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Title	Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib
Author(s)	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', <i>Molecular Pharmaceutics</i> , 17(9), pp. 3202-3213. doi: 10.1021/acs.molpharmaceut.0c00305
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://dx.doi.org/10.1021/acs.molpharmaceut.0c00305 Access to the full text of the published version may require a subscription.
Rights	© 2020, American Chemical Society. This document is the Accepted Manuscript version of a Published Work that appeared in final form in <i>Molecular Pharmaceutics</i> , 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
Embargo information	Access to this article is restricted until 12 months after publication by request of the publisher.
Embargo lift date	2021-07-10
Item downloaded from	http://hdl.handle.net/10468/10661

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

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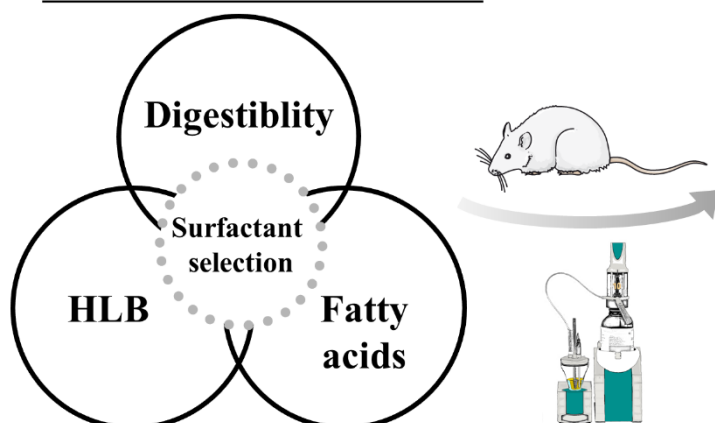
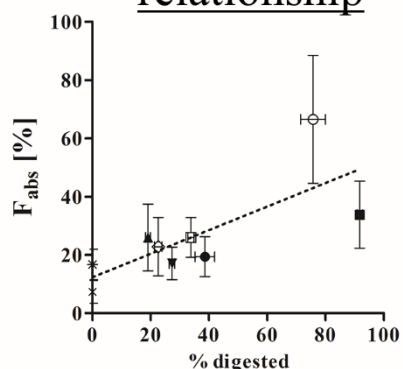
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Nilotinib + SurfactantsSurfactant property relationship

23

24

25 ABSTRACT

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

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

49

50 KEYWORDS

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

52

53 ABBREVIATIONS

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

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61 INTRODUCTION

62 Many emerging drug candidates show poor solubility and/or permeability resulting in a low
63 and variable oral bioavailability when administered in conventional dosage forms.¹ Therefore,
64 there is a need to develop bio-enabling formulation technologies that enhance
65 biopharmaceutical properties and improve oral absorption of these emerging drug candidates.
66 ² The various bio-enabling approaches have been extensively reviewed including solid
67 dispersions,^{3, 4} lipid-based formulations⁵ and nano-sized drug crystals/particles.⁶
68 Interestingly, one of the most common excipients included across most classes of bio-enabling
69 approaches are surfactants, which, from a mechanistic perspective, can impart a variety of
70 biopharmaceutical advantages including promoting supersaturation,^{7, 8} enhancing
71 solubilisation,⁹ stabilisation of colloidal/nano-crystals,¹⁰ increased dissolution rate¹¹ and
72 increasing permeability.¹²⁻¹⁶

73

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

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

101

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

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

117

118 When considering the choice of digestible versus non-digestible surfactants, a key perceived
119 advantage of non-digestible surfactants is in being 'digestion-independent' systems, and
120 therefore *in vitro* characteristics can be readily employed to predict the likely performance *in*
121 *vivo*. Additionally, digestible systems carry the risk that the surfactant may not serve the initial
122 purpose in a post-digestive state. For example, digestion of surfactants may lead to a reduced
123 solubilisation capacity of the colloidal aqueous dispersion and potentially lead to drug
124 precipitation. However, studies have also suggested that digestion may in fact promote
125 transient supersaturation in the intestinal media and therefore may promote absorption.²² In
126 addition, the released FA have shown to increase solubility of poorly soluble drugs as
127 evidenced by *in vitro* measurements of solubility in assembled pre- and post-digestion media
128 with lipid excipients.^{23, 24} Moreover, free fatty acids (FFA) released post digestion may
129 modulate the intestinal permeability. In several studies it was shown that the treatment of cells
130 with medium chain FFA showed an increased paracellular transport *via* tight junction opening.
131²⁵⁻³⁰ In the case of unsaturated long chain FFA, *in vitro* cell experiments showed an increased
132 membrane fluidity³¹⁻³³ as well as opened tight junctions.^{26, 34} These biopharmaceutical effects
133 can further enhance drug absorption from surfactant formulations.

134

135 There is currently a lack of comprehensive knowledge of how surfactant properties translate
136 into *in vivo* bioavailability of poorly water-soluble drugs. The overarching goal of this study
137 was to systematically explore the relationship between surfactant properties and *in vivo*
138 performance, which will support a more science- and risk-based approach to surfactant
139 selection in oral formulations. Nilotinib was chosen as a model poorly water-soluble drug for
140 the study. Nilotinib is practically insoluble in buffer solutions of pH 4.5 and higher (pKa 2.1
141 and 5.4),³⁵ is highly lipophilic (log*P* 4.95) as well as hydrophobic²⁴ and exhibits a moderate
142 permeability across a confluent Caco-2 cell monolayer.^{35, 36} Therefore, nilotinib was
143 categorised as a class IV compound in the biopharmaceutical classification system (BCS).
144 Nilotinib is predominantly metabolised by CYP 3A4³⁵ and is a P-gp substrate.³⁵⁻³⁸ The pre-
145 systemic clearance is high with an AUC increase of 29% after co-administration of nilotinib
146 with grapefruit juice (intestinal CYP 3A4 inhibitor).³⁹ The current commercial formulation,
147 Tasigna®, is a capsule formulation containing the surfactant Pluronic F68 (poloxamer 188).⁴⁰
148 The concentration and the scientific rationale of the addition of Poloxamer 188 is unknown,
149 however it has been reported that the use of surfactants did not increase the dissolution of
150 nilotinib capsules at pH 4.5 and above.³⁶ The marketed formulation of nilotinib showed an
151 absorption of $\geq 30\%$ following a radiolabelled single 400 mg oral dose in humans.⁴¹
152 Additionally, preclinical studies in rats yielded an absolute bioavailability of 34% using a
153 solution with Cremophor, dimethyl acetamide and 5% dextrose (20/10/70 (v/v/v)).³⁵ Using
154 this as a model poorly water-soluble drug, the present study systematically assessed the impact
155 of surfactant properties on oral bioavailability. Surfactants were classified according to FA
156 chain length, HLB value and digestibility and their *in vitro* and *in vivo* performance in rats was
157 investigated.

158

159 MATERIALS AND METHODS

160 *Chemicals and materials*

161 Nilotinib and sorafenib were purchased from Kemprotec Ltd. (UK). Brij O2 (Polyoxyethylene
162 (2) oleyl ether), Brij L23 (Polyoxyethylene (23) lauryl ether), Myrj S40 (Polyoxyethylene (40)
163 stearate), Span 85 (Sorbitan trioleate), Span 80 (Sorbitan monooleate), Tween 80
164 (Polyoxyethylene sorbitan monooleate) and Tween 20 (Polyoxyethylene sorbitan monolaurate)
165 were kindly donated by Croda international Plc (UK). Lipoid E PC S (Phosphatidylcholine)
166 was gifted by Lipoid GmbH (Germany). Taurodeoxycholic acid sodium salt (NaTDC),
167 pancreatic lipase (8 x USP), Cremophor RH40 (Polyoxyl 40 hydrogenated castor oil) and
168 Tween 85 (Polyoxyethylene sorbitan trioleate) were ordered from Sigma-Aldrich (Ireland). A
169 sample of Labrasol (Caprylocaproyl polyoxyl-8 glycerides), Labrafil M1944 CS (Oleoyl
170 polyoxyl-6 glycerides), Labrafil M2125 CS (Linoleoyl polyoxyl-6 glycerides), Plurol Oleique
171 CC 497 (Polyglyceryl-3 dioleate) and Gelucire 44/14 (Lauroyl polyoxyl-32 glycerides) was
172 kindly donated by Gattefossé (France). A sample of Cremophor EL (Polyoxyl 35 castor oil)
173 was kindly donated by BASF (Germany). All other chemicals and solvents were of analytical
174 or high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-
175 Aldrich (Ireland) and used as received.

176

177 *Solubility studies*

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

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

191
192 The samples were analysed as described previously.²⁴ In brief, an Agilent 1200 series HPLC
193 system comprised a binary pump, degasser, autosampler and variable wavelength detector.
194 Data analysis was done with EZChrom Elite version 3.2. Nilotinib was separated with a Zorbax
195 Eclipse Plus-C18 column (5 µm, 4.6 mm x 150 mm) including a Zorbax Eclipse Plus-C18
196 guard column (5 µm, 4.6 mm x 12.5 mm) at 25 °C. The mobile phase consisted of acetonitrile,
197 methanol, water and triethylamine (35:30:34:1 v/v) and was used at a flow rate of 0.9 mL/min.
198 20 µL samples were injected and the detection wavelength was 267 nm. The limit of detection
199 (LOD) and limit of quantification (LOQ) of this method was 4 ng/mL and 12 ng/mL,
200 respectively. Values were determined using the standard error of y-intercept according to the
201 ICH Q2 guideline⁴² and linearity was confirmed between 12 ng/mL and 12 µg/mL drug
202 concentration.

203

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

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

209 operated by the Tiamo 2.2 software. The *in vitro* protocol was amended from Williams *et al.*
210 ^{43,44} In brief, the buffer contained 2 mM TRIS maleate, 150 mM NaCl, 1.4 mM CaCl₂·2H₂O,
211 adjusted to pH 6.5. For the digestion experiments the buffer was supplemented with 3 mM
212 NaTDC and 0.75 mM PC and stirred for 12 hours before further usage. The pancreatin extract
213 was prepared freshly by adding 5 mL of 5 °C buffer to 1 g of porcine pancreatic enzymes (8x
214 USP), which was vortexed thoroughly. The mixture was centrifuged for 15 min at 5 °C, 2800g
215 (Hettich Rotina 380R) and 4 mL of supernatant was recovered and stored at 2 – 8 °C before
216 further usage. The pancreatic extract had a pancreatic lipase activity of ~10 000 TBU/mL (to
217 provide approximately 1000 TBU per mL of digest), where 1 TBU represents the amount of
218 enzyme that liberates 1 μmol of FA from tributyrin per min. ⁴⁵ All experiments were conducted
219 with a stirring speed of 450 rpm.

220
221 For the digestibility study, 1.0 g of blank excipient was dispersed into 36 mL of digestion
222 buffer. The pH was adjusted to 6.5 using 0.2 M, 0.6 M or 1 M NaOH depending on the pH
223 change upon excipient addition. Digestion was initiated by the addition of 4 mL pancreatic
224 enzyme and the pH of 6.5 was maintained using 0.2 M and 0.6 M NaOH for long and medium
225 chain excipients, respectively. After 60 min of digestion the enzymes were inhibited using 1 M
226 4- bromophenylboronic acid in methanol (5 μL per mL media) and the pH was increased to
227 9.0. An additional blank titration using solely the digestion buffer was performed and the
228 released mmol of FFAs from the blank was subtracted from the mmol of FFAs released from
229 the surfactant formulations. The determined amount of FFA was assumed as a surrogate
230 parameter for digestibility. Additionally, the % digested was calculated using the theoretical
231 released FFAs per g of excipient:

$$232 \quad \textit{Theoretical FFA [mmol]} = \frac{SV [mg]}{56.1056 \left[\frac{g}{mol}\right]} \quad (1)$$

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

$$238 \quad \% \textit{ digested} = \frac{\textit{Released FFA [mmol]}}{\textit{Theoretical FFA [mmol]}} \times 100 \% \quad (2)$$

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

242

243 For the *in vitro* lipolysis experiment with nilotinib 1.075 g of lipid formulation was dispersed
244 into 39 mL of digestion buffer for 10 min. Three 1 mL samples were taken at 2.5, 5 and 10 min
245 from the middle of the vessel. pH of the media was adjusted and maintained at 6.5 using 0.2 M
246 NaOH and 0.6 M NaOH for medium and long chain excipients, respectively. To the remaining
247 36 mL (1 g lipid formulation) dispersion 4 mL of pancreatin extract was added to initialize
248 digestion. After 60 min the released non-ionized FFAs were determined by a pH increase of
249 the buffer to pH 9.

250

251 Samples of 1 mL were taken at 5, 10, 15, 30, 45 and 60 min during the digestion experiment
252 from the middle of the vessel. In each sample and after 60 min the enzymes were inhibited by
253 the addition of 1 M 4-bromophenylboronic acid in methanol (5 μ L per mL sample).
254 Additionally, to each 1 mL sample during digestion a 100 μ L sample was taken and added to
255 900 μ L 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 21,000g 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 °C 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

269 **Table 1** Dose:solubility ratio of studied surfactant formulations. The dose was fixed at
270 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

271

272 *In vivo study*

273 The protocol used for the *in vivo* pharmacokinetic study was approved by the institutional
274 animal ethics committee in accordance with Belgian law regulating experiments on animals
275 and in compliance with EC directive 2010/63/EU and the NIH guidelines on animal welfare.
276 Male Sprague-Dawley rats weighing 280-320 g (8-10 weeks of age) on the day of the
277 experiments were purchased from Charles River Laboratories Deutschland (Sulzfeld,
278 Germany) and maintained on standard food and water *ad libitum* in the laboratory for at least
279 5 days before entering the experiment. Food was removed 16-20 h before dosing and water
280 was available *ad libitum* at all times. Parallel groups of animals were administered with each
281 formulation at a volume of 2 mL/kg by oral gavage with a nilotinib dose of 20 mg/kg. By

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

286

287 *Bioanalysis*

288 The plasma concentrations of nilotinib were determined by reversed phase HPLC. The Agilent
289 1260 series HPLC system comprised a binary pump, degasser, temperature controlled
290 autosampler, column oven and diode array detector. The system was controlled, and the data
291 analysed with EZChrom Elite version 3.3.2. The used method was described earlier.²⁴ In brief,
292 a Zorbax Eclipse Plus-C18 column (5 μ m, 4.6 mm x 150 mm) with a Zorbax Eclipse Plus-C18
293 guard column (5 μ m, 4.6 mm x 12.5 mm) was used. The mobile phase consisted of water,
294 methanol, acetonitrile and triethylamine (34:30:35:1 v/v) and was used at a flow rate of 0.9
295 mL/min. The sample and column temperature were set at 5 °C and 25 °C, respectively, and the
296 detection wavelength was 267 nm. Nilotinib was extracted from the plasma samples by liquid-
297 liquid extraction. To 50 μ L of the plasma sample 66 μ L of a methanol acetonitrile mixture
298 (30:35 v/v), containing 1.25 μ g/mL sorafenib as internal standard, was added. The mixture was
299 mixed thoroughly and centrifuged at 22 °C, 11,500g for 9 min. 50 μ L of the supernatant was
300 injected to the HPLC system for analysis. The LOD and LOQ values of this method in plasma
301 were 11 ng/mL and 37 ng/mL, respectively, as determined using the standard error of y-
302 intercept according to the ICH Q2 guideline.⁴² Linearity was confirmed between 37 ng/mL
303 and 4.1 μ g/mL drug concentration.

304

305 *Data Analysis*

306 The pharmacokinetic parameters were calculated using Microsoft Excel. The plasma
307 concentration profiles were analysed by non-compartmental analysis and calculation of AUC
308 by the linear trapezoidal rule. Absolute bioavailability was calculated using previous reported
309 intravenous data.⁴⁶ The statistical analysis for all *in vivo* parameters was performed using a
310 one-way analysis of variance (one-way ANOVA) after using the Bartlett's test to check for
311 equal variance. Groups were pairwise compared using Tukey's multiple comparison test.
312 A one-way ANOVA was also calculated for the lipolysis data using the Tukey post-hoc test to
313 compare the different formulation performances. All statistical analyses were carried out using
314 GraphPad Prism 5.

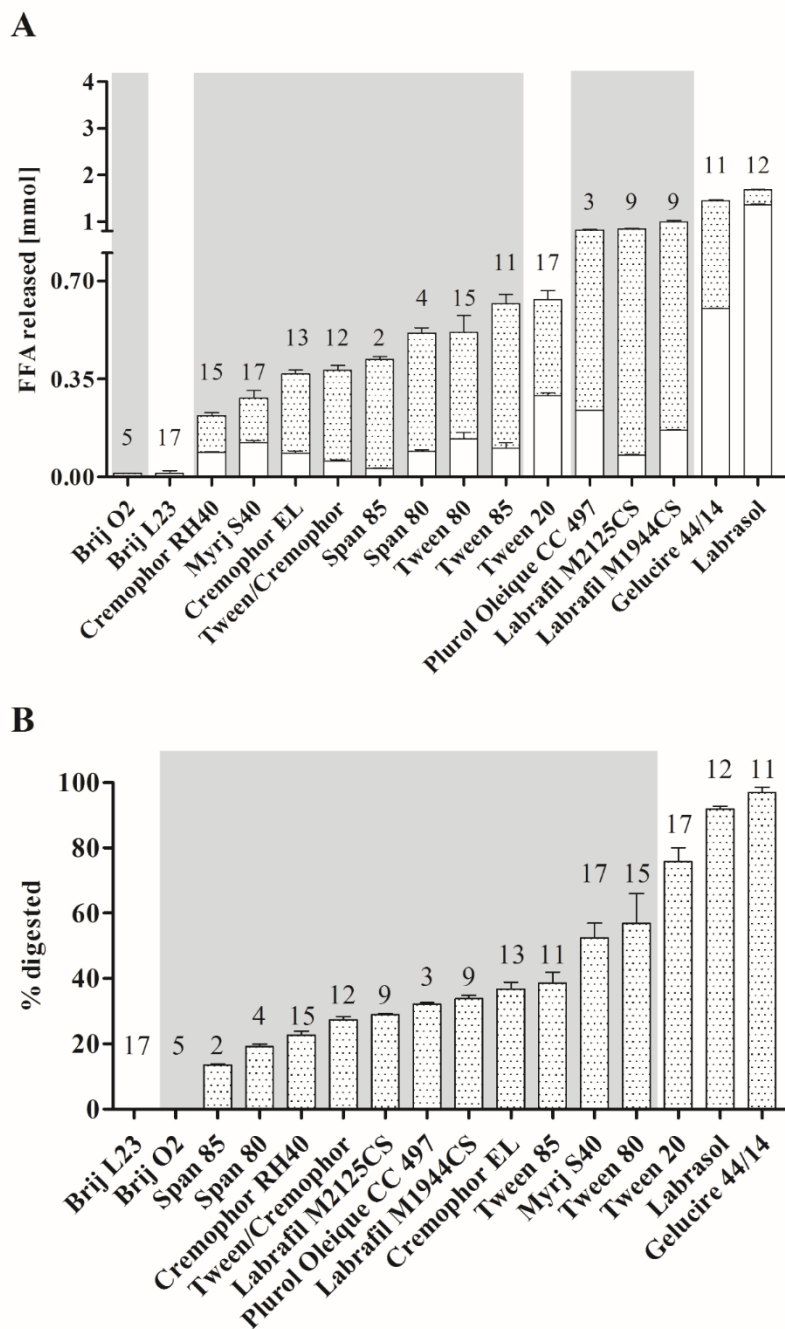
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316 **RESULTS**

317 *Ranking the digestibility of lipid-based surfactants*

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

325



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 \pm SD,
 331 where n=3. A: Free fatty acids (FFA) released. The total FFA released is divided into the

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

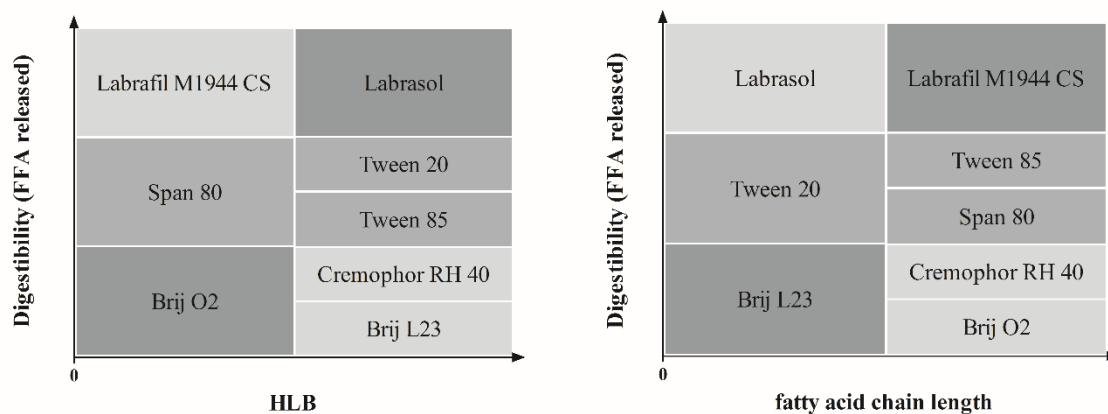
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336 In general, medium chain-based surfactants displayed the highest quantity of FFA released.
337 Labrasol, a medium chain excipient mainly consisting of PEG-8 mono- and diesters of caprylic
338 and capric acid showed the highest release of FFA (Figure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

345

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

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



371
 372 **Figure 2.** Selected excipients for further *in vitro* and *in vivo* evaluation. Excipients were
 373 selected according to the fatty acid chain length, HLB and total free fatty acids (FFA) released
 374 during the lipolysis experiments as a surrogate parameter for digestibility.

375

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

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

382 **Table 2.** Surfactant properties, solubility of nilotinib in surfactants, nilotinib solubilisation in the aqueous phase of the *in vitro* lipolysis test after 60
 383 min, FFA released and % digested after 60 min of digestion and absolute bioavailability of nilotinib formulations in male Sprague-Dawley rats (mean
 384 \pm 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]	<i>In vitro</i> solubilisation [%] ^{b)}	<i>In vivo</i> absolute bioavailability [%]	FFA released [mmol]	% digested
Tween 20	MC	16.7	2.07 \pm 0.22	1.76 \pm 0.11	66.50 \pm 21.96	0.63 \pm 0.04	75.78 \pm 4.25
Labrasol	MC	12	4.12 \pm 0.05	0.66 \pm 0.07	33.82 \pm 11.52	1.69 \pm 0.02	91.81 \pm 0.98
Labrafil M1944 CS	LC	9	0.88 \pm 0.45	0.45 \pm 0.08	26.02 \pm 6.86	0.99 \pm 0.03	33.82 \pm 1.04
Span 80	LC	4.3	0.56 \pm 0.03	1.12 \pm 0.09	25.99 \pm 11.48	0.51 \pm 0.02	19.09 \pm 0.85
Cremophor RH40	LC	15	3.40 \pm 0.41	11.07 \pm 1.40	22.83 \pm 10.00	0.22 \pm 0.01	22.61 \pm 1.25
Tween 85	LC	11	1.27 \pm 0.06	6.40 \pm 0.36	19.38 \pm 6.89	0.62 \pm 0.05	38.61 \pm 3.32
Tween 85/ Cremophor RH40 67:33 (w/w)	LC	12.3	1.88 \pm 0.08	6.94 \pm 0.52	17.08 \pm 5.62	0.38 \pm 0.01	27.33 \pm 0.97
Brij L23	MC	16.9	2.13 \pm 0.03	11.80 \pm 1.78	16.71 \pm 5.27	0.00 \pm 0.01	0.00 \pm 0.00
Brij O2	LC	4.9	0.69 \pm 0.04	0.19 \pm 0.05	7.32 \pm 3.87	- 0.05 \pm 0.02	0.00 \pm 0.00

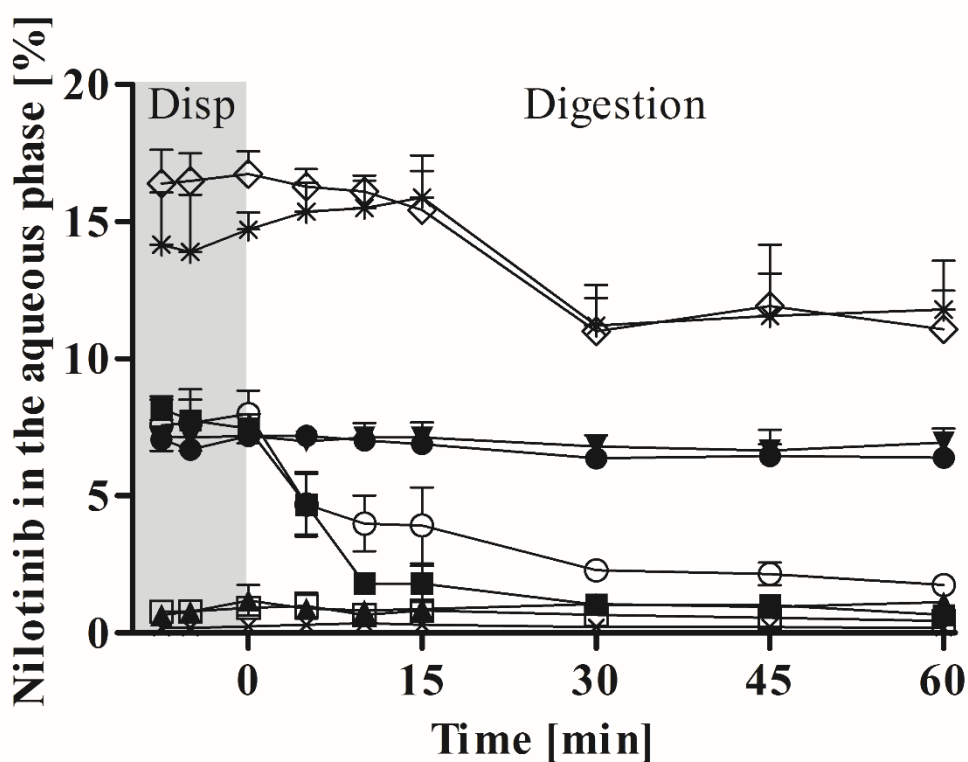
385 ^{a)} MC: medium chain, LC: long chain

386 ^{b)} Aqueous phase concentration in the *in vitro* lipolysis test after 60 min of digestion

387 Comparing nilotinib solubilisation following *in vitro* dispersion and digestion.

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

395



396

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

401 All formulations displayed good dispersion characteristics upon addition to the media. The
402 highest concentration of nilotinib upon dispersion was observed for Cremophor RH40 with 16
403 $\pm 1\%$ of the dose dissolved in the aqueous phase. This was followed by Brij L23 with $14 \pm 2\%$
404 of dissolved nilotinib in the aqueous phase. Approximately 6 - 8% of the nilotinib dose was
405 dissolved in the case of Labrasol, Tween 20, Tween 85 and the Tween 85/Cremophor RH40
406 mixture, which was mid-range relative to the other tested formulations. Poor solubilisation was
407 observed for Span 80, Brij O2 and Labrafil M1944 CS with concentrations $< 1\%$.

408
409 Upon initiation of digestion Cremophor RH40 and Brij L23 were able to maintain relatively
410 high concentrations for 15 min followed by a decrease in concentration to $11 \pm 1\%$ at 30 min,
411 which was maintained throughout the rest of digestion. The Tween 85/Cremophor RH40
412 mixture and Tween 85 maintained the initial dispersion concentration throughout digestion.
413 Labrasol and Tween 20 showed an initial drop in concentration and a further decrease
414 throughout the 60 min resulting in concentrations below 2%. The surfactants that demonstrated
415 very low nilotinib concentrations $< 1\%$ upon dispersion also maintained the low concentrations
416 throughout digestion. The ranking of the nilotinib concentration in the aqueous phase upon
417 dispersion was Cremophor RH40 \geq Brij L23 $>$ Tween 20 \geq Labrasol \geq Tween 85 \geq Tween
418 85/Cremophor RH40 $>$ Span 80 \geq Labrafil M1944 CS $>$ Brij O2. After 60 min of digestion the
419 aqueous phase concentration of nilotinib was ranked Brij L23 \geq Cremophor RH40 $>$ Tween
420 85/Cremophor RH40 mixture \geq Tween 85 $>$ Tween 20 \geq Span 80 \geq Labrasol \geq LabrafilM 1944
421 CS \geq Brij O2. Overall, there was no apparent relationship between the ranking in solubilisation
422 capacity and surfactant HLB FA chain length or digestibility.

423
424 In order to gain insights into the nilotinib distribution post-digestion, all samples were separated
425 into three different phases (solid, aqueous and lipid phase) by centrifugation (Figure S 2). The

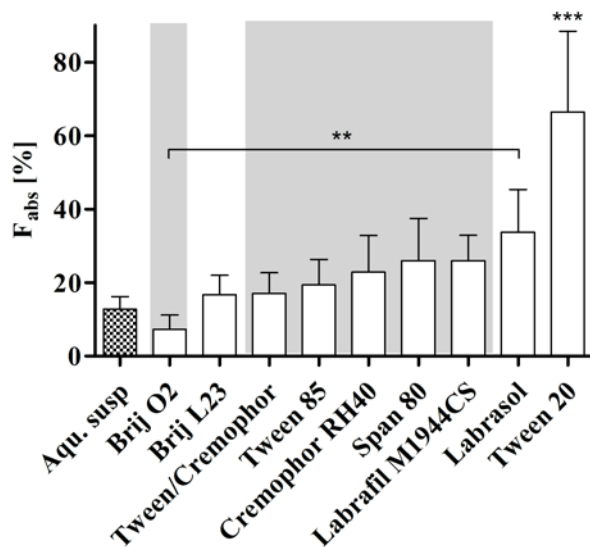
426 solid phase represents the undissolved crystalline drug within the surfactant formulation and all
427 formulations showed the highest amount of nilotinib in the solid phase. Additionally, in the
428 case of Labrafil M1944 CS, Span 80 and Brij O2 an oily phase was detected throughout
429 digestion.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

431

432 *Ranking the in vivo bioavailability of surfactants*

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

438



439

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

444 Cremophor RH40 (67:33 w/w). Shaded area represents long chain excipients and non-shaded
445 area medium chain excipients (mean \pm SD, n = 5).
446
447

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 \pm SD.

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

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

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

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

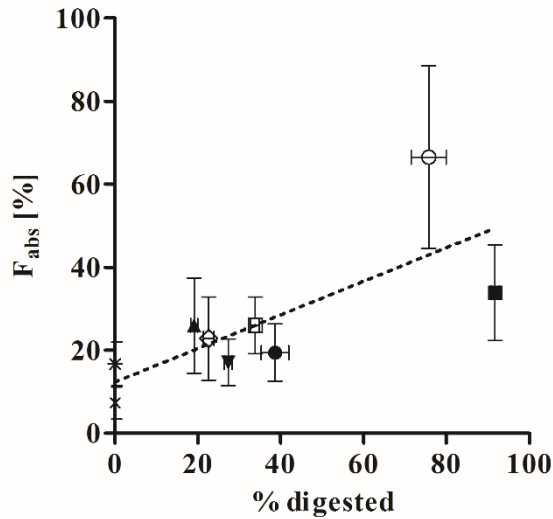
464

465 *Relationship between surfactant digestibility and in vivo bioavailability*

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

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

481



482

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

487

488 DISCUSSION

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

500

501 To the best of our knowledge, this is the first study to compare digestibility of a range of 15
502 commonly used surfactants and hence this allows the establishment of a digestibility databank
503 for surfactants. Overall, the findings suggested that surfactants containing medium chain FAs
504 displayed a higher digestibility compared to surfactants containing long chain FAs. This finding
505 agrees with previous reports of smaller sets of surfactants⁴⁹ as well as glycerides⁵³ and lipid
506 formulations.⁴³ The lower digestibility of surfactants containing long chain FAs can be
507 attributed to the extent of long chain FA ionisation^{43,54} and the limited solubilisation capacity
508 of the digestion media for the long chain FAs.^{43,53} Long chain FAs are ionised to a lower extent
509 relative to medium chain FAs at the pH of 6.5.^{43,53} Therefore, the non-ionised long chain FAs
510 are in need of micellar solubilisation due to a poor solubility in the digestion media. Once the
511 solubilisation capacity of the digestion media for long chain FAs is reached, the long chain FAs
512 accumulate at the droplet/micellar interface, effectively inhibiting further enzyme binding and

513 digestion.⁴³ In addition, the analysis of the surfactant digestibility revealed that digestibility
514 was not influenced by the HLB value. This indicates that surfactant digestibility was not readily
515 predictable and highlights the importance of *in vitro* lipolysis in the characterisation and
516 selection of surfactants in oral dosage forms. It should also be acknowledged that the reported
517 *in vitro* digestibility (FFA released and % digested) might not be entirely translatable to an *in*
518 *vivo* digestibility, as non-ionic surfactants and their lipolytic products can inhibit digestion *in*
519 *vitro* depending on the given conditions. For example, Cremophor RH40, Cremophor EL,
520 Tween 80, Tween 20 and Brij L23 are known to inhibit digestion to varying extents^{51, 55, 56}
521 leading to a lower or slower⁵⁷ digestion compared to *in vivo*, where the inhibiting surfactants
522 and their lipolytic products could be absorbed. Additionally, the type of enzyme or enzymatic
523 extract as well as pH of the media has been shown to influence the enzymatic activity and
524 consequently the overall extent of digestion, as shown for the excipient Labrasol.⁵² Thus, the
525 total amount of released FFA and/or a given % digested depends on the employed *in vitro* or *in*
526 *vivo* system.

527

528 Among the surfactants, the highest absolute bioavailability was observed for the medium chain
529 excipients Tween 20 ($F_{abs} 66.50 \pm 21.96\%$), which was statistically significant higher compared
530 to all other surfactants ($p < 0.05$). The second highest bioavailability was observed for the
531 medium chain surfactant Labrasol, which was statistically significant higher compared to Brij
532 O2 ($p < 0.05$). Both Tween 20 and Labrasol are digestible medium chain-based surfactants
533 indicating that the *in vivo* performance might be influenced by the release of medium chain FA.
534 In terms of the HLB value, a relationship to the *in vivo* performance was not apparent.
535 Additionally, the *in vivo* performance was not influenced by the solubility of nilotinib in the
536 excipients. For example, even though the dose-solubility ratio for Brij L23 (4.7) and Tween 20
537 (4.8) were similar, the bioavailability was significantly higher for the Tween 20 formulation.

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

540

541 Interestingly, the study suggested that the *in vivo* exposure was influenced by the digestibility
542 of the surfactants, and in general highly digestible surfactants such as Labrasol, Tween 20 and
543 Labrafil M1944 CS displayed the highest bioavailability. Similarly, the two least digested
544 surfactants Brij L23 and Brij O2 showed the lowest bioavailability, which may suggest that the
545 drug was trapped within the non-digestible surfactant micelles. This finding is in line with the
546 observation by Berthelsen and co-workers where bioavailability did not increase with
547 increasing surfactant concentration due to an entrapment in Cremophor RH40 micelles (which
548 displayed lower digestibility relative to Cremophor EL). In contrast, in the case of the
549 Cremophor EL surfactant micelles, which displayed higher digestibility, bioavailability
550 increased with increasing surfactant concentration.⁵⁰ It should also be noted that it has been
551 reported in the literature that surfactant only formulations of either Cremophor RH40 or EL did
552 not lead to significant differences in bioavailability of danazol in dogs.⁴⁹ On the contrary, when
553 incorporating surfactants into self-emulsifying lipid-based formulations, the poorly digestible
554 surfactant Cremophor RH40 (55% w/w) displayed higher oral bioavailability of danazol
555 compared to Cremophor EL (55% w/w). Possible reasons for this differing outcome between
556 studies, may reflect the differing role of surfactant between different formulation types.
557 Specifically, in the case of self-emulsifying systems, the surfactants also serve to support self-
558 emulsification and stabilisation of the emulsified oil phase. As such, digestion of surfactants
559 may lead to destabilisation of the emulsion droplet, leading to drug precipitation. Therefore, for
560 oil containing self-emulsifying formulations, it appears that low digestibility surfactants are
561 favoured to improve emulsion stability during digestion and reduce the risk of drug
562 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-

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

593

594 During digestion FFAs are released, which generate a variety of colloidal species in
595 combination with bile salts, phospholipids and lipolytic products, which potentially have a
596 higher solubilisation capacity for drugs. For nilotinib it was previously shown that the solubility
597 in post-digestive media of a lipid formulation was increased.²⁴ In addition, it was reported that
598 the solubility of nilotinib is influenced by the bile salt concentration as evidenced by a higher
599 solubility in FeSSIF ($3.2 \pm 0.1 \mu\text{g/mL}$) compared to FaSSIF ($0.3 \pm 0.03 \mu\text{g/mL}$).²⁴ As FFAs
600 can increase the bile salt release *in vivo*,⁶⁰⁻⁶² digestion appears to be a crucial parameter for the
601 success of the surfactant-only formulations. This was especially apparent for the digestible
602 Tween 20 and non-digestible Brij L23, which both contain the medium chain FA lauric acid.
603 The non-digestible formulation resulted in a significantly lower exposure indicating that the
604 release of lauric acid may have been the driving factor for an improved bioavailability, as FFAs
605 have also shown beneficial effects on drug permeability without pronounced cytotoxic effects.
606^{25, 28-30, 32, 33, 63-65} As nilotinib is passively transported,⁶⁶ the permeability enhancing effects of
607 surfactants¹² and their lipolytic products may have contributed to a higher bioavailability of
608 nilotinib when compared to the aqueous suspension.

609

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

613 15 min of digestion was observed. Similarly, the nilotinib aqueous phase concentration
614 decreased in the case of Labrasol and Tween 20 upon initiation of digestion resulting in a total
615 decrease of approximately 6.2% and 6.8%, respectively. However, the drop in nilotinib
616 concentration in the aqueous phase did not seem to be exclusively related to the digestibility of
617 these surfactants (Figure 1), as Labrasol and Tween 20 are highly digested, but Cremophor
618 RH40 and Brij L23 are poorly or not digested. Furthermore, Labrafil M1944 CS and Tween 85,
619 which are both rapidly and > 33% digested (Figure S 3, Figure 1), did not show a decrease in
620 nilotinib concentration upon initiation of digestion. However, given that Tween 20 and Labrasol
621 are both medium chain-based surfactants, it appears that the initial drop in nilotinib aqueous
622 phase concentration upon initiation of digestion most likely reflects the combination of high
623 digestibility and the release of medium chain FAs. This may reflect the lower solubilisation
624 capacity of medium chain post digestive media, as previously reported.^{67, 68} While there was
625 no direct relationship between the HLB value of the surfactants and the performance in the *in*
626 *vitro* lipolysis, there seemed to be a trend that surfactants with a HLB value > 10 performed
627 better compared to surfactants with a HLB value < 10. For example, Brij L23 (HLB value: 16.9)
628 showed an *in vitro* solubilisation of $11.8 \pm 1.8\%$, whereas Brij O2 (HLB value: 4.9) only
629 reached an *in vitro* solubilisation of $0.2 \pm 0.1\%$. Surfactants with a HLB value > 10 are
630 considered suitable for stabilising oil in water systems, whereas surfactants with a HLB value
631 < 10 are suitable for stabilising water in oil systems. Thus, the higher HLB surfactants seem to
632 stabilise solubilised nilotinib, which is highly lipophilic ($\log P$: 4.95), in the aqueous phase
633 better compared to low HLB surfactants. In contrast to the HLB value trend, the *in vitro*
634 lipolysis test indicated that digestibility of the surfactants is not related to nilotinib solubilisation
635 in the aqueous phase *in vitro*. For example, Brij L23 (non-digestible) and Cremophor RH40
636 (digestible) demonstrated comparable aqueous phase concentrations but different digestibility.
637 Additionally, no trend was established between the FA chain length and the aqueous phase

638 concentration of nilotinib. The aqueous phase concentration of the *in vitro* lipolysis is thought
639 to represent the amount of drug readily available for absorption *in vivo* and is commonly used
640 to rank formulation performances. However, due to the limitations of the *in vitro* systems, such
641 as the lack of an absorptive sink, low media volume in combination with a high drug and
642 excipient load as well as different hydrodynamics and enzyme activity compared to *in vivo*, a
643 relationship between *in vitro* and *in vivo* performance for surfactants^{49,50} or other formulations
644^{24, 69, 70} has been reported difficult in some cases. Also, this study could not demonstrate a
645 correlation between the aqueous phase concentration of the *in vitro* lipolysis test and the *in vivo*
646 performance. For example, the relatively higher *in vitro* solubilisation of Brij L23 and
647 Cremophor RH40 did not correlate with a higher *in vivo* performance, as bioavailability values
648 of Cremophor RH40 and Brij L 23 were in the mid (approximately 23%) and low
649 (approximately 17%) end of the range of absolute bioavailability obtained *in vivo*. An
650 adjustment of the *in vitro* model to rat conditions (e.g. low enzyme activity and low
651 gastrointestinal volumes),^{71, 72} two stages (gastric and intestinal)⁷³⁻⁷⁵ or the addition of an
652 absorptive sink^{76, 77} may offer additional insights into the *in vivo* behaviour.

653

654 CONCLUSION

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

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

668

669 SUPPORTING INFORMATION

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

673

674 ACKNOWLEDGEMENTS

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

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