CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib
Authors	Koehl, Niklas J.;Holm, René;Kuentz, Martin;Jannin, Vincent;Griffin, Brendan T.
Publication date	2020-07-10
Original Citation	Koehl, N. J., Holm, R., Kuentz, M., Jannin, V. and Griffin, B. T. (2020) 'Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib', Molecular Pharmaceutics, 17(9), pp. 3202-3213. doi: 10.1021/acs.molpharmaceut.0c00305
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1021/acs.molpharmaceut.0c00305
Rights	© 2020, American Chemical Society. This document is the Accepted Manuscript version of a Published Work that appeared in final form in Molecular Pharmaceutics, after technical editing by the publisher. To access the final edited and published work see https://pubs.acs.org/doi/abs/10.1021/acs.molpharmaceut.0c00305
Download date	2026-11-01 14:13:26
Item downloaded from	https://hdl.handle.net/10468/10661



UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib

Niklas J. Koehl[1], René Holm[2,3], Martin Kuentz[4], Vincent Jannin[5, †], 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

[5]Gattefossé SAS, 36 Chemin de Genas, 69804, Saint-Priest Cedex, France

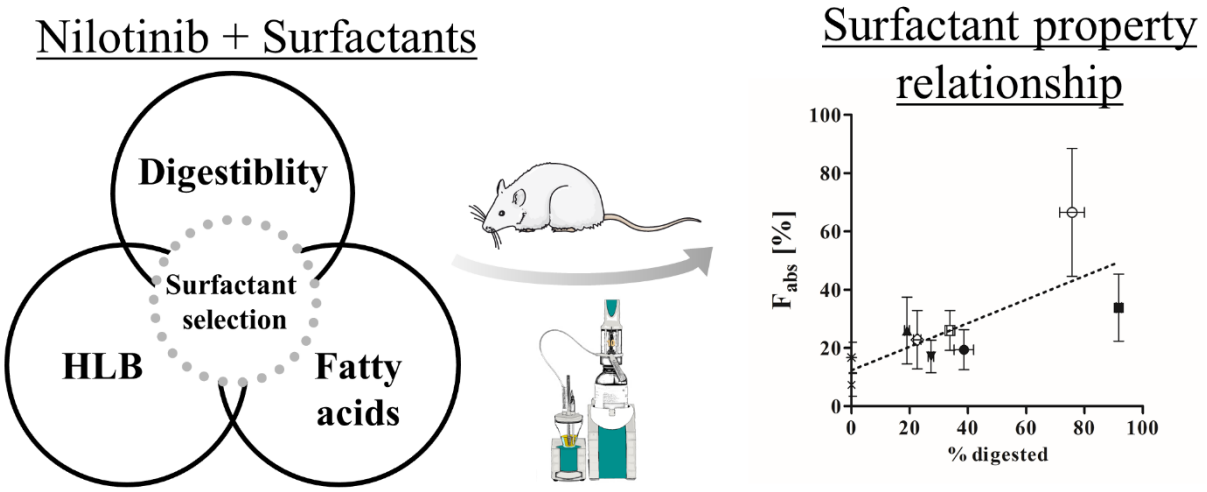
[†]Current address: Lonza Pharma & Biotech, Parc d'Innovation, Rue Tobias Stimmer – BP 30442–67412 Illkirch Graffenstaden, France

*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

TABLE OF CONTENT



ABSTRACT

The scientific rational for selection of surfactant type during oral formulation development requires an in-depth understanding of the interplay between surfactant characteristics and biopharmaceutical factors. Currently, however, there is a lack of comprehensive knowledge of how surfactant properties, such as hydrophilic-lipophilic balance (HLB), digestibility and fatty acid (FA) chain length, translate into *in vivo* performance. In the present study, the relationship between surfactant properties, *in vitro* characteristics and *in vivo* bioavailability was systematically evaluated. An *in vitro* lipolysis model was used to study the digestibility of a variety of non-ionic surfactants. Eight surfactants and one surfactant mixture were selected for further analysis using the model poorly water-soluble drug nilotinib. *In vitro* lipolysis of all nilotinib formulations was performed followed by an *in vivo* pharmacokinetic evaluation in rats. The *in vitro* lipolysis studies showed that medium chain FA based surfactants were more readily digested compared to long chain surfactants. The *in vivo* study demonstrated that a Tween 20 formulation significantly enhanced the absolute bioavailability of nilotinib up to 5.2-fold relative to an aqueous suspension. In general, surfactants that were highly digestible *in*

vitro tended to display higher bioavailability of nilotinib *in vivo*. The bioavailability may additionally be related to the FA chain length of digestible surfactants with an improved exposure in the case of medium chain FA based surfactants. There was no apparent relationship between the HLB value of surfactants and the *in vivo* bioavailability of nilotinib. The impact of this study's findings suggests that when designing surfactant-based formulations to enhance oral bioavailability of the poorly water-soluble drug nilotinib, highly digestible, medium chain-based surfactants are preferred. Additionally, for low permeability drugs such as nilotinib, which is subject to efflux by intestinal P-glycoprotein, the biopharmaceutical effects of surfactants merit further consideration.

KEYWORDS

Surfactants; Digestibility; Nilotinib; Poorly water-soluble drugs; Suspension; Lipolysis

ABBREVIATIONS

AUC, area under the curve; CMC, critical micellar concentration; CYP, cytochrome P450; FA, fatty acid; FFA, free fatty acids; HLB, hydrophilic-lipophilic balance; P-gp, P-glycoprotein

INTRODUCTION

Many emerging drug candidates show poor solubility and/or permeability resulting in a low and variable oral bioavailability when administered in conventional dosage forms. [1] Therefore, there is a need to develop bio-enabling formulation technologies that enhance biopharmaceutical properties and improve oral absorption of these emerging drug candidates. [2] The various bio-enabling approaches have been extensively reviewed including solid dispersions, [3, 4] lipid-based formulations [5] and nano-sized drug crystals/particles. [6] Interestingly, one of the most common excipients included across most classes of bio-enabling approaches are surfactants, which, from a mechanistic perspective, can impart a variety of biopharmaceutical advantages including promoting supersaturation, [7, 8] enhancing solubilisation, [9] stabilisation of colloidal/nano-crystals, [10] increased dissolution rate [11] and increasing permeability. [12-16]

Surfactants can be classified according to the polar head group into ionic (cationic, anionic or zwitterionic) or non-ionic surfactants. [17] Non-ionic surfactants are considered favourable due to a low toxicity and a more readily maintained solubilising power under biorelevant conditions. [18] The most commonly used non-ionic surfactants are based on ethylene oxide / polyoxyethylene and referred to as ethoxylated surfactants e.g. sorbitan ester ethoxylates or fatty amine ethoxylates. [19] Other important non-ionic surfactant classes include polyol-based surfactants (e.g. glycoside, glycol or glycerol esters), amine oxides and sulfinyl surfactants. [19] Ethoxylated and polyol surfactants can be further sub-classified into esters (e.g. glycol, glycerol, sorbitan, fatty acid ethoxylates) and ethers (e.g. poloxamers, ethoxylated fatty alcohol, alkyl phenol ethoxylates). For pharmaceutical applications ethoxylated and polyol esters and ethers such as Tweens (ethoxylated sorbitan esters) or Poloxamers (ethylene oxide-propylene oxide copolymers) are widely utilised. As an excipient class, surfactants can exhibit

a diverse set of properties and characteristics, and hence a variety of physiochemical approaches have been applied for characterising surfactants. These include properties such as hydrophilic-lipophilic balance (HLB) value, molecular weight, chain length, molecular volume, critical micellar concentration (CMC), solubility parameters. [20] While many of these properties have been explored for a specific formulation approach, to date the selection of surfactants remains mostly empirically driven. Additionally, while many surfactants are derived from digestible fats and oils, the impact of digestion on surfactants is commonly not considered in formulation performance. However, digestibility of surfactants can impact many of the aforementioned physico-chemical properties of the excipient, as the chemical structure can change along the transit though the GIT. Furthermore, this illustrates the difficulty in developing a reliable surfactant classification system as properties of the surfactants may be influenced by *in vivo* conditions. [18] The present study aimed to address knowledge gaps in the literature on the relationships between surfactant properties and the biopharmaceutical performance *in vivo*. This study, therefore, provides a basis for establishing a performance-based classification of surfactants in oral drug delivery.

In addition to the solubility enhancing effects, surfactants can interact with lipid bilayer of cell membranes thereby increasing the permeability. [12] Furthermore, surfactants may influence pre-systemic clearance of drugs by modulating transporters and metabolising enzymes. [13] For example, Tween 80 and Cremophor EL increased the uptake of digoxin, a P-glycoprotein (P-gp) substrate, to the same extent as cyclosporin (a commonly used P-gp inhibitor), using the rat everted gut sac method. [14] This indicated that Cremophor EL and Polysorbate 80 can modulate P-gp to improve the bioavailability. Additionally, Pluronic F68, Labrasol, Brij 30 and Tween 20 have also shown to be inhibitors of P-gp *in vitro* using the rat everted gut sac method or various cell lines. [15, 16] In addition to modulation of P-gp efflux activity, a number

of surfactants such as Tween 20, Cremophor EL, Pluronic F68 and Myrj S40 have demonstrated inhibitory effects on cytochrome P450 3A4 (CYP 3A4), [21] a key metabolic enzyme present in intestinal tissue. In the latter study, it was shown that among the tested surfactants Tween 20 showed the highest inhibition resulting in a significantly increased area under the curve (AUC) and a 40% decrease in AUC of the main metabolite of midazolam in rats. [21]

When considering the choice of digestible versus non-digestible surfactants, a key perceived advantage of non-digestible surfactants is in being 'digestion-independent' systems, and therefore *in vitro* characteristics can be readily employed to predict the likely performance *in vivo*. Additionally, digestible systems carry the risk that the surfactant may not serve the initial purpose in a post-digestive state. For example, digestion of surfactants may lead to a reduced solubilisation capacity of the colloidal aqueous dispersion and potentially lead to drug precipitation. However, studies have also suggested that digestion may in fact promote transient supersaturation in the intestinal media and therefore may promote absorption. [22] In addition, the released FA have shown to increase solubility of poorly soluble drugs as evidenced by *in vitro* measurements of solubility in assembled pre- and post-digestion media with lipid excipients. [23, 24] Moreover, free fatty acids (FFA) released post digestion may modulate the intestinal permeability. In several studies it was shown that the treatment of cells with medium chain FFA showed an increased paracellular transport *via* tight junction opening. [25-30] In the case of unsaturated long chain FFA, *in vitro* cell experiments showed an increased membrane fluidity [31-33] as well as opened tight junctions. [26, 34] These biopharmaceutical effects can further enhance drug absorption from surfactant formulations.

There is currently a lack of comprehensive knowledge of how surfactant properties translate into *in vivo* bioavailability of poorly water-soluble drugs. The overarching goal of this study was to systematically explore the relationship between surfactant properties and *in vivo* performance, which will support a more science- and risk-based approach to surfactant selection in oral formulations. Nilotinib was chosen as a model poorly water-soluble drug for the study. Nilotinib is practically insoluble in buffer solutions of pH 4.5 and higher (pKa 2.1 and 5.4), [35] is highly lipophilic (log*P* 4.95) as well as hydrophobic [24] and exhibits a moderate permeability across a confluent Caco-2 cell monolayer. [35, 36] Therefore, nilotinib was categorised as a class IV compound in the biopharmaceutical classification system (BCS). Nilotinib is predominantly metabolised by CYP 3A4 [35] and is a P-gp substrate. [35-38] The pre-systemic clearance is high with an AUC increase of 29% after co-administration of nilotinib with grapefruit juice (intestinal CYP 3A4 inhibitor). [39] The current commercial formulation, Tasigna®, is a capsule formulation containing the surfactant Pluronic F68 (poloxamer 188). [40] The concentration and the scientific rational of the addition of Poloxamer 188 is unknown, however it has been reported that the use of surfactants did not increase the dissolution of nilotinib capsules at pH 4.5 and above. [36] The marketed formulation of nilotinib showed an absorption of ≥ 30% following a radiolabelled single 400 mg oral dose in humans. [41] Additionally, preclinical studies in rats yielded an absolute bioavailability of 34% using a solution with Cremophor, dimethyl acetamide and 5% dextrose (20/10/70 (v/v/v)). [35] Using this as a model poorly water-soluble drug, the present study systematically assessed the impact of surfactant properties on oral bioavailability. Surfactants were classified according to FA chain length, HLB value and digestibility and their *in vitro* and *in vivo* performance in rats was investigated.

MATERIALS AND METHODS

Chemicals and materials

Nilotinib and sorafenib were purchased from Kemprotec Ltd. (UK). Brij O2 (Polyoxyethylene (2) oleyl ether), Brij L23 (Polyoxyethylene (23) lauryl ether), Myrj S40 (Polyoxyethylene (40) stearate), Span 85 (Sorbitan trioleate), Span 80 (Sorbitan monooleate), Tween 80 (Polyoxyethylene sorbitan monooleate) and Tween 20 (Polyoxyethylene sorbitan monolaurate) were kindly donated by Croda international Plc (UK). Lipoid E PC S (Phosphatidylcholine) was gifted by Lipoid GmbH (Germany). Taurodeoxycholic acid sodium salt (NaTDC), pancreatic lipase (8 x USP), Cremophor RH40 (Polyoxyl 40 hydrogenated castor oil) and Tween 85 (Polyoxyethylene sorbitan trioleate) were ordered from Sigma-Aldrich (Ireland). A sample of Labrasol (Caprylocaproyl polyoxyl-8 glycerides), Labrafil M1944 CS (Oleoyl polyoxyl-6 glycerides), Labrafil M2125 CS (Linoleoyl polyoxyl-6 glycerides), Plurol Oleique CC 497 (Polyglyceryl-3 dioleate) and Gelucire 44/14 (Lauroyl polyoxyl-32 glycerides) was kindly donated by Gattefossé (France). A sample of Cremophor EL (Polyoxyl 35 castor oil) was kindly donated by BASF (Germany). 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.

Solubility studies

Equilibrium solubility at 37 °C was determined in Tween 20, Tween 85, Labrasol, Labrafil M1944CS, Span 80, Cremophor RH40, Brij O2, Brij L23, and in a Tween 85/Cremophor RH40 mixture (67:33 w/w) in order to represent the formulations that were used during *in vitro* lipolysis and the *in vivo* study. Solid excipients were melted at 50 °C and cooled to 37 °C, while liquid surfactants were heated to 37 °C before addition of excess nilotinib. The suspensions were stirred at 250 rpm and 37 °C. Samples were taken after 24 h, 48 h, 72 h and centrifuged

at 21,380*g* and 37 °C for 15 min (Mikro 200 R, Hettich GmbH, Germany). The supernatant was transferred to a new tube and centrifuged again under identical conditions. To solubilise the surfactants, the supernatant was diluted in acetonitrile, ethyl acetate (1:4, v/v). Followed by further 1:10 (v/v) dilution with acetonitrile, ethyl acetate (4:1). The obtained samples were diluted appropriately with mobile phase before analysis by reverse phase HPLC. Equilibrium was assumed once two time-points did not differ more than 10%. All samples were run in triplicates.

The samples were analysed as described previously. [24] In brief, an Agilent 1200 series HPLC system comprised a binary pump, degasser, autosampler and variable wavelength detector. Data analysis was done with EZChrom Elite version 3.2. Nilotinib was separated with a Zorbax Eclipse Plus-C18 column (5 μm, 4.6 mm x 150 mm) including a Zorbax Eclipse Plus-C18 guard column (5 μm, 4.6 mm x 12.5 mm) at 25 °C. The mobile phase consisted of acetonitrile, methanol, water and triethylamine (35:30:34:1 v/v) and was used at a flow rate of 0.9 mL/min. 20 μL samples were injected and the detection wavelength was 267 nm. The limit of detection (LOD) and limit of quantification (LOQ) of this method was 4 ng/mL and 12 ng/mL, respectively. Values were determined using the standard error of y-intercept according to the ICH Q2 guideline [42] and linearity was confirmed between 12 ng/mL and 12 μg/mL drug concentration.

In vitro lipolysis: Digestibility and drug solubilisation during formulation dispersion and digestion

In vitro lipolysis was studied using a pH-stat apparatus (Metrohm AG, Herisau, Switzerland) comprising a Titrando 907 stirrer, 804 Ti-stand, a pH electrode (Metrohm, Herisau, Switzerland) and two 800 Dosino dosing units coupled to a 20 mL autoburette. The system was

operated by the Tiamo 2.2 software. The *in vitro* protocol was amended from Williams *et al.* [43, 44] In brief, the buffer contained 2 mM TRIS maleate, 150 mM NaCl, 1.4 mM $CaCl_2 \cdot 2H_2O$, adjusted to pH 6.5. For the digestion experiments the buffer was supplemented with 3 mM NaTDC and 0.75 mM PC and stirred for 12 hours before further usage. The pancreatin extract was prepared freshly by adding 5 mL of 5 °C buffer to 1 g of porcine pancreatic enzymes (8x USP), which was vortexed thoroughly. The mixture was centrifuged for 15 min at 5 °C, 2800*g* (Hettich Rotina 380R) and 4 mL of supernatant was recovered and stored at 2 – 8 °C before further usage. The pancreatic extract had a pancreatic lipase activity of ~10 000 TBU/mL (to provide approximately 1000 TBU per mL of digest), where 1 TBU represents the amount of enzyme that liberates 1 µmol of FA from tributyrin per min. [45] All experiments were conducted with a stirring speed of 450 rpm.

For the digestibility study, 1.0 g of blank excipient was dispersed into 36 mL of digestion buffer. The pH was adjusted to 6.5 using 0.2 M, 0.6 M or 1 M NaOH depending on the pH change upon excipient addition. Digestion was initiated by the addition of 4 mL pancreatic enzyme and the pH of 6.5 was maintained using 0.2 M and 0.6 M NaOH for long and medium chain excipients, respectively. After 60 min of digestion the enzymes were inhibited using 1 M 4- bromophenylboronic acid in methanol (5 µL per mL media) and the pH was increased to 9.0. An additional blank titration using solely the digestion buffer was performed and the released mmol of FFAs from the blank was subtracted from the mmol of FFAs released from the surfactant formulations. The determined amount of FFA was assumed as a surrogate parameter for digestibility. Additionally, the % digested was calculated using the theoretical released FFAs per g of excipient:

$$Theoretical\ FFA\ [mmol] = \frac{SV\ [mg]}{56.1056\ [\frac{g}{mol}]} \quad (1)$$

where FFA are the FFAs that can theoretically be released from the excipient in mmol per g of excipient, SV the saponification value in mg KOH per g of excipient from the certificate of analysis and 56.1056 g/mol the molecular weight of KOH. The absolute amount of theoretical possible released mmol FFA can be calculated by multiplying by the amount of excipient used in this study, i.e 1.0 g. The % digested can be calculated as follows:

$$\%\ digested = \frac{Released\ FFA\ [mmol]}{Theoretical\ FFA\ [mmol]} \times 100\ \% \qquad (2)$$

where the released FFA are the total mmol of FFA released in the digestibility experiment (including the amount detected during back titration to pH 9.0) and theoretical FFA the total mmol of FFA calculated with equation 1.

For the *in vitro* lipolysis experiment with nilotinib 1.075 g of lipid formulation was dispersed into 39 mL of digestion buffer for 10 min. Three 1 mL samples were taken at 2.5, 5 and 10 min from the middle of the vessel. pH of the media was adjusted and maintained at 6.5 using 0.2 M NaOH and 0.6 M NaOH for medium and long chain excipients, respectively. To the remaining 36 mL (1 g lipid formulation) dispersion 4 mL of pancreatin extract was added to initialize digestion. After 60 min the released non-ionized FFAs were determined by a pH increase of the buffer to pH 9.

Samples of 1 mL were taken at 5, 10, 15, 30, 45 and 60 min during the digestion experiment from the middle of the vessel. In each sample and after 60 min the enzymes were inhibited by the addition of 1 M 4-bromophenylboronic acid in methanol (5 µL per mL sample). Additionally, to each 1 mL sample during digestion a 100 µL sample was taken and added to 900 µL of acetonitrile and methanol (35:30 v/v) and mixed. This sample was used to quantify

256 the total drug recovery, which allowed adjustments of inhomogeneous samples. All samples
257 were centrifuged at 37 °C and 21,000*g* for 30 min (Hettich Micro 200R).
258
259 *Formulations for in vivo and in vitro studies*
260 The solubility of nilotinib in the studied surfactants was low resulting in high dose solubility
261 ratios (Table 1) and therefore it was not possible to prepare surfactant solutions at the target
262 dose. It was hence decided to use surfactant suspensions for all experiments, keeping the
263 surfactant and dose load constant. The surfactant suspensions were prepared by combining 10
264 mg nilotinib with 1 mL surfactant excipient followed by an over-night stir at 37 ºC resulting in
265 varying fractions of pre-dissolved nilotinib (Table 1). The suspensions were stirred constantly
266 to prevent sedimentation before usage. Solid excipients were melted prior to nilotinib addition
267 and kept in a liquid form while stirring, addition and *in vivo* administration.
268

Table 1 Dose:solubility ratio of studied surfactant formulations. The dose was fixed at 10 mg/mL.

Formulations for *in vitro* and *in vivo* studies

Excipient	Dose:solubility ratio
Span 80	17.86
Brij O2	14.49
Labrafil M1944 CS	11.36
Tween 85	7.87
Tween 85/ Cremophor RH40 67:33 (w/w)	5.32
Tween 20	4.83
Brij L23	4.69
Cremophor RH40	2.94
Labrasol	2.43

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 280-320 g (8-10 weeks of age) 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. Parallel groups of animals were administered with each formulation at a volume of 2 mL/kg by oral gavage with a nilotinib dose of 20 mg/kg. By

individual tail vein puncture, 200 µL blood samples were collected into plasma collection tubes containing dipotassium EDTA. Samples were taken at 0.5, 1, 2, 4, 6, 8, 10 and 24 h following oral dosing. Plasma was harvested immediately by centrifugation for 10 min at 1,000 *g* and stored at -80 °C until analysis. After the experiment the animals were euthanized.

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 used method was described earlier. [24] 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*g* for 9 min. 50 µL of the supernatant was injected to the HPLC system for analysis. The LOD and LOQ values of this method in plasma were 11 ng/mL and 37 ng/mL, respectively, as determined using the standard error of y-intercept according to the ICH Q2 guideline. [42] Linearity was confirmed between 37 ng/mL and 4.1 µg/mL drug concentration.

Data Analysis

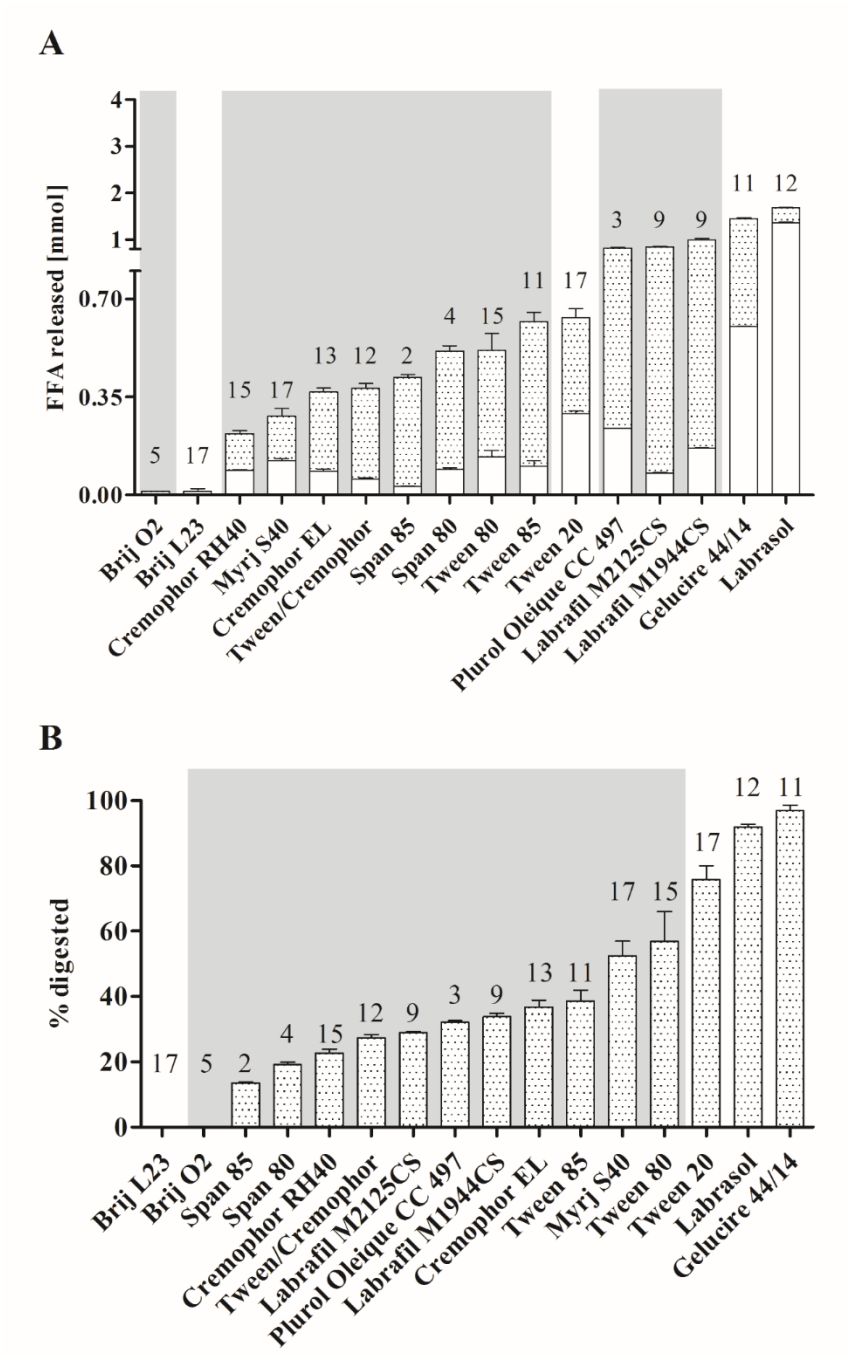
The pharmacokinetic parameters were calculated using Microsoft Excel. The plasma concentration profiles were analysed by non-compartmental analysis and calculation of AUC by the linear trapezoidal rule. Absolute bioavailability was calculated using previous reported intravenous data. [46] The statistical analysis for all *in vivo* parameters was performed using a one-way analysis of variance (one-way ANOVA) after using the Bartlett's test to check for equal variance. Groups were pairwise compared using Tukey's multiple comparison test.

A one-way ANOVA was also calculated for the lipolysis data using the Tukey post-hoc test to compare the different formulation performances. All statistical analyses were carried out using GraphPad Prism 5.

RESULTS

Ranking the digestibility of lipid-based surfactants

The digestibility of 15 surfactants and one surfactant blend commonly used in oral drug formulations was assessed using the *in vitro* lipolysis model. A variety of both medium and long chain FA were selected, with HLB values ranging from 2 to 17. The studied surfactants included stearate, oleate or palmitate, which were considered as long chain FA. Surfactants consisting of FAs like caprylic, capric or lauric acid were considered as medium chain excipients. The FFA based digestibility is shown in Figure 1 A and the % digested based digestibility is shown in Figure 1 B.



326

327 **Figure 1.** Surfactant digestibility in the *in vitro* lipolysis test. Tween/Cremophor is a mixture
328 of Tween 85 and Cremophor RH40 at a ratio of 67:33 (w/w). The shaded area represents long
329 chain excipients and non-shaded areas represent medium chain excipients. The numbers above
330 the bars are the corresponding HLB values of the surfactants. Data is presented as mean ± SD,
331 where n=3. A: Free fatty acids (FFA) released. The total FFA released is divided into the

amount of FFA released during pH stat titrated directly at pH 6.5 (white bars) and the determined amount of FFA during increase of the pH to pH 9.0 after 60 min of digestion (back titration, dotted bars). B: % digested based on the theoretical possible amount of FFA release.

In general, medium chain-based surfactants displayed the highest quantity of FFA released. Labrasol, a medium chain excipient mainly consisting of PEG-8 mono- and diesters of caprylic and capric acid showed the highest release of FFA (Figure 1 A). Gelucire 44/14, which is composed of mostly PEG-32 mono- and diesters of lauric acid, showed the second highest amount of released FFA. Tween 20, a polyoxyethylene sorbitan monoester of lauric acid, released 0.63 ± 0.04 mmol of FFA (Figure 1 A), which reflected a theoretical digestion of 76% (Figure 1 B). Brij L23, a polyoxyethylene lauryl ether, was included as a negative control, as the ether cannot be digested by lipases (a subclass of esterases) [47, 48] and therefore, showed the expected absence of FFA release.

Among the long chain surfactants, Labrafil M1944 CS, which consists of mono-, di- and triglycerides and PEG-6 mono- and diesters of oleic acid, showed the highest released FFA (Figure 1 A). Considering the theoretically possible digestion, only 33.8 ± 1.0% of Labrafil M1944 CS was digested (Figure 1 B). The highest % digested was shown for Tween 80, a surfactant that like Labrafil M1944 CS also contains oleic acid (Figure 1 B). While the lowest % digested was observed for Span 85 (Figure 1 B), the lowest amount of FFA was released in the case of Cremophor RH40 (Figure 1 A). This finding agreed with previous studies that demonstrated a low release of FFAs as well as theoretical digestibility of Cremophor RH40. [49, 50] Brij O2, a polyoxyethylene oleyl ether, did not undergo digestion and was included as a negative control. In fact, long chain surfactants were digested to a lesser extent compared to medium chain surfactants.

Based on the digestibility properties observed, surfactants with a range of digestibility, HLB value and chain length were selected for further *in vitro* and *in vivo* investigations. The study design allowed the evaluation of the influence of digestion, HLB value and FA chain length on the formulation performance *in vivo* (Figure 2). In terms of ranking digestibility, FFA released was chosen as a surrogate parameter for how much the system is changing over time rather than the % digested, as surfactants can show inhibitory effects on lipolysis and the excipient and its lipolytic products can exhibit different activities on the digestive enzymes. [51, 52] Especially in cases with a high amount of possible released FAs the adjustment to % digested may lack information on the amount of liberated FA, which are crucial for the beneficial solubilising and biopharmaceutical effects. Labrasol, Labrafil M1944 CS, Tween 20, Span 80, Brij L23 and Brij O2 were selected as the digestibility was matching with the chain length and HLB value, respectively. Additionally, the two widely used surfactants Tween 85 and Cremophor RH40, as well as a 2:1 (w/w) mixture of both, were included in the *in vivo* study. The study design is shown in Figure 2 and the surfactant properties in Table 2.

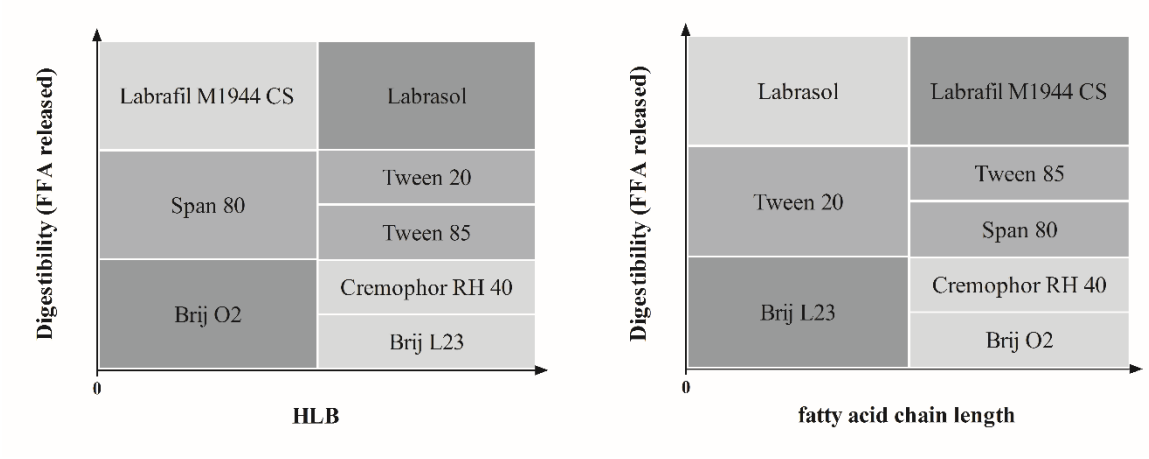


Figure 2. Selected excipients for further *in vitro* and *in vivo* evaluation. Excipients were selected according to the fatty acid chain length, HLB and total free fatty acids (FFA) released during the lipolysis experiments as a surrogate parameter for digestibility.

Comparing nilotinib solubility as a function of lipid-based surfactant type

The solubility of nilotinib in all selected excipients was measured at 37 ºC. The results of the solubility studies are presented in Table 2 and Figure S 1 in the supporting information. Nilotinib displayed the highest solubility in Labrasol and the lowest in Span 80. There were no apparent trends in relationship between solubility and either the FA chain length nor HLB value.

Table 2. Surfactant properties, solubility of nilotinib in surfactants, nilotinib solubilisation in the aqueous phase of the *in vitro* lipolysis test after 60 min, FFA released and % digested after 60 min of digestion and absolute bioavailability of nilotinib formulations in male Sprague-Dawley rats (mean ±SD, n = 3, except *in vivo*, where n = 5). The table is sorted according to the *in vivo* absolute bioavailability from high to low.

	FA type [a)]	HLB	Nilotinib solubility [mg/mL]	*In vitro* solubilisation [%] [b)]	*In vivo* absolute bioavailability [%]	FFA released [mmol]	% digested
Tween 20	MC	16.7	2.07 ±0.22	1.76 ± 0.11	66.50 ± 21.96	0.63 ± 0.04	75.78 ± 4.25
Labrasol	MC	12	4.12 ± 0.05	0.66 ± 0.07	33.82 ± 11.52	1.69 ± 0.02	91.81 ± 0.98
Labrafil M1944 CS	LC	9	0.88 ± 0.45	0.45 ± 0.08	26.02 ± 6.86	0.99 ± 0.03	33.82 ± 1.04
Span 80	LC	4.3	0.56 ± 0.03	1.12 ± 0.09	25.99 ± 11.48	0.51 ± 0.02	19.09 ± 0.85
Cremophor RH40	LC	15	3.40 ± 0.41	11.07 ± 1.40	22.83 ± 10.00	0.22 ± 0.01	22.61 ± 1.25
Tween 85	LC	11	1.27 ± 0.06	6.40 ± 0.36	19.38 ± 6.89	0.62 ± 0.05	38.61 ± 3.32
Tween 85/ Cremophor RH40 67:33 (w/w)	LC	12.3	1.88 ± 0.08	6.94 ± 0.52	17.08 ± 5.62	0.38 ± 0.01	27.33 ± 0.97
Brij L23	MC	16.9	2.13 ± 0.03	11.80 ± 1.78	16.71 ± 5.27	0.00 ± 0.01	0.00 ± 0.00
Brij O2	LC	4.9	0.69 ± 0.04	0.19 ± 0.05	7.32 ± 3.87	- 0.05 ± 0.02	0.00 ± 0.00

[a)] MC: medium chain, LC: long chain

[b)] Aqueous phase concentration in the *in vitro* lipolysis test after 60 min of digestion

Comparing nilotinib solubilisation following in vitro dispersion and digestion.

A range of surfactant-based formulations containing 10 mg/mL nilotinib were prepared and assessed in the *in vitro* dispersion/lipolysis test. The release and dissolution of nilotinib into the different phases were monitored during dispersion and 60 min of digestion. The concentration of nilotinib in the aqueous phase is shown in Figure 3 and Table 2, the distribution across all phases before initiation of digestion (0), 30 min and 60 min after digestion is shown in Figure S 2 in the supporting information and the FFA released versus time profiles in Figure S 3 in the supporting information.

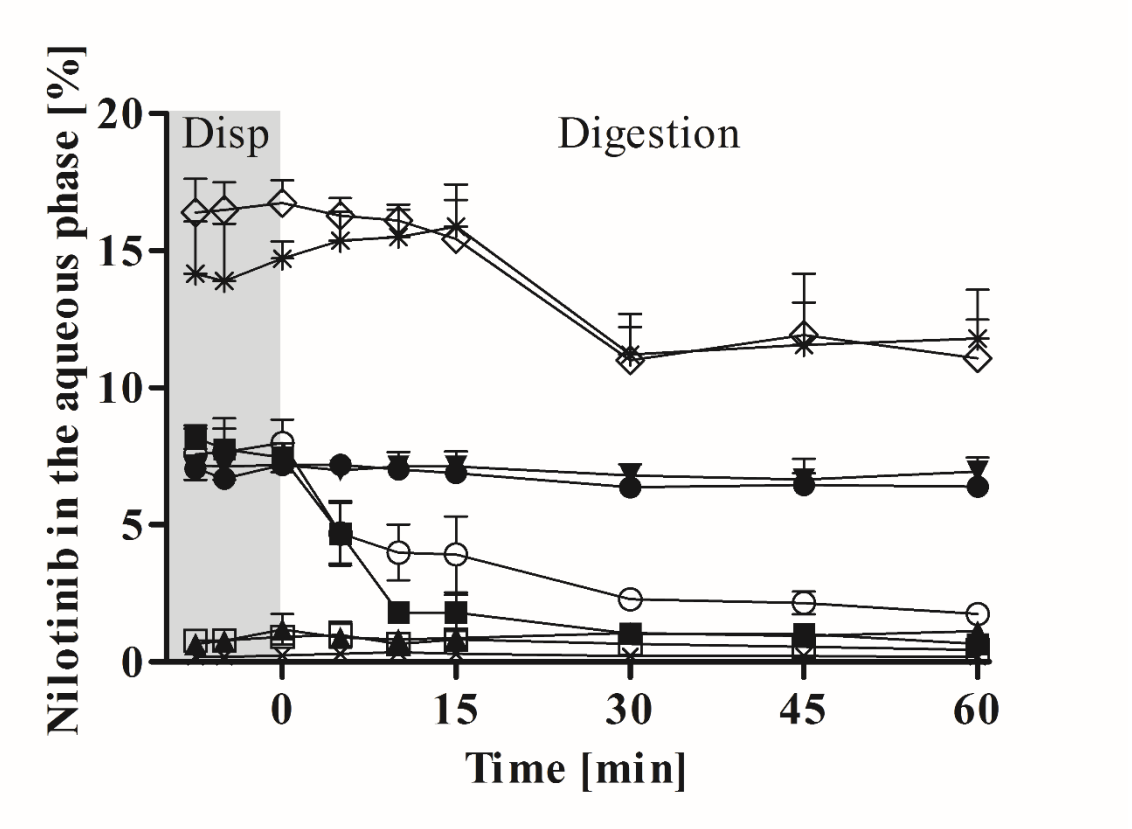


Figure 3. Aqueous phase concentration during 60 min of *in vitro* lipolysis of selected nilotinib suspensions. Cremophor RH40 (◊), Brij L23 (*), Tween 20 (○), Span 80 (▲), Labrasol (■), Labrafil M1944 CS (□), Tween 85 (●), Brij O2 (×), Tween 85/Cremophor RH40 mixture (67:33 w/w) (▼) (mean ± SD, n = 3).

All formulations displayed good dispersion characteristics upon addition to the media. The highest concentration of nilotinib upon dispersion was observed for Cremophor RH40 with 16 ± 1% of the dose dissolved in the aqueous phase. This was followed by Brij L23 with 14 ± 2% of dissolved nilotinib in the aqueous phase. Approximately 6 - 8% of the nilotinib dose was dissolved in the case of Labrasol, Tween 20, Tween 85 and the Tween 85/Cremophor RH40 mixture, which was mid-range relative to the other tested formulations. Poor solubilisation was observed for Span 80, Brij O2 and Labrafil M1944 CS with concentrations < 1%.

Upon initiation of digestion Cremophor RH40 and Brij L23 were able to maintain relatively high concentrations for 15 min followed by a decrease in concentration to 11 ± 1% at 30 min, which was maintained throughout the rest of digestion. The Tween 85/Cremophor RH40 mixture and Tween 85 maintained the initial dispersion concentration throughout digestion. Labrasol and Tween 20 showed an initial drop in concentration and a further decrease throughout the 60 min resulting in concentrations below 2%. The surfactants that demonstrated very low nilotinib concentrations < 1% upon dispersion also maintained the low concentrations throughout digestion. The ranking of the nilotinib concentration in the aqueous phase upon dispersion was Cremophor RH40 ≥ Brij L23 > Tween 20 ≥ Labrasol ≥ Tween 85 ≥ Tween 85/Cremophor RH40 > Span 80 ≥ Labrafil M1944 CS > Brij O2. After 60 min of digestion the aqueous phase concentration of nilotinib was ranked Brij L23 ≥ Cremophor RH40 > Tween 85/Cremophor RH40 mixture ≥ Tween 85 > Tween 20 ≥ Span 80 ≥ Labrasol ≥ LabrafilM 1944 CS ≥ Brij O2. Overall, there was no apparent relationship between the ranking in solubilisation capacity and surfactant HLB FA chain length or digestibility.

In order to gain insights into the nilotinib distribution post-digestion, all samples were separated into three different phases (solid, aqueous and lipid phase) by centrifugation (Figure S 2). The

solid phase represents the undissolved crystalline drug within the surfactant formulation and all formulations showed the highest amount of nilotinib in the solid phase. Additionally, in the case of Labrafil M1944 CS, Span 80 and Brij O2 an oily phase was detected throughout digestion. In all cases the concentration of nilotinib increased in the oily phase as digestion progressed.

Ranking the in vivo bioavailability of surfactants

The *in vivo* performance of nilotinib surfactant formulations was evaluated in male Sprague-Dawley rats. An aqueous suspension containing 0.5% (w/v) methylcellulose (for stabilisation) was used as a non-surfactant control formulation, as previously described. [46] The absolute bioavailability is shown in Figure 4, the plasma concentration versus time profiles are presented in Figure S 4 in the supporting information and the pharmacokinetic parameters in Table 3.

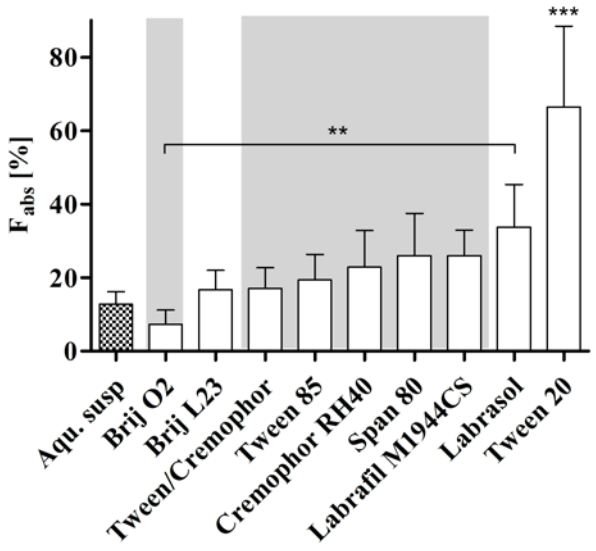


Figure 4. Absolute bioavailability (F_{abs}) of nilotinib suspensions in male Sprague-Dawley rats after oral administration of 20 mg/kg nilotinib and 2 mL/kg excipient in comparison to an aqueous suspension, as previously described. [46] Tween 20 is significantly different to all other tested surfactants and the aqueous suspension. Tween/Cremophor is a mixture of Tween 85 and

Cremophor RH40 (67:33 w/w). Shaded area represents long chain excipients and non-shaded area medium chain excipients (mean ± SD, n = 5).

448 **Table 3.** Pharmacokinetic parameters of nilotinib after oral administration of 20 mg/kg nilotinib and 2 mL/kg excipient to male Spraque-Dawley rats.
449 Nilotinib was administered as surfactant suspensions (n = 5). t_{max}, mean residence time (MRT) and mean absorption time (MAT) are given as median
450 (range), all other parameters as mean ± SD.

	Pharmacokinetic parameters					
	AUC 0 h – inf. [µg*h/mL]	c_{max} [µg/mL]	t_{max} [h]	MRT [h]	MAT [h]	F_{abs} [%] [b)]
Aqu. susp.[a)]	14.33 ± 4.24	2.65 ± 0.68	2 (2-4)	4.14 (3.34 – 4.83)	2.49 (1.69 – 3.18)	12.90 ± 3.34
Tween 20	73.89 ± 24.40	10.28 ± 3.64	2 (2-6)	5.59 (4.97-6.96)	3.94 (3.32-5.32)	66.50 ± 21.96
Tween 85	21.54 ± 7.66	3.13 ± 0.84	4 (2-4)	6.05 (4.56-6.29)	4.40 (2.91-4.64)	19.38 ± 6.89
Labrasol	37.59 ±12.80	4.76 ± 1.83	10 (6-10)	7.62 (6.35-8.00)	5.97 (4.70-6.35)	33.82 ± 11.52
Labrafil M1944 CS	28.91 ±7.63	4.18 ± 1.00	8 (4-10)	7.50 (6.03-8.50)	5.86 (4.38-6.85)	26.02 ± 6.86
Cremophor RH40	25.37 ± 11.11	3.43 ± 1.40	10 (2-10)	7.56 (4.46-7.65)	5.92 (2.82-6.00)	22.83 ± 10.00
Span 80	28.88 ± 12.75	4.30 ± 2.23	8 (8-10)	7.88 (6.65-8.41)	6.23 (5.00-6.77)	25.99 ± 11.48
Brij O2	8.14 ± 4.30	0.98 ± 0.38	4 (1-8)	5.99 (4.58-6.76)	4.35 (2.94-5.12)	7.32 ± 3.87

Brij L23	18.57 ± 5.85	2.77 ± 1.31	10 (6-10)	7.50 (7.26-8.31)	5.85 (5.61-6.67)	16.71 ± 5.27
Tween 85:Cremophor RH40 (67:33 w/w)	18.98 ± 6.24	2.93 ± 1.01	4 (2-8)	5.70 (5.43-7.49)	4.05 (3.78-5.84)	17.08 ± 5.62

a) Data as previously described by Koehl *et al.* [46]

b) Intravenous data obtained from Koehl *et al.* [46]

Interestingly, the highest exposure was observed for the medium chain surfactant Tween 20 with an absolute nilotinib bioavailability of 66.5 ± 22.0%. This was statistically significant higher compared to all other study arms and the aqueous suspension ($p < 0.05$). The Labrasol formulation increased bioavailability to 33.8 ± 11.5%, which was significantly higher relative to the Brij O2, a non-digestible formulation. While there was a trend towards an increased bioavailability for the Labrasol formulation compared to the aqueous suspension, the increases did not reach statistical significance. In contrast, the Brij O2 formulation showed a trend towards a decreased bioavailability of 7.3 ± 3.9% compared to the aqueous suspension, although not statistically significant. All other formulations (Brij L23, Tween 85/Cremophor RH40 mixture, Tween 85, Cremophor RH40, Span 80, Labrafil M1944 CS and the aqueous suspension) displayed similar *in vivo* bioavailability of between 16.7 ± 5.3% to 26.0 ± 6.9%.

Relationship between surfactant digestibility and in vivo bioavailability

The surfactant properties were compared to the absolute bioavailability obtained in the *in vivo* study. Surfactants with different HLB values resulted in a similar absolute bioavailability. Therefore, no relationship between the *in vivo* performance and the HLB value was established. While the two top performing surfactants contain medium chain FA, the third lowest absolute bioavailability was observed for Brij L23, also containing medium chain FA. In the case of the digestion independent Brij L23, however, the FA was not released. The results, therefore, indicate that in terms of released FA during digestion the *in vivo* performance might be related to FA chain length. The most promising *in vitro-in vivo*-relationship was observed between surfactant digestibility and the *in vivo* bioavailability. Figure 5 displays the absolute bioavailability versus % digested and Figure S 5 in the supporting information shows absolute bioavailability versus FFA released. The strongest trend was observed between the absolute bioavailability and the % surfactant digested ($r^2 = 0.5628$). In the case of the *in vitro-in vivo*-

relationship for the absolute bioavailability versus FFA released the overall trend was poor (r^2 = 0.1811), which may reflect that overall the extent of surfactant digestion is more reliable representing the *in vivo* performance than the amount of FFA released.

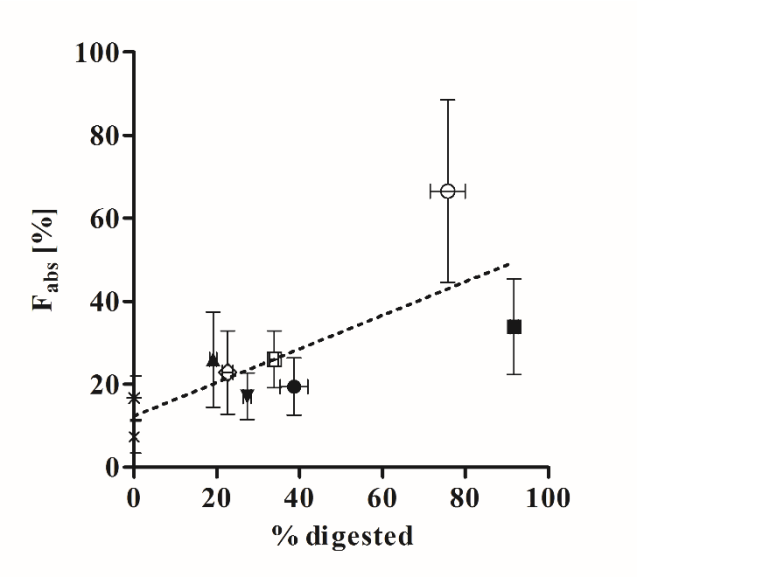


Figure 5. Absolute bioavailability (Fabs) versus % digested of Cremophor RH40 (◊), Brij L23 (*), Tween 20 (○), Span 80 (▲), Labrasol (■), Labrafil M1944 CS (□), Tween 85 (●), Brij O2 (×), Tween 85/Cremophor RH40 mixture (67:33 w/w) (▼) (mean ± SD, Fabs n = 5, % digested n = 3).

DISCUSSION

Selection of surfactants during oral formulation development requires a in depth understanding of the surfactant characteristics both *in vitro* and *in vivo*. Non-ionic surfactants such as Tweens and Poloxamers are widely used excipients in commercial formulations to facilitate a higher dissolution rate and improve solubility. The solubilising effects are typically influenced by digestion and surface-active excipients can affect digestion itself. [51, 52] Surfactants may further affect drug permeation, drug efflux and potentially metabolism in enterocytes. [12, 13, 16, 21] Surfactant selection in oral formulation is largely empirically driven, with limited consideration of the impact of surfactant properties or likely surfactant digestion on the *in vivo* performance. This study, therefore, addresses the need for a systematic comparison of surfactant digestibility and assessment of surfactant properties on *in vivo* performance using nilotinib as a model poorly water-soluble compound.

To the best of our knowledge, this is the first study to compare digestibility of a range of 15 commonly used surfactants and hence this allows the establishment of a digestibility databank for surfactants. Overall, the findings suggested that surfactants containing medium chain FAs displayed a higher digestibility compared to surfactants containing long chain FAs. This finding agrees with previous reports of smaller sets of surfactants [49] as well as glycerides [53] and lipid formulations. [43] The lower digestibility of surfactants containing long chain FAs can be attributed to the extent of long chain FA ionisation [43, 54] and the limited solubilisation capacity of the digestion media for the long chain FAs. [43, 53] Long chain FAs are ionised to a lower extent relative to medium chain FAs at the pH of 6.5. [43, 53] Therefore, the non-ionised long chain FAs are in need of micellar solubilisation due to a poor solubility in the digestion media. Once the solubilisation capacity of the digestion media for long chain FAs is reached, the long chain FAs accumulate at the droplet/micellar interface, effectively inhibiting further enzyme binding and

digestion. [43] In addition, the analysis of the surfactant digestibility revealed that digestibility was not influenced by the HLB value. This indicates that surfactant digestibility was not readily predictable and highlights the importance of *in vitro* lipolysis in the characterisation and selection of surfactants in oral dosage forms. It should also be acknowledged that the reported *in vitro* digestibility (FFA released and % digested) might not be entirely translatable to an *in vivo* digestibility, as non-ionic surfactants and their lipolytic products can inhibit digestion *in vitro* depending on the given conditions. For example, Cremophor RH40, Cremophor EL, Tween 80, Tween 20 and Brij L23 are known to inhibit digestion to varying extents [51, 55, 56] leading to a lower or slower [57] digestion compared to *in vivo*, where the inhibiting surfactants and their lipolytic products could be absorbed. Additionally, the type of enzyme or enzymatic extract as well as pH of the media has been shown to influence the enzymatic activity and consequently the overall extent of digestion, as shown for the excipient Labrasol. [52] Thus, the total amount of released FFA and/or a given % digested depends on the employed *in vitro* or *in vivo* system.

Among the surfactants, the highest absolute bioavailability was observed for the medium chain excipients Tween 20 (F_{abs} 66.50 ± 21.96%), which was statistically significant higher compared to all other surfactants ($p < 0.05$). The second highest bioavailability was observed for the medium chain surfactant Labrasol, which was statistically significant higher compared to Brij O2 ($p < 0.05$). Both Tween 20 and Labrasol are digestible medium chain-based surfactants indicating that the *in vivo* performance might be influenced by the release of medium chain FA. In terms of the HLB value, a relationship to the *in vivo* performance was not apparent. Additionally, the *in vivo* performance was not influenced by the solubility of nilotinib in the excipients. For example, even though the dose-solubility ratio for Brij L23 (4.7) and Tween 20 (4.8) were similar, the bioavailability was significantly higher for the Tween 20 formulation.

This suggests that the solubility in the surfactants was not a limiting factor to the oral bioavailability.

Interestingly, the study suggested that the *in vivo* exposure was influenced by the digestibility of the surfactants, and in general highly digestible surfactants such as Labrasol, Tween 20 and Labrafil M1944 CS displayed the highest bioavailability. Similarly, the two least digested surfactants Brij L23 and Brij O2 showed the lowest bioavailability, which may suggest that the drug was trapped within the non-digestible surfactant micelles. This finding is in line with the observation by Berthelsen and co-workers where bioavailability did not increase with increasing surfactant concentration due to an entrapment in Cremophor RH40 micelles (which displayed lower digestibility relative to Cremophor EL). In contrast, in the case of the Cremophor EL surfactant micelles, which displayed higher digestibility, bioavailability increased with increasing surfactant concentration. [50] It should also be noted that it has been reported in the literature that surfactant only formulations of either Cremophor RH40 or EL did not lead to significant differences in bioavailability of danazol in dogs. [49] On the contrary, when incorporating surfactants into self-emulsifying lipid-based formulations, the poorly digestible surfactant Cremophor RH40 (55% w/w) displayed higher oral bioavailability of danazol compared to Cremophor EL (55% w/w). Possible reasons for this differing outcome between studies, may reflect the differing role of surfactant between different formulation types. Specifically, in the case of self-emulsifying systems, the surfactants also serve to support self-emulsification and stabilisation of the emulsified oil phase. As such, digestion of surfactants may lead to destabilisation of the emulsion droplet, leading to drug precipitation. Therefore, for oil containing self-emulsifying formulations, it appears that low digestibility surfactants are favoured to improve emulsion stability during digestion and reduce the risk of drug precipitation from the oil droplets during lipolysis. However, our study confirmed that in the

563 case of nilotinib surfactant only systems, low digestibility surfactants may have led to lower
564 overall *in vivo* exposure most likely via entrapment within surfactant micelles.
565
566 Compared to the aqueous suspension, Tween 20 was the only surfactant that showed a
567 statistically significant higher bioavailability. All other surfactants displayed a bioavailability
568 between approximately 7.3% and 33.8%, which compared favourably to a previous report of
569 nilotinib bioavailability of 34% in rats using a cremophor based micellar solution. [35] One
570 explanation of the impressive *in vivo* performance of Tween 20 may be additional
571 biopharmaceutical benefits of this excipient. *In vitro* cell assays have shown that Tween 20 is
572 a P-gp (or MDR-1) inhibitor, [15, 16] reducing the efflux into the intestinal lumen. In the case of
573 nilotinib transport by P-gp was demonstrated with an efflux ratio (basolateral/apical) of 3.9-4.1
574 using Caco-2 cells. [35, 37, 38] This indicated that further P-gp inhibition by formulation excipient
575 such as Tween 20 could have contributed positively to nilotinib's bioavailability. A study by
576 Cornaire *et al.* showed that the effectiveness to inhibit P-gp transport for digoxin was Labrasol >
577 Imwitor 742 > Acconon E = Softigen 767 > Cremophor EL > Miglyol > Solutol HS 15 >
578 Sucrose monolaurate > Tween 20 > TPGS > Tween 80 using the rat everted gut sac model. [58]
579 In addition, it was shown that Labrafil M1944 CS was not active as transport enhancer for
580 digoxin. [58] P-gp as well as CYP3A4 share a significant overlap in substrate specificity, [59] which
581 is also the case for nilotinib, which is mainly metabolised by CYP 3A4 in the enterocytes and
582 liver. [35] In fact, co-administering nilotinib with grapefruit juice (intestinal CYP enzyme
583 inhibitor) increased the AUC by 29% [39] showing a significant pre-systemic clearance. A study
584 by Ren and co-workers showed that Tween 20 is a strong CYP 3A4 inhibitor using rat liver and
585 intestinal microsomes. In comparison to the four tested non-ionic surfactants (Tween 20,
586 Cremophor EL, Myrj S40 and Pluronic F68), Tween 20 was the most potent inhibitor.
587 Additionally, the study confirmed a significant higher AUC for diazepam when co-

administered with Tween 20 as well as a decrease in the metabolite (1-hydroxymidazolam) AUC to about 40%. [21] Therefore, the impact of Tween 20 on CYP 3A4 was more distinctive relative to P-gp and overall this would tend to suggest that the effect of CYP 3A4 is more relevant in the case of nilotinib, as also suggested by the significant increased bioavailability in the presence of grapefruit juice. [39]

During digestion FFAs are released, which generate a variety of colloidal species in combination with bile salts, phospholipids and lipolytic products, which potentially have a higher solubilisation capacity for drugs. For nilotinib it was previously shown that the solubility in post-digestive media of a lipid formulation was increased. [24] In addition, it was reported that the solubility of nilotinib is influenced by the bile salt concentration as evidenced by a higher solubility in FeSSIF (3.2 ± 0.1 µg/mL) compared to FaSSIF (0.3 ± 0.03 µg/mL). [24] As FFAs can increase the bile salt release *in vivo*, [60-62] digestion appears to be a crucial parameter for the success of the surfactant-only formulations. This was especially apparent for the digestible Tween 20 and non-digestible Brij L23, which both contain the medium chain FA lauric acid. The non-digestible formulation resulted in a significantly lower exposure indicating that the release of lauric acid may have been the driving factor for an improved bioavailability, as FFAs have also shown beneficial effects on drug permeability without pronounced cytotoxic effects. [25, 28-30, 32, 33, 63-65] As nilotinib is passively transported, [66] the permeability enhancing effects of surfactants [12] and their lipolytic products may have contributed to a higher bioavailability of nilotinib when compared to the aqueous suspension.

The *in vitro* lipolysis of Brij L23 and Cremophor RH40 formulations showed high concentrations of solubilised nilotinib in the aqueous phase upon dispersion and throughout digestion. For both surfactants a drop in nilotinib concentrations of approximately 4.9% after

15 min of digestion was observed. Similarly, the nilotinib aqueous phase concentration decreased in the case of Labrasol and Tween 20 upon initiation of digestion resulting in a total decrease of approximately 6.2% and 6.8%, respectively. However, the drop in nilotinib concentration in the aqueous phase did not seem to be exclusively related to the digestibility of these surfactants (Figure 1), as Labrasol and Tween 20 are highly digested, but Cremophor RH40 and Brij L23 are poorly or not digested. Furthermore, Labrafil M1944 CS and Tween 85, which are both rapidly and > 33% digested (Figure S 3, Figure 1), did not show a decrease in nilotinib concentration upon initiation of digestion. However, given that Tween 20 and Labrasol are both medium chain-based surfactants, it appears that the initial drop in nilotinib aqueous phase concentration upon initiation of digestion most likely reflects the combination of high digestibility and the release of medium chain FAs. This may reflect the lower solubilisation capacity of medium chain post digestive media, as previously reported. [67, 68] While there was no direct relationship between the HLB value of the surfactants and the performance in the *in vitro* lipolysis, there seemed to be a trend that surfactants with a HLB value > 10 performed better compared to surfactants with a HLB value < 10. For example, Brij L23 (HLB value: 16.9) showed an *in vitro* solubilisation of 11.8 ± 1.8%, whereas Brij O2 (HLB value: 4.9) only reached an *in vitro* solubilisation of 0.2 ± 0.1%. Surfactants with a HLB value > 10 are considered suitable for stabilising oil in water systems, whereas surfactants with a HLB value < 10 are suitable for stabilising water in oil systems. Thus, the higher HLB surfactants seem to stabilise solubilised nilotinib, which is highly lipophilic (log*P*: 4.95), in the aqueous phase better compared to low HLB surfactants. In contrast to the HLB value trend, the *in vitro* lipolysis test indicated that digestibility of the surfactants is not related to nilotinib solubilisation in the aqueous phase *in vitro*. For example, Brij L23 (non-digestible) and Cremophor RH40 (digestible) demonstrated comparable aqueous phase concentrations but different digestibility. Additionally, no trend was established between the FA chain length and the aqueous phase

concentration of nilotinib. The aqueous phase concentration of the *in vitro* lipolysis is thought to represent the amount of drug readily available for absorption *in vivo* and is commonly used to rank formulation performances. However, due to the limitations of the *in vitro* systems, such as the lack of an absorptive sink, low media volume in combination with a high drug and excipient load as well as different hydrodynamics and enzyme activity compared to *in vivo*, a relationship between *in vitro* and *in vivo* performance for surfactants [49, 50] or other formulations [24, 69, 70] has been reported difficult in some cases. Also, this study could not demonstrate a correlation between the aqueous phase concentration of the *in vitro* lipolysis test and the *in vivo* performance. For example, the relatively higher *in vitro* solubilisation of Brij L23 and Cremophor RH40 did not correlate with a higher *in vivo* performance, as bioavailability values of Cremophor RH40 and Brij L 23 were in the mid (approximately 23%) and low (approximately 17%) end of the range of absolute bioavailability obtained *in vivo*. An adjustment of the *in vitro* model to rat conditions (e.g. low enzyme activity and low gastrointestinal volumes), [71, 72] two stages (gastric and intestinal) [73-75] or the addition of an absorptive sink [76, 77] may offer additional insights into the *in vivo* behaviour.

CONCLUSION

The present study systematically investigated the relationship between non-ionic surfactant properties and the *in vitro* and *in vivo* performance using nilotinib. Tween 20 demonstrated an impressive 5.2-fold increase in absolute bioavailability when compared to an aqueous suspension. In general, surfactants that displayed high digestibility *in vitro* displayed higher nilotinib bioavailability *in vivo*. Medium chain FA-based surfactants appeared to be favourable to increase bioavailability compared to long chain FA types. However, HLB of the surfactant did not correlate with the *in vivo* performance of nilotinib. The reported additional biopharmaceutical effects of Tween 20, in terms of inhibition of CYP 3A4 and modulation of

P-gp efflux, may explain the impressive increase in bioavailability. Therefore, this study highlights the importance of appropriate surfactant selection to maximise *in vivo* exposure, with careful consideration of solubilisation properties, impact of digestion and biopharmaceutical effects. Further studies using broader range of drugs are therefore merited with the ultimate aim of developing a bio-predictive surfactant classification system.

SUPPORTING INFORMATION

Nilotinib solubility in studied excipients, distribution of nilotinib into different phases during *in vitro* lipolysis, FFA released versus time profiles during *in vitro* lipolysis, plasma concentration versus time profiles of nilotinib in rats, relationship of F_{abs} and FFA released.

ACKNOWLEDGEMENTS

N. J. Koehl, B. T. Griffin, R. Holm and M. Kuentz are part of the PEARRL European Training network, which has received funding from the Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks programme under grant agreement No. 674909.

REFERENCES

1. Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* **2000,** *44*, (1), 235-49.

2. Boyd, B. J.; Bergstrom, C. A. S.; Vinarov, Z.; Kuentz, M.; Brouwers, J.; Augustijns, P.; Brandl, M.; Bernkop-Schnurch, A.; Shrestha, N.; Preat, V.; Mullertz, A.; Bauer-Brandl, A.; Jannin, V. Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. *Eur J Pharm Sci* **2019,** *137*, 104967.

687 3. Singh, A.; Van den Mooter, G. Spray drying formulation of amorphous solid
688 dispersions. *Adv Drug Deliv Rev* **2016,** *100*, 27-50.
689 4. Mooter, G. V. d. The use of amorphous solid dispersions: A formulation strategy to
690 overcome poor solubility and dissolution rate. *Drug Discov Today Technol* **2012,** *9*, (2), e71-
691 e174.
692 5. Feeney, O. M.; Crum, M. F.; McEvoy, C. L.; Trevaskis, N. L.; Williams, H. D.; Pouton,
693 C. W.; Charman, W. N.; Bergstrom, C. A.; Porter, C. J. 50years of oral lipid-based
694 formulations: Provenance, progress and future perspectives. *Adv Drug Deliv Rev* **2016,** *101*,
695 167-94.
696 6. Merisko-Liversidge, E.; Liversidge, G. G.; Cooper, E. R. Nanosizing: a formulation
697 approach for poorly-water-soluble compounds. *Eur J Pharm Sci* **2003,** *18*, (2), 113-20.
698 7. Chen, J.; Ormes, J. D.; Higgins, J. D.; Taylor, L. S. Impact of surfactants on the
699 crystallization of aqueous suspensions of celecoxib amorphous solid dispersion spray dried
700 particles. *Mol Pharm* **2015,** *12*, (2), 533-41.
701 8. Indulkar, A. S.; Mo, H.; Gao, Y.; Raina, S. A.; Zhang, G. G. Z.; Taylor, L. S. Impact
702 of Micellar Surfactant on Supersaturation and Insight into Solubilization Mechanisms in
703 Supersaturated Solutions of Atazanavir. *Pharm Res* **2017,** *34*, (6), 1276-1295.
704 9. Vinarov, Z.; Katev, V.; Radeva, D.; Tcholakova, S.; Denkov, N. D. Micellar
705 solubilization of poorly water-soluble drugs: effect of surfactant and solubilizate molecular
706 structure. *Drug Dev Ind Pharm* **2018,** *44*, (4), 677-686.
707 10. Wu, L.; Zhang, J.; Watanabe, W. Physical and chemical stability of drug nanoparticles.
708 *Adv Drug Deliv Rev* **2011,** *63*, (6), 456-69.
709 11. Park, S. H.; Choi, H. K. The effects of surfactants on the dissolution profiles of poorly
710 water-soluble acidic drugs. *Int J Pharm* **2006,** *321*, (1-2), 35-41.

12. Heerklotz, H. Interactions of surfactants with lipid membranes. *Q Rev Biophys* **2008,** *41*, (3-4), 205-64.

13. Al-Ali, A. A. A.; Nielsen, R. B.; Steffansen, B.; Holm, R.; Nielsen, C. U. Nonionic surfactants modulate the transport activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC): Relevance to oral drug absorption. *Int J Pharm* **2019,** *566*, 410-433.

14. Cornaire, G.; Woodley, J. F.; Saivin, S.; Legendre, J. Y.; Decourt, S.; Cloarec, A.; Houin, G. Effect of polyoxyl 35 castor oil and Polysorbate 80 on the intestinal absorption of digoxin in vitro. *Arzneimittelforschung* **2000,** *50*, (6), 576-9.

15. Wasan, K. M., *Role of lipid excipients in modifying oral and parenteral drug delivery : basic principles and biological examples*. Wiley-Interscience: Hoboken, N.J., 2007; p x, 205 p.

16. Ma, L.; Wei, Y.; Zhou, Y.; Ma, X.; Wu, X. Effects of Pluronic F68 and Labrasol on the intestinal absorption and pharmacokinetics of rifampicin in rats. *Arch Pharm Res* **2011,** *34*, (11), 1939-43.

17. Myers, D., *Surfactant science and technology*. 3rd ed.; J. Wiley: Hoboken, N.J., 2006; p xvi, 380 p.

18. Vinarov, Z.; Katev, V.; Burdzhiev, N.; Tcholakova, S.; Denkov, N. Effect of Surfactant-Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media. *Mol Pharm* **2018,** *15*, (12), 5741-5753.

19. Tadros, T. F., *Applied Surfactants: Principles and Applications*. Wiley: 2006.

20. Xavier-Junior, F. H.; Huang, N.; Vachon, J. J.; Rehder, V. L.; do Egito, E. S.; Vauthier, C. Match of Solubility Parameters Between Oil and Surfactants as a Rational Approach for the Formulation of Microemulsion with a High Dispersed Volume of Copaiba Oil and Low Surfactant Content. *Pharm Res* **2016,** *33*, (12), 3031-3043.

21. Ren, X.; Mao, X.; Cao, L.; Xue, K.; Si, L.; Qiu, J.; Schimmer, A. D.; Li, G. Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity in vitro and in vivo. *Eur J Pharm Sci* **2009,** *36*, (4-5), 401-11.

22. Anby, M. U.; Williams, H. D.; McIntosh, M.; Benameur, H.; Edwards, G. A.; Pouton, C. W.; Porter, C. J. Lipid digestion as a trigger for supersaturation: evaluation of the impact of supersaturation stabilization on the in vitro and in vivo performance of self-emulsifying drug delivery systems. *Mol Pharm* **2012,** *9*, (7), 2063-79.

23. Gautschi, N.; Bergstrom, C. A.; Kuentz, M. Rapid determination of drug solubilization versus supersaturation in natural and digested lipids. *Int J Pharm* **2016,** *513*, (1-2), 164-174.

24. Koehl, N. J.; Holm, R.; Kuentz, M.; Griffin, B. T. New Insights into Using Lipid Based Suspensions for "Brick Dust' Molecules: Case Study of Nilotinib. *Pharm Res-Dordr* **2019,** *36*, (4).

25. Lindmark, T.; Kimura, Y.; Artursson, P. Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *J Pharmacol Exp Ther* **1998,** *284*, (1), 362-9.

26. Higaki, K.; Yata, T.; Sone, M.; Ogawara, K.; Kimura, T. Estimation of absorption enhancement by medium-chain fatty acids in rat large intestine. *Res Commun Mol Pathol Pharmacol* **2001,** *109*, (3-4), 231-40.

27. Aungst, B. J. Intestinal permeation enhancers. *J Pharm Sci* **2000,** *89*, (4), 429-42.

28. Lindmark, T.; Nikkila, T.; Artursson, P. Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers. *J Pharmacol Exp Ther* **1995,** *275*, (2), 958-64.

29. Anderberg, E. K.; Lindmark, T.; Artursson, P. Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. *Pharm Res* **1993,** *10*, (6), 857-64.

30. Lindmark, T.; Schipper, N.; Lazorova, L.; de Boer, A. G.; Artursson, P. Absorption enhancement in intestinal epithelial Caco-2 monolayers by sodium caprate: assessment of molecular weight dependence and demonstration of transport routes. *J Drug Target* **1998,** *5*, (3), 215-23.

31. Kamp, F.; Guo, W.; Souto, R.; Pilch, P. F.; Corkey, B. E.; Hamilton, J. A. Rapid flip-flop of oleic acid across the plasma membrane of adipocytes. *J Biol Chem* **2003,** *278*, (10), 7988-95.

32. Ibarguren, M.; Lopez, D. J.; Escriba, P. V. The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochim Biophys Acta* **2014,** *1838*, (6), 1518-28.

33. Cerezo, J.; Zuniga, J.; Bastida, A.; Requena, A.; Ceron-Carrasco, J. P. Atomistic molecular dynamics simulations of the interactions of oleic and 2-hydroxyoleic acids with phosphatidylcholine bilayers. *J Phys Chem B* **2011,** *115*, (40), 11727-38.

34. Pabla, D.; Akhlaghi, F.; Zia, H. Intestinal permeability enhancement of levothyroxine sodium by straight chain fatty acids studied in MDCK epithelial cell line. *Eur J Pharm Sci* **2010,** *40*, (5), 466-72.

35. EMEA Tasigna (Nilotinib) European public assessment report. https://www.ema.europa.eu/en/documents/scientific-discussion/tasigna-epar-scientific-discussion_en.pdf (December 02),

36. FDA Nilotinib (Tasigna) - Clinical Pharmacology and Biopharmaceutics Review. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068toc.cfm (October 01),

37. Yamakawa, Y.; Hamada, A.; Uchida, T.; Sato, D.; Yuki, M.; Hayashi, M.; Kawaguchi, T.; Saito, H. Distinct interaction of nilotinib and imatinib with P-Glycoprotein in intracellular

783 accumulation and cytotoxicity in CML Cell Line K562 cells. *Biol Pharm Bull* **2014,** *37*, (8),
784 1330-5.
785 38. Mahon, F. X.; Hayette, S.; Lagarde, V.; Belloc, F.; Turcq, B.; Nicolini, F.; Belanger,
786 C.; Manley, P. W.; Leroy, C.; Etienne, G.; Roche, S.; Pasquet, J. M. Evidence that resistance
787 to nilotinib may be due to BCR-ABL, Pgp, or Src kinase overexpression. *Cancer Res* **2008,** *68*,
788 (23), 9809-16.
789 39. Yin, O. Q.; Gallagher, N.; Li, A.; Zhou, W.; Harrell, R.; Schran, H. Effect of grapefruit
790 juice on the pharmacokinetics of nilotinib in healthy participants. *J Clin Pharmacol* **2010,** *50*,
791 (2), 188-94.
792 40. EMEA Annex I Summary of product characteristics.
793 https://www.ema.europa.eu/en/documents/product-information/tasigna-epar-product-
794 information_en.pdf (Januar 01),
795 41. Tian, X.; Zhang, H.; Heimbach, T.; He, H.; Buchbinder, A.; Aghoghovbia, M.;
796 Hourcade-Potelleret, F. Clinical Pharmacokinetic and Pharmacodynamic Overview of
797 Nilotinib, a Selective Tyrosine Kinase Inhibitor. *J Clin Pharmacol* **2018,** *58*, (12), 1533-1540.
798 42. ICH, G. u. i. d. e. l. i. n. e. In *Validation of analytical procedures: text and methodology*
799 *Q2 (R1)*, International conference on harmonization, Geneva, Switzerland, 2005; ICH
800 harmonised tripartite guideline.
801 43. Williams, H. D.; Sassene, P.; Kleberg, K.; Bakala-N'Goma, J. C.; Calderone, M.;
802 Jannin, V.; Igonin, A.; Partheil, A.; Marchaud, D.; Jule, E.; Vertommen, J.; Maio, M.; Blundell,
803 R.; Benameur, H.; Carriere, F.; Mullertz, A.; Porter, C. J.; Pouton, C. W. Toward the
804 establishment of standardized in vitro tests for lipid-based formulations, part 1: method
805 parameterization and comparison of in vitro digestion profiles across a range of representative
806 formulations. *J Pharm Sci* **2012,** *101*, (9), 3360-80.

807 44. Williams, H. D.; Sassene, P.; Kleberg, K.; Calderone, M.; Igonin, A.; Jule, E.;
808 Vertommen, J.; Blundell, R.; Benameur, H.; Mullertz, A.; Pouton, C. W.; Porter, C. J.;
809 Consortium, L. Toward the establishment of standardized in vitro tests for lipid-based
810 formulations, part 3: understanding supersaturation versus precipitation potential during the in
811 vitro digestion of type I, II, IIIA, IIIB and IV lipid-based formulations. *Pharm Res* **2013,** *30*,
812 (12), 3059-76.
813 45. Sek, L.; Porter, C. J.; Charman, W. N. Characterisation and quantification of medium
814 chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with
815 in situ densitometric analysis. *J Pharm Biomed Anal* **2001,** *25*, (3-4), 651-61.
816 46. Koehl, N. J.; Holm, R.; Kuentz, M.; Griffin, B. T. Chase Dosing of Lipid Formulations
817 to Enhance Oral Bioavailability of Nilotinib in Rats. *Pharm Res-Dordr* **2020,** *37*, (7), 124.
818 47. Gandhi, N. N. Applications of lipase. *Journal of the American Oil Chemists' Society*
819 **1997,** *74*, (6), 621-634.
820 48. Larsen, A. T.; Sassene, P.; Mullertz, A. In vitro lipolysis models as a tool for the
821 characterization of oral lipid and surfactant based drug delivery systems. *Int J Pharmaceut*
822 **2011,** *417*, (1-2), 245-255.
823 49. Cuine, J. F.; McEvoy, C. L.; Charman, W. N.; Pouton, C. W.; Edwards, G. A.;
824 Benameur, H.; Porter, C. J. Evaluation of the impact of surfactant digestion on the
825 bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to
826 dogs. *J Pharm Sci* **2008,** *97*, (2), 995-1012.
827 50. Berthelsen, R.; Holm, R.; Jacobsen, J.; Kristensen, J.; Abrahamsson, B.; Mullertz, A.
828 Kolliphor surfactants affect solubilization and bioavailability of fenofibrate. Studies of in vitro
829 digestion and absorption in rats. *Mol Pharm* **2015,** *12*, (4), 1062-71.

51. Christiansen, A.; Backensfeld, T.; Weitschies, W. Effects of non-ionic surfactants on in vitro triglyceride digestion and their susceptibility to digestion by pancreatic enzymes. *Eur J Pharm Sci* **2010,** *41*, (2), 376-82.

52. Fernandez, S.; Jannin, V.; Rodier, J. D.; Ritter, N.; Mahler, B.; Carriere, F. Comparative study on digestive lipase activities on the self emulsifying excipient Labrasol, medium chain glycerides and PEG esters. *Biochim Biophys Acta* **2007,** *1771*, (5), 633-40.

53. Sek, L.; Porter, C. J.; Kaukonen, A. M.; Charman, W. N. Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J Pharm Pharmacol* **2002,** *54*, (1), 29-41.

54. Kanicky, J. R.; Shah, D. O. Effect of degree, type, and position of unsaturation on the pKa of long-chain fatty acids. *J Colloid Interface Sci* **2002,** *256*, (1), 201-7.

55. Li, Y.; McClements, D. J. Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions. *Eur J Pharm Biopharm* **2011,** *79*, (2), 423-31.

56. Feeney, O. M.; Williams, H. D.; Pouton, C. W.; Porter, C. J. 'Stealth' lipid-based formulations: poly(ethylene glycol)-mediated digestion inhibition improves oral bioavailability of a model poorly water soluble drug. *J Control Release* **2014,** *192*, 219-27.

57. Vithani, K.; Hawley, A.; Jannin, V.; Pouton, C.; Boyd, B. J. Inclusion of Digestible Surfactants in Solid SMEDDS Formulation Removes Lag Time and Influences the Formation of Structured Particles During Digestion. *Aaps J* **2017,** *19*, (3), 754-764.

58. Cornaire, G.; Woodley, J.; Hermann, P.; Cloarec, A.; Arellano, C.; Houin, G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. *Int J Pharm* **2004,** *278*, (1), 119-31.

59. Wacher, V. J.; Silverman, J. A.; Zhang, Y.; Benet, L. Z. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* **1998,** *87*, (11), 1322-30.

60. Porter, C. J. H.; Trevaskis, N. L.; Charman, W. N. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat Rev Drug Discov* **2007,** *6*, (3), 231-248.

61. McLaughlin, J.; Lucà, M. G.; Jones, M. N.; D'Amato, M.; Dockray, G. J.; Thompson, D. G. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology* **1999,** *116*, (1), 46-53.

62. McLaughlin, J. T.; Lomax, R. B.; Hall, L.; Dockray, G. J.; Thompson, D. G.; 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)*, 11-8.

63. Kataoka, M.; Ohi, Y.; Sakanoue, K.; Minami, K.; Higashino, H.; Yamashita, S. Impact of dietary intake of medium-chain triacylglycerides on the intestinal absorption of poorly permeable compounds. *Mol Pharm* **2019**.

64. Aspenstrom-Fagerlund, B.; Ring, L.; Aspenstrom, P.; Tallkvist, J.; Ilback, N. G.; Glynn, A. W. Oleic acid and docosahexaenoic acid cause an increase in the paracellular absorption of hydrophilic compounds in an experimental model of human absorptive enterocytes. *Toxicology* **2007,** *237*, (1-3), 12-23.

65. Tomita, M.; Hayashi, M.; Awazu, S. Absorption-enhancing mechanism of sodium caprate and decanoylcarnitine in Caco-2 cells. *J Pharmacol Exp Ther* **1995,** *272*, (2), 739-43.

66. Blay, J. Y.; von Mehren, M. Nilotinib: a novel, selective tyrosine kinase inhibitor. *Semin Oncol* **2011,** *38 Suppl 1*, S3-9.

876 67. Pouton, C. W. Formulation of poorly water-soluble drugs for oral administration:
877 physicochemical and physiological issues and the lipid formulation classification system. *Eur*
878 *J Pharm Sci* **2006,** *29*, (3-4), 278-87.
879 68. Williams, H. D.; Anby, M. U.; Sassene, P.; Kleberg, K.; Bakala-N'Goma, J. C.;
880 Calderone, M.; Jannin, V.; Igonin, A.; Partheil, A.; Marchaud, D.; Jule, E.; Vertommen, J.;
881 Maio, M.; Blundell, R.; Benameur, H.; Carriere, F.; Mullertz, A.; Pouton, C. W.; Porter, C. J.
882 Toward the establishment of standardized in vitro tests for lipid-based formulations. 2. The
883 effect of bile salt concentration and drug loading on the performance of type I, II, IIIA, IIIB,
884 and IV formulations during in vitro digestion. *Mol Pharm* **2012,** *9*, (11), 3286-300.
885 69. Thomas, N.; Richter, K.; Pedersen, T. B.; Holm, R.; Mullertz, A.; Rades, T. In vitro
886 lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery
887 systems containing fenofibrate. *Aaps J* **2014,** *16*, (3), 539-49.
888 70. Griffin, B. T.; Kuentz, M.; Vertzoni, M.; Kostewicz, E. S.; Fei, Y.; Faisal, W.; Stillhart,
889 C.; O'Driscoll, C. M.; Reppas, C.; Dressman, J. B. Comparison of in vitro tests at various levels
890 of complexity for the prediction of in vivo performance of lipid-based formulations: Case
891 studies with fenofibrate. *Eur J Pharm Biopharm* **2014,** *86*, (3), 427-437.
892 71. Siqueira Jorgensen, S. D.; Al Sawaf, M.; Graeser, K.; Mu, H.; Mullertz, A.; Rades, T.
893 The ability of two in vitro lipolysis models reflecting the human and rat gastro-intestinal
894 conditions to predict the in vivo performance of SNEDDS dosing regimens. *Eur J Pharm*
895 *Biopharm* **2018,** *124*, 116-124.
896 72. Anby, M. U.; Nguyen, T. H.; Yeap, Y. Y.; Feeney, O. M.; Williams, H. D.; Benameur,
897 H.; Pouton, C. W.; Porter, C. J. An in vitro digestion test that reflects rat intestinal conditions
898 to probe the importance of formulation digestion vs first pass metabolism in Danazol
899 bioavailability from lipid based formulations. *Mol Pharm* **2014,** *11*, (11), 4069-83.

73. Bakala-N'Goma, J. C.; Williams, H. D.; Sassene, P. J.; Kleberg, K.; Calderone, M.; Jannin, V.; Igonin, A.; Partheil, A.; Marchaud, D.; Jule, E.; Vertommen, J.; Maio, M.; Blundell, R.; Benameur, H.; Mullertz, A.; Pouton, C. W.; Porter, C. J.; Carriere, F. Toward the establishment of standardized in vitro tests for lipid-based formulations. 5. Lipolysis of representative formulations by gastric lipase. *Pharm Res* **2015,** *32*, (4), 1279-87.

74. Fernandez, S.; Chevrier, S.; Ritter, N.; Mahler, B.; Demarne, F.; Carriere, F.; Jannin, V. In vitro gastrointestinal lipolysis of four formulations of piroxicam and cinnarizine with the self emulsifying excipients Labrasol and Gelucire 44/14. *Pharm Res* **2009,** *26*, (8), 1901-10.

75. Christophersen, P. C.; Christiansen, M. L.; Holm, R.; Kristensen, J.; Jacobsen, J.; Abrahamsson, B.; Mullertz, A. Fed and fasted state gastro-intestinal in vitro lipolysis: In vitro in vivo relations of a conventional tablet, a SNEDDS and a solidified SNEDDS. *Eur J Pharm Sci* **2014,** *57*, 232-9.

76. Alvebratt, C.; Keemink, J.; Edueng, K.; Cheung, O.; Stromme, M.; Bergstrom, C. A. S. An in vitro dissolution-digestion-permeation assay for the study of advanced drug delivery systems. *Eur J Pharm Biopharm* **2020,** *149*, 21-29.

77. Keemink, J.; Martensson, E.; Bergstrom, C. A. S. Lipolysis-Permeation Setup for Simultaneous Study of Digestion and Absorption in Vitro. *Mol Pharm* **2019,** *16*, (3), 921-930.