (log*P* 4.95), but also a pronounced hydrophobicity.

Additionally, nilotinib showed a positive food effect (82 % increase in AUC after a high fat meal (27)) indicating a benefit from lipid excipients. For this high log*P* and melting point drug, solubility in lipid excipients was reported to be low (14). We have previously applied a lipid suspension approach to assess the impact of lipid excipients on the bioavailability of nilotinib in rats. However, despite high lipophilicity and a pronounced food effect, lipid suspensions based on MC and LC excipients did surprisingly not increase the bioavailability of nilotinib relative to an aqueous suspension. In the case of the TG formulations, bioavailability was even significantly reduced compared to an aqueous suspension (14). We hypothesised that the nilotinib particles were entrapped in the lipid excipient so that poor dispersion and a remaining lipid film on the particulate surface would kinetically hinder a favourable solubilization interaction with bile salts, phospholipids and other post-digestive components. Thus, a separation of drug and lipid excipient by chase dosing, i.e. a concomitant administration of a lipid, could prevent an entrapment and harness the aforementioned other *in vivo* benefits of lipid excipients.

In comparison to the lipid suspension results (14), the chase dosing resulted in a significant increase in nilotinib bioavailability for LC TG (7-fold), MC TG (4-fold) and LC MG (2-fold) ($p < 0.05$). The highest increase in bioavailability between the lipid suspensions and the chase dosing was observed for the TG formulations, whereas in the case of the MG formulations the difference was low or indeed in the case of MC MG no difference was observed. Therefore, in the case of poorly dispersible formulations chase dosing of the drug with the LBF is preferred to prevent a masking of the bio-enabling effects. In fact, the drug load in the formulation and the solubility in the lipid excipient was not limiting the bioavailability once the formulation was chase dosed. The chase dosing also confirmed the previously hypothesized hampered

dissolution due to an entrapment of the nilotinib particles by the vehicle of the lipid suspensions (14).

Chase dosing of the LBFs enhanced the bioavailability of nilotinib compared to the aqueous suspension and was in good agreement with previously published data (10, 11, 16). An improved bioavailability was reported for the co-administration of danazol (in rats), cinnarizine (in rats and humans), halofantrine (in rats) and carvedilol (in dogs) with different lipid excipients (10, 11, 16). The *in vivo* results also matched with the previously reported nilotinib solubility data in the post digestive state. Compared to fasted state simulated intestinal fluid (FaSSIF) the simulated LC TG and MC TG post digestion media had an approximately 17- and 22-fold higher solubilisation capacity indicating that digestion was vital for an increased solubilisation capacity. Also, Kaukonen et al. showed that the exposure to post digestive products for highly hydrophobic drugs was of importance, because the solubilizing capacity of the post digestion phase was much higher compared to the drug that could be dissolved in the lipid excipient (28). Therefore, a rapid *in vitro* screening tool with simulated post digestive media could be helpful in early screenings as a model to predict a higher *in vivo* exposure by co-administering lipids.

While the co-administration of LC based LBFs showed a statistically significant 2-fold increase compared to an aqueous suspension ($p < 0.05$), the MC based LBFs did not result in an increased bioavailability relative to the aqueous suspension. This fatty acid chain length effect was unexpected for nilotinib due to a higher solubility in MC dispersions pre- and post-digestion compared to LC using simulated post-digestive media and *in vitro* lipolysis (14). However, the results of this study were in good agreement with previously reported effects that long chain fatty acids (digestion products of tri- and diglycerides) with a chain length of C12

or higher are able to increase cholecystokinin (CCK) plasma concentrations in humans (20, 29). A similar effect of fatty acids with a chain length of C12 or higher was demonstrated *in vitro* using the mouse cell line STC-1, which is able to release CCK (21). Especially in the present study, where a lipid load of 2 mL/kg was used (resulting in approximately 0.23 – 0.57 mol/L free fatty acids released upon digestion, depending on the available gastrointestinal volume), a stimulation of CCK was likely. CCK is a peptide hormone of the gastrointestinal tract, which stimulates the release of digestive enzymes and bile, which may increase the solubilisation of poorly water-soluble drugs in the intestinal fluids. In the case of nilotinib, an increased solubility was observed in the presence of higher bile salt concentrations in fed state simulated intestinal fluid (FeSSIF) (3.2 ± 0.1 µg/mL) when compared to FaSSIF (0.3 ± 0.03 µg/mL) (14). The MC excipients in the present study had a chain length of C8 and C10 and were not suspected to increase CCK plasma levels, which may explain the observed differences between MC and LC based excipients *in vivo*. In addition, the results in this study are in line with previous reports for LC based LBFs (Labrafil) that were co-administrated with danazol and cinnarizine. Compared to an aqueous suspension the co-administration for both drugs showed a 2-fold increase in bioavailability in rats (10). It is also interesting to note that nilotinib displayed a pronounced increase in bioavailability in the fed state in humans, and while the possible reasons for this are varied, this study suggested that dietary LC lipids may have a role in the improved bioavailability in the post prandial state.

The results from deconvolution showed that the absorption of nilotinib from LBF suspensions appeared slower with less than 1 % of the total administered dose absorbed after 1 h. These results were in agreement with the *in vitro* lipolysis results reported for the lipid suspension study, where the total drug amount was below 1.1 % after 60 min of lipolysis (14). Also, the chase dosed LBFs showed a slow absorption with a total amount absorbed < 1 % after 1 h.

However, the deconvolution further showed a trend that the absorption from the chase dosed LBFs was prolonged. In the case of chase dosing, the initial 25 % and 50 % of nilotinib in the plasma was absorbed approximately 1 h later when compared to the initial 25 % and 50 % of nilotinib in plasma from the corresponding lipid suspensions. This kinetic advantage of the lipid suspensions did not result in an enhanced *in vivo* performance, but rather a similar or significantly decreased bioavailability. This difference most likely arose as a result of a decreased release of nilotinib from the LBF suspensions due to the compound's high lipophilicity. The previously reported greater wettability of nilotinib crystals by lipid vehicles compared to aqueous vehicles suggested pronounced hydrophobic interactions between the lipids and nilotinib crystals (14). We hypothesised that these interactions favoured the formation of a lipid film around the nilotinib crystals, which remained intact even after dispersion in aqueous media and during the initial phases of digestion, and hence may be the most important contributing factor to the poor *in vivo* performance of the lipid suspensions.

Administering LBFs resulted in a delayed absorption relative to the aqueous suspension. This was also evident by a longer t_{max} and MAT for the LBFs. A reason for the delayed absorption with lipid excipients may be the delayed gastric emptying. It was reported that the half-life ($t_{1/2}$) of gastric emptying was approximately 77 min after a high caloric liquid meal in rats (30). Lipid excipients mimic fed state conditions (31) and may have delayed the gastric emptying when compared to the aqueous suspension, which should not affect gastric emptying. An additional factor contributing to a slower initial absorption may be digestion of the lipid excipients. This was especially prominent for the co-administered LBFs with LC TG, LC MG and MC TG. Despite the delayed initial absorption, over the next 4 h, extensive absorption was observed, which resulted in an overall increase in bioavailability compared to the aqueous suspension. This suggested that the access to post-digestive products of the lipid excipients as

well as to bile salts and phospholipids was more readily available for the chase dosed LBFs. It seemed that the MC MG was not able to generate a solubility increasing environment, which in part may be due to a very rapid digestion (14) and subsequent absorption of the excipient resulting in the same performance as the aqueous suspension.

5 Conclusion

The present study investigated the potential merits of lipid excipients to increase bioavailability of nilotinib, a 'brick dust' drug, by applying a chase dosing approach. Chase dosing of LBF was found to increase the bioavailability by up to 7-fold compared to the individual lipid suspensions. Also using this chase dosing approach, a significant bio-enabling effect of LC was observed with a 2-fold increased exposure compared to a non-lipid aqueous suspension. This study showed that the drug load in the lipid formulations may not be limiting in the context of the bio-enabling benefits of LBFs. The *in vivo* study also revealed that LC excipients performed better than MC excipients. Additionally, the study confirmed that dosing lipid suspensions of poorly dispersible LBF excipients like TGs should be avoided, whereas chase dosing of lipid excipients appears to be a viable approach to (a) explore the bio-enabling effects of lipid excipients and (b) overcome dispersibility issues of lipid suspensions for hydrophobic and/or lipophilic drugs.

Acknowledgements

This work was kindly supported by funding from the Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks programme under grant agreement No. 674909. The authors are part of the PEARRL European Training network, which is funded under this MSCA programme.

References

1. Savla R, Browne J, Plassat V, Wasan KM, Wasan EK. Review and analysis of FDA approved drugs using lipid-based formulations. Drug Dev Ind Pharm. 2017:1-16.
2. Feeney OM, Crum MF, McEvoy CL, Trevaskis NL, Williams HD, Pouton CW, Charman WN, Bergstrom CA, Porter CJ. 50years of oral lipid-based formulations: Provenance, progress and future perspectives. Adv Drug Deliv Rev. 2016;101:167-194.
3. O' Driscoll CM, Griffin BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility--the potential impact of lipid-based formulations. Adv Drug Deliv Rev. 2008;60(6):617-624.
4. Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliver Rev. 1997;25(1):103-128.
5. Alskar LC, Porter CJ, Bergstrom CA. Tools for Early Prediction of Drug Loading in Lipid-Based Formulations. Mol Pharm. 2016;13(1):251-261.
6. Morgen M, Saxena A, Chen XQ, Miller W, Nkansah R, Goodwin A, Cape J, Haskell R, Su C, Gudmundsson O, Hageman M, Kumar A, Chowan GS, Rao A, Holenarsipur VK. Lipophilic salts of poorly soluble compounds to enable high-dose lipidic SEDDS formulations in drug discovery. Eur J Pharm Biopharm. 2017;117:212-223.
7. Sahbaz Y, Williams HD, Nguyen TH, Saunders J, Ford L, Charman SA, Scammells PJ, Porter CJ. Transformation of poorly water-soluble drugs into lipophilic ionic liquids enhances oral drug exposure from lipid based formulations. Mol Pharm. 2015;12(6):1980-1991.
8. Larsen A, Holm R, Pedersen ML, Mullertz A. Lipid-based Formulations for Danazol Containing a Digestible Surfactant, Labrafil M2125CS: In Vivo Bioavailability and Dynamic In Vitro Lipolysis. Pharm Res-Dordr. 2008;25(12):2769-2777.

9. Dahan A, Hoffman A. The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats. Eur J Pharm Biopharm. 2007;67(1):96-105.

10. Larsen AT, Holm R, Mullertz A. Solution or suspension - does it matter for lipid based systems? In vivo studies of chase dosing lipid vehicles with aqueous suspensions of a poorly soluble drug. Eur J Pharm Biopharm. 2017;117:308 - 314.

11. Christiansen ML, Holm R, Abrahamsson B, Jacobsen J, Kristensen J, Andersen JR, Mullertz A. Effect of food intake and co-administration of placebo self-nanoemulsifying drug delivery systems on the absorption of cinnarizine in healthy human volunteers. Eur J Pharm Sci. 2016;84:77-82.

12. Thomas N, Richter K, Pedersen TB, Holm R, Mullertz A, Rades T. In vitro lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery systems containing fenofibrate. Aaps J. 2014;16(3):539-549.

13. Carrigan PJ, Bates TR. Biopharmaceutics of Drugs Administered in Lipid-Containing Dosage Forms .1. Gi-Absorption of Griseofulvin from an Oil-in-Water Emulsion in Rat. J Pharm Sci-Us. 1973;62(9):1476-1479.

14. Koehl NJ, Holm R, Kuentz M, Griffin BT. New Insights into Using Lipid Based Suspensions for "Brick Dust' Molecules: Case Study of Nilotinib. Pharm Res-Dordr. 2019;36(4).

15. Rolan PE, Mercer AJ, Weatherley BC, Holdich T, Meire H, Peck RW, Ridout G, Posner J. Examination of some factors responsible for a food-induced increase in absorption of atovaquone. Br J Clin Pharmacol. 1994;37(1):13-20.

16. Alskar LC, Parrow A, Keemink J, Johansson P, Abrahamsson B, Bergstrom CAS. Effect of lipids on absorption of carvedilol in dogs: Is coadministration of lipids as efficient as a lipid-based formulation? Journal of Controlled Release. 2019;304:90-100.

17. ICH Guideline. Validation of analytical procedures: text and methodology Q2 (R1). In.International conference on harmonization, Geneva, Switzerland: ICH harmonised tripartite guideline; 2005.

18. Langenbucher F, Mysicka J. Invitro and Invivo Deconvolution Assessment of Drug Release Kinetics from Oxprenolol Oros Preparations. Brit J Clin Pharmaco. 1985;19:S151-S162.

19. Henze LJ, Griffin BT, Christiansen M, Bundgaard C, Langguth P, Holm R. Exploring gastric emptying rate in minipigs: Effect of food type and pre-dosing of metoclopramide. Eur J Pharm Sci. 2018;118:183-190.

20. McLaughlin J, Lucà MG, Jones MN, D'Amato M, Dockray GJ, Thompson DG. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. Gastroenterology. 1999;116(1):46-53.

21. McLaughlin JT, Lomax RB, Hall L, Dockray GJ, Thompson DG, Warhurst G. Fatty acids stimulate cholecystokinin secretion via an acyl chain length-specific, Ca2+-dependent mechanism in the enteroendocrine cell line STC-1. J Physiol. 1998;513 (Pt 1):11-18.

22. Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6(3):231-248.

23. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29(3-4):278-287.

24. Ditzinger F, Price DJ, Ilie AR, Kohl NJ, Jankovic S, Tsakiridou G, Aleandri S, Kalantzi L, Holm R, Nair A, Saal C, Griffin B, Kuentz M. Lipophilicity and hydrophobicity considerations in bio-enabling oral formulations approaches - a PEARRL review. J Pharm Pharmacol. 2018.

25. Thomas N, Holm R, Garmer M, Karlsson JJ, Mullertz A, Rades T. Supersaturated self-nanoemulsifying drug delivery systems (Super-SNEDDS) enhance the bioavailability of the poorly water-soluble drug simvastatin in dogs. Aaps J. 2013;15(1):219-227.

26. Thomas N, Holm R, Mullertz A, Rades T. In vitro and in vivo performance of novel supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS). J Control Release. 2012;160(1):25-32.

27. FDA. Nilotinib (Tasigna) - Clinical Pharmacology and Biopharmaceutics Review. U.S. Department of Health and Human Services. 2018 October 01. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068toc.cfm

28. Kaukonen AM, Boyd BJ, Charman WN, Porter CJH. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. Pharm Res-Dordr. 2004;21(2):254-260.

29. Feinle C, Grundy D, Fried M. Modulation of gastric distension-induced sensations by small intestinal receptors. Am J Physiol Gastrointest Liver Physiol. 2001;280(1):G51-57.

30. Qualls-Creekmore E, Tong M, Holmes GM. Gastric emptying of enterally administered liquid meal in conscious rats and during sustained anaesthesia. Neurogastroenterol Motil. 2010;22(2):181-185.

31. O'Shea JP, Holm R, O'Driscoll CM, Griffin BT. Food for thought: formulating away the food effect - a PEARRL review. J Pharm Pharmacol. 2018.