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**University College Cork**
**School of Pharmacy**




# Exploring the utility of lipid-based formulation technology to enhance oral bioavailability of BCS class IV molecules

Thesis presented by

**Niklas J. Köhl**

*In fulfilment of the requirements for the degree of*

**Doctor of Philosophy**

*under the supervision of*
**Prof. Dr. Brendan T. Griffin (University College Cork)**

**Prof. Dr. René Holm (Janssen Pharmaceutica NV)**
*and*
**Prof. Dr. Martin Kuentz (FHNW)**

*Head of School:*
Prof. Dr. Stephen Byrne
submitted: April 2020









**It is important to make a dream of life and a dream reality.**

Pierre Curie

Pierre Curie: With Autobiographical Notes by Marie Skłodowska Curie (1924)

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# Declaration

This thesis has not been previously submitted, in part or in whole, to this or any other university for any degree and is, except where duly noted and acknowledged, the original work of the author.

## Author Contribution

All work was performed independently by the author, with the following exceptions:

**Chapter 2, Chapter 3 and Chapter 4**

The oral bioavailability studies were conducted at Janssen Pharmaceutica NV, Beerse, Belgium and facilitated by Dr. René Holm. This included preparation of suspensions for the *in vivo* studies, oral gavage and blood collection as well as plasma harvesting.

**Chapter 5**

Dr. Brendan Griffin, Mr. Vincent Mehigan, Ms. Laura Henze assisted during the oral bioavailability study. Ms Laura Henze additionally assisted during formulation development.

**Chapter 6**

Dr. Brendan Griffin, Ms. Harriet Bennett-Lenane assisted during the oral bioavailability study. Mr. Daniel Price conducted the *in silico* screening.

**Chapter 7**

Dr. John J. Keating assisted in the development of the salt formation protocol and the analysis of NMR data. Dr. Andreas Marx (Merck KGaA), Dr. Denis Lynch performed the NMR analysis. Mr. John Meehan performed the elemental analysis. Dr. Waleed Faisal assisted with the PXRD analysis. Dr. Thomas De Vijlder performed the LC-MS analysis. Dr. Brendan Griffin and Ms. Harriet Bennett-Lenane assisted during the oral bioavailability study.

Signed: _____ Date: 30/04/2020

# Author publications

i. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

Research paper titled 'New Insights into Using Lipid Based Suspensions for 'Brick Dust' Molecules: Case Study of Nilotinib', published 22nd of February 2019 in Pharmaceutical Research. DOI: 10.1007/s11095-019-2590-y.

ii. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

Research paper titled 'Chase dosing of lipid formulations to enhance oral bioavailability of nilotinib in rats', published 22nd of February 2019 in Pharmaceutical Research. DOI: 10.1007/s11095-020-02841-9

iii. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

Research paper titled 'Exploring impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib', published 10th of July 2020 in Molecular Pharmaceutics. DOI: 10.1021/acs.molpharmaceut.0c00305

iv. Niklas J. Koehl, Laura J. Henze, Martin Kuentz, René Holm, Brendan T. Griffin

Research paper titled 'Supersaturated lipid-based formulations to enhance oral bioavailability of venetoclax', published 18th June 2020 in Pharmaceutics. DOI: 10.3390/pharmaceutics12060564

v. Niklas J. Koehl, Laura J. Henze, Harriet Bennett-Lenane, Daniel J. Price, René Holm, Martin Kuentz, Brendan T. Griffin

Research paper titled '*In silico*, *in vitro* and *in vivo* evaluation of precipitation inhibitors in supersaturated lipid-based formulations of venetoclax', submitted 17th June 2020 to Molecular Pharmaceutics

vi. Niklas J. Koehl, Laura J. Henze, René Holm, Martin Kuentz, JJ. Keating, Brendan T. Griffin

Research paper titled ‘Lipophilic salts and lipid-based formulations for bridging the food effect gap of venetoclax’, submitted 02nd of March 2021 to Journal of Pharmaceutical Sciences

# Conference presentations

i. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

'Lipid suspensions for highly lipophilic 'brick dust' molecules – case study: Nilotinib'

Oral presentation given at the 2nd all WG UNGAP Meeting, Sofia, 12th – 13th of February 2019.

ii. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

'Lipid suspensions for highly lipophilic 'brick dust' molecules – case study: Nilotinib'

Poster presentation given at the 2nd all WG UNGAP Meeting, Sofia, 12th – 13th of February 2019.

iii. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

'New insights into using lipid-based suspensions for 'brick dust' molecules: case study of Nilotinib'

Poster presentation given at the 3rd European Conference on Pharmaceutics, Bologna, 25th – 26th of March 2019.

iv. Niklas J. Koehl, René Holm, Martin Kuentz, Brendan T. Griffin

'Lipid suspensions versus chase dosing for increasing oral delivery of 'brick dust' molecules: Case study Nilotinib'

Poster presentation given at the Keystone symposia on delivering therapeutics across biological barriers, Dublin, 6th – 9th of May 2019.

v. Niklas J. Koehl, Laura J. Henze, René Holm, Martin Kuentz, Brendan T. Griffin

'Exploring precipitation inhibitors in supersaturated lipid-based formulations: case study venetoclax'

Poster presentation given at the AAPS PharmSci360, San Antonio, TX, 3rd – 6th of November 2019.

## Concomitant Peer Reviewed Publications

i. Laura J. Henze, Niklas J. Koehl, Joseph P. O'Shea, René Holm, Brendan T. Griffin

Review titled 'The pig as a preclinical model for predicting oral bioavailability and *in vivo* performance of pharmaceutical oral dosage forms: a PEARRL review', published 10th of April 2018 in the Journal of Pharmacy and Pharmacology.

DOI: 10.1111/jphp.12912.

ii. Daniel J. Price, Felix Ditzinger, Niklas J. Koehl, Sandra Jancovic, Georgia Tsakiridou, Anita Nair, René Holm, Martin Kuentz, Jennifer B. Dressman, Christoph Saal

Review titled 'Approaches to increase mechanistic understanding and aid in the selection of precipitation inhibitors for supersaturating formulations - a PEARRL review', published 16th of May 2018 in the Journal of Pharmacy and Pharmacology. DOI: 10.1111/jphp.12927.

iii. Felix Ditzinger, Daniel Price, Alexandra-Roxana Ilie, Niklas J. Koehl, Sandra Jankovic, Georgia Tsakiridou, Simone Aleandri, Lida Kalantzi, René Holm, Anita Nair, Christoph Saal, Brendan T. Griffin, Martin Kuentz

Review titled 'Lipophilicity and hydrophobicity considerations in bio-enabling oral formulations approaches - a PEARRL review' published 2nd of August 2018 in the Journal of Pharmacy and Pharmacology. DOI: 10.1111/jphp.12984.

iv. Sandra Jankovic, Georgia Tsakiridou, Felix Ditzinger, Niklas J. Koehl, Daniel J. Price, Alexandra-Roxana Ilie, Lida Kalantzi, Kristof Kimpe, René Holm, Anita Nair, Brendan T. Griffin, Christoph Saal, Martin Kuentz

Review titled 'Application of the solubility parameter concept to assist with oral delivery of poorly water-soluble drugs - a PEARRL review', published 5th of July 2018 in the Journal of Pharmacy and Pharmacology. DOI: 10.1111/jphp.12948.

v. Laura J. Henze, Niklas J. Koehl, Joseph P. O'Shea, René Holm, Maria Vertzoni, Brendan T. Griffin

Research article titled 'Toward the establishment of a standardized pre-clinical porcine model to predict food effects – Case studies on fenofibrate and paracetamol', published 1st of December 2019 in the International Journal of Pharmaceutics. DOI: 10.1016/j.ijpx.2019.100017

vi. Laura J. Henze, Niklas J. Koehl, Regina Jansen, René Holm, Maria Vertzoni, Phil D. Whitfield, Brendan T. Griffin

Research article titled 'Development and evaluation of a biorelevant medium simulating porcine gastrointestinal fluids', published 1st of September 2020 in the European Journal of Pharmaceutics and Biopharmaceutics. DOI: 10.1016/j.ejpb.2020.06.009

vii. Laura J. Henze, Niklas J. Koehl, Harriet Bennett-Lenane, René Holm, Michael Grimm, Felix Schneider, Werner Weitschies, Mirko Koziolek, Brendan T. Griffin

Research article titled 'Characterization of gastrointestinal transit and luminal conditions in pigs using a telemetric motility capsule', published 1st of January 2021 in the European Journal of Pharmaceutical Sciences. DOI: 10.1016/j.ejps.2020.105627

# Abstract

**Purpose** Increasing numbers of poorly water-soluble drugs emerging from discovery pipelines have led to formulation delivery challenges. Poorly water-soluble drugs that exhibit limited *in vivo* bioavailability generate a need for bio-enabling formulation approaches, such as lipid-based formulations (LBF), to ensure maximal *in vivo* exposure. While traditionally, highly lipophilic drugs (i.e. 'grease ball' drugs) were considered to be suitable candidates for a LBF approach, to date few studies have attempted to explore the biopharmaceutical benefits of LBFs for 'brick dust' drug candidates due to excessive hydrophobicity which tends to lead to insufficient solubility in lipid excipients and dose loading limitations in LBF solutions. Therefore, the increasing pool of hydrophobic and lipophilic drug candidates emerging from discovery are traditionally not considered for a LBF approach. This creates a need for advanced LBF types to address the dose loading limitations and potentially unleash the additional lipid mediated effects for enhancing oral absorption such as increased post-digestive intestinal solubilisation, increased permeability and promotion of lymphatic transport. While there are a range of advanced LBFs available, studies to date have mostly been demonstrated with model drugs that no longer truly reflect the emerging drug discovery space and hence the applicability of these alternative LBF approaches to the current 'brick dust' pipeline drugs is unknown. The aim of this thesis was to explore the utility of advanced LBFs to overcome solubility limited oral absorption of poorly water-soluble 'brick dust' drugs, as well as the establishment of an industrial developability guide for LBF development for hydrophobic, lipophilic and high molecular weight drugs.

**Methods** The suitability of LBF suspensions, supersaturated LBF solutions (sLBF), LBF solutions using lipophilic salts and the chase dosing approach for current pipeline drugs was evaluated using the model 'brick dust' drugs nilotinib and venetoclax. In addition, the incorporation of precipitation inhibitors (PIs) into sLBFs was investigated. The formulations

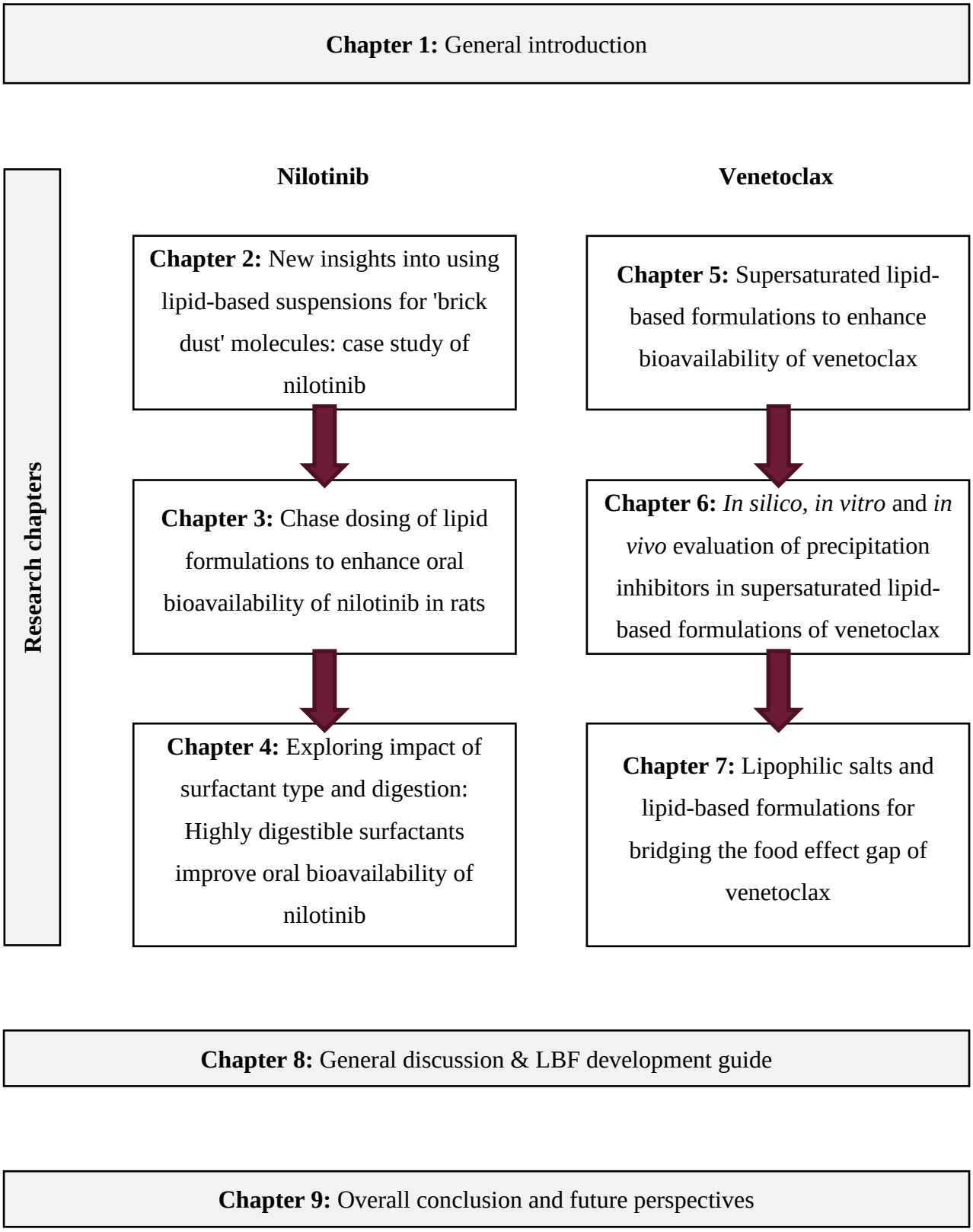
were assessed *in vitro* using the standard pH stat lipolysis setup as well as quick screening tools such as simulated post-digestive media or a solvent shift based supersaturation/precipitation assay. All tested formulations were subsequently evaluated *in vivo* either in rats or landrace pigs. Subsequently, based on the findings in these studies and current literature a LBF development guide was proposed.

**Results** This thesis has firstly demonstrated that while surfactant suspensions increased the bioavailability, poorly dispersible oil-only suspensions should be avoided, as it may lead to particle entrapment in the lipid vehicle. It was further shown that the concomitant administration (chase dosing) of the 'brick dust' drug, nilotinib, with LBF excipients increased the bioavailability in rats and was able to overcome the limitations of the poorly dispersible oily suspension approach. Thirdly it was demonstrated that highly digestible surfactants resulted in a higher bioavailability of nilotinib relative to low or non-digested surfactants using surfactant-only suspensions. Furthermore, it was shown that a thermally induced supersaturation approach achieved a higher drug loading in oily lipid excipients, which facilitated the use of sLBFs in pre-clinical *in vivo* studies. Subsequently, it was demonstrated that sLBFs increased the bioavailability of venetoclax *in vivo*. In addition, it was demonstrated that the use of PIs in such supersaturated lipid systems did not increase the bioavailability but rather showed a decreased bioavailability. Moreover, the thesis showed that a thermodynamically stable LBF solution, which was obtained via synthesis of lipophilic salts of venetoclax, increased the bioavailability significantly relative to the commercialised solid dispersion. The combined lipophilic salt-LBF solution achieved a bioavailability similar to the commercial solid dispersion in the fed state. Ultimately, the thesis also demonstrated that standard *in vitro* testing was useful in identifying LBF potential and in explaining observed formulation performance *in vivo*. However, the employed *in vitro* tools were not in all cases

predictive of the *in vivo* situation, generating the need for more, fast and high throughput tools for these advanced LBF formulation approaches.

**Conclusion** This thesis demonstrated the utility of LBFs for drugs displaying both poor water and lipid solubility and therefore exhibit dose loading limitations in conventional lipid vehicles. A bioavailability increase was achieved with all LBF approaches, showing that dose limitations in a lipid vehicle due to excessive hydrophobicity do not preclude a LBF approach. However, it was also demonstrated that care should be taken in terms of the excipient choice and existing biopharmaceutical knowledge should be integrated to achieve the most effective LBF. A Lipid Formulation Developability Guide (LFDG) was proposed to provide industrial guidance on the development of LBFs for such drug candidates. Overall, the impact of this thesis is that the range of application for LBFs can be extended to include difficult to formulate 'brick dust' molecule throughout the development process.

# Thesis overview



# 1 Chapter 1: Introduction

This chapter contains material adapted from the following publications:

1. Approaches to increase mechanistic understanding and aid in the selection of precipitation inhibitors for supersaturating formulations - a PEARRL review

   Daniel J. Price, Felix Ditzinger, Niklas J. Koehl, Sandra Jancovic, Georgia Tsakiridou, Anita Nair, René Holm, Martin Kuentz, Jennifer B. Dressman, Christoph Saal

   Journal of Pharmacy and Pharmacology; DOI: 10.1111/jphp.12927.
2. Lipophilicity and hydrophobicity considerations in bio-enabling oral formulations approaches - a PEARRL review

   Felix Ditzinger, Daniel Price, Alexandra-Roxana Ilie, Niklas J. Koehl, Sandra Jankovic, Georgia Tsakiridou, Simone Aleandri, Lida Kalantzi, René Holm, Anita Nair, Christoph Saal, Brendan T. Griffin, Martin Kuentz

   Journal of Pharmacy and Pharmacology; DOI: 10.1111/jphp.12984.
3. Application of the solubility parameter concept to assist with oral delivery of poorly water-soluble drugs - a PEARRL review

   Sandra Jankovic, Georgia Tsakiridou, Felix Ditzinger, Niklas J. Koehl, Daniel J. Price, Alexandra-Roxana Ilie, Lida Kalantzi, Kristof Kimpe, René Holm, Anita Nair, Brendan T. Griffin, Christoph Saal, Martin Kuentz

   Journal of Pharmacy and Pharmacology; DOI: 10.1111/jphp.12948.

## 1.1 Emerging compounds in drug discovery

It is increasingly being recognised that there is a need to explore new strategies that increase the developability of new molecules to medicines and exploring novel ways of expediting the drug formulation development to facilitate a faster access to the market for emerging drug molecules. A particularly challenging barrier in the design of oral formulations is that most new drug candidates display poor solubility. In cases where the solubility of the drug in the gastrointestinal tract is limited, the drug is predisposed to slow or incomplete dissolution and consequentially low and variable bioavailability [1]. These solubility and/or dissolution limitations can lead to variability in the *in vivo* efficacy of the drug. The fraction absorbed will be highly influenced by both the dosing conditions (i.e. fasted versus fed state) and inter-subject variability in intestinal conditions [2]. Studies showed that between 70% to 90% of drug candidates under development display low solubility, and are classified as BCS classes II and IV [3; 4]. This challenge in developability is further evident by the observation that the % drugs in BCS class II/IV is greater for drugs under development than for licensed oral medicines [3]. The need to develop bio-enabling formulations tailored to increase solubility and increase oral bioavailability is evident.

The increasing poor solubility of drug candidates in the development pipeline are mainly due to hydrophobic and lipophilic characteristics of the drugs. The most accessible surrogate parameters for hydrophobicity and lipophilicity are the melting point ($T_m$) and the partitioning coefficient between octanol and water (log$P$). There are manifold reasons for such drug characteristics including chemoinformatic-aided drug design, high throughput screening, addition of lipophilic substituents during lead optimization or a shift in therapeutic target identification [3]. Therapeutic target shift is of particular interest in the strive for more safe and effective medicines. New target identification techniques focus on the design of more complex

molecules that are able to overcome physiological barriers to target new intracellular sites. The more challenging and often intracellular new targets such as nuclear receptors, ion channels or kinases require more lipophilic characteristics to enable crossing of the cellular bilayer. Moreover, the target classes are often in need of stronger intermolecular interactions at the target site leading also to more interactions between the drug molecules within the crystal [3]. A consequence of the drive to these new therapeutic targets is that drug characteristics become more hydrophobic i.e. higher crystal lattice energies and lipophilic with an overall lower aqueous solubility. In addition, a trend towards increasing molecular weight is evident in the new drug entities, leading to lower aqueous solubility as the energy requirement to create a solvent cavity and to be inserted into the solvent phase during the solvation process increases [3]. All these aforementioned predominant drug properties of drugs in the pipelines indicate a shift of the chemical space of drug compounds towards increasingly difficult to formulate molecules in the future.

As solubility is a major stifling molecule characteristic during development, it is crucial to consider the underlying cause for a limited solubility in order to address it appropriatly during the development process. Such a molecule characteristic guided approach may in fact reduce the development time and allow a faster and more efficient access to new medicines. In general, the solubility of any given drug molecule depends on the properties of the drug molecule as well as on the solvent and the resulting mixture of both solvent and drug (solute). In detail, the thermodynamic solubility depends on the free energy difference in solution and the crystalline state upon mixing [5] (Equation 1-1).

$$\Delta G_{Mixing} = G_{Mixture} - (G_{Solute} + G_{Solvent}) \qquad (1\text{-}1)$$

Where $\Delta G_{Mixing}$ is the free energy difference upon dissolution and G is the Gibbs energy of either the mixture, solute or solvent. The free energy difference should be negative to be in favour of mixing. Equation 1-1 shows that further knowledge about the energy of the solution and of both the solvent and the crystalline solute is needed to understand the solubility of a drug. In the case of a crystalline solute, the high energy intermolecular bonds need to be split in order to break the crystal lattice by overcoming the crystal lattice energy. Consequently, the solute molecules need to be solvated by the solvent molecules. This can be described by the sum of the energy difference between the crystalline state and an intermediate state of a free molecule, and the energy difference between the intermediate state and the solution in the vehicle [5]. Energy is also required in the solvent; a cavity needs to be formed in order to accommodate a molecule of the drug [6]. This is also followed by the formation of new intermolecular bonds between the solvent and the solute. Consequently, the balance between the crystal lattice energy and the interaction between the solvent and the solute are the key determinants of drug solubility in a lipid solvent. The solubility can be either 'solid state limited', (i.e. 'brick dust') if the energy needed to break the crystal lattice and to create the solvent cavity is too high, or 'solvation limited', (i.e. 'grease ball') if the drug exhibits limited ability to form favourable interactions with the solvent.

However, the concept of 'brick dust' and 'grease balls' does not apply to all molecules. A mixture of both extremes is possible for drugs with a high $T_m$ and an intermediate to high lipophilicity. These drugs are usually highly aromatic structures hindered in their solubility by both their solid state properties and poor hydration. These drugs need hydrogen bonds or π-π interactions, besides the lipophilic interactions (mostly London dispersion forces) with the solvent. Most likely these molecules are categorized as a 'brick dust', as it will be the first challenge to overcome the lattice energy during the dissolution process. An analysis by

Mao *et al.* showed that 56.2% of approved drugs had a $T_m$ above 150 °C [7]. Analysing the dataset for only orally administered drugs increased the proportion to 61.6% (Figure 1-1 A). For the same dataset of oral drugs, the lipophilicity was analysed and resulted in a clog*P* > 2 for 61.6% of the drugs (Figure 1-1 B). Thus, most of the approved drugs show a pronounced hydrophobicity and lipophilicity and therefore limited solubility already nowadays.

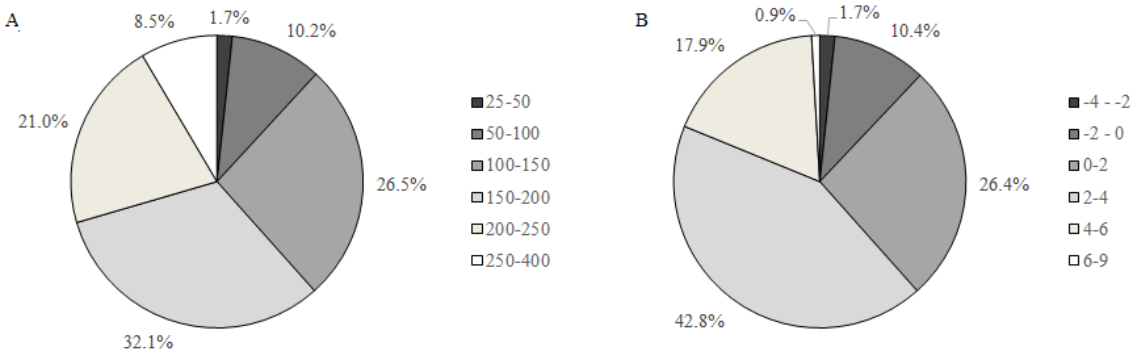



Figure 1-1: A: $T_m$ [°C] distribution of orally administered, approved and discontinued drugs. B: clog*P* distribution of orally administered and approved drugs. For both a new analysis of the drug database by Mao *et al.* was conducted [7].

## 1.2 Lipid-based systems as a bio-enabling formulation strategy

The strategies employed to overcome the solubility and dissolution rate limitations of low soluble drugs are broad and well described [8; 9]. In general, drugs that are solely dissolution rate limited merit from a reduction in particle size, which is generally sufficient to overcome this limitation. Drugs which are truly solubility limited, however, require more complex formulations [10]. These formulation techniques focus on two methods of enhancing solubilisation: altering the physiochemical properties of a compound or co-administering solubilising excipients.

Lipid-based formulations (LBF), which belong to the latter formulation technique, offer a potential bio-enabling benefit for such poorly water-soluble drugs. The approach is now well recognised and fostered the development of several drug products on the market [11]. Oral LBFs are defined as delivery systems, which present the drug in a mixture of excipients consisting of triglyceride oils, partial glycerides, surfactants or co-surfactants and co-solvents [12]. The field of LBFs include different types of drug delivery systems. Commonly, the various types of LBFs are classified in accordance with the Lipid Formulation Classification System (LFCS) [13]. Based on the LFCS, type I formulations consists of pure oils as triglycerides, diglycerides, monoglycerides or a mixture thereof, whereas type IV formulations contain no lipids but surfactants and co-solvents. type II, IIIA and IIIB contain different kinds of lipids, surfactants and co-solvents [13]. While type II formulations contain water insoluble surfactants (hydrophilic-lipophilic-balance (HLB) < 12), type III formulations contain water soluble surfactants (HLB > 12) resulting in greater self-emulsifying potential. Of particular interest in the field of LBFs, is the utility of self-emulsifying and self-nano-emulsifying drug delivery systems (SEDDS and SNEDDS), which readily self-emulsify on administration within the gastrointestinal fluids to form kinetically stable emulsions and/or nano-emulsions. The key merits of SEDDS is the ability to maintain drug solubilisation on dispersion within the complex aqueous environment of the gastrointestinal tract, and various SEDDS are available commercially [14; 11].

The original rationale for the investigation of LBFs to improve absorption of poorly soluble drugs was the observation that numerous drugs showed favourable increases in oral bioavailability when co-administered with food. However, clinically co-administration with food is inherently variable and unpredictable due to variability in food ingestion and meal composition [8]. The identification and comprehension of the physiological and

biopharmaceutical properties underpinning this effect lead to a co-administration of poorly soluble drugs with formulated lipids, mostly derived from food oils, providing a predictable, reproducible, safe and effective route to the advantages of lipid co-administration. The key advantages of LBFs can be categorised into an increase in solubilisation/dissolution and an increase in absorption as illustrated in Figure 1-2.

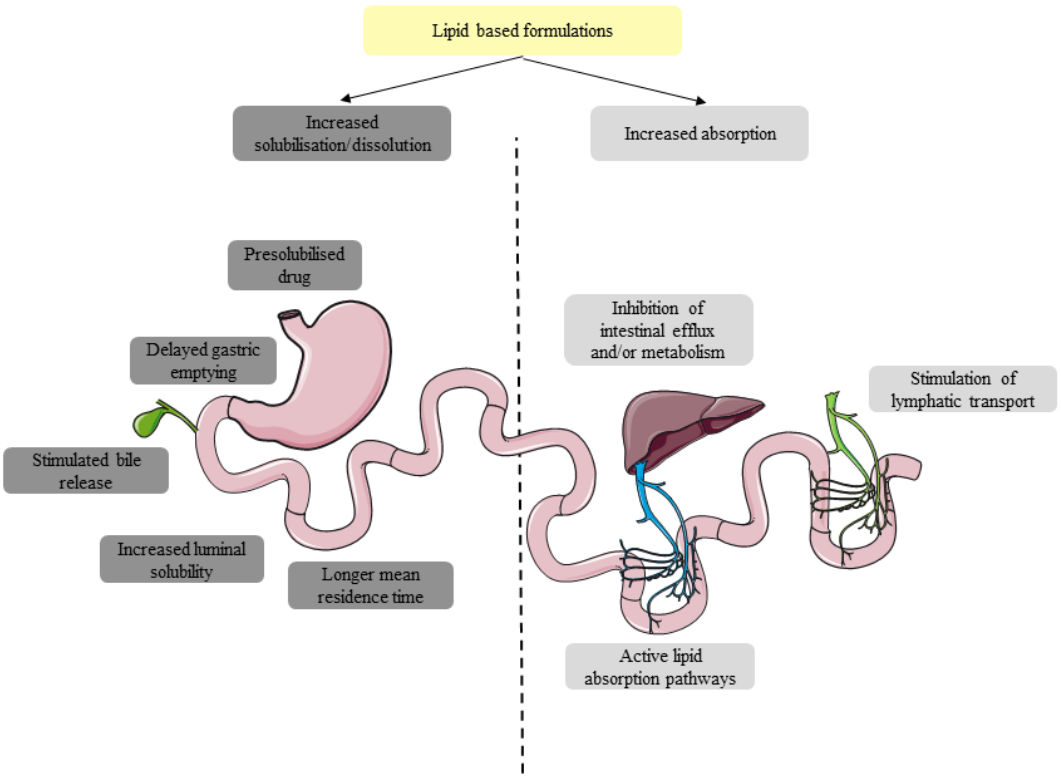



Figure 1-2 Illustration of the potential LBF benefits for poorly soluble drugs in the intestine. Solubility and dissolution effects on the left panel and permeability/absorption benefits on the right panel. Schematic illustration was adapted from O'Driscoll and Griffin [15].

A crucial advantage of LBFs is the ability to pre-solubilise the drug in the lipid excipients, allowing the delivery of a lipid solution. Such lipid solutions offers an advantage for drugs that show a dissolution rate limited bioavailability, as it bypasses the drug dissolution step that is normally required for a drug in a crystalline state [14]. Additionally the lipid excipients can

improve the solubilisation capacity of the drug in the gastrointestinal fluids [15]. Lipid excipients, like dietary lipids, stimulate the release of biliary salts, lipids and lipase enzymes. These enzymes digest triglycerides to monoglycerides and fatty acids, which are solubilized in bile salt mixed micelles, from which absorption can occur [16]. However, a solubility gain depends on the microstructure of the mixture [5], i.e. upon digestion and the introduction of bile salts the mixture of drug, lipids, surfactants and co-solvents constantly changes forming lamellar or hexagonal phases and last mixed micelles [17], which in general have a higher solubilisation capacity compared to fasted state gastrointestinal fluids and might prevent or delay precipitation [18-20].

LBF excipients, such as lipids and surfactants, have all shown to impact intestinal permeability. In particular, permeability enhancing effects of various end-stage lipid digestion products, exogenic surfactants and bile salts are well known [21]. These include inhibiting efflux transporters and intestinal enzyme activity [22; 23], altering tight junction integrity [24-27] and increasing transcellular flux by promoting membrane solubilization and a membrane fluidity increase [28]. Once the lipid is in the enterocyte, it can enter the systemic circulation either via the portal vein or the intestinal lymphatic system, while the first pass metabolism is circumvented by the elevation of lymphatic uptake. However, targeting the lymphatic drug uptake is a function of both the type of lipid excipient and the physiochemical characteristics of the drug [29; 30]. It was reported that for an effective lymphatic transport, long chain excipients are beneficial and the triglyceride solubility and log*P* should be > 50 mg/mL and > 5, respectively [29]. Overall, LBFs as a bio-enabling formulation strategy offer a potential benefit for molecules with a limited aqueous solubility by solubilising the drug in the formulation excipients, increasing their solubility in the gastrointestinal tract and improving the absorption process [15].

While the aforementioned benefits of LBFs are well recognised, it is also apparent that the conventional approach of lipid solutions cannot be applied to all potential drug candidates. Normally, lipid solutions are beneficial for 'grease ball' molecules with a high lipophilicity and a weak crystal lattice, which exhibit poor aqueous solubility. These molecules can be easier dissolved in lipids due to the favoured interactions between the solvent and the solute. However, a poor water solubility does not translate to a good lipid solubility, limiting the approach of lipid solutions to a small subset of potential drug candidates. In recent times, a major challenge in oral drug product development are drugs with high $T_m$, which inherently display both poor water and poor lipid solubility. For such drug candidates more advanced LBFs are needed that broaden the application of lipid formulations to the majority of difficult to formulate drug candidates such as supersaturated LBFs (sLBFs) [31-34], ionic liquids/lipophilic salts [35-43], suspensions [44; 45], or hybrid [46-51] systems which are described further below [52].

## 1.3 Hydrophobicity and lipophilicity considerations in the design of LBFs

When a LBF strategy is considered for a new lead drug candidate, both hydrophobic and lipophilic characteristics of a drug need careful consideration. While the drug ideally should display highly lipophilic characteristics to ensure high solubility in the lipid vehicles (and hence good dose loading), high hydrophobic interactions of the drug may limit the solubility in the lipid vehicles. Specifically, the forces within the crystal lattice can be a key determinant of the suitability of a drug for a LBF, as these must be overcome prior to drug solvation in the LBF. It has been reported that compounds with a $T_m$, which is a surrogate parameter for the molecules hydrophobicity, < 150 °C show a reasonable high solubility in glycerides [53]. In the dataset of 35 compounds a clear trend of decrease in solubility in lipid excipients with increasing $T_m$ was

observed. Similar observations were observed in other studies of 30 and 16 compounds, respectively [54; 20]. Nevertheless, Persson *et al.* also observed limitations of $T_m$ in models used to estimate the solubility in ethoxylated excipients [54]. $T_m$ is an easy way to describe the crystallinity of a drug, however, it lacks information about polymorphism, molecular flexibility and the change in entropy of fusion. In the study it was reported that the accuracy of the solubility predictions slightly increased, when including $T_m$ and the change in entropy of fusion at the same time. In another model solely based on $T_m$ to estimate the solubility in Captex® 1000 and 8000, outliers in the dataset were observed, which also show the limitations of $T_m$ as a descriptor for solubility [20]. Consequently, solubility estimations solely based on $T_m$ exhibit high variability and may only be valid for a narrow chemical space or a specific dataset. Nonetheless, $T_m$ should be understood as an important descriptor in solubility models and a guiding predictor in the decision making of formulation design. Thus, solid state characterization is important for high melting drugs. Especially a compound with a high $T_m$ combined with a low entropy of fusion is less favourable to dissolve in pure triglycerides [53]. Exceptions may be considered for ethoxylated excipients and PEGs [54; 53]. In these cases the crystal lattice energy appears to be less relevant [53] and additional work is needed to increase the understanding of the balance between lipophilicity and hydrophobicity in determining the solubility in these excipients.

Secondly a thermodynamically favourable solvation process is crucial for the solubility in any solvent [6]. In lipids, this is commonly associated with a high log*P* value. In the specific literature it is proposed that a minimum log*P* of 4 is needed to achieve good solubility in pure triglycerides, while an intermediate log*P*, between 2 and 4, ensures good solubility in LBFs containing triglycerides, surfactants and co-solvents [12]. Compounds with a log*P* of approximately 2 exhibit a poor solubility in aqueous and lipid environment and are limited in

LBFs [12]. The analysis of FDA approved LBFs reviled that the log*P* value had an interquartile range from 3.66 – 5.97 (full range 0.79 – 7.45) with a median of 4.85. Except for three compounds (aprenavir, ranitidine and topotecan) the log*P* was > 2. Furthermore, analysing the dataset of 35 compounds by Alskär *et al.* [53], which have a log*P* > 2, showed that there is a trend of solubility increase in soybean oil and Captex® 355 for compounds from a log*P* of 2 to 5. In the cases of both excipients the solubility decreased for compounds with a log*P* > 5 (Figure 1-3 and Figure 1-4). Moreover, a drug molecule with a log*P* of 5 and a solubility > 50 mg/g in triglycerides is associated with a higher lymphatic uptake of the drug, which increases the bioavailability [29]. It was further reported that the lymphatic transport of drugs was 15.8%, 5.5% and 2.2% after administration with a long chain, medium chain and short chain triglyceride, respectively [55]. Therefore, lipophilicity of the drug and the excipients are not only crucial for the solubility in the LBF excipients, but also for the bioavailability of the drug. However, in principal it cannot be generalized that an increase in hydrophobic and lyophobic properties will decrease the success as a LBF. For example, topotecan with a log*P* of 1.84 and a $T_m$ of 213 – 218 °C has been formulated as a LBF and is approved by the FDA, even though there is no apparent formulation rational from the lipophilicity and hydrophobicity perspective [11].

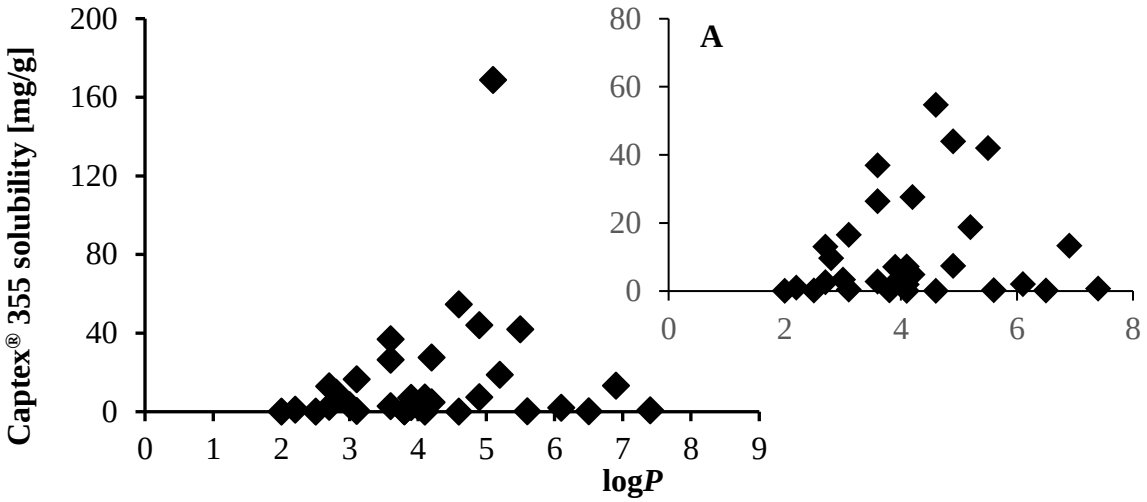



Figure 1-3 Solubility in Captex® 355 plotted against the log*P*. Dataset from Alskär *et al.* [53] Insert A: Close up with fenofibrate omitted. The $T_m$ values of all used drugs were < 150 °C.

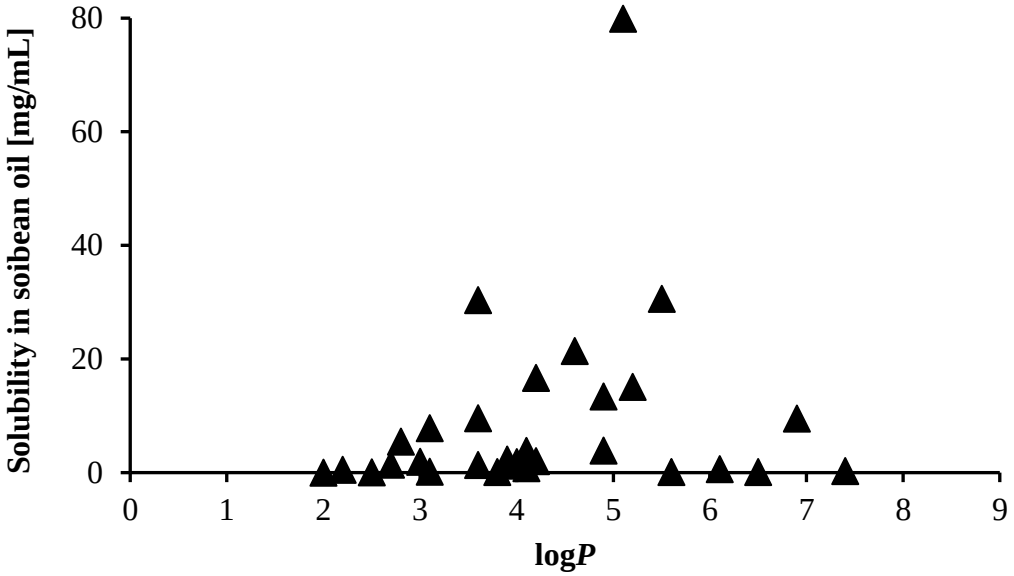



Figure 1-4 Solubility in soybean oil plotted against the log*P*. Dataset from Alskär *et al.* [53] The $T_m$ values of all used drugs were < 150 °C.

Besides the drug molecular properties, the excipients chosen in a LBF are also an important consideration. The more lipophilic a drug molecule is the more likely it will dissolve in type I, II and IIIA formulations. A more hydrophobic molecule favours a type IIIB or IV formulation [13]. In general, it was shown in two studies that the solubility of a dataset of 10 and 35

structurally divers compounds followed a general solubility ranking of long chain triglycerides < medium chain triglycerides < surfactant [56; 53]. Furthermore, several excipient properties can be used to predict and guide the choice of lipids, surfactants and co-solvents [57]. It was shown that the polarity expressed as the dielectric constant, HLB, ester concentration, solubility parameter, surface tension, partitioning coefficient, chain length, molecular weight and the molecular volume are important [57]. These excipient characteristics describe the lipophilicity and hydrophilicity of the solvent. As described above the interaction between the drug (solute) and the excipient (solvent) is a major aspect for solubilization besides the interaction of the solute and solvent itself. The polarity of a compound and the excipients is of utmost importance for the solubility in a LBF. The polarity describes the capability of a drug or solvent to interact through dipole interactions, especially through hydrogen-bond formation, and is often described with the dielectric constant and related to the HLB of a molecule [57]. In formulation design, to choose the optimal excipient for a LBF, it is highly important to know if the compound of interest needs to form polar and/or non-polar interaction with the selected excipients. These two characteristics of a drug molecule and the excipient are well captured in the HLB-value and used in molecular dynamic simulations to improve the understanding where the drug molecule resides in a LBF and in the complex mixture after dispersion [20; 58]. Moreover, it has been shown that the HLB also influences the release rate of the drug from LBFs [16]. Nowadays, the use of different solubility parameters such as the Hansen solubility parameter could facilitate the choice of the LBF according to the drug and excipients [59].

## 1.4 How to improve drug loading in LBFs – change the drug or the delivery system?

### 1.4.1 Selecting alternative drug candidates

#### 1.4.1.1 Lipophilic salts and ionic liquids

For drugs that are challenging to formulate in LBFs new formulation approaches have emerged over the past years, in order to meet the patients as well as pre-clinical need of bio-enabling formulations. An approach that increases the overall lipophilicity and decreases the hydrophobicity of a drug substance has recently been investigated. In the study, lipophilic and bulky counter ions were used to form lipophilic salts [38]. This approach reduced the crystalline forces and consequently $T_m$ of the drug-ion-pair and in addition the higher lipophilicity promoted the solute-solvent interaction. Consequently, this approach improved two major factors of lipid solubility that have been outlined above. The solubility of the atazanavir lipid salt in an unoptimized LBF vehicle increased 3- to 5-fold compared with the free base (Table 1-1) [38]. Another study showed that the solubility in LBF was significantly increased by using lipophilic counter ions for itraconazole, halofantrine and cinnarizine [37] (Table 1-1). Ionic liquids were synthesized, which are organic salts with a $T_m$ < 100 °C by using bulky and highly lipophilic counter ions. In the case of itraconazole an approximately 49-fold solubility increase with a docusate salt in a long chain LBF containing 30% lipid, 60% surfactant and 10% co-solvent was observed [37]. The approach of lipid salts or ionic liquids is in use for a long time, however, it was mainly used to optimize solvents or lubricants for their use [35]. Only in recent years drugs have been altered to make their properties more favourable, which has been reviewed by Williams *et al.* [43]. Table 1-1 illustrates the drugs and counterions that have been used in various studies. It shows that in most cases the solubility of the lipophilic salt was increased relative to the drug free form. The analysis of the studies in Table 1-1 demonstrated

a median solubility increase of the lipophilic salts in the LBFs of 6.6-fold with a range of 1.3-fold - 50.0-fold relative to the drug free form. However, it is also illustrated that an increase in solubility was not possible for some drug-counterion pairs. The median decrease in solubility of this dataset was 0.5-fold with a range of 0.2-fold - 0.7-fold. In addition to the use of 'classical' counterions listed in Table 1-1, the possibility of salts consisting of two pharmaceutical active ingredients as cation and anion was reported [60; 35]. Besides the pharmacological benefit of a combination, this could lead to drug-drug combinations that enhance the drug delivery of poorly water- and lipid-soluble drugs (solid state limited dissolution molecules).

Table 1-1 Overview of solubility of lipophilic drug salts in LBFs as previously reported. In the case of solubility measurements in several LBFs, the medium chain LBFs are presented in this table.

**Solubility of lipophilic drug salts in LBFs**

| Drug | Solubility free drug [mg/g] | Counterion | Solubility lipophilic salt [mg/g] [a] | Fold difference [b] |
|---|---|---|---|---|
| Atazanavir [38] | 0.75 mg/mL [c] | docusate | 2.8 mg/mL [c] | 3.7 |
| | | 2-naphthalene sulfonic acid | 4.3 mg/mL [c] | 5.7 |
| Amlodipine [41] | 50 – 100 [d] | besylate | 40 – 50 [d] | 0.6 |
| | | lauryl sulfate | > 125 [d] | 1.7 |
| | | docusate | > 200 [d] | 2.7 |
| Fexofenadine [36; 41] | < 10 [d] [41] | lauryl sulfate | > 75 [d] [41] | 7.5 |
| | | decylsulfate | > 250 [e] | 25.0 |
| | | octadecylsulfate | 100 – 200 [e] | 15.0 |
| | | 7-ethyl-2-methyl-4-undecylsulfate (Niaproof) | > 250 [e] | 25.0 |
| | | docusate | >250 [e] | 25.0 |
| | | oleate | >100 [e] | 10.0 |
| | | 3,7-dimethyloctanesulfate | 100 – 200 [e] | 15.0 |
| | | nonylsulfate | > 250 [e] | 25.0 |
| | | adamantylsulfate | 100 – 200 [e] | 15.0 |
| | | octylsulfonate | > 250 [e] | 25.0 |
| Ranitidine [41] | < 5 [f] | oleate | > 250 [f] | 50.0 |
| Metformin [41] | < 10 [d] | docusate | > 75 [d] | 7.5 |
| Erlotinib [40] | - | docusate | - | |
| Gefitinib [40] | - | docusate | - | |
| Cabozantinib [40] | - | docusate | - | |
| Ceritinib [40] | - | docusate | - | |
| Cinnarizine [37] | 43.2 [e] | decylsulfate | ≥ 330 [e] | 7.6 |
| | | dodecyl(lauryl)sulfate | ≥ 221 [e] | 5.1 |
| | | octadecylsulfate | 63.2 [e] | 1.5 |

**Solubility of lipophilic drug salts in LBFs**

| Drug | Solubility free drug [mg/g] | Counterion | Solubility lipophilic salt [mg/g] [a] | Fold difference [b] |
|---|---|---|---|---|
| | | 7-ethyl-2-methyl-4-undecylsulfate (Niaproof) | ≥ 266 [e] | 6.2 |
| | | oleate | 93.7 [e] | 2.2 |
| | | stearate | 80.6 [e] | 1.9 |
| | | triflimide | ≥ 306 [e] | 7.1 |
| Halofantrine [36; 37] | 74.9 [e] | decylsulfate | 129 [e] | 1.7 |
| | | dodecyl(lauryl)sulfate | 141 [e] | 1.9 |
| | | oleate | ≥ 323 [e] | 4.3 |
| | | triflimide | ≥ 345 [e] | 4.6 |
| Itraconazole [36; 37] | 2.7 [e] | dodecyl(lauryl)sulfate | 25.7 [e] | 9.5 |
| | | 7-ethyl-2-methyl-4-undecylsulfate (Niaproof) | 75.7 [e] | 28.0 |
| | | docusate | 115.1 [e] | 42.6 |
| Lumefantrine [42] | 15 – 20 [g] | dodecyl sulfate | ~ 35 [g] | 2.0 |
| | | docusate | > 175 [g] | 10.0 |
| Tolfenamic acid [39] | 42.7 [h] | dodecylammonium | 54.7 [h] | 1.3 |
| | | octadecylammonium | 7.2 [h] | 0.2 |
| | | spermine | 82.0 [h] | 1.9 |
| | | *N*-butyl-*N*-dodecyl-*N*,*N*-dimethylammonium | ≥ 245 [h] | 5.7 |
| | | *N*,*N*-didecyl-*N*,*N*-dimethylammonium | ≥ 236.2 [h] | 5.5 |
| | | *N*,*N*-dioctadecyl-*N*,*N*-dimethylammonium | ≥ 162.8 [h] | 3.8 |
| | | *N*,*N*-didecyl-*N*-methylammonium | ≥ 302.5 [h] | 7.1 |
| | | benzalkonium | ≥ 234.1 [h] | 5.5 |

**Solubility of lipophilic drug salts in LBFs**

| Drug | Solubility free drug [mg/g] | Counterion | Solubility lipophilic salt [mg/g] [a)] | Fold difference [b)] |
|---|---|---|---|---|
| Diclofenac [36; 39] | 63.2 [h)] | *N*,*N*-didecyl-*N*,*N*-dimethylammonium | ≥ 238 [h)] | 3.8 |
| | | benzalkonium | ≥ 236 [h)] | 3.7 |
| Meclofenamic acid [36; 39] | 23.4 [h)] | 1-hexadecyl-3-methylpyridinium | ≥ 239 [h)] | 10.2 |
| | | *N*,*N*-didecyl-*N*,*N*-dimethylammonium | ≥ 237 [h)] | 10.1 |
| | | *N*-butyl-*N*,*N*-dimethyldodecyl ammonium | > 220 [e)] | 9.4 |
| | | *N*-decyl-*N*,*N*-dimethyldodecyl ammonium | > 240 [e)] | 10.3 |
| Ibuprofen [39] | 287.9 [h)] | *N*,*N*-didecyl-*N*,*N*-dimethylammonium | ≥ 195 [h)] | 0.7 |
| Dextromethorphan [36] | < 50 [e)] | decylsulfate | 50 – 100 [e)] | 1.5 |
| | | dodecylsulfate | 10 – 25 [e)] | 0.4 |

a) Solubility given as amount of free drug equivalents
b) Fold differences between the solubility of the free drug and lipophilic salts. In the case of reported solubility ranges the mean values were used for the fold difference calculation. In the case of solubility reported as >, <, ≥, ~, the reported solubility value was used for the fold difference calculation.
c) LBF consisted of 35% Miglyol® 812, 15% Capmul MCM® C10 and 50% Tween® 80 (w/w).
d) LBF consisted 15% Mygliol® 812, 15% glyceryl monocaprylate (Imwitor® 308), 60% Kolliphor® EL and 10% ethanol (w/w).
e) LBF consisted of 15% Captex® 355, 15% Capmul MCM®, 60% Kolliphor® EL, 10% ethanol (w/w).
f) LBF consisted of Mygliol® 812.
g) LBF consisted of 25% Capmul MCM®, 50% Kolliphor® RH40 and 25% ethanol (w/w).
h) LBF consisted of 30% soybean oil, 30% Maisine® 35-1, 30% Kolliphor® EL and 10% ethanol (w/w).

Besides a higher solubility in the formulation, LBFs with lipophilic salts also promoted drug absorption. The results of several pre-clinical rat studies with lipophilic salts are summarised in Table 1-2. In general, it should be noted that the lipophilic salt increases the solubility in LBFs making LBF solutions for the poorly lipid-soluble drugs accessible, however, the observed bioavailability impact may originate from the formulation excipients rather than the lipophilic salt itself.

Table 1-2 Overview of *in vivo* tested LBF solutions of lipophilic drug salts as previously reported.

**LBF solutions of lipophilic salts**

| Drug | Animal model | Counterion | Dose [mg/kg] | *In vivo* results |
|---|---|---|---|---|
| Atazanavir [38] a) | rats | docusate | 10 | LBF susp. (free base) = aqueous solution (free base) = LBF (free base) = LBF (docusate) = LBF (2-naphthalene sulfonic acid) |
| | | 2-naphthalene sulfonic acid | 30 | aqueous solution (free base) > LBF (2-naphthalene sulfonic acid) ≥ LBF (docusate) = LBF (free base) |
| | | | 60 | LBF (2-naphthalene sulfonic acid) ≥ PEG400 solution (free base) |
| Cinnarizine [37] b) | rats | decylsulfate | 35 | LBF (free base) = LBF (decylsulfate) > aqueous susp. (free base, 125 mg/kg) |
| | | | 125 | LBF (decylsulfate) > LBF susp. (free base) > aqueous susp. (free base) |
| Cabozantinib [40] c) d) | rats | docusate | 25 | LC-LBF (docusate) = LC-LBF susp. (free base) ≥ MC-LBF (docusate) ≥ aqueous susp. (free base) |
| Erlotinib [40] d) | rats | docusate | 12.5 | LBF (docusate) = aqueous susp. (HCl salt) |
| | | | 25 | LBF (docusate) = aqueous susp. (HCl salt) |
| | | | 100 | LBF (docusate) > aqueous susp. (HCl salt) |
| Itraconazole [37] b) e) | rats | docusate | 20 | LBF (docusate) > Sporanox® > LBF PM susp. (free base) > LBF susp. (free base) = aqueous susp. (free base) |
| Lumefantrine [42] f) | rats | docusate | 50, 65, 85 | LC-IIIB-LBF (docusate) > MC-IIIB-LBF (docusate) > MC-II-LBF (docusate) ≥ IV-LBF (docusate) ≥ LC-IIIB-LBF susp. (free base) ≥ aqueous susp. (free base) |
| Tolfenamic acid [39] g) | rats | *N*-butyl-*N*-dodecyl-*N*,*N*-dimethylammonium | 18 | LBF (free acid) = LBF (*N*-butyl-*N*-dodecyl-*N*,*N*-dimethylammonium) |
| | | *N*,*N*-didecyl-*N*,*N*- | 100 | aqueous susp. (free acid) ≥ LBF susp. (free acid) = LBF (*N*,*N*-didecyl-*N*,*N*-dimethylammonium) ≥ LBF (*N*,*N*-didecyl-*N*- |

**LBF solutions of lipophilic salts**

| Drug | Animal model | Counterion | Dose [mg/kg] | *In vivo* results |
|---|---|---|---|---|
| | | dimethylammonium<br><br>*N*,*N*-didecyl-*N*-methylammonium | | methylammonium) = LBF (*N*-butyl-*N*-dodecyl-*N*,*N*-dimethylammonium) |

a) LBF consisted of 35% Miglyol® 812, 15% Capmul MCM® C10 and 50% Tween® 80 (w/w).
b) LBF consisted of 15% Captex® 355, 15% Capmul MCM®, 60% Kolliphor® EL, 10% ethanol (w/w).
c) LC (long chain) LBF consisted of 30% corn oil, 30% Maisine® 35-1, 30% Kolliphor® EL and 10% ethanol (w/w).
d) MC (medium chain) LBF consisted of 20% glyceryl monocaprylate (Imwitor® 308), 40% propylene glycol monocaprylate (Capryol® 90) and 40% Kolliphor® EL.
e) LBF PM susp. was a physical mixture of itraconazole free base and docusate in the LBF.
LBF susp. and aqueous susp. were below the limit of quantification.
Sporanox® (Janssen Cilag) is the commercially available product. An amorphous solid dispersion with HPMC, spray dried on a sugar sphere.
Sporanox® (29 mg) was suspended in 0.5 mL of water and dosed at 20 mg/kg.
f) LC-IIIB-LBF consisted of 25% Maisine® 35-1, 50% Kolliphor® RH40, 25% ethanol.
MC-IIIB-LBF consisted of 25% Capmul MCM® C10, 50% Kolliphor® RH40, 25% ethanol.
MC-II-LBF consisted of 32.5% Captex® 355, 32.5% Capmul MCM®, 35% Tween® 85.
IV LBF consisted of 50% Kolliphor® RH40, 50% ethanol.
g) LBF consisted of 30% soybean oil, 30% Maisine® 35-1, 30% Kolliphor® EL and 10% ethanol (w/w).

#### 1.4.1.2 Lipophilic prodrugs

An alternative strategy is to select lipophilic prodrugs. The prodrug approach may reduce first pass metabolism, polarity and add lipophilicity [61] and there is even potential to improve the solid state characteristics to favour the formation of lipid solutions. According to Feeney *et al.* it is rarely used to specifically enhance the lipid solubility, but it is most likely the case and has potential being used in LBFs [61]. The higher lipophilicity further promotes the lymphatic uptake and the ability of being dosed with long chain triglycerides that enhance this particular LBF benefit even further. A study with a range of prodrugs for an anti-cancer drug (SN38) showed a significant solubility increase in soybean oil of 11 - 444-fold, a reduction in cytotoxicity and an increase in permeability [62]. Interestingly the solubility of the prodrugs with medium chain fatty acids revealed the highest increase in solubility in soybean oil compared to the long chain fatty acid derivates [62]. Thus, the most soluble prodrug had not the highest log*P* value. This is in line with the finding in Figure 1-3 and Figure 1-4. Indeed, the solubility in soybean oil was decreasing with higher clog*P* values. In addition, the study reported an increase in permeability of the prodrugs [62]. However, it needs a good understanding of the metabolic mechanisms to create the right prodrug that is stable enough in the intestinal fluids but also readily metabolisable to release the parent drug after absorption. Another research group encountered the problem of stability and poor permeability for some of the used prodrugs [63]. However, an increase in lymphatic transport was still detectable. Overall, more work is needed to investigate the benefits of lipophilic prodrugs in LBFs.

### 1.4.2 Advanced lipid-based delivery systems

#### 1.4.2.1 Supersaturated lipid-based formulations

In cases where a solution is limited by the thermodynamic solubility of the drug in LBF vehicles, a sLBF can be used. Compounding of sLBF mostly uses heat to overcome the crystal lattice energy. For example, the sLBF for the drug simvastatin was first vortexed followed by a heating process to 60 °C for 3 h [32]. A similar procedure was followed in the case of halofantrine [31]. Both sLBFs were cooled down to 37 °C overnight and both exceeded the equilibrium solubility by 50%. In most cases the increased solubility is maintained after cooling and the formulation is kinetically stable depending on the level of supersaturation, lipid vehicle and storage conditions [32; 34]. In general, supersaturated lipid systems show promising *in vivo* results as illustrated in Table 1-3 and proved beneficial in pre-clinical settings. For example, in the case of halofantrine, *in vivo* studies showed a 2.5 – 3 fold increase in area under the curve (AUC) for the sLBF compared to a normal lipid solution (SEDDS) [31]. Nevertheless, these kinetically stable supersaturated systems come with challenges such as a limited stability precluding long shelf life and indicating regulatory challenges to obtain a market authorisation. Therefore, the use of sLBFs has currently only demonstrated potential applications in the pre-clinical space, where maximal exposure is needed in safety/toxicological evaluations.

Table 1-3 Overview of *in vivo* tested supersaturated LBFs (sLBF) previously reported in literature.

**Supersaturated LBFs**

| Drug | Animal model | Solubility [mg/g] | Lipid formulation (saturation level) [a] | *In vivo* results |
|---|---|---|---|---|
| Halofantrine [64] | rats | 64.0 in LC LBF [b] [31] | LBF (75% $S_{eq}$), sLBF (150% $S_{eq}$), LBF and sLBF with orlistat [c] | sLBF with orlistat ≥ sLBF ≥ LBF = LBF with orlistat |
| Halofantrine [31] | dogs | 47.0 in MC LBF [d]<br>64.0 in LC LBF [b] | MC and LC LBF (85% $S_{eq}$), MC and LC sLBF (150% $S_{eq}$) | LC sLBF = LC LBF > MC sLBF = MC LBF (1.7-fold) |
| Simvastatin [32] | dogs | 112.9 in in LBF [b] | LBF (75% $S_{eq}$)<br>sLBF (150% $S_{eq}$) | sLBF > LBF (1.8-fold) |
| Fenofibrate [33] | minipigs | 108.8 in LBF [e] | LBF (75% $S_{eq}$), sLBF (150% $S_{eq}$), LBF susp. (150% $S_{eq}$) | sLBF = LBF susp. = LBF = Lipanthyl® [f] |
| Cinnarizine [65] | rats | 25 in LBF [b] | LBF (80% $S_{eq}$), sLBF (200% $S_{eq}$), LBF susp. (200% $S_{eq}$), aqueous susp. with LBF (chase) | LBF ≥ LBF chase ≥ LBF susp. ≥ sLBF ≥ aqueous susp. |
| R3040 [66] | rats | 205 in LBF [b] | LBF (80% $S_{eq}$), sLBF (200% $S_{eq}$), LBF susp. (200% $S_{eq}$), aqueous susp. with LBF (chase) | sLBF = LBF = LBF susp. ≥ LBF chase > aqueous susp. |

[a] Saturation level is given as % of equilibrium solubility ($S_{eq}$).
[b] LBF consisted of 27.5% soybean oil, 27.5% Maisine® 35-1, 35% Kolliphor® RH40, 10% ethanol (w/w). LC: long chain.
[c] Orlistat was added to the LBFs to inhibit lipid digestion in order to evaluate whether lipid digestion is important for oral bioavailability.
[d] LBF consisted of 27.5% Captex® 300, 27.5% Capmul MCM®, 35% Kolliphor® RH40, 10% ethanol (w/w). MC: medium chain.
[e] LBF consisted of 24% soybean oil, 32.2% Maisine® 35-1, 30% Kolliphor® RH40 and 13.8% ethanol.
[f] Lipanthyl® 200 M is a micronized formulation.

### 1.4.2.2 Lipid suspensions

As an alternative to pure lipid solutions, there have also been attempts to utilise lipid suspensions as a bio-enabling strategy. However, suspensions are more complicated to stabilise and can show high variability [37]. Table 1-4 gives an overview of previously reported lipid suspensions that were evaluated *in vivo*. In general, it was reported that lipid suspensions may perform as good as a comparable lipid solution [45]. Carrigan and Bates were the first to investigate lipid suspensions *in vivo* 45 years ago. Both demonstrated that griseofulvin suspended in corn oil resulted in a higher bioavailability compared to an aqueous suspension, when dosed to rats orally [67]. From then onwards an *in vivo* benefit for lipid suspensions compared to aqueous suspensions has been shown for many drugs such as phenytoin, diacerein, halofantrine, danazol, cinnarizine or fenofibrate [68; 45; 33; 69; 70]. For halofantrine it was reported that a long chain triglyceride suspension showed a higher AUC and peak plasma concentration ($c_{max}$) than an equivalent solution [71]. A further positive result was reported for fenofibrate. Thomas *et al.* reported that a SEDDS suspension was similar to the fully solubilised supersaturated SEDDS and a classical SEDDS solution [33]. All the LBFs showed a higher rate of drug absorption compared to the marketed Lipanthyl® 200 M formulation (micronised fenofibrate). However, the faster onset of the LBFs was not sufficient to result in an improved bioavailability after 72 h [33]. Dahan *et al.* investigated the impact of the type of lipid on the bioavailability of lipid suspensions in rats and reported for griseofulvin that medium chain triglycerides resulted in a higher bioavailability than long chain and short chain triglycerides [44]. In general, the performance of a LBF depends on the physicochemical properties, digestibility and absorption pathways of the lipid excipients and drug [14]. As this also applies to lipid suspensions, the excipients need to be carefully selected and more work is needed to understand the influence of excipients in lipid suspensions.

Table 1-4 Overview of *in vivo* tested lipid suspensions previously reported in literature.

**Lipid suspensions**

| Drug | Animal model | Solubility | Lipid vehicle | *In vivo* results |
|---|---|---|---|---|
| Phenytoin [68] | rats | - | corn oil susp. & emulsion | emulsion > oil susp. > aqueous susp. |
| Atovaquone [72] | human | 4 mg/mL in Miglyol® | Miglyol® susp. | aqueous susp. = oil susp.<br>$c_{max}$: aqueous susp. > oil susp. |
| Griseofulvin [44] | rats | - | LC, MC, SC triglyceride susp. a) | MC susp. > LC susp. > SC susp. > aqueous susp. a) |
| Diacerein [69] | rats | 6.5 mg/mL in LBF b) | SNESNS c) | SNESNS > aqueous susp. |
| Danazol [45] | rats | 8.3 mg/mL in Labrafil® | Labrafil® M2125CS susp. & solution | LBF solution (7 mg/mL) = LBF susp. (14 mg/mL) > LBF susp. (28 mg/mL) > aqueous susp. |
| Fenofibrate [33] | minipigs | 108.8 mg/g in LBF d) | LBF solution, sLBF, LBF susp. | sLBF = LBF susp. = LBF solution = Lipanthyl® e) |
| R3040 [66] | rats | 205 mg/g in LBF f) | LBF solution, sLBF, LBF susp., aqueous susp. with LBF (chase) | sLBF = LBF = LBF susp. ≥ LBF chase > aqueous susp. |
| Dexamethasone [44] | rats | - | LC, MC, SC triglyceride susp. a) | MC susp. = LC susp. = SC susp. a) |

a) LC: long chain, MC: medium chain, SC: short chain. Tested excipients: peanut oil (LC), Captex® 355 (MC) and triacetin (SC).
b) LBF consisted of 10% Maisine®, 70% Tween® 80 and 20% Transcutol® (w/w)
c) SNESNS: self-nanoemulsifying self-nanosuspension [69], a LBF suspension, that reduces particle size upon dispersion due to a higher solubilisation capacity. Lipid vehicle is a LBF as described in footnote b.
d) LBF consisted of 24% soybean oil, 32.2% Maisine® 35-1, 30% Kolliphor® RH40 and 13.8% ethanol
e) Lipanthyl® 200 M is a micronized formulation.
f) LBF consisted of 27.5% soybean oil, 27.5% Maisine® 35-1, 35% Kolliphor® RH40, 10% ethanol (w/w)

### 1.4.2.3 Solid lipid-based systems

Solidifying LBFs [49] or using solid LBFs [73] is another approach to increase drug loading, improve drug solubilisation, dissolution, safety and processing on an industrial and commercial level as well as the option to control drug release [49]. Solid LBFs can be obtained by various method such as spray cooling, spray drying, melt granulation, extrusion, supercritical fluids or adsorption on solid inert carriers [73]. For example, O'Shea and co-workers evaluated a spray dried lipidic dispersion containing polyvinylpyrrolidone (PVP), long chain triglycerides, surfactants and fenofibrate *in vitro, in vivo* and *in silico* [74]. In the case of the lipid dispersion, the *in vitro* dissolution under biorelevant conditions in the fasted and fed state were similar, this was also indicated by the *in silico* predicted plasma concentration versus time curves. In landrace pigs, it was demonstrated that the lipid dispersion showed a higher bioavailability in the first 8 h. This difference was lost considering the whole plasma profile due to a food induced higher absorption 8 h post dosing [74].

Another example is the adsorption of LBFs onto solid carriers, which gained attention in recent years [46-48; 50; 51]. In liquid LBFs drug loading is low at usually around 80% of the thermodynamic solubility of the drug in the LBF to prevent precipitation upon dispersion and digestion as well as to increase stability of the formulation during the shelf life. Using sLBFs in combination with solid carriers such as mesoporous silica, can increase the drug load while increasing the stability of the formulation. These hybrid systems can be prepared either by pre-loading the inert carrier with drug prior to the combination with the LBF or by preparation of a sLBF and the adsorbing the sLBF onto a solid carrier [49]. In a recent study, ibuprofen was investigated with different drug loads and preparation methods. Silica-lipid-hybrids were prepared using a spray drying technique or by adding mesoporous silica to a sLBF. While it was shown that the silica-lipid-hybrids at 10% and 20% drug load showed a higher oral

bioavailability in rats when compared to the commercial product Nurofen® (tablets, Reckitt Benckiser), a silica-lipid-hybrid with a drug load of 30% showed a similar bioavailability compared to Nurofen® [48; 50]. Higher drug loads showed crystallisation of ibuprofen in the hybrid formulation [48; 50], demonstrating that there is a trade-off between high drug loads, stability and formulation performance. The study also showed that a spray dried silica-lipid-hybrid formulation exhibited a similar bioavailability when compared to the silica produced with a sLBF [50]. Considering these investigations, it seems that the formulation success depends on the stabilisation of the drug within/on the silica in a molecular dispersed manner, regardless of the preparation method.

## 1.5 *In vitro* characterisation methods for LBFs

In order to adequately assess the suitability of a LBF, bio-predictive *in vitro* tests are required to estimate the performance *in vivo*. In the field of LBFs the *in vitro* digestion model is widely employed, which simulates the impact of lipolysis on LBFs [75]. The employed different models have in common that the formulation is dispersed in a biorelevant media (in most cases reflecting the fasted state), while continuously stirred. Digestion is initiated by the addition of a pancreatic extract and the pH is kept constant throughout the experiment [75]. Samples are taken at various timepoints and centrifuged to separate the main phases present [76]. There are generally three phases obtained after centrifugation: a lipid, aqueous and solid phase. The drug content is analysed in all three phases, solid state analysis can be carried out in the solid phase and e. g. cryogenic electron microscopy can be utilized to analyse the different colloidal species in the aqueous phase [75]. One of the main limitations of the digestion model is the missing absorption step. While in the classic lipolysis model the drug absorption is not considered, the absorption of the free fatty acids (FFA) is resembled by the addition of calcium to precipitate the released FFA. However, also bile salts as well as phospholipids can precipitate and lower

the solubility of the drug in these models [75]. Therefore, precipitation of the drug in the digestion model should be evaluated with care. Additionally, the *in vitro* lipolysis only considers intestinal fasted conditions neglecting the pH change upon gastric emptying as well as gastric digestion. Especially for weak bases, which show a higher solubility at lower pHs, the addition of a gastric step could improve the predictability of the *in vitro* model. It should also be noted that the drug and lipid load in the standard *in vitro* lipolysis setup is relatively high (1 g of LBF in 40 mL), potentially leading to a false increase in solubilisation capacity or high degree of precipitation (especially in light of the missing absorption). Consequently, in some cases the results of these models may not be predictive of *in vivo* results [77] and the results should be handled with care.

In an attempt to overcome the limitations of the standard *in vitro* digestion model, gastric lipolysis was evaluated [78; 79] and the two stage models were developed simulating gastrointestinal digestion [80; 33]. Christophersen and co-workers developed a two stage model with a gastric compartment simulating the fasted and fed state [80]. *Candida antarctica* lipase A was used for gastric digestion at pH 2 (fasted) or pH 6 (fed). Additionally, the gastric emptying was modelled by prolonging the gastric digestion from 30 min in the fasted state to 60 min in the fed state. The intestinal step was similar to the classic *in vitro* lipolysis test with a pH of 6.5, pancreatic lipase and a digestion over approximately 2 h [80]. With this model two LBFs (a LBF solution in a capsule and a solidified LBF using an inert carrier) were evaluated and compared to a commercially available cinnarizine product. It was demonstrated that the *in vitro* model in the fasted state reliably predicted the *in vivo* performance in dogs [81; 80]. In the fed state a similar aqueous phase concentration *in vitro* did not correlate with the significant better performance of the LBF solution *in vivo*. However, the *in vitro* model correctly predicted the non-existence of a food effect for the LBFs *in vivo* [81; 80]. In another study by

Thomas *et al.* a similar gastrointestinal lipolysis model was utilized to study a LBF solution, sLBF, and LBF suspension containing fenofibrate [33]. From the *in vitro* results it was assumed that the tested LBFs should exhibit a higher bioavailability compared to the commercially available Lipanthyl® 200 M (capsules, Abbott AG, Baar Switzerland). Among the LBFs, the LBF solution was expected to be superior to the sLBF, which was expected to yield similar bioavailability compared to the LBF suspension. However, *in vivo* administration of the LBFs resulted in a similar *in vivo* performance for all formulations. Therefore, the *in vivo* performance was not related to the *in vitro* findings [33]. This miss-match may be a drug specific effect and further studies on gastric lipolysis are needed to assess the impact on the predictability of *in vitro* models.

Further refined *in vitro* digestion models were recently proposed by adding an absorptive element using for example a Permeapad® membrane [82], a Caco-2 cell monolayer [83] or a decanol layer [84]. The artificial cell free permeation membrane Permeapad® is a lipophilic membrane consisting of two hydrophilic, polymeric carrier membranes (cellulose hydrate), with soy phosphatidylcholine as the lipid layer in the middle [85; 86]. The integrity and stability of the membrane has been evaluated in a side-by-side permeation setup (ussing chamber) in the presence of lipid excipients and digestion products (including free enzymes) using calcein as a hydrophilic marker (which would show higher permeability in case of lipid membrane integrity loss). It was shown that the Permeapad® membrane was not affected by the excipients or media [82]. Bibi and co-workers postulated that the use of this system for LBFs may translate to a better *in vitro-in vivo* relationship. Besides artificial, biomimicking membranes, Caco-2 cell monolayers have also been employed in digestion-permeation setups. However, Caco-2 cells are not compatible with the pancreatic enzyme extract, which is usually used in *in vitro* lipolysis setups. Therefore, immobilised lipase (bound lipase to inert carrier) was used. In

general, the immobilised lipase shows a lower extent of digestion compared to the pancreatic extract, which also results in a different drug distribution across the different phases (aqueous, lipid and solid phase) when compared to the standard lipolysis model [83]. Using a Franz-cell like setup with caco-2 cells, Keemink and co-workers were able to demonstrate that the amount of permeated drug was predictive of the *in vivo* performance of different LBFs using fenofibrate [83]. It was further shown that the aqueous phase of the i*n vitro* lipolysis test (which was not bio-predictive) did not correlate with the amount of permeated drug across the cell monolayer in the case of fenofibrate and danazol [83]. This observation was also evident in a different study by Alvebratt *et al.*, who used Caco-2 cells in the µFlux® apparatus (Pion Inc.) to study various LBFs containing felodipine [87]. A benefit of the µFlux setup is that the amount of drug was measured using *in situ* fluorescence fibre optics, allowing for a faster throughput relative to the standard digestion setup. In a different approach by O'Dwyer and co-workers, which used the inForm® system (Pion Inc.), a decanol layer was used as an absorption compartment. The model also incorporated a gastric step, which allowed the model to potentially capture supersaturation and precipitation of ionisable drugs as they transit from the acidic gastric to the rather neutral intestinal environment [84]. The system was tested for various LBF solutions and suspensions containing nilotinib, danazol and fenofibrate, which had previously been published and tested *in vivo*. In three out of four studies the novel two-stage biphasic *in vitro* lipolysis model more reliably predicted *in vivo* performance relative to the standard *in vitro* lipolysis model. Also, in this setup fibre optics was used for drug quantification in the decanol layer making this system efficient and high-throughput relative to other setups [84]. However, in these 'side-by-side' setups the area-to-volume ratio is low, i.e. the area is too small, and does not represent *in vivo* conditions. Therefore, the dynamic nature of dispersion, digestion, precipitation and re-dissolution of LBFs might not be fully captured. And while it has previously been shown that lipid digestion is initiated in the stomach and that

gastric lipases remain active in the small intestine [79], it must be acknowledged that in these advanced models gastric lipolysis has not been taken into account by the above-mentioned models. Therefore, the implementation of gastric lipolysis in *in vitro* assays including an absorptive sink may be beneficial [79; 88].

## 1.6 Summary

LBFs have evolved substantially since their first introduction as a bio-enabling formulation technology. As LBFs gained greater interest, the knowledge increased and the array of LBF technologies was continuously extended. To date a variety of LBF approaches are at hand to meet the need of a range of drug substances. However, the development of LBFs can be complex, different LBF types may be required for different drugs, and the adoption of LBF strategies in the industry is largely empirical in nature, as opposed to scientifically informed based on the specific drug properties. A key limitation to the more wider application of LBFs in early drug product development is that there is a perception that LBFs are complex in combination with the formulation development tending to be overly reliant on the principle of 'as simple as possible, but as complex as necessary'. In addition, the relatively hydrophobic drugs in the current development pipeline fall outside the traditionally set specifications for LBFs. In practice, many studies in the literature are conducted with drugs that exhibit a low $T_m$ and a relatively high log*P* that favour lipophilic milieus (e.g. fenofibrate, halofantrine, cinnarizine) and precaution should be taken when generalising findings of studies with these drugs, as the LBF development could be misguided. To improve the uptake of LBFs by the pharmaceutical industry further there is a need for a routine fast throughput *in vitro* test method or a set of tools with varying complexity to assess the feasibility of LBFs during the early stages of formulation development/screening. Additionally, there is a need for studies involving compounds that represent the challenging chemical space of the pipeline to date (i.e. recently

licenced drugs) as well as a direct comparison of the various LBF technologies to assess the suitability of LBFs for such drugs using *in vitro*, *in vivo* as well as *in silico* tools. This thesis attempts to address these gaps as outlined in the primary aims.

## 1.7 Model compounds

In order to assess the utility of the above described approaches, a variety of chemically divers model compounds that represent the current pipeline molecules, i.e. recently licenced drugs, are needed. In this thesis two poorly water- and lipid-soluble compounds that exhibit a pronounced food dependent bioavailability and lipophilicity were used to investigate the aims (section 1.8).

### 1.7.1 Nilotinib

Nilotinib is a tyrosine kinase inhibitor taken by the oral route, which was approved for the treatment of chronic myelogenous leukaemia in 2007. The molecular structure of nilotinib is depicted in Figure 1-5. The therapeutic dose of nilotinib is 300 – 400 mg twice daily. It is practically insoluble in buffer solutions of pH 4.5 and higher [89], exhibits a high lipophilicity (log*P* 4.95) as well as hydrophobicity ($T_m$ 236 ºC) and exhibits a moderate permeability across a confluent Caco-2 cell monolayer [89; 90]. Therefore, nilotinib was categorised as a class IV compound in the BCS. Nilotinib demonstrated a pronounced food effect with a 29% increased AUC after a light meal and an 82% increase in AUC after a high fat meal [90]. The physicochemical characteristics of nilotinib indicate that it is a ‘brick dust’ molecule, i.e. the solubility is highly solid state limited, and leads to the assumption that the solubility in lipid vehicles will be low. Up to date, nilotinib has not been evaluated in LBFs. A benefit from lipid

exhibits may be expected due to the food dependent bioavailability and the evident lipophilicity.

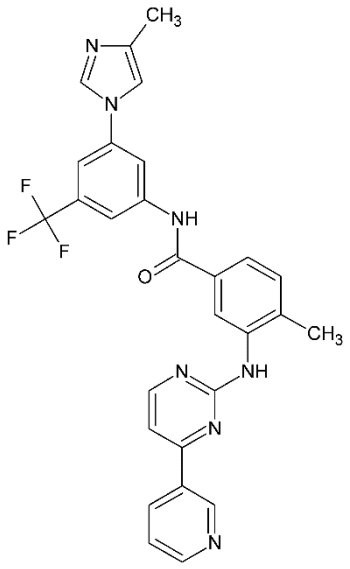



Figure 1-5 Chemical structure of nilotinib.

Nilotinib is predominantly metabolised by CYP 3A4 [89] and is a P-gp substrate [89-92]. As a result of the pre-systemic metabolism, the AUC increases about 29% after co-administration of nilotinib with grapefruit juice (intestinal CYP 3A4 inhibitor) [93]. These pre-systemic effects may be leveraged using lipid excipients as previously described in section 1.2. The current commercial formulation, Tasigna® (Novartis Pharmaceuticals Corporation), is a capsule formulation containing the surfactant Pluronic® F68 (Poloxamer® 188) [94]. The concentration and the scientific rational of the addition of Pluronic® F68 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 [90]. The marketed formulation of nilotinib showed an absorption of ≥ 30% following a radiolabelled single 400 mg oral dose in humans [95]. Additionally, pre-clinical 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)) [89].

## 1.7.2 Venetoclax

Venetoclax is an orally taken selective B-cell lymphoma-2 inhibitor licenced for the treatment of leukaemia in 2016. The chemical structure is illustrated in Figure 1-6. It is highly lipophilic (log*P* 5.5), displays a high molecular weight (868.44 g/mol) [96] and a mid-range $T_m$ (138 ºC onset [97], or 136 ºC [98]). Venetoclax is classified as a BCS class IV [99] drug and shows a solubility in aqueous buffer of < 0.0042 µg/mL at pH 7.4 [96] and 2.24 mg/mL in soybean oil [98]. The commercialised formulation, Venclyxto® (AbbVie Ltd.), displays a substantial food effect with a 3.4-fold increase in oral bioavailability after a low-fat meal and a 5-fold increase after a high fat meal compared to the fasted state [96]. Venclyxto® is a solid dispersion in copovidone [97] and must be taken with food to achieve an adequate bioavailability [97] suggesting that dietary lipids may be beneficial for improving oral absorption of venetoclax.

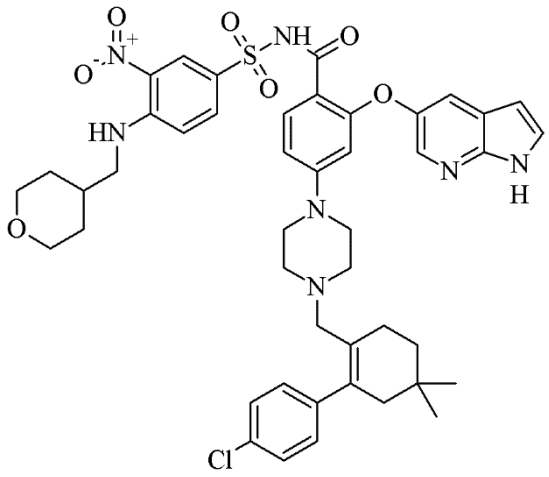



Figure 1-6 Chemical structure of venetoclax.

Additionally, venetoclax displays pronounced lymphatic transport (43 ± 8% of the total absorbed dose) when administered in the fed state in dogs [98]. This could be beneficial for the approach of LBFs and may be leveraged with long chain-based lipid excipients. A venetoclax

tablet manufactured by Abbott Park (USA) showed an absolute bioavailability of 38.8 ± 7.2% in dogs [98].

## 1.8 Aim of the thesis

The aim of this thesis was to explore the utility of LBFs to overcome solubility limited oral absorption of poorly water-soluble drugs, with a focus on 'brick dust' molecules. Furthermore, the thesis explored the use of a range of LBFs for the model drugs using *in silico*, *in vitro* and *in vivo* evaluations to assess the benefit of advanced and non-conventional LBFs for hydrophobic, lipophilic and high molecular weight drugs. The specific objectives were as follows:

i) To mechanistically assess LBF suspensions ranging from oil-only to surfactant-only systems to enhance oral bioavailability.

ii) To assess the impact of excipient type (e.g. surfactant type) on the bioavailability *in vivo*.

iii) To assess the propensity of poorly lipid-soluble drugs to supersaturate in lipid excipients and the impact of sLBFs on the bioavailability *in vivo*.

iv) To assess the utility of precipitation inhibitors (PIs) in sLBFs to prolong supersaturation and improve bioavailability *in vivo*.

v) To assess the ability of LBFs and lipophilic salts to overcome the food depended bioavailability of highly lipophilic, but poorly lipid-soluble drugs.

A final goal was to develop an industrial developability guide for the formulation design of advanced LBFs.

# 2 Chapter 2: New insights into using lipid-based suspensions for 'brick dust' molecules: case study of Nilotinib

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

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

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

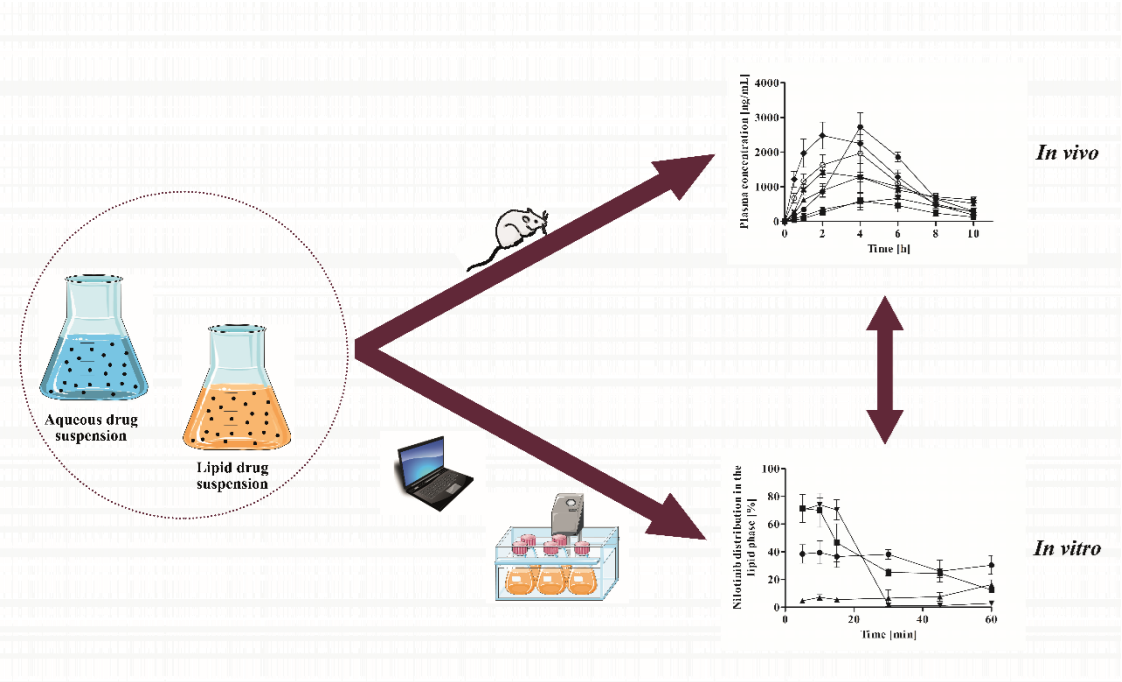
[3] Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark

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

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## 2.1 Abstract




Graphical abstract 2-1 *In vitro* and *in vivo* evaluation of lipid suspensions for the 'brick dust' molecule nilotinib.

**Purpose** Lipid suspensions have been shown to be a suitable bio-enabling formulation approach for highly lipophilic or 'grease ball' drug molecules, but studies on 'brick dust' drugs are lacking. This study explored the utility of lipid suspensions for enhancing oral bioavailability of the rather hydrophobic drug nilotinib *in vivo* in rats.

**Methods** Four lipid suspensions were developed containing long chain triglycerides, medium chain triglyceride, long chain monoglycerides and medium chain monoglycerides and *in vivo* bioavailability was compared to an aqueous suspension. Additionally, *in vitro* lipolysis and wettability tests were conducted.

**Results** Nilotinib bioavailability was lower for triglyceride suspensions, relative to both monoglyceride and an aqueous suspension. The long chain monoglyceride displayed a significantly higher bioavailability relative to the triglycerides. The slower *in vivo* absorption observed for triglyceride formulations may be explained by *in vitro* lipolysis results, which

suggested entrapment of nilotinib crystals within poorly dispersible triglycerides, leading to slower nilotinib release. This was further supported by higher wettability of nilotinib by lipids. **Conclusion** Monoglycerides improved oral bioavailability of nilotinib in rats, relative to triglycerides. For 'brick dust' drugs formulated as lipid suspensions, poorly dispersible formulations may delay the release of drug crystals from the formulation leading to reduced absorption.

## 2.2 Introduction

In recent years, there has been an emerging trend towards the discovery of drug candidates that display sub-optimal developability characteristics [10]. A key shift is the increasing number of lead drug candidates displaying poor aqueous solubility, where it is estimated that up to 75% of drugs in development are classified as Class II/IV in the BCS [3]. There is a general increase in molecular weight [100; 3] as well as lipophilicity [101], with the intention to improve target receptor selectivity and maximise potency. These highly lipophilic drug candidates may consequently display solubility limitations and hence require bio-enabling formulation approaches such as nanosizing or LBFs to ensure sufficient oral absorption. In particular, LBFs have demonstrated commercial potential for delivery of drugs with high lipophilicity. For such so-called 'grease ball' drug candidates, LBFs are considered favourable to increase drug solubilization in the intestinal tract and, in general, good dose loading capacity can be achieved within lipid vehicles. It has been suggested that a drug with a log*P* of > 4 would be best to achieve adequate solubility in pure triglycerides, while an intermediate log*P*, between 2 and 4, may result in a suitable solubility in mixtures of lipids including mono-, di- and triglycerides, hydrophilic surfactants and water-soluble co-solvents, dependent on the dose [12].

While poor aqueous solubility driven by high lipophilicity provides good drug candidates for LBFs, the situation is more complex in the case of drugs displaying high hydrophobicity [52]. Such high melting drug candidates are often formulated using amorphous solid dispersions to diminish the impact of the solid state on the dissolution [6]. Thus, the forces within the crystal lattice can also be a key determinant for the suitability of a drug using LBF, as these must be overcome prior to drug solvation in the LBF. Therefore, for molecules that display 'brick dust' characteristics, dose loading in LBFs is limited by the high crystal lattice energy. It has been reported that compounds with a $T_m$ > 150 °C display poor solubility in triglycerides [53], theoretically limiting the classical approach of lipid solutions to low hydrophobic and high lipophilic molecules. While such $T_m$ and log*P* based guides are helpful, it should also be kept in mind that the majority of drugs emerging from drug discovery display $T_m$ > 150 °C and clog*P* > 2. For example, a recent study on the $T_m$ distribution of globally available drugs suggests that > 61% of drugs have a $T_m$ > 150 °C and clog*P* > 2 [7]. Nevertheless, numerous drugs with a $T_m$ > 150 °C have benefited from LBFs and more recent lipid-based formulation approaches have the potential to overcome dose-loading limitations, such as super-SNEDDS [31; 32; 52], ionic liquids [60; 35] and lipid suspensions [70; 52].

Lipid suspensions, where crystalline drug is dispersed in a lipid vehicle within an oral capsule, offer a scalable approach for oral administration, with the potential to enhance oral absorption via excipient-mediated effects on solubilisation within the intestine. Additionally, the excipients in lipid suspensions may offer the benefit of increased intestinal permeability and/or promotion of intestinal lymphatic transport [15; 102]. Lipid suspensions offer the potential benefit for sustained delivery via particle size mediated control of the dissolution rate of the suspended drug particles. In general, lipid suspensions may be particularly useful in a pre-clinical setting

for poor soluble drug candidates, where high dosing in rodent models is necessary for early stage toxicological evaluation [103].

Lipid suspensions have been investigated with different excipients and drugs for their benefit *in vivo* with the general experience that in most performed studies beneficial effects have been observed. Drugs such as griseofulvin ($T_m$ 220 °C, log*P* 2.2), atovaquone ($T_m$ 216 - 219 °C, log*P* 5.8) [104], phenytoin ($T_m$ 295 °C, log*P* 2.5), diacerein ($T_m$ 217 °C, log*P* 2.0), danazol ($T_m$ 227 °C, log*P* 4.9), or fenofibrate ($T_m$ 79 °C, log*P* 5.1) have been investigated [68; 72; 44; 45; 33; 69]. For example, a griseofulvin corn oil suspension resulted in a higher bioavailability compared to an aqueous suspension, when dosed orally to rats [67]. In the case of drugs with sufficient lipid solubility like danazol and fenofibrate, the dose loading enables either a lipid drug solution or suspension. In the latter case, the lipid suspensions are generated by reducing the amount of lipid excipients.

In terms of 'brick dust' molecules with a high hydrophobicity as well as lipophilicity similar to the used model drug in this study, there are limited reports in the literature exploring the utility of lipid suspensions. Danazol showed a 4 - 9-fold increase in bioavailability in rats was achieved using a Labrafil® M2125CS suspension compared to an aqueous suspension. Furthermore, one of the tested Labrafil® suspensions showed equivalent exposure to the Labrafil® solution [45]. Roland *et al.* employed a lipid suspension approach for atovaquone, a potent antiprotozoal drug [72]. The bioavailability of atovaquone is 3.3-fold higher after a high fat meal, however, the drug displays limited solubility in medium chain triglycerides (~ 4 mg/mL). In *in vivo* studies in humans, atovaquone bioavailability was similar for a lipid suspension (500 mg in 30 mL medium chain triglycerides) and an aqueous suspension (500 mg in 30 mL of 0.25% methyl cellulose solution). Moreover, the lipid suspension absorption was

prolonged as evident by longer $t_{max}$ and lower $c_{max}$ compared to the aqueous suspension [72]. Thus, the potential benefit of lipid suspensions for highly lipophilic and hydrophobic drugs is not clear and merits further investigation.

Nilotinib is a tyrosine kinase inhibitor which was approved for the treatment of chronic myelogenous leukemia in 2007. Nilotinib displays high lipophilicity (log$P$ ~ 5) and higher bioavailability after ingestion of a high fat meal (> 80%), which are both considered favourable characteristics from a LBF perspective. However, the $T_m$ of nilotinib is 236 °C hence the expected solubility in lipids is solid state limited. Therefore, nilotinib was chosen as a model 'brick dust' drug for the present study, where the aim was to investigate the potential benefit of a lipid suspension as formulation approach. The *in vivo* bioavailability of a series of lipid suspensions was compared to an aqueous suspension. In addition, the *in vitro* lipolysis model was employed to provide mechanistic insights on the formulation performance.

## 2.3 Materials and methods

### 2.3.1 Chemicals and materials

Nilotinib and sorafenib were purchased from Kemprotec Ltd. (UK). Olive oil, highly refined and low acidity, capric acid, L-α-phosphatidylcholine type XI-E (PC) (768 g/mol), taurodeoxycholic acid (NaTDC) and pancreatic lipase (8 x USP) were obtained from Sigma-Aldrich (Ireland). Capmul MCM® and Captex® 1000 were kindly donated by Abitec corporation (USA). Monocaprin was obtained from TCI (Germany) and oleic acid was received from VWR (Ireland). A sample of Peceol® was kindly donated by Gattefossé (France) and fasted state simulating intestinal fluid (FaSSIF) powder version 1 was kindly donated by biorelevant.com (UK). All other chemicals and solvents were of analytical or high-performance

liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 2.3.2 Particle size measurements

Wet laser diffraction analysis was performed using a Mastersizer® 3000 (Malvern Instruments Limited, UK), equipped with a Hydro MV medium automated dispersion unit with 120 mL dispersant volume. Nilotinib sample solution was prepared by adding excess nilotinib to HPLC grade water. The suspension was ultrasonicated for 5 sec. before the measurement. A refractive index of 1.4 was used for water as a reference index for statistical calculation using the particle sizing program. A refractive index value of 1.65 [105], absorption index of 0.1 and density of 1.362 g/cm$^3$ were used for particle size distribution analysis of nilotinib. The nilotinib sample was added dropwise into the saturated wet dispersion unit containing approximately 100 mL of dispersant (water) until obscuration reached between 1.2% and 5.4%, at a stirring speed of 1250 rpm. D10, D50, D90 are reported for all the samples, where n = 3. The results of the laser diffraction analysis were confirmed by optical microscopy using an Olympus BX51 (Olympus, Ireland) equipped with an Olympus BC 100 (Olympus, Ireland) camera. Measurements were done at 40 x magnification with Olympus Stream Start® version 1.7.

### 2.3.3 Solubility studies

Equilibrium solubility was determined in olive oil, Captex® 1000, Peceol® and Capmul MCM® using the shake flask method. In brief, an excess of nilotinib was added to 2 mL of the excipients, thoroughly mixed and shaken in a water bath shaker at 37 °C (GLS400, Grant Instruments, UK). Samples were taken after 24 h, 48 h, 72 h and centrifuged at 21,380*g* (Mikro 200 R, Hettich GmbH, Germany) and 37 °C for 15 min. The supernatant was transferred to a

new tube and centrifuged again under identical conditions. In order to solubilise the oily excipient, the supernatant was diluted approximately 1:5 - 1:50 with a mixture of tetrahydrofuran (THF) and dimethylformamide (DMF) (50:50, v/v), followed by further dilution with DMF and dimethyl sulfoxide (DMSO). The obtained samples were analysed by reverse phase HPLC, as described below. Equilibrium was assumed once two time-points had a variation of less than 10%. All samples were run in triplicates.

#### 2.3.3.1 Biorelevant solubility and dispersion

FaSSIF and fed state simulated intestinal fluid (FeSSIF) were prepared according to the instructions by biorelevant.com. FeSSIF was used directly, whereas FaSSIF was left at room temperature for 2 h prior further usage. Nilotinib's equilibrium solubility in a biorelevant dispersion of the lipid formulation was simulated by adding 2 g of olive oil (FaSSIF $_{\text{olive oil}}$) or Captex® 1000 (FaSSIF $_{\text{Captex® 1000}}$) to 80 mL of prepared FaSSIF. The mixture was stirred at 37 °C for 40 min prior to the addition of excess nilotinib to 2 mL of media.

The post-digestion equilibrium solubility of the triglyceride formulations was simulated by adding the expected lipolysis components to FaSSIF media, similar to the artificial digestion media suggested by Gautschi and co-workers [20]. The measured equilibrium solubility resembled the maximum solubility increase upon complete digestion of the triglyceride excipients. Oleic acid and α-monooleat (FaSSIF $_{\text{olive oil dig}}$) or capric acid and α-monocaprin (FaSSIF $_{\text{Captex® 1000 dig}}$) in a molar ratio of 2:1 (mol/mol) were added to FaSSIF in order to simulate the digestion of long chain or medium chain triglycerides, respectively. Where necessary, the excipients were melted first and mixed thoroughly before 2 g of this mixture was added to 80 mL of medium. The dispersion was stirred at 37 °C for 40 min and the pH was adjusted to 7.5 prior to the addition of excess nilotinib.

After the addition of excess nilotinib to 2 mL of the dispersions all samples were placed in a water bath shaker at 200 shakes/min and 37 °C (GLS400, Grant Instruments, UK). After 3 h, 6 h and 24 h samples were taken and analysed. All taken samples were processed like the lipid solubility samples. The resulting supernatant was diluted with a mixture of THF, DMF and DMSO (1.25:23.75:75, v/v) before analysis.

The samples were analysed using an Agilent 1200 series HPLC system (Agilent Technology Inc., USA) that comprised a binary pump, degasser, autosampler and variable wavelength detector. Data analysis was done with EZChrom Elite® version 3.2. In order to separate the lipids/surfactants from nilotinib 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 20 mM phosphate buffer pH 2 and methanol (53:47 v/v) and was used at a flow rate of 1 mL/min. The column temperature was set to 25 °C and the detection wavelength was 255 nm. The lower limit of quantification (LOQ) for this method was 25 ng/mL.

### 2.3.4 *In vitro* evaluation: Drug solubilization during formulation dispersion and digestion

*In vitro* lipolysis was performed using a pH-stat apparatus (Metrohm AG, Herisau, Switzerland) comprising a Titrando® 907 stirrer, 804 Ti-stand®, a pH electrode (Metrohm AG, Herisau, Switzerland) and two 800 Dosino® dosing units coupled to a 20 mL autobuerette. The system was operated by the Tiamo® 2.2 software. The *in vitro* protocol was amended from Williams *et al.* [106; 76] except that the overall volume of the buffer was increased to allow for a higher sample yield. The ratio of formulation (1.583 g) to digestion buffer (57 mL) remained

constant. In brief, the buffer contained 2 mM TRIS maleate, 150 mM NaCl, 1.4 mM $CaCl_2 \cdot 2H_2O$, adjusted to pH 7.5. For the digestion experiments the buffer was supplemented with 3 mM NaTDC and 0.75 mM PC (digestion buffer) and stirred for 12 h 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* (Rotina 380 R, Hettich GmbH, Germany) and 4 mL of supernatant were recovered and stored at 2 – 8 °C before further usage.

For the *in vitro* lipolysis experiment 1.583 g of suspension (10 mg/mL) was dispersed into 57 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 7.5 throughout digestion using the pH stat method of the Titrando® device with 0.2 M NaOH and 0.6 M NaOH for long and medium chain formulations, respectively. The amount of dispensed NaOH was recorded by the system. To the remaining 54 mL (1.5 g lipid formulation) dispersion, 6 mL of pancreatic extract was added to initialize digestion. After 60 min the released non-ionized free fatty acids were determined by a pH increase of the buffer to pH 9.

Samples of 4.9 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). All samples containing a lipid phase were centrifuged at 37 °C and 400,000*g* for 30 min (Beckman Coulter Optima® L-90K, Rotor: VTI 65.2). Samples, that did not contain a lipid phase (aqueous suspension) were centrifuged at 37 °C and 21,000*g* for 30 min using a benchtop centrifuge (Micro 200R, Hettich GmbH, Germany).

### 2.3.5 Contact angle measurements

Nilotinib's wettability was determined using the contact angle measurement by the sessile drop technique. Nilotinib disks were prepared according to Muster *et al.* [107]. In brief, 40 mg nilotinib were compacted for 1 min with a pressure of approximately 210 MPa (Star Specac® manual hydraulic press). 6 µL of Peceol®, Capmul MCM®, olive oil, Captex® 1000, 0.5% (w/v) methylcellulose in water and pure water, respectively, were placed on the pressed disk using a fully automated optical tensiometer (Theta Attention® by Biolin Scientific). After the drop was released the contact angle was captured using 76 frames per sec (FPS) for 20 sec followed by 7.6 FPS for 100 sec. The contact angle was calculated directly, 0.5 sec, 60 sec and 120 sec after the drop release using the fit of the droplet's shape to the Young-Laplace equation. The contact angle for one measurement was the mean of the individual calculated angles of each side of the droplet. All measurements were done on 3 disks and consisted of at least 5 measurements per time point.

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

The lipid formulations were prepared by combining 10 mg nilotinib with 1 mL lipid excipient followed by an overnight stir prior to dosing. The aqueous formulation was prepared by adding 10 mg of nilotinib to 1 mL of the aqueous 0.5% (w/v) methylcellulose solution and mixed thoroughly. In order to decrease the powder agglomerates the suspension was placed in an ultrasonic bath for 5 sec and vortexed again afterwards.

### 2.3.7 *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 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. For the fasted study legs food was removed 16 - 20 h before dosing and water was available *ad libitum* at all times. In the case of the fed study leg, food was available throughout the study and was not removed. 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 ethylenediaminetetraacetic acid (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.

### 2.3.8 Bioanalysis

The plasma concentrations of nilotinib were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., USA) 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 modified from Pirro *et al.* [108]. 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 LOQ in plasma by this method was 10 ng/mL and linearity was confirmed between 10 ng/mL and 4 µg/mL. The extraction efficiency was found to be > 92.5% across the concentration range and the intra- and inter-day variability was 4.2% and 5.4% at maximum, respectively.

### 2.3.9 Data analysis

After using the Bartlett's test to check for equal variance a one-way analysis of variance (ANOVA) was performed for the lipolysis data using a Tukey post-hoc test to compare the different formulation performances. The solubility limited absorption dose (SLAD) was calculated for the biorelevant media and dispersions according to the following equation [10]:

$$SLAD = S_{Si} \times V \times M_P \qquad (2\text{-}1)$$

where $S_{Si}$ is the solubility in the different media, V the fluid volume available in the intestine (500 mL) and $M_p$ is the permeability dependent multiplier, which for low permeable drugs like nilotinib was kept at unity.

The pharmacokinetic parameters were calculated using Microsoft® Excel®. The plasma concentration profiles were analysed by non-compartmental analysis and calculation of each AUC was based on the linear trapezoidal rule. Mean residence time (MRT) was calculated according to the following equation:

$$MRT = \frac{AUMC_{0-inf}}{AUC_{0-inf}} \qquad (2\text{-}2)$$

where $AUMC_{0\text{-}inf}$ is the area under the first moment curve from timepoint 0 to infinity and $AUC_{0\text{-}inf}$ is the area under the curve from timepoint 0 to infinity.

The statistical analysis for all *in vivo* parameters was performed using a one-way ANOVA after using the Bartlett's test to check for equal variance. The Gaussian distribution of the data was tested with the Kolmogorov-Smirnov test and the pairwise comparison of the groups was done using Tukey's multiple comparison test. All statistical analyses were carried out using GraphPad Prism® 5 (GraphPad Software, USA).

## 2.4 Results

### 2.4.1 Solubility in lipid excipients

Nilotinib is a high $T_m$ and high log*P* compound, hence displaying properties of a 'brick dust' molecule (Table 2-1). Initial solubility screening in pure lipid excipient indicated that nilotinib was practically insoluble [109] in long chain and medium chain triglycerides (Figure 2-1 A). The solubility was higher in monoglycerides compared to triglycerides. Within the triglycerides and monoglycerides, a higher solubility was observed for the medium chain lipids compared to the long chain excipients. Overall, the percent of the therapeutic dose (300 mg) that would be dissolved in 1 mL lipid ranged between 0.01-1.5% (Figure 2-1 A). This confirms that despite a high log*P* for nilotinib, the use of a classical lipid solution approach was not feasible, and hence lipid suspensions were developed to evaluate if lipids could still have a bio-enhancing influence on nilotinib.

Table 2-1 Physicochemical properties and *in vivo* behaviour of nilotinib.

| | |
|---|---|
| **Literature data** | |
| Log*P* [110] | 4.95 |
| Molecular weight [g/mol] [90] | 565.98 |
| $pK_a$ values [90] | 2.1, 5.4 |
| BCS class [111]<br>DCS class | II/IV<br>IIb/IV |
| Food effect [90] | 29% AUC increase with a light meal<br>82% AUC increase with a high fat meal |
| **Experimental data** | |
| $T_m$ [°C] (by DSC) | 236.42 ± 0.22 |
| FaSSIF solubility [µg/mL]<br>FeSSIF solubility [µg/mL] | 0.32 ± 0.03<br>3.16 ± 0.09 |
| *In vitro* FeSSIF/FaSSIF ratio | 9.98 |
| Olive oil solubility [µg/mL]<br>Peceol® solubility [µg/mL]<br>Captex® 1000 solubility [µg/mL]<br>Capmul MCM® solubility [µg/mL] | 6.82 ± 0.45<br>928.76 ± 35.24<br>50.19 ± 8.15<br>3361.21 ± 318.01 |
| Employed particle size [µm] | 1.31 ± 0.40 (D10)<br>7.41 ± 4.06 (D50)<br>197.88 ± 22.81 (D90) |

Subsequently, nilotinib solubility was determined under biorelevant conditions. Solubility in FaSSIF was low at 0.0001% of a 300 mg dose, whereas it increased approximately 10-fold in simulated fed state media (Figure 2-1 B, Table 2-1). Indeed, nilotinib's bioavailability is reported to be higher in the fed state (82% increase in AUC after a high fat meal). Moreover, solubility was screened in lipidic dispersions to subsequently assess the nilotinib solubility on aqueous dispersion of lipid formulations in biorelevant media. Overall the solubility increases in the pure triglyceride dispersions ($FaSSIF_{olive\ oil}$ and $FaSSIF_{Captex^{®}\ 1000}$) were relatively low,

whereas lipid excipients that simulate post-digestive intestinal conditions ($FaSSIF_{olive\ oil\ dig}$ and $FaSSIF_{Captex^{®}\ 1000\ dig}$) suggested significantly higher solubilisation capacity for nilotinib. The post-digestive media showed an increase in the SLAD from 0.16 in FaSSIF to 2.81 and 3.57 for $FaSSIF_{olive\ oil\ dig}$ and $FaSSIF_{Captex^{®}\ 1000\ dig}$, respectively. Despite this increase in SLAD, the overall SLAD obtained was substantially lower than the therapeutic dose.

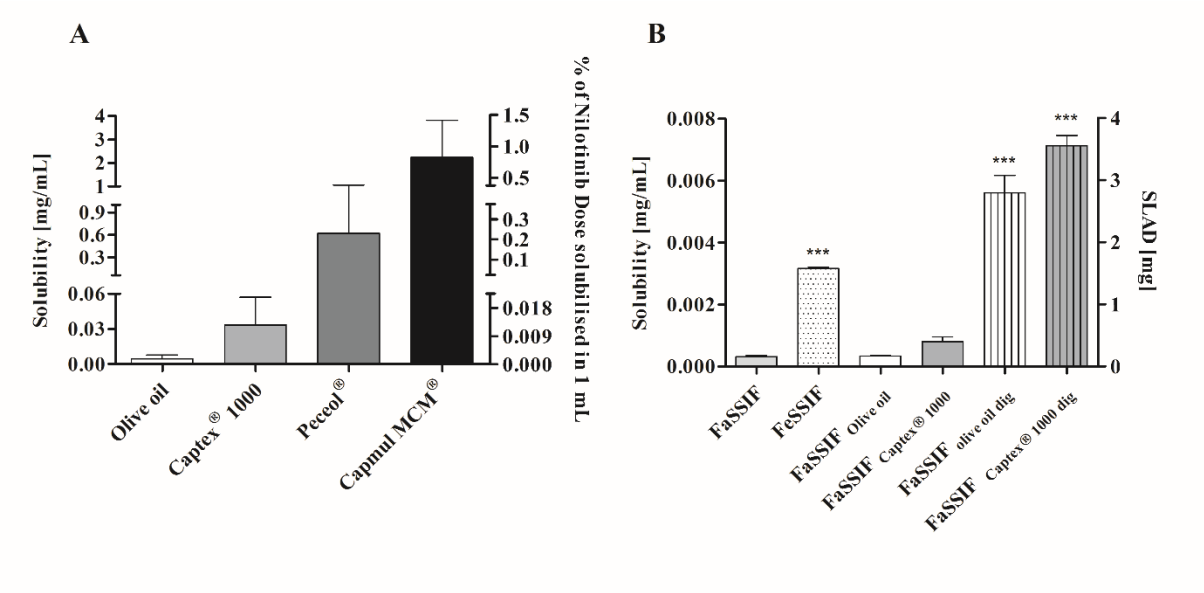



Figure 2-1 A: Nilotinib equilibrium solubility in Olive oil, Captex® 1000, Peceol®, Capmul MCM® (n = 3) and the % of a 300 mg dose solubilised in 1 mL of lipid excipient at 37 °C. B: Nilotinib solubility in FaSSIF, FeSSIF and biorelevant lipid dispersions in FaSSIF containing olive oil, Captex® 1000, as well as simulated digested olive oil and Captex® 1000 (n ≥ 3). And the amount of drug that can be dissolved in 500 mL of a biorelevant lipid dispersion utilizing the solubility limited absorption dose (SLAD). (mean ± SD).

## 2.4.2 *In vivo* bioavailability of nilotinib

Nilotinib suspensions were prepared in olive oil, Captex® 1000, Peceol® and Capmul MCM® and bioavailability was assessed *in vivo* in rats. The dose and lipid amount were fixed at 20 mg/kg and 2 mL/kg, respectively. The amount of nilotinib present in these lipid suspensions exceeded the equilibrium solubility in the lipid vehicles 1471-fold for the olive oil formulation, 199-fold for the Captex® 1000 formulation, 11-fold for the Peceol® formulation and 3-fold for

the Capmul MCM® formulation. An aqueous nilotinib suspension was dosed as a comparator, and additionally nilotinib's food effect was investigated by the administration of an aqueous suspension in the fed state. The mean plasma concentration versus time profiles are presented in Figure 2-2 and the AUC from 0 h to infinity and MRT for nilotinib after oral administration of the lipid and aqueous suspensions are shown in Figure 2-3. Table 2-2 presents a summary of the pharmacokinetic parameters obtained.

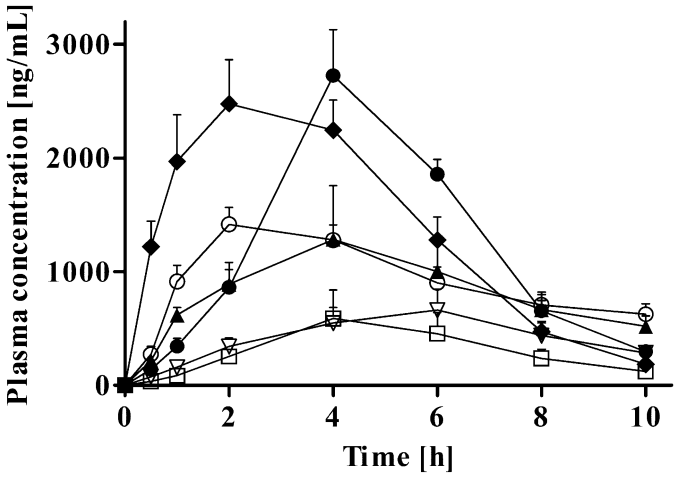



Figure 2-2 Plasma concentration profiles as a function of time (mean ± SEM for n = 5) for a crude aqueous suspension in the fasted state (♦), crude aqueous suspension in the fed state (○), Peceol® (●), olive oil (□), Capmul MCM® (▲) and Captex® 1000 (▽) suspension in male Sprague Dawley rats.

Among the lipid formulations, the performance ranking of the LBF suspensions showed that the highest exposure was achieved for Peceol® followed by Capmul MCM®, Captex® 1000 and olive oil, i.e. Peceol® ≥ Capmul MCM® ≥ Captex® 1000 = olive oil. The Peceol® suspensions showed a significantly higher AUC than the triglyceride suspensions ($p \leq 0.05$) and the Capmul MCM® suspension showed a significant higher AUC than the olive oil suspension, whereas there was no statistically significant difference between the other lipid suspensions

(Table 2-2). Additionally, a trend towards increased $t_{max}$ was observed in cases where lipid excipients were used indicating that solubilizing benefits of the lipids were time delayed.

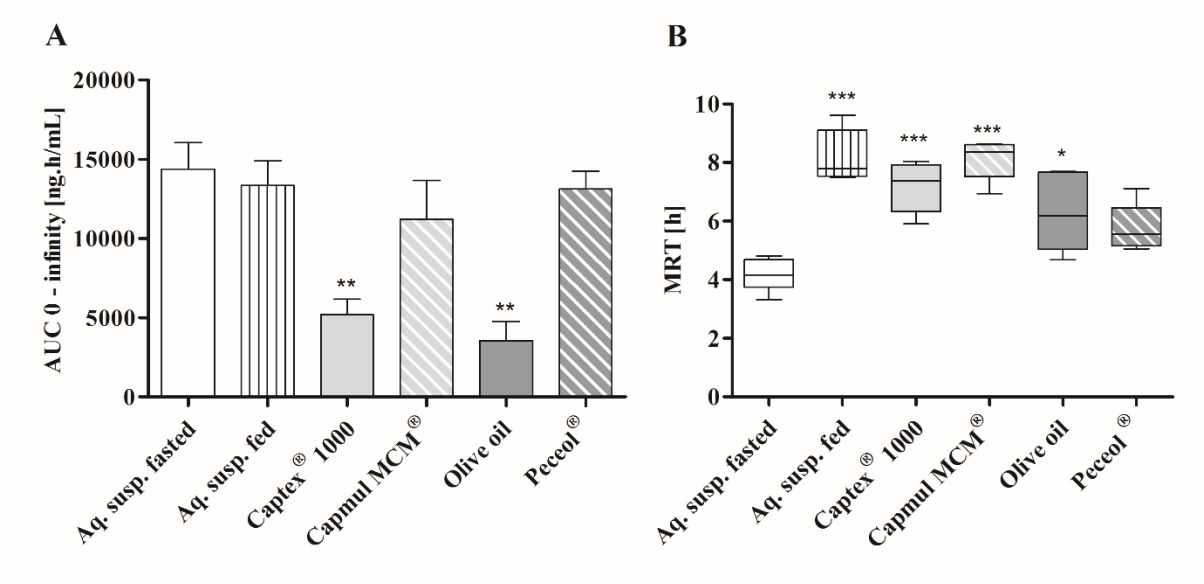



Figure 2-3 Results of the *in vivo* study of nilotinib dosed in an aqueous suspension in the fasted and fed state, a Captex® 1000, Capmul MCM®, olive oil and Peceol® suspension to male Sprague Dawley rats (n = 5) The given statistical significance was compared to the aqueous suspension in the fasted state. A: AUC 0 h - infinity (mean ± SEM) and B: Mean residence time (MRT) (whiskers: min. to max. value).

The aqueous nilotinib suspension in the fasted state led to an AUC of 14369 ± 3747 ng.h/mL. In the cases of the lipid suspensions, the highest overall AUC was observed for the Peceol® (AUC of 13103 ± 2557 ng.h/mL) and Capmul MCM® (AUC of 11210 ± 5476 ng.h/mL), which were not statistically dissimilar from the aqueous suspension. Critically however, no bioavailability enhancement was evident for any of the lipid suspension. On the contrary the Captex® 1000 showed a significant 2.8-fold decrease and the olive oil a significant 4.0-fold decrease ($p < 0.01$). Thus, relative to the aqueous suspension in the fasted state both triglyceride formulations showed a significant reduced bioavailability.

Dosing nilotinib to rats with free access to food resulted in a similar AUC compared to the dosing in the fasted rats. Thus, the profound food effect observed in humans was not evident in the employed rat model. It is notable that the MRT was prolonged in the fed state study, with the MRT being comparable to the medium chain suspension study group (Figure 2-3 B). A MRT performance ranking of aqueous suspension $_{(fasted)}$ = Peceol® ≤ olive oil ≤ Captex® 1000 = Capmul MCM® = aqueous suspension $_{(fed)}$ was observed.

Table 2-2 Pharmacokinetic parameters of nilotinib after oral administration of 20 mg/kg to male Sprague Dawley rats (n = 5). $t_{max}$ and MRT given as median (range), all other parameters as mean ± SD, n = 5.

| **Pharmacokinetic parameters** | | | | | | |
|---|---|---|---|---|---|---|
| | Aq. suspension Fasted | Aq. suspension Fed | Captex® 1000 | Olive oil | Capmul MCM® | Peceol® |
| $c_{max}$ [ng/mL] | 2648 ± 676 | 1423 ± 321 | 774 ± 345 | 605 ± 550 | 1321 ± 1038 | 2801 ± 756 |
| $t_{max}$ [h] (range) | 2 (2 - 4) | 2 (2 - 4) | 6 (4 - 10) | 4 (2 - 6) | 4 (1 - 6) | 4 (4 - 6) |
| AUC 0 - 10 h [ng*h/mL] | 13984 ± 3576 | 9350 ± 2431 | 4264 ± 1949 | 3151 ± 2516 | 8323 ± 4873 | 12393 ± 2666 |
| AUC 0 h - inf. [ng*h/mL] | 14369 ± 3747 | 13335 ± 3487 | 5168 ± 2197 | 3548 ±2711 | 11210 ± 5476 | 13103 ± 2557 |
| MRT [h] (range) | 4.17 (3.32 - 4.81) | 7.79 (7.49 - 9.60) | 7.37 (5.91 - 8.04) | 6.18 (4.67 - 7.70) | 8.35 (6.94 - 8.64) | 5.57 (5.05 - 7.12) |
| $F_{rel}$ [%] [a)] | 100 | 92.80 ± 24.27 | 35.96 ± 15.29 | 24.69 ± 18.87 | 78.01 ± 38.12 | 91.19 ± 17.80 |

[a)] Relative to the aqueous suspension in the fasted state.

## 2.4.3 Drug Solubilization during *in vitro* dispersion and digestion

In order to provide an improved mechanistic understanding of the *in vivo* pharmacokinetics, further *in vitro* studies were undertaken. Thus, the lipid suspensions were assessed using the dynamic *in vitro* lipolysis model. Lipid suspensions were dispersed initially in biorelevant buffer representing the fasted state for 10 min prior to initiation of the digestion by the addition of lipase. The release of nilotinib into the aqueous phase during the dispersion and digestion is shown in Figure 2-4 A.

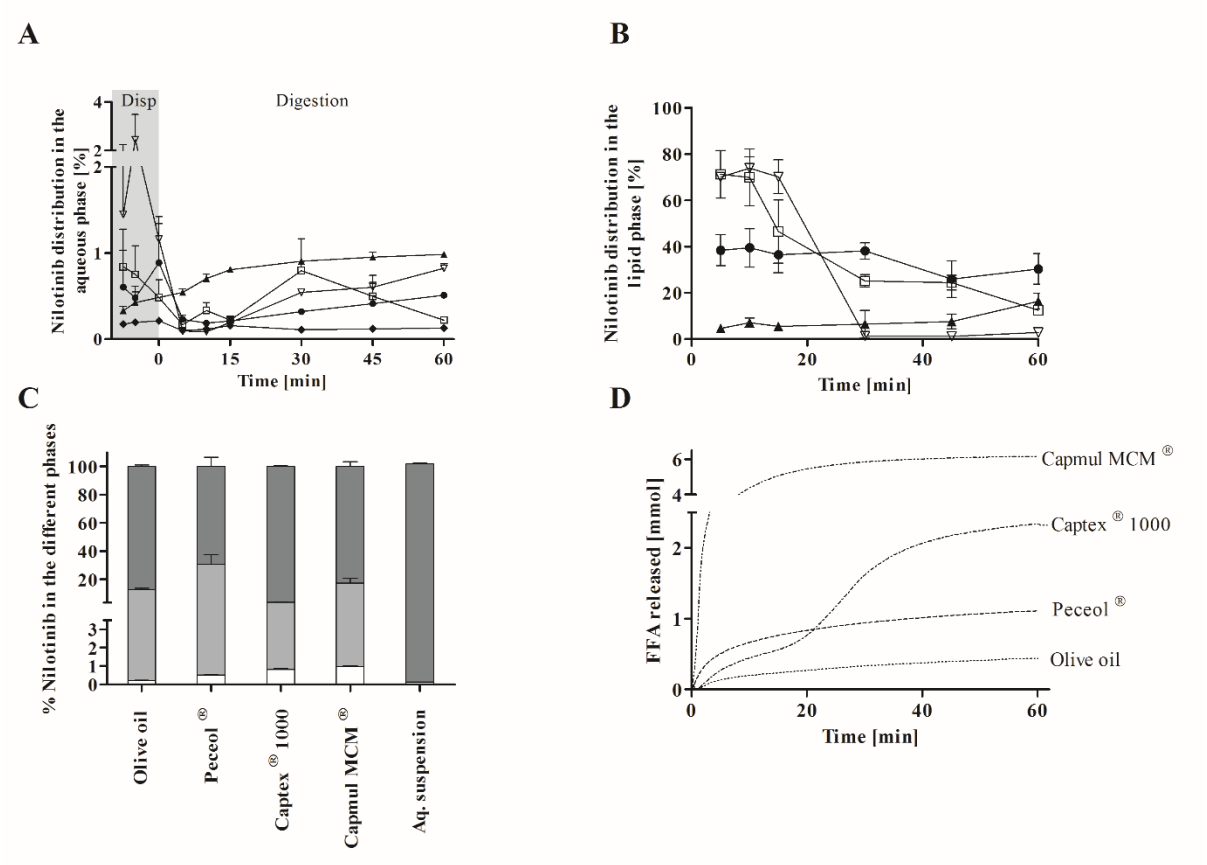



Figure 2-4 Results of the *in vitro* lipolysis (mean ± SEM; n = 3) for the aqueous suspension (♦), Peceol® (●),olive oil (□), Capmul MCM® (▲), Captex® 1000 (▽). A: Percent of nilotinib in the aqueous phase, B: % of nilotinib in the calculated lipid phase, C: Distribution of nilotinib across the aqueous (white), calculated lipid (light grey) and solid phase (dark grey) after 60 min of digestion, D: Free fatty acids (FFA) released over time for the studied lipid-based formulations corrected for a blank during 60 min of digestion.

Overall for the four lipid suspensions, the extent of drug solubilised in the aqueous phase was higher compared to the aqueous suspension throughout dispersion and digestion. However, the % of the dose solubilised in the aqueous phase was low at between 0.1 and 1.1% of the dose. The rank order of the five tested formulations was Capmul MCM® = Captex® 1000 > Peceol® > olive oil = aqueous suspension. Upon dispersion of the lipid suspensions the highest concentration in the aqueous phase was observed for the Captex® 1000 suspension with 2.5 ± 1.8% of the dose solubilised, whereas at the end of digestion the highest nilotinib concentration of 1.0 ± 0.1% was observed for the Capmul MCM® formulation. It was notable that upon the start of digestion the initial solubilisation capacity for nilotinib was reduced for the Captex® 1000, olive oil and Peceol® suspensions. However, for these three lipid suspensions an increase of the nilotinib concentration was observed after the initial drop, indicating that the post-digestive products aid the solubilisation of nilotinib. In the case of the Capmul MCM® suspension the nilotinib concentration in the aqueous phase steadily increased during dispersion and digestion. These observations translated to a SLAD from 0.16 for the aqueous suspension to 0.28 – 1.09 for the lipid suspensions after 60 min of digestion.

Figure 2-4 C presents the distribution of drug between the aqueous phase, pellet phase and 'oil' phase after 60 min of digestion of the sample. As expected, most of nilotinib was recovered in the solid phase for all five suspension formulations, which mainly reflects suspended drug particles. In the case of the poorly dispersible triglyceride suspensions, an oily lipid phase was particularly evident during the initial stages of digestion. This oily phase most likely reflected undispersed and undigested or partially digested lipids in the formulation. The quantity of drug in this phase was theoretically calculated using a mass balance approach i.e. by subtracting the quantity of drug determined analytical in the pellet and aqueous phase samples from the total amount of drug present. Interestingly, at the initial phase of digestion, a greater amount of drug

was calculated to be within this oil phase for the triglyceride formulations relative to the monoglyceride formulations (Figure 2-4 B). Up to 85% and 83% of nilotinib's dose was theoretically calculated to reside within this oil phase on top of the media in the lipolysis vessel for the olive oil and Captex® 1000 formulations, respectively. These amounts exceeded the equilibrium solubility of the drug within these oils significantly indicating that nilotinib was likely to be present as suspended drug crystals within this phase. By comparison, for the monoglyceride formulations much lower amounts of drug were present in this initial phase of digestion, with 52% and 10% in Peceol® and Capmul MCM®, respectively. Therefore, it would appear that the formulations that performed poorest *in vivo* displayed the greatest amount of drug within this oil phase in the initial phase of digestion. As digestion proceeded, the amount of drug within this oil phase decreased, most likely reflecting the digestion of these lipid formulations, which was mirrored by the increase in FFA released (Figure 2-4 D). In particular, Captex® 1000 distinct increase in FFA generated between 15-30 min of digestion corresponded to the decrease in nilotinib concentrations in the oil phase from 70.2 ± 12.7% at 15 min to 1.4 ± 1.0% at 30 min. A similar, albeit less dramatic, decrease in the amount of drug estimated in the oil phase was observed for the olive oil formulation between 15 to 30 min. However, the overall extent of digestion for the olive oil suspension was lower relative to the other formulations. Following completion of a back titration to pH 9 to adjust for the non-ionised FFA the rank order of digestibility was olive oil (1.30 mM FFA released) < Peceol® (2.22 mM FFA released) ≤ Captex® 1000 (2.93 mM FFA released) < Capmul MCM® (6.27 mM FFA released).

### 2.4.4 Wettability of nilotinib crystals with lipid and aqueous media

In order to probe whether differing wetting characteristics of nilotinib crystals between the various formulations could be used to explain lower bioavailability of the triglyceride

formulations, the wettability of nilotinib by the five formulation vehicles and pure water was determined utilizing the sessile drop technique. The results are presented in Figure 2-5. The equilibrium contact angle was reached after 60 sec of the measurement. Water was used as a reference which confirmed the hydrophobic nature of nilotinib with contact angles of up to 80°. Additionally, the four lipid excipients and the 0.5% methyl cellulose vehicles from the *in vivo* study were tested. It was observed that all lipid vehicles used in this study wetted nilotinib better than the aqueous 0.5% methyl cellulose with contact angles between 10.1° and 12.5°. It was further observed that the lipids penetrated the nilotinib disk much faster covering the nilotinib crystals in a lipid film.

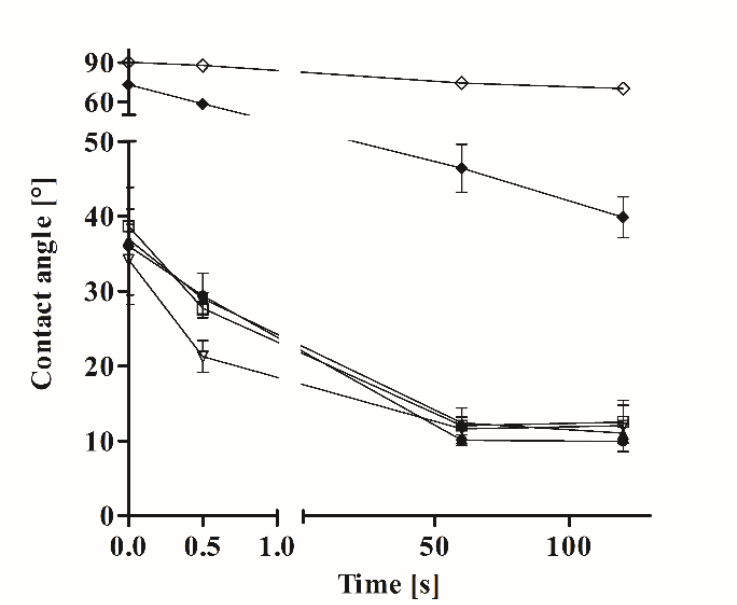



Figure 2-5 Wettability of nilotinib by water (◊), 0.5% methyl cellulose solution (♦), Peceol® (●), olive oil (□), Capmul MCM® (▲), Captex® 1000 (▽). Measurements for each time point are done on 3 disks (n ≤ 5).

## 2.5 Discussion

Lipid excipients have shown great potential to enhance oral bioavailability by increasing solubilisation in intestinal fluids and improving intestinal permeability/uptake [15]. Lipid suspensions have been investigated for a number of drugs as an approach to enhance *in vivo* bioavailability with the overall experience that in the majority of reported studies a benefit was observed [68; 44; 45; 33; 69]. However, the utility of any LBF as a bio-enabling strategy is highly dependent on the molecular properties of the candidate drug, and in particular both hydrophobic and lipophilic properties of the drug. In the case of highly hydrophobic 'brick dust' molecules, there are knowledge gaps in the literature on the usefulness of lipid suspensions and there is a need for more studies involving high $T_m$ and high log*P* drugs to assess potential *in vivo* merits.

Nilotinib is a hydrophobic compound ($T_m$ of 236 °C), but it is also highly lipophilic (log*P* 4.95) and displays an 82% higher bioavailability on administration with a fat rich meal [90]. In this study, the solubility of nilotinib in lipids was found to be very low. While solubility increased in more polar oils such as medium chain-based oils and monoglycerides, the overall solubility in lipids was insufficient to solubilise the dose. Solubility screening in biorelevant media confirmed a higher solubility in the fed state intestinal fluids with a FeSSIF/FaSSIF ratio of approximately 10. However, overall with a SLAD of 0.16 in FaSSIF, oral absorption of nilotinib is clearly solubility limited. Subsequent biorelevant solubility screening that mimicked the post-digestive state appeared to lead to further enhancements in *in vivo* solubilisation, which may in part explain the increased bioavailability observed clinically in humans in the fed state. It might therefore have been anticipated that the *in vivo* study would show increased bioavailability in the fed state as well as an increased exposure following dosing as lipid suspensions. However, in rat model, a food effect was not observed most likely

reflecting limitations of this model, which are described in further detail below. Additionally, despite the above *in vitro* results the lipid suspensions did not show an increased exposure compared to the aqueous suspension.

The *in vivo* study results showed that both monoglyceride and triglyceride suspension did not result in a bioavailability enhancement. Both monoglyceride suspensions were comparable to the aqueous suspension and a trend towards a better performance of the monoglyceride suspensions compared to the triglyceride suspensions was observed. While only the Peceol® suspension was statistically significant different from both triglyceride suspensions ($p < 0.01$), the Capmul MCM® suspension was significantly different from the olive oil suspension ($p < 0.05$). In the case of the triglyceride suspensions, bioavailability was significantly lower relative to the Peceol® and aqueous suspension ($p < 0.01$), respectively. While the previous biorelevant dispersion experiments suggested a higher solubilisation for the medium chain formulations relative to the long chain formulation, this performance was not evident in the *in vivo* study in case of the monoglycerides. The better performance of the Peceol® versus Capmul MCM® suspension may reflect other effects of long chain versus medium chain lipids on intestinal uptake and/or absorption. While the impact of chain length remains unclear, it appears that the long chain digestion products more readily maintain solvation capacity [61]. Additionally, it was interesting to note that the MRT of the Peceol® suspension was significantly lower compared to the MRT of the Capmul MCM® suspension (i.e. 5.57 h versus 8.35 h) indicative of a faster absorption process for the Peceol® suspension ($p < 0.01$). In general, MRT is an indicator of the average time a drug molecule spends in the body. As none of the excipients used in this study are known to significantly alter distribution, metabolism or excretion, an increased MRT between study groups is indicative of a delayed absorption phase, most likely reflecting prolonged drug residence time in the gastrointestinal tract.

Further *in vitro* experiments were conducted to provide mechanistic insights into the results obtained *in vivo*. The formulations were therefore tested in an *in vitro* digestion and dispersion experiment to explore the changes in solvents solvation capacity over time as possible causes for a lower dissolution rate and bioavailability of the lipid formulations (Figure 2-4). The dissolution/release into the aqueous phase was limited in all tested formulations (Figure 2-4 A). Both medium chain formulations and the Peceol® formulation performed better than the aqueous suspension. The drug amount in the aqueous phase after 60 min of digestion was influenced by the excipients chain length with medium chain excipients resulting in higher concentrations than long chain excipients. However, the performance ranking observed based on drug concentrations in the aqueous phase after 60 min digestion, did not match the *in vivo* performance ranking. In fact, *in vivo* the aqueous suspension showed similar or higher bioavailability compared to the lipid suspensions. This suggests that the amount of drug in the aqueous phase may not be a strong predictor of lipid suspension performance, but rather other factors that govern drug dissolution and release from lipid suspensions may be relevant such as limited solubility in lipids, drug-excipient interactions and crystalline particle characteristics. While the aqueous phase data seemed to overestimate the *in vivo* performance of lipid suspensions, it is also possible that the test setup underestimated the aqueous suspension. The saturation levels in such closed *in vitro* test settings are quickly reached for low soluble drugs limiting the dissolution and release into the aqueous phase. Clearly, the presence of digestible lipid excipients increased nilotinib's solubilisation post-digestion as shown by the solubility studies in the artificial post-digestive media. However, it is not clear whether the enhanced solubilisation will lead to increased absorption or whether release and/or dissolution of drug crystals is the rate limiting step to absorption. An additional absorption step would allow further insights and the evaluation of the release and dissolution rate for such low soluble compounds like nilotinib [20; 112]. Therefore, it may also reflect the limitations of the

standard *in vitro* lipolysis test [77; 112]. Furthermore, nilotinib is a weak base that showed increasing solubility with decreasing pH of the media. The better solubility in a gastric media may generate higher initial concentrations in the intestine leading to a better absorption. A two-step gastrointestinal lipolysis may be beneficial for weakly basic compounds, like nilotinib, to get a better match with the *in vivo* data [80].

While most of nilotinib was recovered in the solid phase (Figure 2-4 C), it appeared that for the triglyceride-based suspensions nilotinib concentrations in the lipid phase remained high in the initial stages of digestion. At the start of digestion approximately 70% of the nilotinib dose resided within the lipid triglyceride phase in the vessel decreasing to approximately 20% at the end of digestion (Figure 2-4 B). This was equivalent to approximately 1244-fold and 165-fold excess of equilibrium drug solubility in the olive oil and Captex® 1000 phase, respectively. Such high amounts of drug within the triglycerides indicated that nilotinib crystals remained unreleased within the oil phase on top of the vessel and were not sampled (the samples were taken from the middle of the vessel) and consequently not recovered in the solid pellet phase that was collected after ultra-centrifugation. In fact, for the triglyceride suspensions, a distinct undispersed 'oil' phase was evident at the top of the vessel on dispersion and during the initial phases of digestion. As samples were collected from the middle of the vessel, drug crystals that were retained within the oil phase were not sampled. Consequently, the major reason for the higher amount of drug in the oil phase was the poor dispersibility of the triglyceride-based suspensions. This behaviour of nilotinib indicated a pronounced lipophilic interaction between the triglyceride excipients and nilotinib crystals, potentially delaying the release of nilotinib crystals into aqueous media which may lead to slower overall dissolution. Such a kinetic effect was likely of relevance for the *in vivo* performance of the formulations. In the case of the monoglyceride excipients that displayed greater dispersibility in the biorelevant media relative

to triglycerides, only a minor (Peceol®) or no (Capmul MCM®) lipid layer was evident in the vessel. The overall amount of drug estimated to reside within the lipid layer for these monoglyceride formulations was lower than the triglyceride formulations and was not influenced to any great extent by digestion. Collectively, these observations may explain the lower bioavailability observed for the triglyceride formulations relative to the monoglyceride formulations, where nilotinib crystals were not released from the undispersed oil phase leading to a delayed release of nilotinib crystals within the intestine which may have reduced absorption overall.

The propensity for nilotinib to be released and to dissolve in any solvent is fundamentally determined by the balance between the crystal lattice energy and the interactions with the solvent. Additionally, the rate of dissolution depends on a number of factors including particle size, viscosity of the solvent, hydrodynamics, the overall available volume of solvent, the solvents solvation capacity and wettability of the crystalline particles [113]. When comparing the lipid and aqueous suspensions used *in vivo* in this study, all of these factors were expected to remain constant except for wettability, as the addition of lipids alters the effective wettability of nilotinib crystals. For all lipid excipients a lower contact angle of 10.1° - 12.5° was observed, whereas for 0.5% methyl cellulose, a higher contact angle of 46.4° was obtained. This indicated a stronger interaction between nilotinib crystals and the lipid vehicles compared to the aqueous vehicle. Thus, greater wettability of the nilotinib crystals within the lipid suspensions may have been a contributor to the *in vivo* performance. The observations of greater wettability in lipids further supported the slower release of crystals from the undispersed oil layer observed in the *in vitro* lipolysis test. Therefore, we suggest that the pronounced hydrophobic interactions between the lipids and nilotinib crystal will favour the formation of a lipid film around the nilotinib crystals, which remains intact even after dispersion in aqueous media and during the

initial phases of digestion. This surface bound lipid film may delay wetting of nilotinib crystals by aqueous media and therefore result in delayed dissolution of the drug. In addition, the partition of drug through the lipid layer into the aqueous media will be limited by the inherent low solubility of this drug within lipids. Over time the lipid film will gradually be removed, either via dispersion of the lipid excipient into the aqueous media or digestion of the lipids, leading to complete wetting of the drug crystals by the aqueous media and dissolution of the crystals in aqueous media can proceed. A delayed release from the formulation would also explain the significant exposure decrease for the triglyceride formulations. The delayed release from lipid suspensions was further supported by observations from the *in vivo* MRT. The MRT was significantly longer for the olive oil, Captex® 1000 and Capmul MCM® formulations, relative to the aqueous suspensions ($p < 0.05$). It therefore seems as if nilotinib dissolution and absorption from the triglyceride suspensions, and to a lesser extent Capmul MCM® suspension, was slower and may have led to a reduction of bioavailability for the triglyceride formulations. Overall, a performance increase might be achieved by preventing the lipid film formation utilizing for example a chase dosing regimen, in which the lipid and drug are administered consecutively [70] or in a capsule-in-capsule approach. Additionally, the use of nilotinib with more polar and amphiphilic excipients such as surfactants may result in a better performance.

While the study demonstrated that rats did not show a food effect for nilotinib in this specific model, this may be related to the model itself or species-specific physiological differences, and in particular differences regarding bile secretion and/or gastrointestinal volumes [114]. One limitation of the fed state study leg employed in this study is that the rats had free access to food throughout the experimental procedure. As a result, this may have led to variability in the amount of food present in the stomach and intestine of rats. While it has been demonstrated that Sprague Dawley rats can be used to predict food effects in humans for several drugs, it is

recommended to use a specific protocol involving a homogenized Food and Drug Administration (FDA) style breakfast [115]. However, due to logistical constraints an FDA style fed state protocol was not employed in this study. Nevertheless, it was noteworthy that the MRT in the fed state was significantly longer than the MRT in the fasted state, which might reflect a delayed absorption process in the fed state. Interestingly, relative to the aqueous suspension in the fasted state, the Capmul MCM®, Captex® 1000 and olive oil suspensions displayed a longer MRT, and comparable to that obtained for the aqueous suspension in the fed state. This may indicate that these lipid excipients mimicked fed state conditions in terms of a slower absorption, due to the digestion process [116].

## 2.6 Conclusion

This study focused on providing new *in vivo* and *in vitro* insights for the 'brick dust' drug nilotinib in LBFs. *In vivo* neither triglycerides nor monoglyceride suspensions resulted in an increase in bioavailability of nilotinib relative to an aqueous suspension. Nevertheless, it was demonstrated that dispersibility of the lipids was a major contributing factor to the performance of nilotinib lipid suspensions. A higher *in vivo* exposure was observed for nilotinib lipid suspensions based on monoglycerides compared to triglycerides. The poorly dispersible triglyceride suspensions resulted in a significantly reduced bioavailability compared to the aqueous suspension. Subsequent, *in vitro* studies suggested that the lower bioavailability observed for both triglyceride suspensions most likely reflected a slower release of nilotinib from these formulations which were also reflected by a slower *in vivo* absorption. The key determinants for the success of a lipid suspension using a 'brick dust' molecule appeared to be the dispersibility and release from the lipid excipient.

# 3 Chapter 3: Chase dosing of lipid formulations to enhance oral bioavailability of nilotinib in rats

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

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

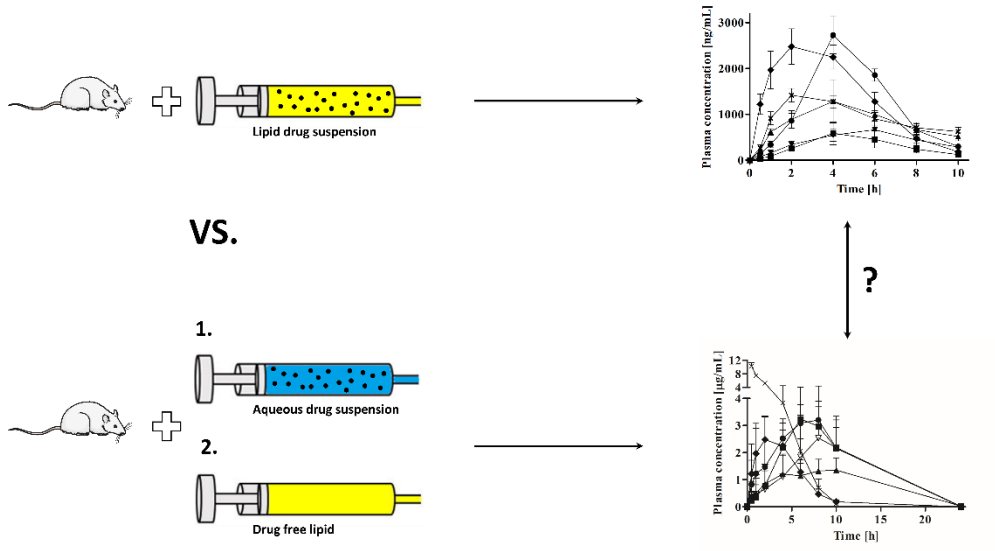
[2] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

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

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

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## 3.1 Abstract




Graphical abstract 3-1 Investigation of lipid chase dosing to overcome particle entrapment in lipid nilotinib suspensions.

**Purpose** LBFs have shown oral bioavailability enhancement of lipophilic drugs, but not necessarily in the case of hydrophobic drugs. This study explored the potential of lipid vehicles to improve the bioavailability of the hydrophobic drug nilotinib comparing a chase dosing approach and lipid suspensions.

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

**Results** Compared to the lipid suspensions, the chase dosed lipids showed a 2- to 7-fold higher bioavailability. Both long chain chase dosed excipients also significantly increased the bioavailability up to 2-fold compared to the aqueous suspension. Deconvolution of the

pharmacokinetic data indicated that chase dosing of nilotinib resulted in prolonged absorption compared to the aqueous suspension.

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

## 3.2 Introduction

The oral bioavailability of many poorly water-soluble drugs is hindered by low solubility and slow dissolution in the gastrointestinal tract fluids. LBFs are one option to improve the oral bioavailability of poorly water-soluble drugs and have shown numerous commercial success [61; 11]. In particular, drugs in the BCS class II and IV can benefit from usage of lipid excipients to enhance oral delivery. One of the primary biopharmaceutical advantages of LBFs is the ability to deliver the drug dissolved in lipid excipients and therefore avoiding the drug dissolution process. In addition, LBFs have been shown to increase drug solubilisation within the intestinal fluids, as well as intestinal permeability and promoting intestinal lymphatic uptake [117; 15]. To date, the majority of successful examples of enhanced *in vivo* bioavailability via a LBF approach have been demonstrated for poorly water-soluble drugs with highly lipophilic characteristics (i.e. high log*P*). Such drugs tend to display high solubility in lipid excipients which supports high dose loading in LBFs. However, poor solubility in water is not always reflective of a good solubility in lipid vehicles. Solubility in lipid vehicles can be solid state limited due to a strong crystal lattice, i.e. so called 'brick dust' molecules. For example, it has been reported that poorly water-soluble drugs with a $T_m$ > 150 °C display an insufficient solubility in glycerides [53], and hence lipid vehicles, which limits the aforementioned advantage in terms of bypassing drug dissolution via a LBF. However, for 'brick dust' drugs

displaying both hydrophobic and high lipophilic properties it remains unclear whether the additional bio-enabling effects of lipid excipients can increase oral bioavailability. For such challenging compounds exhibiting low aqueous and lipid solubility the formulation as LBF is often disregarded. Recently, formulation strategies such as designing lipophilic salts (Table 1-1and Table 1-2) or sLBFs (Table 1-3) have been proposed as an approach to increase the drug loading in a LBF solution [31; 32; 37; 38]. However, LBF suspensions [44; 45] and concomitant administration of drug and LBF excipients (chase dosing) [118-120] have also shown promise in terms of enhancing the *in vivo* exposure. In the latter chase dosing, crystalline drug and the lipid excipient (or LBF) are administered immediately following each other. This can be done *ad hoc* via gavaging in rodents an aqueous suspension followed by the drug-free lipid vehicle or as a capsule-in-capsule approach in humans or larger animals. Lipid and aqueous suspensions are particularly useful in a pre-clinical setting, offering a balance in terms of ease of administration in rodent models, and also the potential of the lipid excipient to increase *in vivo* exposure which is a key consideration for example in the early stage of toxicological drug evaluation. This is particularly relevant, as large resource investment in formulation design and optimisation in pre-clinical development is risky given the high attrition rate of drug candidates.

Lipid suspensions have shown potential to enhance oral bioavailability for a number of poorly water-soluble drugs including danazol, fenofibrate and griseofulvin [67; 44; 45; 33]. For example, in the case of danazol, a bioavailability increase of 4 - 9-fold was observed when dosed in a Labrafil® M2125CS suspension compared to an aqueous suspension [45]. However, in the case of 'brick dust' drugs like atovaquone and nilotinib, lipid excipients did not improve the bioavailability (Chapter 2) [72; 121]. Atovaquone shows a positive food effect, high lipophilicity, high hydrophobicity and has a limited solubility in medium chain triglycerides of ~ 4 mg/mL.

The bioavailability of an atovaquone medium chain triglyceride suspension was similar to the bioavailability of an aqueous suspension in humans [72]. In the case of nilotinib, which is highly lipophilic and hydrophobic with a medium chain triglyceride solubility of ~ 0.05 mg/mL and a food effect of approximately 80% (increase in AUC after a high fat meal), a similar bioavailability for both medium chain and long chain monoglyceride suspensions was observed. Interestingly, for nilotinib there was a significantly reduced bioavailability observed in case of both medium chain and long chain triglyceride suspensions compared to an aqueous suspension (Chapter 2) [121]. In the latter study, it was hypothesized that the main factor for the poor *in vivo* performance of the lipid suspensions was a kind of entrapment of the nilotinib drug crystal by a persistent lipid film due to a low dispersibility of the triglycerides. Such an entrapment may have hampered the drug interactions with the post-digestive colloids in the gastrointestinal tract and hence drug solubilisation. Consequently, it was suggested that the separation of the drug and the lipid excipient would avoid such entrapment. The current study was hence designed to assess whether the concomitant administration of nilotinib as an aqueous suspension and a drug free LBF (chase dosing) would influence oral bioavailability relative to administration of either an aqueous or lipid-based drug suspension.

Lipid co-administration or chase dosing has been shown to successfully enhance the bioavailability compared to an aqueous suspension or even lipid solution across different pre-clinical species and in humans [118-120]. Additionally, co-administration avoids any risk of formulation or active pharmaceutical ingredient instability. Larsen *et al.* demonstrated in rats that for danazol, halofantrine and cinnarizine a co-administered lipid resulted in a similar bioavailability compared to a lipid solution, which was higher than the bioavailability of an aqueous suspension [119]. In humans the co-administration of the commercial cinnarizine tablets with a placebo-LBF was tested in the fasted and fed state [118]. In the fasted state the

co-administration showed a tendency to increase the bioavailability compared to the commercialized tablets. In the fed state a decreased food effect was observed based on the individual profiles [118]. In a different study the co-administration of carvedilol was studied in dogs [120]. Co-administering carvedilol with a drug-free-LBF showed a similar bioavailability compared to a LBF solution and a higher bioavailability compared to an aqueous suspension [120].

Given that most research on LBFs is focused on a relatively limited number of model poorly water-soluble drugs, there is a need to study other more recently licenced drugs, such as the tyrosine kinase inhibitor nilotinib. Nilotinib is considered a 'brick dust' molecule based on its high lipophilicity (log*P* 4.95) and high $T_m$ of 236 ºC and hence displays low solubility in lipid excipients. The present study investigated the hypothesis that nilotinib would benefit from co-administration with lipids rather than its direct formulation as a lipid suspension. To compare the results of the previously published lipid suspensions in chapter 2 to the present chase dosing approach, the previously tested lipid excipients olive oil, Captex® 1000, Peceol® and Capmul MCM® were also evaluated *in vivo*.

## 3.3 Materials and methods

### 3.3.1 Chemicals and materials

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

### 3.3.2 Formulations for *in vivo* studies

For the lipid co-administration, an aqueous suspension with 20 mg/mL nilotinib was prepared by adding 20 mg nilotinib to 1 mL of the aqueous 0.5% (w/v) methylcellulose solution and mixed thoroughly. To decrease any powder aggregates, the suspension was placed in an ultrasonic bath for 5 sec and vortexed again afterwards. For the lipid suspensions the lipid excipients were melted as needed and 10 mg/mL nilotinib was added and stirred overnight. To ensure a homogeneous distribution of nilotinib, all suspensions were stirred prior to administration.

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

### 3.3.3 *In vivo* study

The protocol used for the *in vivo* pharmacokinetic study was approved by the institutional animal ethics committee in accordance with Belgian law regulating experiments on animals and in compliance with EC directive 2010/63/EU and the NIH guidelines on animal welfare. Male Sprague Dawley rats weighing 250 - 320 g on the day of the experiments were purchased from Charles River Laboratories Deutschland (Sulzfeld, Germany) and maintained on standard food and water *ad libitum* in the laboratory for at least 5 days before entering the experiment. Food was removed 16 - 20 h before dosing and water was available *ad libitum* at all times. For the oral study arms, parallel groups of animals were administered an aqueous suspension at a volume of 1 mL/kg by oral gavage with a nilotinib dose of 20 mg/kg immediately followed by oral administration of the drug free lipid excipient at a volume of 2 mL/kg. For the intravenous

part, animals were slowly injected 5 mL/kg of the intravenous formulation (2.5 mg/kg nilotinib) via the tail vein. 200 µL blood samples were collected into plasma collection tubes containing EDTA by individual tail vein puncture. Samples were taken at 0.5, 1, 2, 4, 6, 8, 10 and 24 h following oral dosing and at 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 h following intravenous dosing. Plasma was harvested immediately by centrifugation for 10 min at 4 °C and 1,900*g* and stored at -20 °C until analysis. After the experiment the animals were euthanized.

### 3.3.4 Bioanalysis

The plasma concentrations of nilotinib were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., US) comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was controlled, and the data analysed with EZChrom Elite® version 3.3.2. The employed method was described previously (chapter 2). 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 lower limit of detection (LOD) and lower LOQ in plasma by this method was 11 ng/mL and 37 ng/mL, respectively, determined using the standard error of y-intercept according to International council for Harmonisation of Technical Requirements

for Pharmaceuticals for Human Use (ICH) Q2 guideline [122]. Linearity was confirmed between 37 ng/mL and 4.1 µg/mL.

### 3.3.5 Deconvolution

Concentration-time-profiles were deconvoluted to obtain information about the absorption process as an *in vivo* absorption function over time. This was done in Microsoft® Excel® by means of system analysis and required no compartmental pharmacokinetic modelling or curve fitting [123; 124]. The response observed after oral administration of nilotinib, i.e. the plasma concentration time profile, can be treated as the response function R(t). Additionally, a weighting function W(t) is needed, which can be obtained from intravenous or oral bolus administration. The correlation between both functions R(t) and W(t) depends on the input and can be described by the following integral [123; 124]:

$$R(t) = \int_0^t I(\vartheta) W(t-\theta) d\theta \qquad (3\text{-}1)$$

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

$$R(t) = I(t) * W(t) \qquad (3\text{-}2)$$

where * is the convolution operator. The process to obtain I(t) is called deconvolution and was described by Langenbucher *et al.* as follows [123]:

$$I(x_i) = \frac{[\frac{R(t)}{T} - \sum_{k-1}^{n} I(x_k) * W(x_{n-k+1})}{W(x_i)} \quad (3\text{-}3)$$

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

$$W(T) = e^{(m*T+n)} \quad (3\text{-}4)$$

where T is the time interval ($t_2$-$t_1$), m the slope of the intravenous data and n the intercept of the intravenous data. With the information of W(T), I(t) can be calculated as follows [123; 124]:

$$I(t) = \frac{R(t)}{W(T)} \quad (3\text{-}5)$$

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

$$\%\ drug\ released\ and\ absorbed = \frac{F_{abs}\ or\ 100\%}{\sum_{t\ last}^{0} I(t)} * \sum_{t}^{0} I(t) \quad (3\text{-}6)$$

### 3.3.6 Data analysis

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

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

## 3.4 Results

### 3.4.1 Long chain lipid-based formulations increased bioavailability of nilotinib

Nilotinib was orally dosed as an aqueous suspension followed by oral administration of the pure lipid excipients olive oil, Captex® 1000, Peceol® or Capmul MCM®. Additionally, an intravenous formulation was administered to allow absolute bioavailability to be determined. The mean plasma concentration versus time profiles (after oral administration) are presented in Figure 3-1 together with the relative bioavailability ($F_{rel}$) compared to the aqueous suspension. The mean plasma versus time profile for the intravenous formulation are shown in Figure 3-2. Table 3-1 presents a summary of the pharmacokinetic parameters obtained for all dosed formulations.

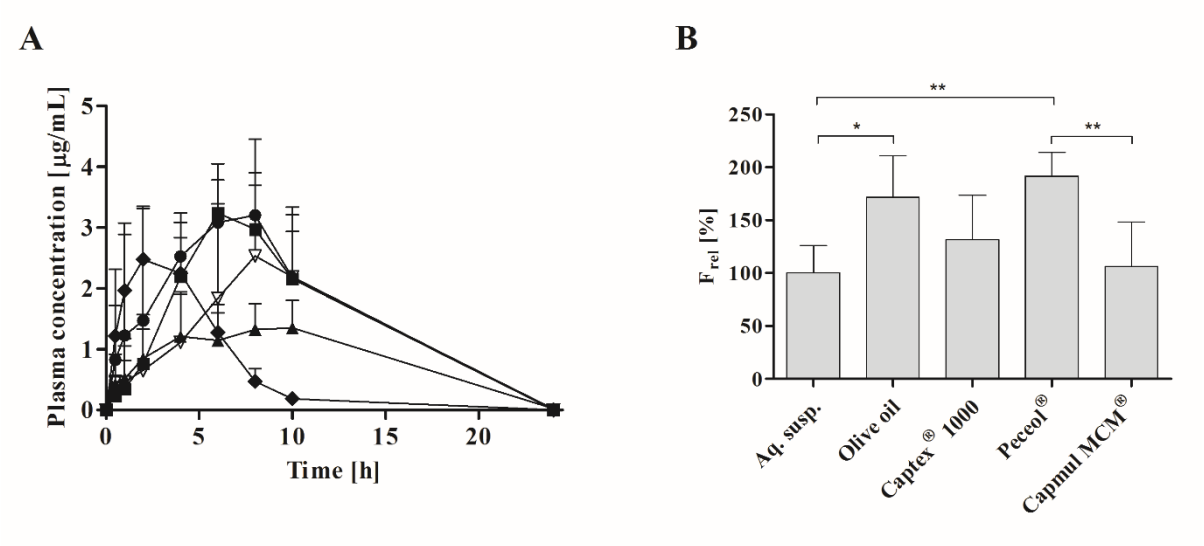



Figure 3-1 Plasma concentration profiles as a function of time (mean ± SD, n = 5) for nilotinib aqueous suspension with a dose of 20 mg/kg co-administered with Peceol® (●), Olive oil® (■), Capmul MCM® (▲) and Captex® 1000 (▽) in male Sprague Dawley rats. Additionally, a nilotinib aqueous suspension (♦) with 20 mg/kg nilotinib in the same species previously reported [121] (mean ± SD, n = 5). B: Relative bioavailability of co-administered lipid excipients compared to the aqueous suspension previously published in chapter 2 [121].

Co-administration of long chain-based LBFs resulted in a significant increase in nilotinib bioavailability relative to that obtained for an aqueous suspension alone ($p \leq 0.05$). The highest relative bioavailability was observed for the Peceol® formulation (191 ± 22%) followed by olive oil as vehicle (171 ± 39%). Additionally, the Peceol® formulation showed the highest $c_{max}$ with 4.12 ± 0.67 µg/mL and the lowest variability for AUC and $c_{max}$. In the case of the medium chain formulations, a relative bioavailability of 131 ± 42% for Captex® 1000 and 106 ± 42% for Capmul MCM® was observed, which were not statistically different to the aqueous nilotinib suspension alone.

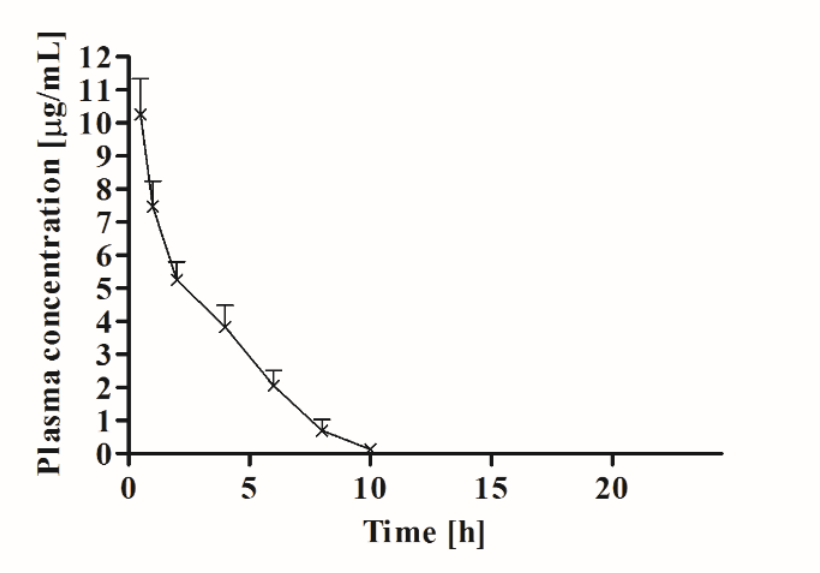



Figure 3-2 Plasma concentration profiles as a function of time for an intravenous nilotinib formulation dosed at 2.5 mg/kg in male Sprague Dawley rats (mean ± SD, n = 6).

Table 3-1 Pharmacokinetic parameters of an orally nilotinib aqueous suspension chase dosed with olive oil, Captex® 1000, Peceol® and Capmul MCM® and the corresponding lipid suspensions as well as an aqueous suspension previously reported in chapter 2 [121] (reanalysed with the intravenous data obtained in the present study). All oral parameters were obtained at a nilotinib dose of 20 mg/kg and n = 5. Pharmacokinetic parameters of an intravenous formulation were obtained with a nilotinib dose of 2.5 mg/kg and n = 6. All studies were performed in male Sprague Dawley rats. Mean residence time (MRT), mean absorption time (MAT) and the time to reach maximum plasma concentration ($t_{max}$) are given as median (range), all other parameters as mean ± SD.

| | **Pharmacokinetic parameters** | | | | | |
|---|---|---|---|---|---|---|
| | $c_{max}$ [µg/mL] | $t_{max}$ [h] (range) | AUC 0 h - inf. [µg*h/mL] | MRT [h] (range) | MAT [h] (range) | $F_{abs}$ [%] |
| Aq. susp. | 2.65 ± 0.68 | 2 (2 - 4) | 14.33 ± 3.71 | 4.14 (3.34 - 4.83) | 2.49 (1.69 - 3.18) | 12.90 ± 3.34 |
| Lipid susp. Olive oil | 0.61 ± 0.56 | 4 (2 - 6) | 3.60 ±2.82 | 5.64 (4.42 - 6.42) | 4.00 (2.78 - 4.77) | 3.24 ± 2.54 |
| Aq. susp., Olive oil (chase dosed) | 3.48 ± 0.65 | 6 (4 – 8) | 24.57 ± 5.65 | 7.87 (5.80 - 7.87) | 6.22 (4.16 - 6.29) | 22.11 ± 5.08 |
| Lipid susp. Captex® 1000 | 0.77 ± 0.35 | 6 (4 - 10) | 5.10 ± 2.35 | 6.18 (5.34 - 9.62) | 4.54 (3.70 - 7.97) | 4.58 ± 2.12 |
| Aq. susp., Captex® 1000 (chase dosed) | 2.93 ± 1.09 | 8 (0.5 – 10) | 18.86 ± 6.01 | 8.15 (5.47 - 8.72) | 6.50 (3.82 - 7.07) | 16.97 ± 5.41 |
| Lipid susp. Peceol® | 2.80 ± 0.76 | 4 (4 - 6) | 13.63 ± 2.70 | 5.29 (4.85 - 6.16) | 3.64 (3.21 - 4.51) | 12.26 ± 2.43 |
| Aq. susp., Peceol® (chase dosed) | 4.12 ± 0.67 | 6 (2 - 8) | 27.47 ± 3.22 | 7.25 (4.56 - 7.98) | 5.60 (2.92 - 6.33) | 24.72 ± 2.90 |

| Pharmacokinetic parameters | | | | | | |
|---|---|---|---|---|---|---|
| | $c_{max}$ [µg/mL] | $t_{max}$ [h] (range) | AUC 0 h - inf. [µg*h/mL] | MRT [h] (range) | MAT [h] (range) | $F_{abs}$ [%] |
| Lipid susp. Capmul MCM® | 1.32 ± 1.04 | 4 (1 - 6) | 9.58 ± 5.50 | 5.78 (5.17 - 6.14) | 4.13 (3.53 - 4.49) | 8.62 ± 4.95 |
| Aq. susp., Capmul MCM® (chase dosed) | 1.76 ± 0.47 | 8 (4 - 10) | 15.18 ± 6.05 | 7.35 (5.49 - 8.24) | 5.70 (3.85 - 6.59) | 13.66 ± 5.44 |
| intravenous formulation | - | - | 22.31 ± 4.50 [a)]<br>5.56 ± 1.19 [b)] | 1.70 (1.19 - 2.39) | - | - |

[a)] Dose [mg] corrected
[b)] Dose [mg/kg] corrected

Among the various lipid formulations evaluated, while there was a trend towards a lower bioavailability for the Captex® 1000 excipient, only the Capmul MCM® formulation was significantly lower compared to Peceol® formulation ($p \leq 0.05$). The general ranking among the four formulations was Peceol® ≥ olive oil ≥ Captex® 1000 ≥ Capmul MCM®. These results indicated that long chain excipients performed better than medium chain excipients, which may be related to a long chain fatty acid mediated increase in bile salt release [125; 126]. Additionally, the results showed that *in vivo* exposure was not influenced by whether the LBF was administered as either a monoglyceride or a triglyceride, when chase dosed.

### 3.4.2 Chase dosing leads to higher increases in bioavailability

The results of the chase dosing study conducted in the present work were compared to a previously published *in vivo* study in rats (chapter 2) that investigated lipid suspensions for nilotinib [121]. In both studies olive oil, Captex® 1000, Peceol® and Capmul MCM® were administered at the same dose and lipid volume [121]. The previously reported aqueous and lipid nilotinib suspensions [121] were re-analysed using the intravenous data obtained in the present study. The absolute bioavailability ($F_{abs}$) is shown in Figure 3-3 and the pharmacokinetic parameters are summarised in Table 3-1.

In comparison to the lipid suspensions (chapter 2) [121] the chase dosing approach of Peceol®, olive oil and Captex® 1000 resulted in a statistically significant increase in the absolute bioavailability ($p \leq 0.05$). The largest increase was observed for olive oil, where the chase dosing increased the absolute bioavailability of nilotinib by approximately 22%. This translates to a 6.8-fold increase in bioavailability, relative to the $F_{abs}$ of 3% for the olive oil suspension. For the Captex® 1000 and Peceol® formulations, chase dosing increased bioavailability 3.7-fold and 2-fold, respectively, compared to the suspension approach. While the

Capmul MCM® formulation displayed an increase of approximately 5% (1.6-fold), the difference was not significant indicating that relative to this excipient, a lipid suspension approach already reached a reasonably high bioavailability. The rank order of bioavailability increases of the chase dosed formulations relative to the lipid suspensions (i.e. non-chase dosed) was TG > MG (LC TG > MC TG > LC MG > MC MG). Therefore, the greatest impact of chase dosing was observed for the least dispersible formulations, when compared to the lipid suspensions. For more readily dispersible formulations, chase dosing offered a relatively lower increase. These findings supported the hypothesis from the previous study in chapter 2 in that the lowest bioavailability was observed for the least dispersible formulations [121].

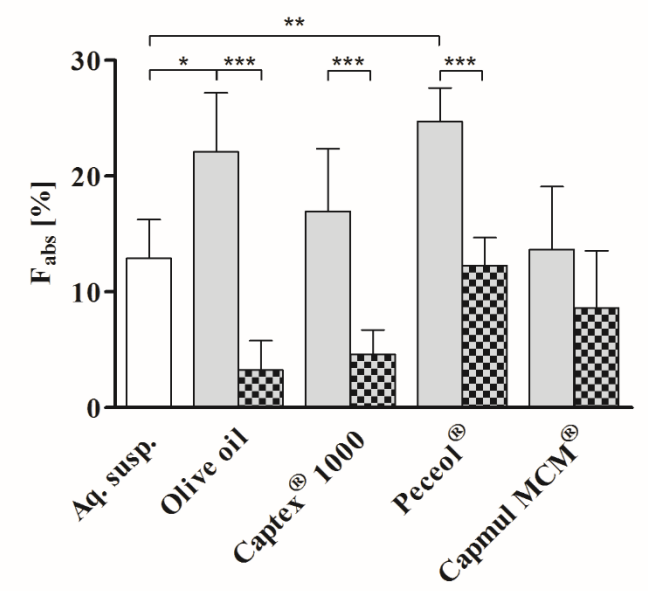



Figure 3-3 *In vivo* absolute bioavailability of nilotinib co-administered with olive oil, Captex® 1000, Peceol® and Capmul MCM® in male Sprague Dawley rats (grey bars) in comparison to an aqueous suspension (white bar) and the four corresponding lipid suspensions (checked bars) in male Sprague Dawley rats, which were reported previously (chapter 2) [121]. All study legs are presented as mean ± SD, where n = 5.

In the chase dosing study, the pharmacokinetic evaluation showed that the nilotinib absorption was prolonged. Relative to the lipid suspensions, there was a trend towards a prolonged time

to reach the maximum plasma concentration ($t_{max}$), mean absorption time (MAT) and MRT. A consistent increase of 2 h in $t_{max}$ from lipid suspensions to chase dosing was observed for olive oil, Peceol® and Captex® 1000. The same observation was made for MAT and MRT with an increase of approximately 2 h when the lipid excipient was chase dosed. For the medium chain monoglyceride (Capmul MCM®) excipient an increase of 4 h was observed for $t_{max}$ and an increase of 2 h was observed for MRT, while MAT was only prolonged by 1 h. While these trends for prolonged absorption were not statistically significant, reflecting a high variability in rate of absorption *in vivo*, the data suggested that the prolonged absorption may be a factor in explaining the improved bioavailability for chase dosing.

### 3.4.3 Deconvolution to explore differences in absorption

To further investigate the absorption from the tested formulations, deconvolution was performed according to Langenbucher and Mysicka as described above [123]. Using a numerical deconvolution technique, information about the *in vivo* drug absorption as a function of time was obtained. Deconvolution was calculated up to 8 h, which was the last plasma concentration within the limits of quantification of the bioanalysis. Normally, the cumulative integral for the last time point is set to 100% drug absorbed [123; 124]. Additionally, in this study, the cumulative integral for the last time point was set equal to the absolute bioavailability, as this approach provided insights on the extent and kinetics of the drug absorption process. The results of the deconvolution for the aqueous suspension, chase dosing and lipid suspensions of olive oil, Peceol®, Captex® 1000 and Capmul MCM®, respectively, are shown in Figure 3-4. Additionally, Table 3-2 presents a summary of the time needed to absorb 25% and 50% of the maximum absorbed amount of nilotinib.

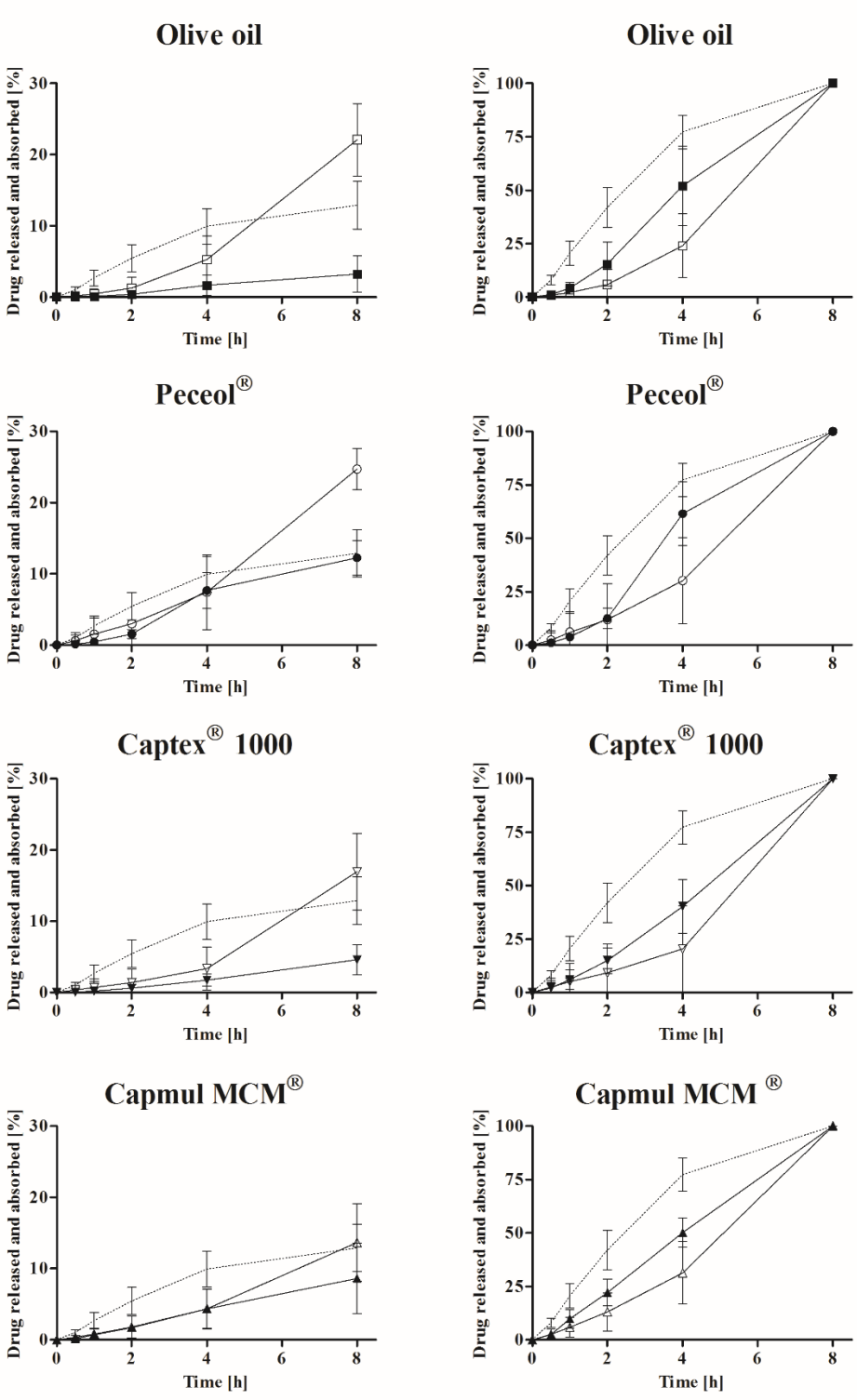



Figure 3-4 Numerical deconvolution of the aqueous suspension (dotted line, no symbol), chase dosing (open symbol) and the lipid suspensions (filled symbol) for Peceol® (●), Olive oil® (■), Capmul MCM® (▲) and Captex® 1000 (▼). On the left absolute bioavailability was set as maximum drug released and absorbed, on the right maximum drug absorption was set to 100% (mean ± SD, n = 5).

All chase dosed excipients increased the absorption rate of nilotinib *in vivo* compared to the lipid suspensions between 4 and 8 h. The rate of absorption from the olive oil, Captex® 1000 and Capmul MCM® suspensions were linear from 0 – 8 h. While for the Peceol® suspension a linear rate of absorption was observed in the first 2 h after administration, the absorption rate increased between 2 and 4 h. In case of the chase dosed formulations, the absorption rate was lower compared to the lipid suspensions up to 4 h post-dosing. However, the rate of absorption increased between 4 and 8 h, which was in agreement with the observation of a longer MAT. This increased absorption rate was also evident in the absorbed extent of nilotinib, which distinctly increased 4 h post-dosing. Thus, the apparently slower initial absorption rate did not translate to an overall lower amount of nilotinib being absorbed. For example, in the case of the long chain monoglyceride Peceol® the higher absorption rate for the lipid suspension between 2 and 4 h resulted in the same amount absorbed after 4 h compared to the chase dosing.

Table 3-2 Time to absorb 25% and 50% of the total fraction of absorbed drug for both lipid suspensions and chase dosed excipients with olive oil, Captex® 1000, Peceol® and Capmul MCM® (mean ± SD, n = 5).

| **Time to absorb 25% of the drug [h]** | | |
|---|---|---|
| | lipid suspension | chase dosed |
| Olive oil | 2.68 ± 0.78 | 3.97 ± 0.87 |
| Captex® 1000 | 2.92 ± 0.70 | 3.94 ± 1.46 |
| Peceol® | 2.58 ± 0.39 | 3.49 ± 1.40 |
| Capmul MCM® | 2.20 ± 0.38 | 3.44 ± 1.00 |
| Aq. susp. | 1.24 ± 0.29 | |
| **Time to absorb 50% of the drug [h]** | | |
| Olive oil | 3.99 ± 0.97 | 5.26 ± 0.71 |
| Captex® 1000 | 4.57 ± 0.69 | 5.29 ± 0.99 |
| Peceol® | 3.28 ± 0.96 | 4.89 ± 1.22 |
| Capmul MCM® | 4.00 ± 0.47 | 4.97 ± 0.80 |
| Aq. susp. | 2.50 ± 0.43 | |

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

## 3.5 Discussion

LBFs have numerous biopharmaceutical benefits including the delivery of dissolved drug, a potential to promote lymphatic transport, improved intestinal permeability and most importantly an increased extent of *in vivo* solubilisation upon luminal dispersion and digestion of the formulation [117; 15]. Many of the benefits of lipids in bio-enabling formulations are commonly demonstrated with drugs displaying high lipophilicity, i.e. 'grease ball' molecules, where the dose is soluble in the lipid vehicle to form a lipid solution. It has been reported that as a rule of thumb for a LBF approach a drug candidate should have a log$P$ > 2 [13] and a $T_m$ < 150 ºC [53]. However, it is increasingly recognised that the hydrophobic characteristics of the drug merit consideration [52]. For example, it has been reported that drugs with a $T_m$ > 150 ºC may be less suited for formulation as lipid formulations, due to a high crystal lattice energy, which can lead to dose loading limitations in LBF [53]. However, the merits of a LBF approach for drugs displaying both high hydrophobic and high lipophilic properties, may be theoretically limited due to the inability to dissolve drug in a reasonable lipid volume of the final dosage form. It remains unclear whether the additional biopharmaceutical benefits of lipid excipients for highly lipophilic drugs (e.g. *in vivo* solubilisation in post-digestive micellar fluids and/or increased permeability) may still lead to improved bioavailability. Therefore, for 'brick dust' drugs, strategies focused on increasing dose loading in the lipid formulation have been reported such as sLBFs (Table 1-3) [32] or a lipophilic salt approach (Table 1-1, Table 1-2) [38]. Alternatively, the potential to administer a drug as a lipid suspension has been explored as a bio-enabling approach [44; 45; 31; 32; 121].

Nilotinib, a tyrosine kinase inhibitor used in the treatment of leukaemia that was licenced in 2007, shows a high lipophilicity (log$P$ 4.95), but also a pronounced hydrophobicity. Additionally, nilotinib showed a positive food effect (82% increase in AUC after a high fat

meal [90]) indicating a benefit from lipid excipients. For this high log*P* and $T_m$ drug, solubility in lipid excipients was reported to be low (chapter 2) [121]. We have previously applied a lipid suspension approach to assess the impact of lipid excipients on the bioavailability of nilotinib in rats. However, despite high lipophilicity and a pronounced food effect, lipid suspensions based on medium chain and long chain excipients did surprisingly not increase the bioavailability of nilotinib relative to an aqueous suspension. In the case of the triglyceride formulations, bioavailability was even significantly reduced compared to an aqueous suspension (chapter 2) [121]. We hypothesised that the nilotinib particles were entrapped in the lipid excipient so that poor dispersion and a remaining lipid film on the particulate surface would kinetically hinder a favourable solubilization interaction with bile salts, phospholipids and other post-digestive components. Thus, a separation of drug and lipid excipient by chase dosing, i.e. a concomitant administration of a lipid, could prevent an entrapment and harness the aforementioned other *in vivo* benefits of lipid excipients.

In comparison to the lipid suspension results in chapter 2 [121], the chase dosing resulted in a significant increase in nilotinib bioavailability for olive oil (7-fold), Captex® 1000 (4-fold) and Peceol® (2-fold) ($p < 0.05$). The highest increase in bioavailability between the lipid suspensions and the chase dosing was observed for the triglyceride formulations, whereas in the case of the monoglyceride formulations the difference was low or indeed in the case of Capmul MCM® no difference was observed. Therefore, in the case of poorly dispersible formulations chase dosing of the drug with the LBF is preferred to prevent a masking of the bio-enabling effects. In fact, the drug load in the formulation and the solubility in the lipid excipient was not limiting the bioavailability once the formulation was chase dosed. The chase dosing also confirmed the previously hypothesized hampered dissolution due to an entrapment of the nilotinib particles by the vehicle of the lipid suspensions (chapter 2) [121].

Chase dosing of the LBFs enhanced the bioavailability of nilotinib compared to the aqueous suspension and was in good agreement with previously published data. An improved bioavailability was reported for the co-administration of danazol (in rats), cinnarizine (in rats and humans), halofantrine (in rats) and carvedilol (in dogs) with different lipid excipients [118-120]. The *in vivo* results also matched with the previously reported nilotinib solubility data in the post-digestive state. Compared to FaSSIF the simulated olive oil and Captex® 1000 post-digestion media had an approximately 17- and 22-fold higher solubilisation capacity indicating that digestion was vital for an increased solubilisation capacity. Also, Kaukonen *et al.* showed that the exposure to post-digestive products for highly hydrophobic drugs was of importance, because the solubilizing capacity of the post-digestion phase was much higher compared to the drug that could be dissolved in the lipid excipient [127]. Therefore, a rapid *in vitro* screening tool with simulated post-digestive media could be helpful in early screenings as a model to predict a higher *in vivo* exposure by co-administering lipids.

While the co-administration of long chain-based LBFs showed a statistically significant 2-fold increase compared to an aqueous suspension ($p < 0.05$), the medium chain-based LBFs did not result in an increased bioavailability relative to the aqueous suspension. This fatty acid chain length effect was unexpected for nilotinib due to a higher solubility in medium chain-based dispersions pre- and post-digestion compared to long chain using simulated post-digestive media and *in vitro* lipolysis (chapter 2) [121]. However, the results of this study were in good agreement with previously reported effects that long chain fatty acids (digestion products of tri- and diglycerides) with a chain length of C12 or higher are able to increase cholecystokinin (CCK) plasma concentrations in humans [126; 128]. A similar effect of fatty acids with a chain length of C12 or higher was demonstrated *in vitro* using the mouse cell line STC-1, which is able to release CCK [125]. Especially in the present study, where a lipid load of 2 mL/kg was

used (resulting in approximately 0.23 – 0.57 mol/L free fatty acids released upon digestion, depending on the available gastrointestinal volume), a stimulation of CCK was likely. CCK is a peptide hormone of the gastrointestinal tract, which stimulates the release of digestive enzymes and bile, which may increase the solubilisation of poorly water-soluble drugs in the intestinal fluids. In the case of nilotinib, an increased solubility was observed in the presence of higher bile salt concentrations in fed state simulated intestinal fluid (FeSSIF) (3.2 ± 0.1 µg/mL) when compared to FaSSIF (0.3 ± 0.03 µg/mL) [121]. The medium chain excipients in the present study had a chain length of C8 and C10 and were not suspected to increase CCK plasma levels, which may explain the observed differences between medium chain and long chain-based excipients *in vivo*. In addition, the results in this study are in line with previous reports for long chain-based LBFs (Labrafil®) that were co-administered with danazol and cinnarizine. Compared to an aqueous suspension the co-administration for both drugs showed a 2-fold increase in bioavailability in rats [119]. It is also interesting to note that nilotinib displayed a pronounced increase in bioavailability in the fed state in humans, and while the possible reasons for this are varied, this study suggested that dietary long chain lipids may have a role in the improved bioavailability in the post-prandial state.

The results from deconvolution showed that the absorption of nilotinib from LBF suspensions appeared slower with less than 1% of the total administered dose absorbed after one hour. These results were in agreement with the *in vitro* lipolysis results reported for the lipid suspension study, where the total drug amount was below 1.1% after 60 min of lipolysis (chapter 2) [121]. Also, the chase dosed LBFs showed a slow absorption with a total amount absorbed < 1% after one hour. However, the deconvolution further showed a trend that the absorption from the chase dosed LBFs was prolonged. In the case of chase dosing, the initial 25% and 50% of nilotinib in the plasma was absorbed approximately one hour later when

compared to the initial 25% and 50% of nilotinib in plasma from the corresponding lipid suspensions. This kinetic advantage of the lipid suspensions did not result in an enhanced *in vivo* performance, but rather a similar or significantly decreased bioavailability. This difference most likely arose as a result of a decreased release of nilotinib from the LBF suspensions due to the compound's high lipophilicity. The previously reported greater wettability of nilotinib crystals by lipid vehicles compared to aqueous vehicles suggested pronounced hydrophobic interactions between the lipids and nilotinib crystals (chapter 2) [121]. We hypothesised that these interactions favoured the formation of a lipid film around the nilotinib crystals, which remained intact even after dispersion in aqueous media and during the initial phases of digestion, and hence may be the most important contributing factor to the poor *in vivo* performance of the lipid suspensions.

Administering LBFs resulted in a delayed absorption relative to the aqueous suspension. This was also evident by a longer $t_{max}$ and MAT for the LBFs. A reason for the delayed absorption with lipid excipients may be the delayed gastric emptying. It was reported that the half-life ($t_{1/2}$) of gastric emptying was approximately 77 min after a high caloric liquid meal in rats [129]. Lipid excipients mimic fed state conditions [116] and may have delayed the gastric emptying when compared to the aqueous suspension, which should not affect gastric emptying. An additional factor contributing to a slower initial absorption may be digestion of the lipid excipients. This was especially prominent for the co-administered LBFs with olive oil, Peceol® and Captex® 1000. Despite the delayed initial absorption, over the next four hours, extensive absorption was observed, which resulted in an overall increase in bioavailability compared to the aqueous suspension. This suggested that the access to post-digestive products of the lipid excipients as well as to bile salts and phospholipids was more readily available for the chase dosed LBFs. It seemed that the Capmul MCM® was not able to generate a solubility increasing

environment, which in part may be due to a very rapid digestion (chapter 2) [121] and subsequent absorption of the excipient resulting in the same performance as the aqueous suspension.

## 3.6 Conclusion

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

# 4 Chapter 4: Exploring 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

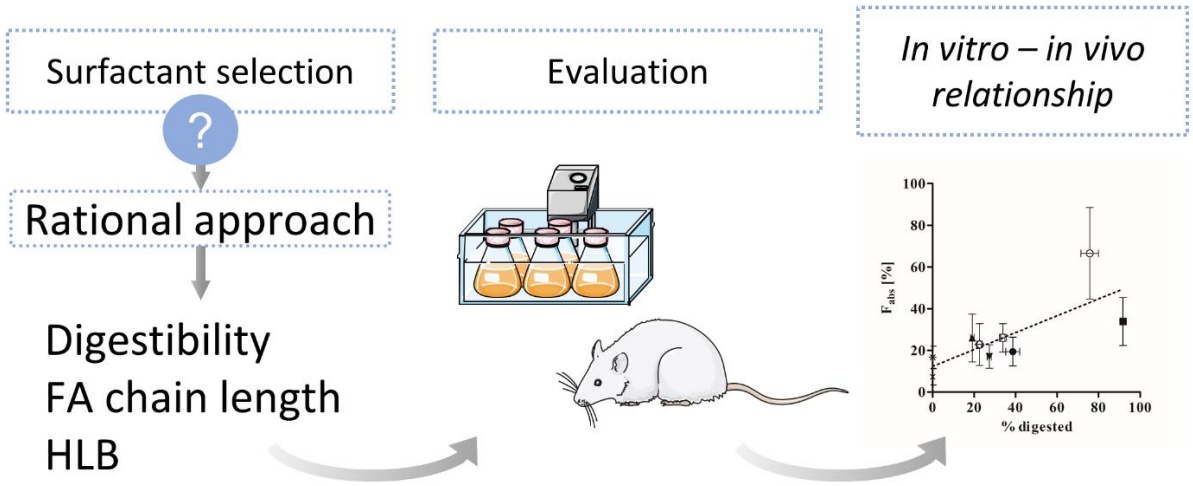
[4] University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharma Technology, Muttenz, Switzerland

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

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## 4.1 Abstract




Graphical abstract 4-1 Systematic evaluation of the relationship between surfactant properties, *in vitro* characteristics and *in vivo* bioavailability.

**Purpose** 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 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.

**Methods** 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.

**Results** 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 *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.

**Conclusion** The impact of this study's findings suggests that when designing surfactant-based formulations to enhance oral bioavailability of poorly water-soluble drugs, 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 (P-gp), the biopharmaceutical effects of surfactants merit further consideration.

## 4.2 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 [100]. Therefore, there is a need to develop bio-enabling formulation technologies that enhance biopharmaceutical properties and improve oral absorption of these emerging drug candidates [130]. The various bio-enabling approaches have been extensively reviewed including solid dispersions [131; 132], LBFs [61] and nano-sized drug crystals/particles [133]. 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 [134; 135], enhancing solubilisation [136], stabilisation of colloidal/nano-crystals [137], increased dissolution rate [138] and increasing permeability [139; 22; 28; 140; 23].

Surfactants can be classified according to the polar head group into ionic (cationic, anionic or zwitterionic) or non-ionic surfactants [141]. Non-ionic surfactants are considered favourable due to a low toxicity and a more readily maintained solubilising power under biorelevant conditions [142]. 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 [143]. Other important non-ionic surfactant classes include polyol-based surfactants (e.g. glycoside, glycol or glycerol esters), amine oxides and sulfinyl surfactants [143]. 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 HLB value, molecular weight, chain length, molecular volume, critical micellar concentration (CMC) and solubility parameters [144]. 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 gastrointestinal tract. Furthermore, this illustrates the difficulty in developing a reliable surfactant classification system as properties of the surfactants may be influenced by *in vivo* conditions [142]. 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 [28]. Furthermore, surfactants may influence pre-systemic clearance of drugs by modulating transporters and metabolising enzymes [23]. For example, Tween® 80 and Cremophor® EL increased the uptake of digoxin, a P-gp substrate, to the same extent as cyclosporin (a commonly used P-gp inhibitor), using the rat everted gut sac method [139]. This indicated that Cremophor® EL and Tween® 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 [22; 140]. 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) [145], 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 AUC and a 40% decrease in AUC of the main metabolite of midazolam in rats [145].

When considering the choice of digestible versus non-digestible surfactants, 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 [146]. 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 [20; 121]. Moreover, 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 [147; 24-26; 148; 149]. In the case of unsaturated long chain FFA, *in vitro* cell experiments showed an increased membrane fluidity [150-152] as well as opened tight junctions [149; 153]. 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 [89], is highly lipophilic (log*P* 4.95) as well as hydrophobic [121] and exhibits a moderate permeability across a confluent Caco-2 cell monolayer [89; 90]. Therefore, nilotinib was categorised as a class IV compound in the BCS. Nilotinib is predominantly metabolised by CYP 3A4 [89] and is a P-gp substrate [89-92]. The pre-systemic clearance is high with an AUC increase of 29% after co-administration of nilotinib with grapefruit juice (intestinal CYP 3A4 inhibitor) [93]. The current commercial formulation, Tasigna®, is a capsule formulation containing the surfactant Pluronic® F68 (Poloxamer® 188) [94]. 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 [90]. The

marketed formulation of nilotinib showed an absorption of ≥ 30% following a radiolabelled single 400 mg oral dose in humans [95]. Additionally, pre-clinical 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) [89]. 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.

## 4.3 Materials and methods

### 4.3.1 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® M1944CS (Oleoyl polyoxyl-6 glycerides), Labrafil® M2125CS (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 HPLC grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 4.3.2 Solubility studies

Equilibrium solubility at 37 ºC 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 brief, an excess of nilotinib was added to 2 mL excipients and stirred at 250 rpm (25% power) (MIXdrive 15 HT, 2Mag AG, Germany) at 37 °C. Samples were taken after 24 h, 48 h, 72 h and centrifuged at 21,380*g* (Mikro 200 R, Hettich GmbH, Germany) for 15 min and 37 ºC. The supernatant was transferred to a new tube and centrifuged again under identical conditions. To solubilise the oily excipient, the supernatant was diluted in a mixture of acetonitrile and ethyl acetate (1:3, v/v). Followed by further 1:10 (v/v) dilution with a mixture of acetonitrile and ethyl acetate (3:1, v/v). The obtained samples were diluted 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 [121]. In brief, an Agilent 1200 series HPLC system (Agilent Technology Inc., US) 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 LOD and 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 [122] and linearity was confirmed between 12 ng/mL and 12 µg/mL drug concentration.

### 4.3.3 *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 AG, 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.* [106; 76]. 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 h 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* (Rotina 380 R, Hettich GmbH, Germany) 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 [154]. 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 FFAs released from the LBFs. 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:

$$Theoretical\ FFA\ [mmol] = \frac{SV\ [mg]}{56.1056\ [\frac{g}{mol}]} \quad (4\text{-}1)$$

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:

$$\%\ digested = \frac{Released\ FFA\ [mmol]}{Theoretical\ FFA\ [mmol]} \times 100\% \quad (4\text{-}2)$$

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 4-1.

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 the total drug recovery, which allowed adjustments of inhomogeneous samples. All samples were centrifuged at 37 °C and 21,000*g* for 30 min (Mikro 200 R, Hettich GmbH, Germany).

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

The solubility of nilotinib in the studied surfactants was low resulting in high dose solubility ratios (Table 4-1). It was hence decided to use surfactant suspensions for all *in vivo* and *in vitro* experiments keeping the lipid and dose load constant, while varying the fraction of solubilised nilotinib in the surfactants. The surfactant suspensions were prepared by combining 10 mg nilotinib with 1 mL surfactant excipient followed by an over-night stir resulting in varying fractions of pre-dissolved nilotinib (Table 4-1). The suspensions were stirred constantly to prevent sedimentation before usage. Solid excipients were melted prior to nilotinib addition and kept in a liquid form while stirring, addition and *in vivo* administration.

Table 4-1 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® M 1944 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 |

### 4.3.5 *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 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.

### 4.3.6 Bioanalysis

The plasma concentrations of nilotinib were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., US) 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 [121]. 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 [122]. Linearity was confirmed between 37 ng/mL and 4.1 µg/mL drug concentration.

### 4.3.7 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 (chapter 3). The statistical analysis for all *in vivo* parameters was performed using a 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 (GraphPad Software, USA).

## 4.4 Results

### 4.4.1 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 4-1 A and the % digested based digestibility is shown in Figure 4-1 B.

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 4-1 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 4-1 A), which reflected a theoretical digestion

of 76% (Figure 4-1 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) [155; 75] and therefore, showed the expected absence of FFA release.

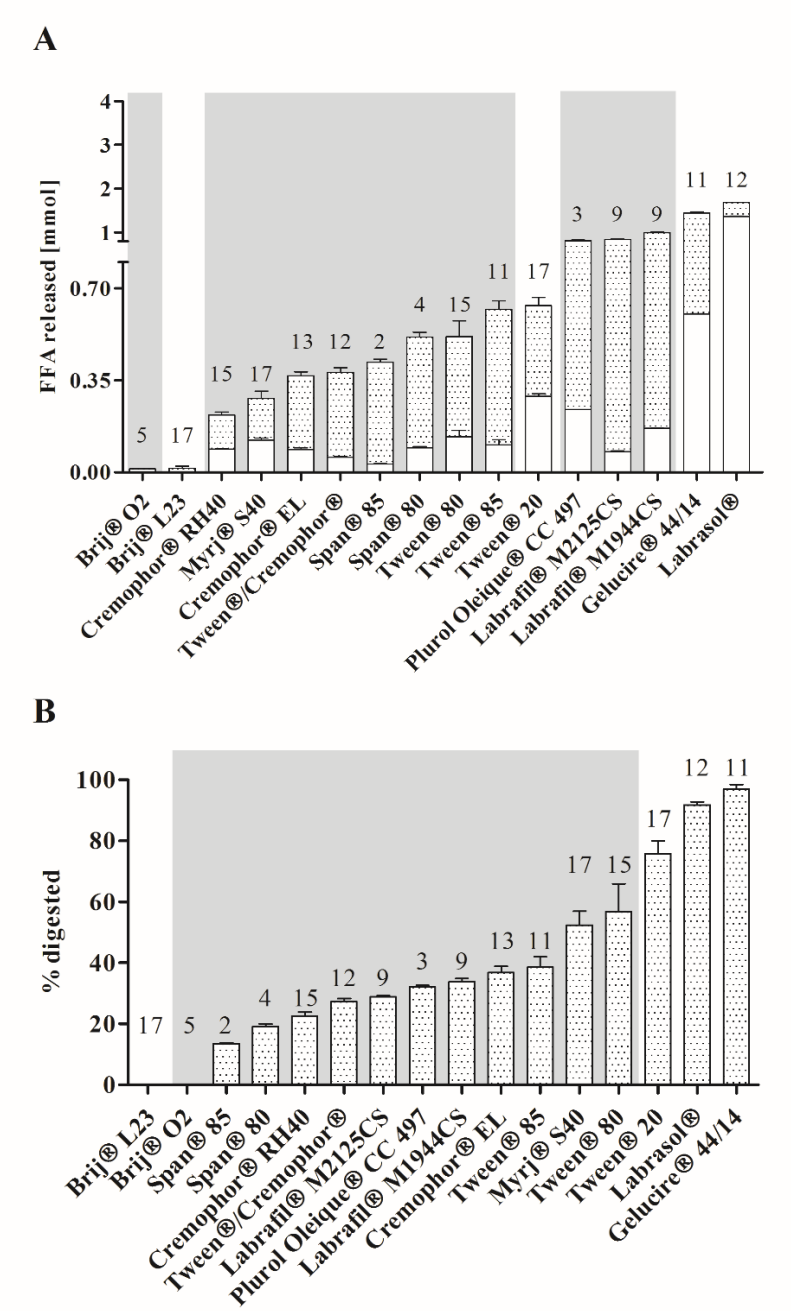



Figure 4-1 Surfactant digestibility in the *in vitro* lipolysis test. Tween®/Cremophor® is a mixture of Tween® 85 and Cremophor® RH40 at a ratio of 67:33 (w/w). The shaded area represents long chain excipients and non-shaded areas represent medium chain excipients. The numbers above the bars are the corresponding HLB values

of the surfactants. Data is presented as mean ± SD, 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.

Among the long chain surfactants, Labrafil® M 1944 CS, which consists of mono-, di- and triglycerides and PEG-6 mono- and diesters of oleic acid, showed the highest released FFA (Figure 4-1 A). Considering the theoretically possible digestion, only 33.8 ± 1.0% of Labrafil® M 1944 CS was digested (Figure 4-1 B). The highest % digested was shown for Tween® 80, a surfactant that like Labrafil® M 1944 CS also contains oleic acid (Figure 4-1 B). While the lowest % digested was observed for Span® 85 (Figure 4-1 B), the lowest amount of FFA was released in the case of Cremophor® RH40 (Figure 4-1 A). This finding agreed with previous studies that demonstrated a low release of FFAs as well as theoretical digestibility of Cremophor® RH40 [156; 157]. 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 4-2). 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 [158; 159]. 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® M 1944 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 4-2 and the surfactant properties in Table 4-2.

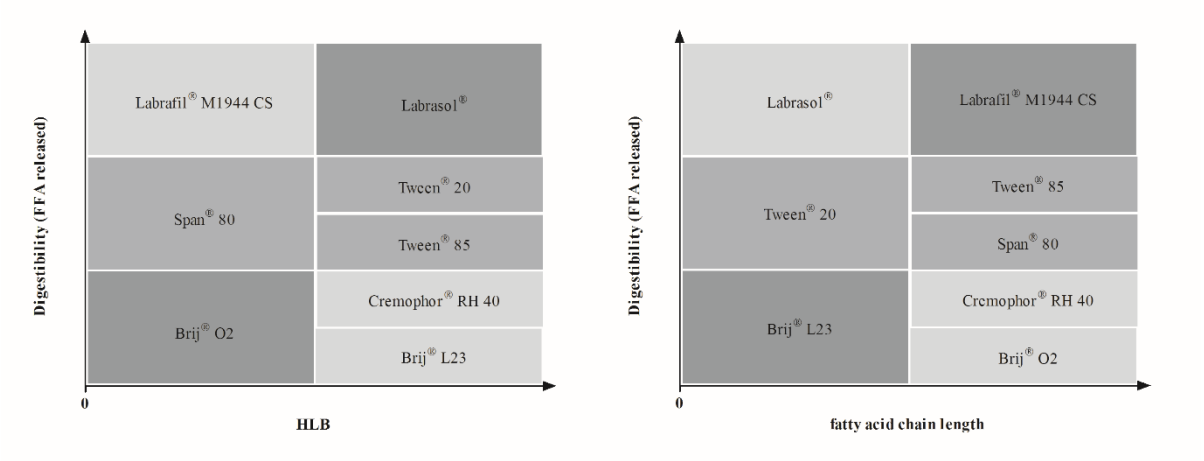



Figure 4-2 Selected excipients for further *in vitro* and *in vivo* evaluation. Excipients were selected according to the fatty acid chain length, HLB and total free fatty acids (FFA) released during the lipolysis experiments as a surrogate parameter for digestibility.

### 4.4.2 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 4-2 and Figure I-1 in the appendix. 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 4-2 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® M 1944 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 (m/m) | 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

### 4.4.3 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 4-3 and Table 4-2 and the distribution across all phases before initiation of digestion (0), 30 min and 60 min after digestion is shown in Figure I-2 in the appendix.

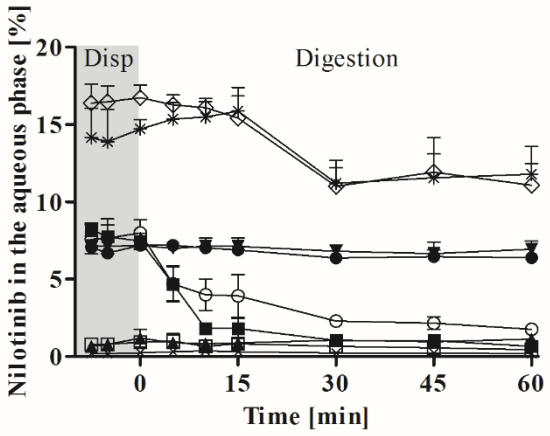



Figure 4-3 Aqueous phase concentration during 60 min of *in vitro* lipolysis of selected nilotinib suspensions. Cremophor® RH40 (◊), Brij® L23 (*), Tween® 20 (○), Span® 80 (▲), Labrasol® (■), Labrafil® M1944 CS (□), Tween® 85 (●), Brij® O2 (×), Tween® 85/Cremophor® RH40 mixture (67:33 w/w) (▼) (mean ± 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® M 1944 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® M 1944 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® ≥ Labrafil® M 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 I-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® M 1944 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.

### 4.4.4 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 (chapter 3). The absolute bioavailability is shown in Figure 4-4 and the pharmacokinetic parameters in Table 4-3.

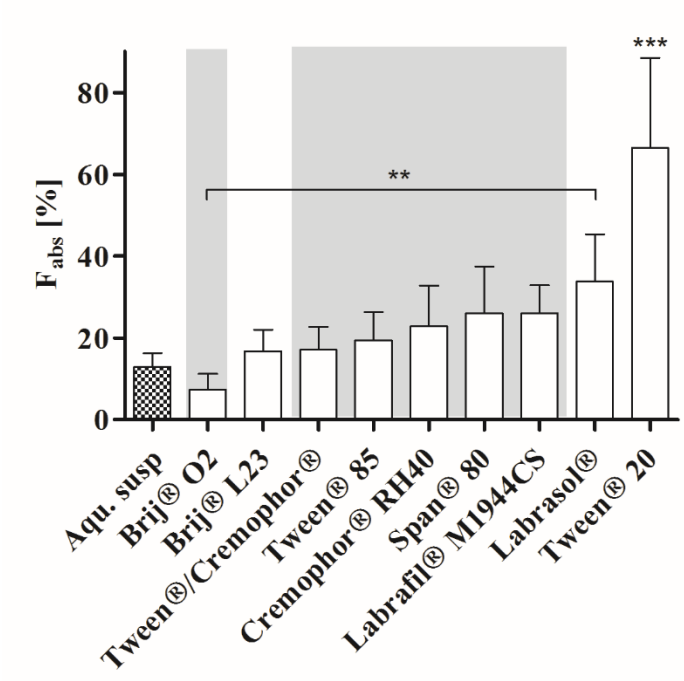



Figure 4-4 Absolute bioavailability ($F_{abs}$) of nilotinib suspensions in male Sprague Dawley rats after oral administration of 20 mg/kg nilotinib and 2 mL/kg excipient in comparison to an aqueous suspension, as previously described (chapter 3). Tween® 20 is significantly different to all other tested 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).

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® M 1944 CS and the aqueous suspension) displayed similar *in vivo* bioavailability of between 16.7 ± 5.3% to 26.0 ± 6.9%.

Table 4-3 Pharmacokinetic parameters of nilotinib after oral administration of 20 mg/kg nilotinib and 2 mL/kg excipient to male Spraque Dawley rats. Nilotinib was administered as surfactant suspensions (n = 5). $t_{max}$, mean residence time (MRT) and mean absorption time (MAT) are given as median (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 |

| | Pharmacokinetic parameters | | | | | |
|---|---|---|---|---|---|---|
| | AUC 0 h – inf. [µg*h/mL] | $c_{max}$ [µg/mL] | $t_{max}$ [h] | MRT [h] | MAT [h] | $F_{abs}$ [%] [b)] |
| Tween® 85 Cremophor® RH40 (67:33 m/m) | 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.* (chapter 3)
[b)] Intravenous data obtained from Koehl *et al.* (chapter 3)

### 4.4.5 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 4-5 displays the absolute bioavailability versus % digested and Figure I-3 in the appendix 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.

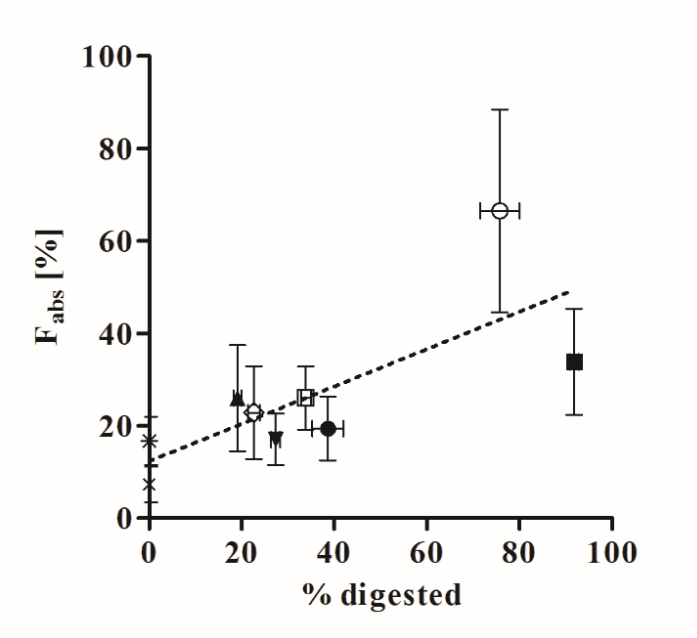



Figure 4-5 Absolute bioavailability ($F_{abs}$) versus % digested of Cremophor® RH40 (◊), Brij® L23 (*), Tween® 20 (○), Span® 80 (▲), Labrasol® (■), Labrafil® M1944 CS (□), Tween® 85 (●), Brij® O2 (×), Tween® 85/Cremophor® RH40 mixture (67:33 m/m) (▼) (mean ± SD, $F_{abs}$ n = 5, % digested n = 3).

## 4.5 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 [158; 159]. Surfactants may further affect drug permeation, drug efflux and potentially metabolism in enterocytes [28; 145; 140; 23]. 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. The analysis 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 [159-161] leading to a lower or slower [162] 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® [158]. 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® M 1944 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 [157]. 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 [156]. On the contrary, when incorporating surfactants into self-emulsifying LBFs, 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 surfactant 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 case of surfactant only systems, low digestibility surfactants may have led to lower overall *in vivo* exposure most likely via entrapment within surfactant micelles.

Compared to the aqueous suspension, Tween® 20 was the only surfactant that showed a statistically significant higher bioavailability. All other surfactants displayed a bioavailability between approximately 7.3% and 33.8%, which compared favourably to a previous report of nilotinib bioavailability of 34% in rats using a Cremophor® based micellar solution [89]. One explanation of the impressive *in vivo* performance of Tween® 20 may be additional biopharmaceutical benefits of this excipient. *In vitro* cell assays have shown that Tween® 20 is

a P-gp (or MDR-1) inhibitor [22; 140], reducing the efflux into the intestinal lumen. In the case of nilotinib transport by P-gp was demonstrated with an efflux ratio (basolateral/apical) of 3.9-4.1 using Caco-2 cells [89; 91; 92]. This indicated that further P-gp inhibition by formulation excipient such as Tween® 20 could have contributed positively to nilotinib's bioavailability. P-gp as well as CYP3A4 share a significant overlap in substrate specificity [163], which is also the case for nilotinib, which is mainly metabolised by CYP 3A4 in the enterocytes and liver [89]. In fact, co-administering nilotinib with grapefruit juice (intestinal CYP enzyme inhibitor) increased the AUC by 29% [93] showing a significant pre-systemic clearance. A study by Ren and co-workers showed that Tween® 20 is a strong CYP 3A4 inhibitor using rat liver and intestinal microsomes. In comparison to the four tested non-ionic surfactants (Tween® 20, Cremophor® EL, Myrj® S40 and Pluronic® F68), Tween® 20 was the most potent inhibitor. 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% [145].

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 [121]. In addition, it was reported that the solubility of nilotinib is influenced by bile salt concentrations as evident due to a higher solubility in FeSSIF (3.2 ± 0.1 µg/mL) compared to FaSSIF (0.3 ± 0.03 µg/mL) [121]. As FFAs can increase the bile salt release and increase permeability without pronounced cytotoxic effects [147; 24; 164; 25-27; 151; 152; 165], 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 nilotinib is passively transported [166], the permeability enhancing effects of surfactants [28] 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. 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 compared to *in vivo*, a relationship between *in vitro* and *in vivo* performance for surfactants [156; 157] or other formulations [77; 33; 121] 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*.

## 4.6 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 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. The reported additional biopharmaceutical effects of Tween® 20, in terms of 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.

# 5 Chapter 5: Supersaturated lipid-based formulations to enhance oral bioavailability of venetoclax

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

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

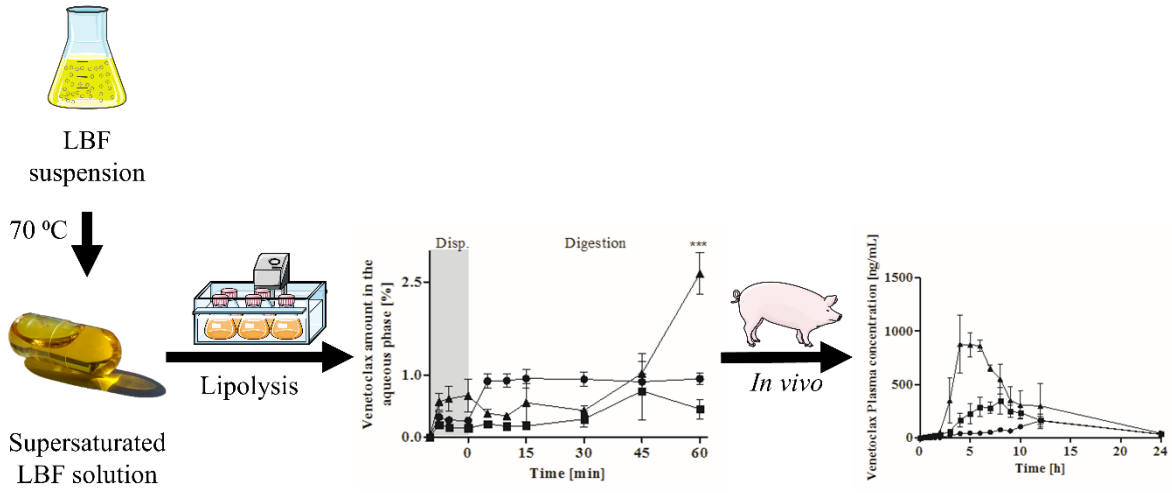
[2] University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharma Technology, Muttenz, Switzerland

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

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

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## 5.1 Abstract




Graphical abstract 5-1: Investigation of supersaturated lipid solutions *in vitro* and *in vivo*.

**Purpose** Increasing numbers of beyond Rule-of-Five drugs are emerging from discovery pipelines, generating a need for bio-enabling formulation approaches, such as LBFs, to ensure maximal *in vivo* exposure. However, many drug candidates display insufficient lipid solubility leading to dose loading limitations in LBFs. The aim of this study was to explore the potential of sLBFs for the model beyond Rule-of-Five drug venetoclax.

**Methods** Temperature induced sLBFs of venetoclax were obtained in olive oil, Captex® 1000, Peceol® and Capmul MCM®, respectively. A Peceol®-based sLBF displayed the highest drug loading and was therefore evaluated further.

**Results** *In vitro* lipolysis demonstrated that the Peceol®-based sLBF was able to generate higher venetoclax concentrations in the aqueous phase compared to a Peceol®-based suspension and aqueous suspension. A subsequent bioavailability study in pigs demonstrated for sLBF a 3.8-fold and 2.1-fold higher bioavailability compared to the drug powder and Peceol®-based suspension, respectively.

**Conclusion** In conclusion, sLBFs are a promising bio-enabling formulation approach to enhance *in vivo* exposure of beyond Rule-of-Five drugs, such as venetoclax. The *in vitro* lipolysis results correctly predicted a higher exposure of the sLBF *in vivo*. The findings of this study are of particular relevance to pre-clinical drug development where maximum exposure is required.

## 5.2 Introduction

Over the past few decades, it is well recognised that the oral bioavailability of drug candidates is negatively influenced by trends in drug discovery for candidates with increasing molecular size, lipophilicity and hydrophobicity [100; 3; 167]. In addition, increasingly new drug candidates fall outside the classical Rule-of-Five drug discovery space, resulting in solubility and/or permeability challenges [100; 167]. Such beyond Rule-of-Five drugs necessitate a bio-enabling formulation approach addressing both solubility and permeability limitations simultaneously. One formulation approach addressing both limitations is a LBF. LBFs may improve oral bioavailability via a number of mechanisms including avoidance of a drug dissolution step, an increased extent of drug solubilisation in intestinal fluids, lipid excipient mediated increased intestinal permeability as well as possible enhancement of lymphatic uptake from the gastrointestinal tract [18; 15]. However, the LBF development is complicated by the fact that for many beyond Rule-of-Five drug substances it is not possible to solubilise the dose in a relevant amount of lipid vehicle due an inherent low drug solubility in lipid vehicles. For such challenging drug candidates, displaying both poor water and lipid solubility, advanced formulation strategies that increase drug loading in lipid vehicles, such as lipophilic salts/ionic liquids [60; 35; 37; 38; 43] or sLBFs [31; 32; 34], have recently been proposed.

sLBFs are lipid-based solutions that contain drug concentration above the thermodynamic solubility of the drug in the lipid vehicle. Such supersaturated formulations are commonly prepared using heat [31; 32; 34]. While supersaturated formulations may display kinetic stability, thermodynamics dictate that precipitation will eventually occur depending on the drug properties, lipid vehicle, supersaturation ratio and storage conditions. sLBFs offer significant advantages in a pre-clinical drug product development setting, offering a balance between the potential to maximise oral exposure and facilitating ease of preparation and administration in pre-clinical models, which is particularly relevant in early toxicological drug evaluations [168]. In the literature, sLBFs of small and highly lipophilic drugs such as halofantrine [31], simvastatin [32], fenofibrate [33], cinnarizine [65] or R3040 [66] have been reported. In most cases, a higher oral bioavailability was observed in comparison to either an aqueous suspension, commercial product or an undersaturated lipid solution. While sLBF have been successfully used for small molecules that are highly lipophilic and show low-moderate hydrophobicity, it remains unclear, whether the formulation concept is also applicable to larger molecular weight (e.g. > 500 g/mol) and/or moderate-high hydrophobic drug candidates.

Venetoclax, a selective B-cell lymphoma-2 inhibitor licenced in 2016 for the treatment of leukaemia, displays a high lipophilicity (log*P* 5.5), high molecular weight (868.44 g/mol) [96] and a mid-range $T_m$ (138 ºC onset) [97]. It is classified as a BCS class IV [99] drug and exhibits a solubility in aqueous buffer of < 0.0042 µg/mL at pH 7.4 [96]. The commercialised formulation, Venclyxto®, exhibits a substantial food dependent bioavailability with a 3.4-fold increase in oral bioavailability after a low-fat meal and a 5-fold increase after a high fat meal compared to the fasted state [96]. Venclyxto® is considered to be a solid dispersion in copovidone [97] and must be taken with food to achieve an adequate bioavailability [97] suggesting that dietary lipids may be beneficial for improving oral absorption of venetoclax.

Venetoclax was chosen as a model drug for the present study, to explore whether it can be supersaturated in a lipid vehicle and whether a sLBF improves *in vivo* bioavailability using landrace pigs as animal model.

## 5.3 Materials and methods

### 5.3.1 Chemicals and materials

Two venetoclax batches were purchased from Kemprotec Ltd. (UK) (batch 1: #1705150, batch 2: #180620). One further venetoclax batch was ordered from Sigma Aldrich (Ireland) (batch 3: #11777). Olive oil, highly refined and low acidity, capric acid, L-α-Phosphatidylcholine type XI-E (PC) (768 g/mol), taurodeoxycholic acid (NaTDC) and pancreatic lipase (8 x USP) were ordered from Sigma-Aldrich (Ireland). Capmul MCM® and Captex® 1000 were kindly donated by Abitec corporation. A sample of Peceol® was kindly provided by Gattefossé (France) and FaSSIF/FeSSIF powder version 1 was kindly donated by biorelevant.com (UK). All other chemicals and solvents were of analytical or HPLC grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 5.3.2 Differential scanning calorimetry (DSC)

The thermal behaviour of venetoclax was studied using a TA Q1000 with a TA Refrigerated Cooling system 90 (TA Instruments, New Castle, DE). The cell was purged with nitrogen at 50 mL/min. The melting temperature was measured by modulated DSC. Venetoclax (5 mg) were weighed into a T-zero pan and heated from 20 ºC to 200 ºC at 3 ºC/min. The modulation amplitude was set to ± 1.0 ºC and the modulation time to 60 sec.

The crystallisation behaviour of venetoclax was studied using the protocol by Baird *et al.* [169]. In brief, 2 mg venetoclax was weighed into a T-zero pan and heated to 165 ºC at 10 ºC/min, held isothermally for 3 min, cooled to -75 ºC at 20 ºC/min and reheated to 160 ºC at 10ºC/min. As no crystallisation was observed during cooling and reheating, the reheating rate was lowered to 2 ºC/min. The experiments were run in triplicates. The glass forming ability of drugs was categorised according to Baird *et al.* into class I (crystallisation during cooling prior to the glass transition temperature ($T_g$)), class II (no crystallisation during cooling, but crystallisation was observed upon reheating above $T_g$) and class III (no crystallisation observed during cooling nor reheating to its $T_m$) [169]. Additionally, the absence of crystals after the heating and upon cooling was confirmed by hot-stage microscopy. All DSC data is presented in the appendix (Figure II-2, Figure II-3).

### 5.3.3 Hot stage microscopy

Samples were placed in a glass crucible onto a Linkam THMS 600 hot stage connected to a TP94 temperature controller (Linkam Scientific Instruments, Tadworth, UK). Samples were heated to 165 ºC at 10 ºC/min, held isothermally for 3 min, cooled to 25 ºC at 20 ºC/min and continuously monitored using Olympus BX51 with an Olympus SC100 camera operated by Olympus Stream essentials 2.3.3. Images were collected at 40x magnification and the light was polarized using the polarizer U-POT and analysed with the analyser U-ANT. The absence of crystals was assumed, if no birefringence was observed. The images are presented in the appendix II (Figure II-4).

### 5.3.4 Cross-polarised light microscopy

The absence of crystals in the supersaturated solution was confirmed by means of cross-polarised light microscopy using an Olympus BX51 with an Olympus SC100 camera operated by Olympus Stream essentials 2.3.3. The light was polarized using the polarizer U-POT and analysed with the analyser U-ANT. The absence of crystals was assumed, if no birefringence was observed.

### 5.3.5 Solubility studies

The solubility was determined in olive oil, Captex® 1000, Peceol® and Capmul MCM®. Excess of venetoclax was added to 2 mL of the excipients and stirred at 200 rpm (25% power) (MIXdrive 15 HT, 2Mag AG, Germany) at 37 °C. Samples were taken after 24 h, 48 h, 72h. In the case of Peceol® and Capmul MCM®, a high amount of venetoclax dissolved instantly upon addition to the excipient (exceeding the thermodynamic solubility), which lead to precipitation after 24 h - 48 h. Therefore, Peceol® and Capmul MCM® samples were left at 37 °C up to 48 h before the first sample was taken. All samples were centrifuged at 21,380*g* (Mikro 200 R, Hettich GmbH, Germany) and 37 ºC for 15 min. The supernatant was transferred to a new sample tube and centrifuged again under identical conditions. To solubilise the oily excipient, the supernatant was diluted in acetonitrile, ethyl acetate (1:3, v/v), followed by further 1:10 (v/v) dilution with acetonitrile:ethyl acetate (3:1, v/v). The obtained samples were diluted appropriately with mobile phase before analysis by reverse phase HPLC as described below. All samples were run in triplicates.

#### 5.3.5.1 Biorelevant solubility

FaSSIF and FeSSIF were prepared according to the instructions by biorelevant.com. Solutions of FeSSIF were prepared and used directly, whereas FaSSIF was left at room temperature for 2 h prior to usage. Excess of venetoclax was added to 2 mL FaSSIF and FeSSIF, respectively. The suspensions were placed in a water bath shaker with 200 shakes/min at 37 ºC (GLS400, Grant Instruments, UK). Samples were taken at 3 h, 6 h and 24 h and centrifuged at 21,380*g* (Mikro 200 R, Hettich GmbH, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again under identical conditions. Samples were diluted 1:10 (v/v) with mobile phase before analysis.

The samples were analysed using an Agilent 1200 series HPLC system (Agilent Technology Inc., US) that comprised a binary pump, degasser, column oven, autosampler and variable wavelength detector. Data analysis was done with EZChrom Elite® version 3.2. Venetoclax was separated from the sample matrix 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 40 °C. The mobile phase consisted of (a) acetonitrile with 0.5% (v/v) trifluoroacetic acid (TFA) and (b) water with 0.5% (v/v) TFA at a ratio of 53:47 (a:b, v/v) and was used at a flow rate of 1 mL/min. The injection volume was 20 µL and the detection wavelength was set to 316 nm. The LOD was 20 ng/mL and the LOQ 65 ng/mL determined using the standard error of y-intercept according to the ICH Q2 guideline [122].

### 5.3.6 *In vitro* lipolysis: Drug solubilization during formulation dispersion and digestion

*In vitro* lipolysis was performed using a pH-stat apparatus (Metrohm AG, Herisau, Switzerland) comprising a Titrando® 907 stirrer, 804 Ti-stand, a pH electrode (Metrohm AG, Herisau, Switzerland) and two 800 Dosino® dosing units coupled to a 20 mL automatic burette. The system was operated by the Tiamo® 2.2 software. The *in vitro* protocol was amended from Williams *et al.* [106; 76] as reported previously [121]. 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 (digestion buffer) and stirred for 12 h before further usage. The pancreatin extract was prepared freshly by adding 5 mL of 5 °C digestion 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* (Rotina 380 R, Hettich GmbH, Germany) and 4 mL of supernatant was recovered and stored on ice before further usage.

For the *in vitro* lipolysis experiment 1.583 g of lipid formulation was dispersed into 57 mL of digestion buffer for 10 min at a stirring speed of 450 rpm, which was maintained throughout the experiment. 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. To the remaining 54 mL (1.5 g lipid formulation) dispersion 6 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 4.9 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). All samples containing a lipid phase were centrifuged at 37 °C and 400,000*g* for 30 min (Beckman Coulter Optima L-90K, Rotor: VTI 65.2). Samples, that did not contain a lipid phase (aqueous suspension) were centrifuged at 37 °C and 21,000*g* for 30 min using a benchtop centrifuge (Micro 200 R, Hettich GmbH, Germany). The lipid phase was dissolved in a mixture of ethyl acetate and acetonitrile (3:1 v/v) and diluted with 1:10 (v/v) with a mixture of ethyl acetate and acetonitrile (1:3 v/v). The solid phase was added to 2 mL of a mixture of ethyl acetate and acetonitrile (3:7 v/v) and ultrasonicated for 10 min. The aqueous phase was diluted 1:10 (v/v) with mobile phase. The resulting lipid, solid and aqueous phase samples were subsequently centrifuged at 37 °C and 21,000*g* for 30 min. The resulting supernatants were diluted further with mobile phase and analysed by HPLC as stated above.

### 5.3.7 Formulation development of supersaturated LBFs

The increase of venetoclax concentration above its solubility in the excipients was achieved by incorporating venetoclax at an elevated temperature, i.e. the formulations were heated, kept at elevated (isothermal) temperature and cooled to room temperature. In detail, excess venetoclax was added to Peceol®, olive oil, Capmul MCM®, Capex® 1000, respectively, and suspended at 600 rpm (Stuart CD 162 heat-stir, Cole-Parmer, UK). The vial was sealed with parafilm and a continuous nitrogen stream was purged through the vial to prevent any oxidation during the heating and cooling process. While continuously stirring at 600 rpm the suspensions were heated to 70 °C and kept at 70 °C for 10 min (Stuart CD 162 heat-stir, Cole-Parmer, UK) followed by cooling to 23 °C ± 2 °C by elevating the vial from the hot plate. Once the mixtures reached a temperature below 25 °C a 0.5 mL sample was taken, and the remaining mixture was heated and cooled again using the same protocol described above. After this second cycle a

second 0.5 mL sample was taken, and the mixture was heated and cooled a third time using the same conditions as described above followed by a final 0.5 mL sample. During all heating and cooling cycles excess venetoclax was present to investigate the maximum achievable concentration with this protocol. The samples were centrifuged at 21,380*g* and 37 ºC (Mikro 200 R, Hettich GmbH, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again using identical conditions. The supernatant was used for HPLC analysis using the same treatment as described for the solubility studies in the lipid excipients above. The remaining suspension after the third heating cycle was centrifuged under identical conditions as stated above and transferred to a new sample tube for stability testing and stored at room temperature. Stability of promising formulations was evaluated using polarised light (Allen Viewer type LV28, P.W. Allen & Co Ltd, UK). Due to the high drug amount needed these formulations and stability tests were conducted once.

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

For the *in vivo* used powder venetoclax formulation, 100 mg venetoclax were weighed into hard gelatine capsules size 00. For the *in vivo* used Peceol® suspension, 50 mg venetoclax was weight into hard gelatine capsules size 000 and 1 mL of Peceol® (melted at 50 ºC and cooled to 37 ºC) was added. Venetoclax was dispersed by vigorous shaking.

In the *in vitro* experiments the powder formulation was resembled by an aqueous suspension. The aqueous suspension was prepared by adding 50 mg venetoclax to 1 mL water followed by ultrasonication. The suspensions were stirred constantly to prevent sedimentation before usage. The Peceol® suspension was prepared by melting Peceol® at 50 ºC and cooling it to 37 ºC before adding 50 mg venetoclax to 1 mL Peceol® followed by an over-night stir.

The supersaturated lipid solution was prepared by adding 300 mg venetoclax to 6 mL Peceol® (50 mg/mL). The mixture was stirred at 600 rpm (Stuart CD 162 heat-stir, Cole-Parmer, UK) and sealed with parafilm. A continuous nitrogen stream into the vial removed oxygen throughout the manufacturing process. After suspending the drug particles, the obtained suspension was slowly heated to 70 °C (Stuart CD 162 heat-stir, Cole-Parmer, UK). The mixture was kept at 70 °C for 10 min and cooled to 25 °C while continuously stirring at 600 rpm. Subsequently, the mixture was heated a second time under the same conditions as stated above and cooled to room temperature. The absence of crystals was confirmed using cross-polarized light microscopy. For the *in vivo* study, sLBF was administered in hard gelatine capsules size 000 (1 mL per capsule).

### 5.3.9 *In vivo* study

All experiments were approved and conducted with licences issued by the Health Product Regulatory Authority, Ireland (project licence AE19130/P058) as directed by the EU Statutory instruments of the EU directive 2010/63/EU (Protection of Animals used for Scientific Purposes). Local ethical approval was granted by University College Cork Animal Experimentation Ethics Committee (AEEC). A randomised, 3-way crossover study in 3 male landrace pigs (15 – 17 kg) was conducted and each pig received a single dose of 100 mg venetoclax. Pigs were fed approximately 175 g of standard weanling pig pellet feed twice daily. In the fasted study legs the final feed of 175 g was given 24 h prior to dosing. As part of the study design any remaining food was removed 16 h before dosing, however, no food remained at this point in any of the groups. On day 1 of the experiment, an intravenous catheter was inserted from the ear vein into the jugular vein under general anaesthesia, which was used for blood sampling throughout the study. On day 3, following an overnight fast of 16 h, pigs were

administered either a reference capsule with venetoclax powder or two capsules of either venetoclax Peceol® suspension or a venetoclax sLBF with the aid of a dosing gun followed by 50 mL of water via a syringe. In order to control the water intake with the dosage forms, the water availability was restricted for 3 h post-dosing. At all other times water was available *ad libitum*. To facilitate handling during the oral administration, an intramuscular dose of ketamine (5mg/kg) and xylazine (1mg/kg) was administered. Blood samples were collected after 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24 h in heparinised tubes. Upon collection, blood samples were immediately centrifuged at 3000*g*, 4 ºC for 5.5 min (Eppendorf 5702 R, Rotor A-4-38, Eppendorf Ltd., UK). The supernatant plasma was harvested and stored at -20 ºC until further analysis. A 6-day washout period was maintained between the study legs. Due to a rupture and spillage of one sLBF capsule during oral dosing to one of the pig's, this experimental unit was excluded, resulting in a n = 2 for the sLBF study group.

### 5.3.10 Bioanalysis

The plasma concentrations of venetoclax were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., US) comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was operated, and the data analysed with EZChrom Elite® version 3.3.2. 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 for the separation of venetoclax. The mobile phase consisted of water and acetonitrile with 0.5% (v/v) TFA at a ratio of 47:53 (v/v) and was used at a flow rate of 1.0 mL/min. The sample and column temperature were set at 5 °C and 40 °C, respectively, and the detection wavelength was set to 250, 290 and 316 nm. Venetoclax was extracted from the plasma samples by liquid-liquid extraction. To 500 µL of pig plasma 50 µL vemurafenib (internal standard dissolved in acetonitrile) and 450 µL acetonitrile was

added. The mixture was mixed thoroughly, and 1 mL of ethyl acetate was added followed by mixing for 15 sec. The mixture was centrifuged at 25 °C, 11,500*g* for 5 min (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany). The supernatant (1.5 mL) was recovered and transferred to a new sample tube. Subsequently, the supernatant was dried at 60 °C under a nitrogen stream. To the remaining plasma sample 1 mL of ethyl acetate was added and the mixture was thoroughly mixed and centrifuged using the same condition as above. The supernatant was recovered and transferred to the same sample tube that contained the supernatant of the plasma sample after the first centrifugation (which was dried as described above). After drying of the supernatant, the residues were reconstituted in 100 µL mobile phase (excluding TFA) followed by centrifugation at 25 °C, 11,500*g* for 5 min (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany). The injection volume used for HPLC analysis of the supernatant was 50 µL. The LOD and LOQ in plasma by this method was 6 ng/mL and 20 ng/mL, respectively, determined using the standard error of y-intercept according to ICH Q2 guideline [122]. Linearity was confirmed between 25 ng/ml and 2.5 µg/ml.

### 5.3.11 Data analysis

A one-way ANOVA was performed for the lipolysis data using a Tukey post-hoc test to compare the different formulation performances and the Bartlett's test to check for equal variance. The pharmacokinetic parameters were calculated using Gastro Plus® version 9.5 and in-house intravenous data [170]. The plasma concentration profiles were analysed by non-compartmental analysis. The statistical analysis for all *in vivo* parameters was performed using a one-way ANOVA after using the Bartlett's test to check for equal variance. The pairwise comparison of the groups was based on Tukey's multiple range test. All statistical analyses were calculated using GraphPad Prism® 5 (GraphPad Software, USA).

## 5.4 Results

### 5.4.1 Solubility in lipid excipients and biorelevant media

Venetoclax is a structural bulky and high molecular weight drug, displaying a mid-range $T_m$ and high log*P* (Table 5-1, Figure 1-6, Figure II-2). Initially, three separate batches of the drug substance were sourced, in order to assess inter-batch variability of *in vitro* characteristics. Solubility was measured at 37 ºC in olive oil, Captex® 1000, Peceol® and Capmul MCM®, respectively. Additionally, venetoclax solubility was measured in FaSSIF and FeSSIF. Venetoclax solubility in lipid excipients is shown in Table 5-2 and venetoclax biorelevant solubility is presented in Table 5-3.

While venetoclax solubility in biorelevant buffers was consistent between the three batches, variability was observed for venetoclax solubility in the lipid vehicles. Solubility of different batches of venetoclax in Peceol® varied 6.7-fold from 2.9 ± 0.2 mg/mL to 19.4 ± 2.0 mg/mL. For olive oil a variation of 9.2-fold, for Capmul MCM® a 1.6-fold variation and for Captex® 1000 a 2.3-fold variation was observed across the different venetoclax batches. While two venetoclax batches showed a comparable solubility within an approximately 2-fold difference (batch 2 and 3), batch 1 was considerably different in the case of both long chain excipients Peceol® and olive oil. Despite the variability, the solubility screening in pure lipid excipient indicated that venetoclax showed a higher solubility in monoglycerides than triglycerides. Additionally, a dose loading comparable to the marketed product of 100 mg/tablet was not reached in any of the studied excipients precluding the use of a classical lipid solution approach, i.e. not a supersaturated and hence stable system, for venetoclax in these lipid vehicles.

Table 5-1 Physicochemical properties and *in vivo* data of venetoclax.

**Physicochemical characteristics**

| | |
|---|---|
| Log*P* [a] | 5.5 |
| Molecular weight [g/mol] [a] | 868.44 |
| BCS class [b] | IV |
| Food effect [a] | 3.4-fold $c_{max}$ and AUC increase with a light meal<br>5-fold $c_{max}$ and AUC increase with a high fat meal |
| $T_m$ [°C] | 138.61 ± 0.12 °C [c]<br>138 ºC onset [d] |
| Glass forming ability [c] | III |
| Venclyxto® tablet core excipients [e] | Copovidone<br>Polysorbate 80<br>Colloidal anhydrous silica<br>Anhydrous calcium hydrogen phosphate<br>Sodium stearyl fumerate |

[a] Obtained from FDA Venclexta® (venetoclax) clinical pharmacology and biopharmaceutics review [96]
[b] Obtained from Emami Riedmaier *et al.* [99]
[c] Experimentally determined by DSC for venetoclax batch 1 (Figure II-2, Figure II-3, Figure II-4)
[d] Obtained from EMA, CHMP assessment report, Procedure No. EMEA/H/C/004106/000 [97]
[e] Obtained from section 6.1 of summary of product characteristics of Venclyxto® as of 09/2018

It became apparent during solubility screening of venetoclax in monoglyceride vehicles (i.e. Peceol® and Capmul MCM®) at 37 ºC, that the drug appeared to spontaneously oversaturate following addition of excess drug to the lipid, which visually appeared to dissolve. However, over time venetoclax precipitated and theoretically equilibrium was achieved. This phenomenon was also demonstrated quantitatively, where higher drug concentrations were observed in the first 24-48 h, compared to concentrations at later time points. In both the monoglyceride vehicles, this oversaturated state was stable for up to 48 h. Such atypical behaviour of venetoclax displayed challenges for the methodology of determining thermodynamic solubility of venetoclax in the lipid vehicles. In fact, while drug solubility at 37 ºC was monitored for up to 72 h post-precipitation, the solubility determined in the lipid

excipients may not reflect true 'thermodynamic' equilibrium but rather a higher energy intermediate state. As a result of this behaviour, where excess drug appeared to dissolve initially and precipitated over 12-48 h, in the solubility studies presented in Table 5-2, the first concentrations were only determined (i.e. first sampling point t = 24 h) after drug had precipitated from an oversaturated system. It is likely that thermodynamic equilibrium would be achieved over an extended storage period (i.e. weeks). However, in this study with a 72 h study period, equilibrium solubility may not have been achieved, hence apparent solubilities are reported. The thermodynamic equilibrium may have been further influence by the viscosity of the lipid excipients and the glass forming ability of venetoclax (class III, Table 5-1, Figure II-3). Nevertheless, this venetoclax specific effect indicated that monoglycerides may be a good solubilising vehicle to promote and maintain drug supersaturation.

Table 5-2 Apparent solubility of venetoclax in lipid excipients at 37 ºC (mean ± SD, n = 3).

| **Solubility in lipid excipients [mg/mL]** | | | |
|---|---|---|---|
| | Batch 1 | Batch 2 | Batch 3 |
| Olive oil | 2.5 ± 0.2 | 0.3 ± 0.1 | 0.4 ± 0.1 |
| Peceol® | 19.4 ± 2.0 | 2.9 ± 0.2 | 4.3 ± 0.5 |
| Captex® 1000 | 0.4 ±0.1 | 0.7 ± 0.1 | 1.0 ± 0.2 |
| Capmul MCM® | 6.1 ±0.4 | 3.7 ± 0.5 | 5.9 ± 0.4 |

In order to further investigate the atypical behaviour of venetoclax the thermal behaviour of the different batches was studied. The results are presented in Table II-1 and Figure II-2 and showed that venetoclax batch 1 showed a $T_m$ of 138.6 ± 0.1 ºC, which was similar to the reported onset of the $T_m$ of 138 ºC (Table 5-1) [97], an enthalpy of fusion of 19.9 ± 0.7 kJ/mol and entropy of fusion of 4.8 ± 0.2 x$10^{-2}$ kJ/mol/K. Batch 2 showed similar results with a $T_m$ of 140.2 ± 0.1 ºC, entropy of fusion of 18.4 ± 0.4 kJ/mol and an entropy of fusion of 4.5 ± 0.1 x$10^{-2}$ kJ/mol/K. However, batch 3 showed two endothermal events with one major

peak and one minor peak indicating that several crystalline structures could be present. While the major peak showed a similar $T_m$ compared to the other batches, the entropy and enthalpy of fusion was different when compared to batch 1 and 2. While batch 3 showed a distinct different thermal behaviour compared to batch 1 and 2, the solubility of batch 3 was similar to the solubility of batch 2 and different to batch 1.

In addition, the glass forming ability of venetoclax batch 1 was determined by DSC. The results are presented in Figure II-3 and Table 5-1. The first heating cycle confirmed the $T_m$ of venetoclax batch 1 as described above. In the second cycle, the venetoclax melt was cooled at a rate of 20 ºC/min in order to generate amorphous venetoclax. No crystallisation event was observed during cooling. Hot stage cross-polarised microscopy confirmed the generation of amorphous venetoclax and revealed no crystallisation during cooling to room temperature (25 ºC) (Figure II-4). In the reheating cycle, no endothermic peak was present showing that venetoclax did not tend to recrystallise, categorising venetoclax as a class III glass former, according to Baird and co-workers definition [169].

Table 5-3 Biorelevant solubility of venetoclax at 37 ºC (mean ± SD, n = 3).

| **Biorelevant solubility [µg/mL]** | | | |
|---|---|---|---|
| | Batch 1 | Batch 2 | Batch 3 |
| FaSSIF | 5.5 ± 1.0 | 5.2 ± 0.8 | 4.8 ± 0. 6 |
| FeSSIF | 26.3 ± 1.8 | 30.7 ± 4.0 | 28.2 ± 0.7 |

The solubility values in FaSSIF were similar for all studied batches with approximately 1.4 - 1.7% drug dissolved of a 100 mg dose in 250 mL of gastrointestinal fluid. In the fed state the solubility increased 4.8-fold to approximately 6.6 - 7.7% of a 100 mg dose in 250 mL of gastrointestinal fluid, which is therefore in line with the *in vivo* food effect reported for

venetoclax (Table 5-1). In contrast to the atypical solubility behaviour of venetoclax in lipid vehicles (i.e. high inter-batch variability and oversaturation), venetoclax solubility in biorelevant media was similar between the three batches. This most likely reflects the ability to more quickly achieve equilibrium solubility in aqueous buffers due to a lower viscosity, higher wettability and the presence of bile salts/phospholipids to aid solubilisation.

### 5.4.2 Supersaturation of venetoclax in lipid excipients

Applying a temperature induced supersaturation approach, suspensions of venetoclax in lipid excipients were heated to 70 °C, kept at 70 °C for 10 min followed by cooling to 25 °C. This protocol was repeated up to three times. An excess of drug (i.e. a solid phase) was present at all times during heating and cooling. The incorporated concentration of venetoclax, i.e. kinetic solubility, was measured after cooling in olive oil, Captex® 1000, Peceol® and Capmul MCM®, respectively. At the end of the experiment, the samples were centrifuged, and the supernatants of promising formulations were stored at room temperature to evaluate the stability of the supersaturated solutions. Due to the large quantities of venetoclax required in this screening study, an n = 1 was used at each sampling timepoint. The venetoclax concentration for all three batches in Peceol®, Capmul MCM®, olive oil and Captex® 1000 is shown in Figure 5-1. The fold difference between the solubility at 37 ºC and the maximum achieved concentration at 70 ºC is shown in Table 5-4.

In all excipients, relatively higher concentrations were reached after the heating cycles compared to the solubility at 37 °C. While in the case of olive oil and Captex® 1000 the concentration increases were high ranging from 1.3– to 12.5-fold for olive oil and 3.7- to 28.2-fold in the case of Captex® 1000, the total dissolved amount was in most cases below 5 mg/mL, which is insufficient dose loading and precludes the use of triglycerides for

the sLBF approach. For Capmul MCM®, venetoclax concentrations increased between 5.9- and 22.3-fold, however, only batch 1 was able to generate venetoclax concentrations above 50 mg/mL. Additionally, Capmul MCM® showed poor stability of < 1 day limiting the use of this sLBF to possible *ad hoc* preparation in pre-clinical development. In the case of Peceol® a concentration > 50 mg/mL was achieved with all studied batches, resulting in an increase in venetoclax concentration of 3.5- to 67.9-fold in the supersaturated state. Interestingly, for Peceol®, supersaturated concentration of venetoclax was maintained in the formulation for in excess of 2 weeks.

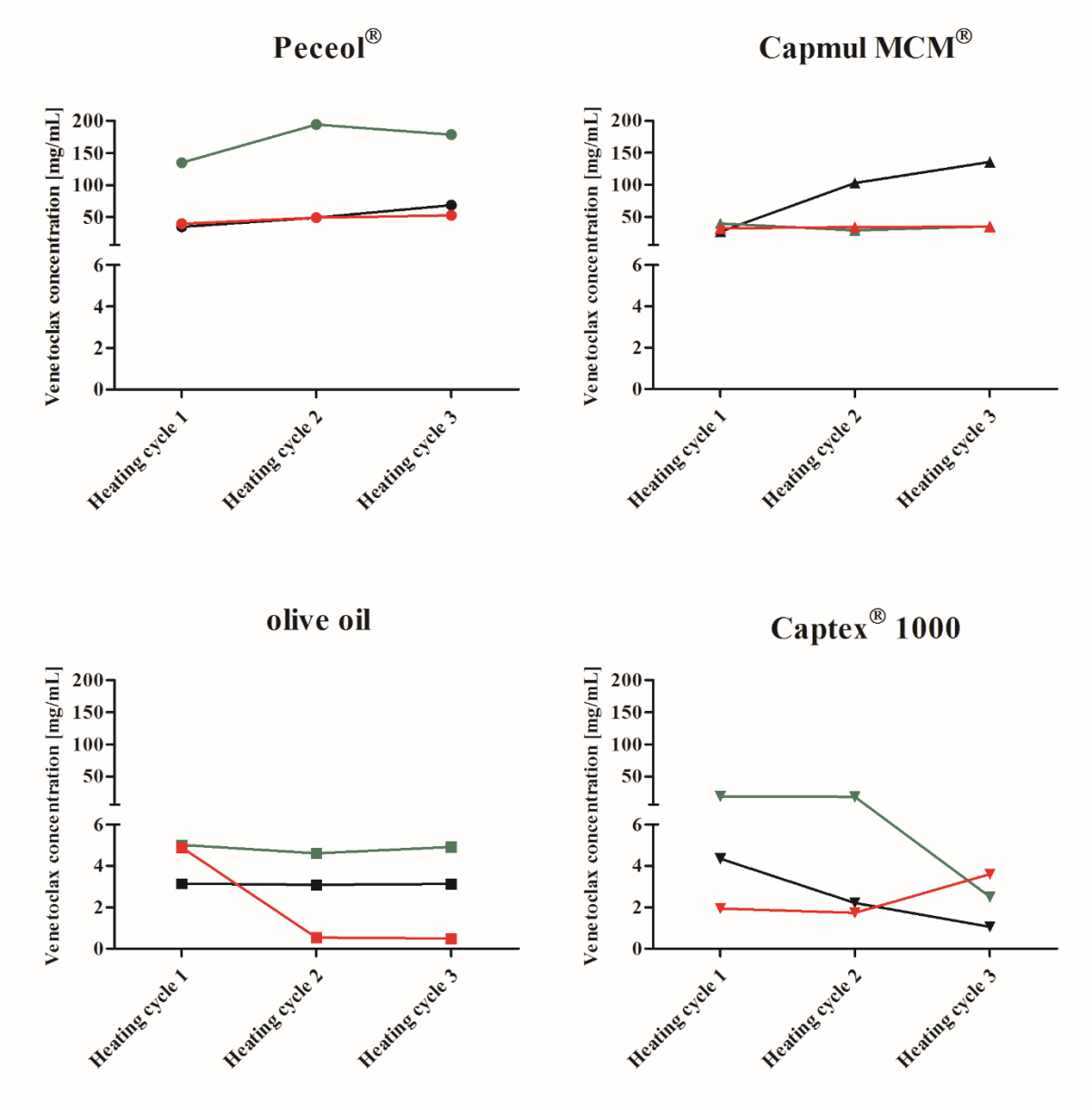



Figure 5-1 Venetoclax concentrations after each heating-holding (10 min)-cooling cycle to 70 ºC in Peceol® (●), Capmul MCM® (▲), olive oil (■) and Captex® 1000 (▼). Batch 1 (black), batch 2 (green) and batch 3 (red), where n = 1 for initial screening.

The consecutive heating cycles were designed to investigate whether further thermal exposure would increase venetoclax concentrations in the excipients. In the case of Peceol®, venetoclax concentrations increased, while in the case of Capmul MCM® an increase was observed in only one case (batch 1) with the other batches showing a similar venetoclax concentration

throughout the heating cycles. Interestingly, in the case of triglycerides venetoclax concentration decreases in some cases upon each heating cycle. The initial supersaturated Captex® 1000 showed a decrease in the amount of venetoclax with each further heating cycle for batch 1 and 2. A similar decrease was observed for batch 3 in olive oil with the second heating cycle.

Table 5-4 Fold difference between the supersaturated venetoclax concentrations reached at 70 ºC and the measured solubility for olive oil, Peceol®, Captex® 1000 and Capmul MCM®, where n = 1 for initial screening.

| **Fold difference** | | | |
|---|---|---|---|
| | Batch 1 | Batch 2 | Batch 3 |
| Olive oil | 1.3 | 18.6 | 12.5 |
| Peceol® | 3.5 | 67.9 | 12.1 |
| Captex® 1000 | 10.1 | 28.2 | 3.7 |
| Capmul MCM® | 22.3 | 10.6 | 5.9 |

As a result of these initial screening trials, a Peceol® sLBF was used for further *in vitro* and *in vivo* evaluation due to the high and maintained concentrations of drug in the vehicle over 2 weeks. The drug amount in the formulation was fixed at 50 mg/mL, which was below the maximum achievable concentration in Peceol® (batch 1: 68.3 mg/mL, batch 2: 178.62 mg/mL, batch 3: 52.4 mg/mL). Batch 1 was chosen for further studies, based on displaying the lowest fold increase in supersaturation at 50 mg/mL (i.e. 2.5-fold) and hence a lower risk of precipitation relative to batch 2 (17.5-fold) and batch 3 (11.6-fold). The sLBF was produced using two heating cycles rather than three to reduce the thermal exposure to drug and excipient. Short term storage studies on sLBFs produced under these conditions confirmed the concentration of venetoclax in the Peceol® sLBF (batch 1) was maintained at 50 mg/mL for up to 20 days with no macroscopic signs of instability.

### 5.4.3 Drug solubilisation during *in vitro* dispersion and digestion

In order to study how digestion affects the supersaturated formulation, sLBF (50 mg/mL venetoclax supersaturated in Peceol®, (5% w/v) was assessed using the dynamic *in vitro* lipolysis model and compared to a Peceol® suspension (50 mg/mL venetoclax mixed in Peceol® 5% w/v) and an aqueous suspension (5% w/v). Initially, lipid formulations were dispersed in biorelevant buffer representing the fasted state for 10 min prior to initiation of the digestion by the addition of porcine pancreatic lipase. The recovery of venetoclax in the aqueous phase, lipid phase and solid phase during dispersion and digestion is shown in Figure 5-2.

The amount of venetoclax recovered in the aqueous phase is shown in Figure 5-2 A. Upon dispersion and after 60 min of digestion the highest venetoclax concentration in the aqueous phase was observed for sLBF. However, upon initiation of digestion the sLBF showed a decrease in venetoclax concentration in the aqueous phase, which correlated well with the slow increase of the solid phase Figure 5-2 C. From 30 min of digestion onwards, the venetoclax concentration in the aqueous phase for sLBF started to increase from 0.4 ± 0.1% to 2.6 ± 0.3% of the added venetoclax dose, which was significantly different 60 min after initiation of digestion to all other formulations ($p < 0.05$). The time delay of 30 min to increase the venetoclax concentration in the aqueous phase suggested that digestion of the sLBF may be a key contributor to the high concentration of venetoclax at the end of the *in vitro* lipolysis. Additionally to the aqueous phase, for sLBF a drug rich aqueous phase was present after ultracentrifugation during digestion (Figure II-1). In the case of the aqueous suspension, upon dispersion a lower venetoclax concentration was observed in the aqueous phase when compared to sLBF. Interestingly, upon initiation of digestion an approximately 3-fold increase in venetoclax concentration in the aqueous phase was observed for the aqueous suspension. This concentration of 0.9 ± 0.1% was maintained throughout digestion. The overall lowest

concentration was observed for the Peceol® suspension throughout dispersion and digestion, which resulted in 0.5 ± 0.2% of venetoclax dose solubilised after 60 min of digestion.

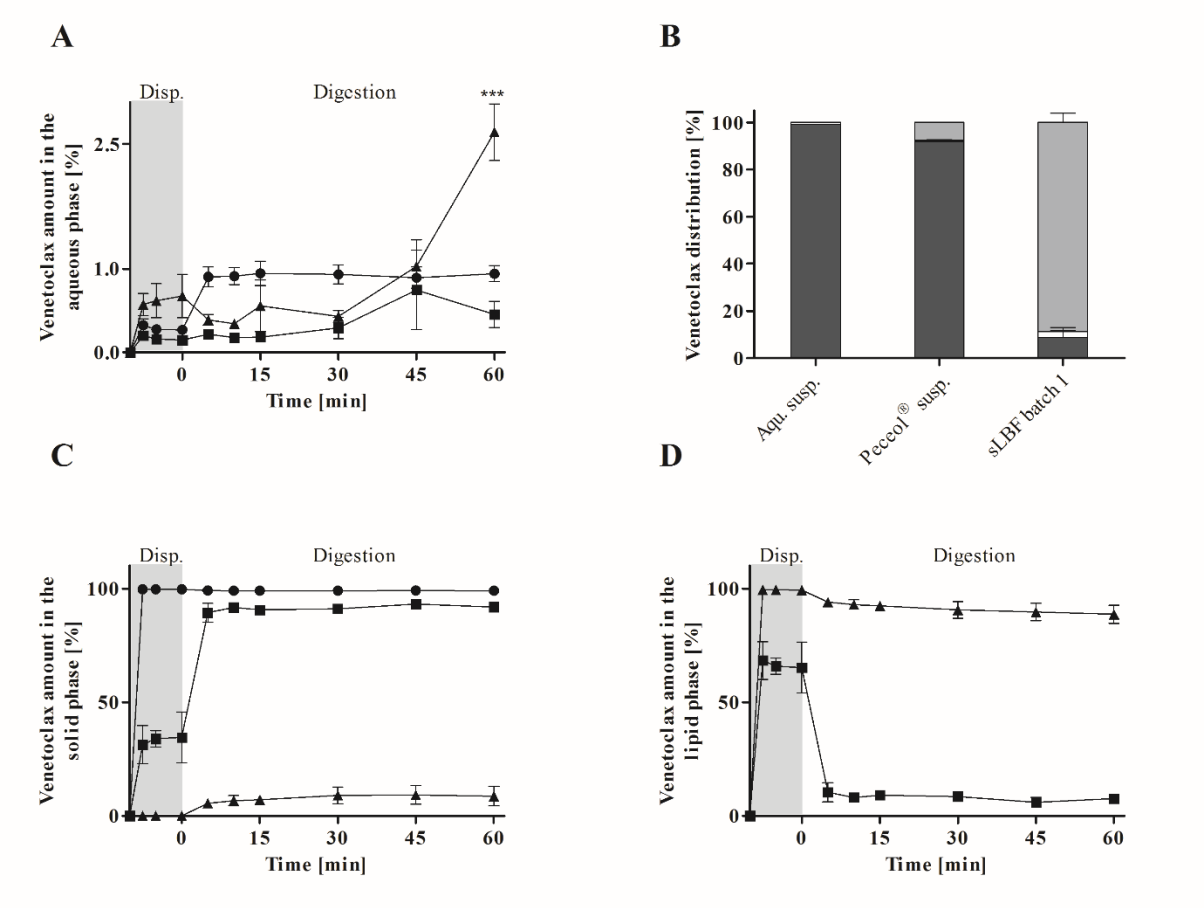



Figure 5-2 *In vitro* lipolysis of venetoclax formulated as aqueous suspension (5% w/v) (●), Peceol® suspension (5% w/v) (■), supersaturated Peceol® solution (sLBF) (5% w/v) (▲). A: % of venetoclax in the aqueous phase. Statistical significance is compared to Peceol® and aqueous suspension at the same time point, B: Distribution of venetoclax into the different phases after 60 min of lipolysis, C: % of venetoclax in the solid phase, D: % of the venetoclax in the lipid phase. All experiments were run with n = 3 and results are shown as mean ± SD.

The amount of venetoclax in the lipid phase for sLBF was significantly higher compared to the amount of venetoclax in the solid and aqueous phase ($p < 0.05$) as presented in Figure 5-2 D. On average 99.2 ± 0.2% of the venetoclax dose resided within the lipid phase during dispersion. The venetoclax dose in the lipid phase is considered absorbable as the drug can partition into

the aqueous phase, be absorbed through the lipid absorption pathway and be released by digestion of the lipid phase. The lipid phase of the sLBF demonstrated only an 8% decrease in venetoclax concentration over 60 min of digestion in the lipolysis experiment. In the case of the Peceol® suspension, the venetoclax amount in the lipid phase was higher during dispersion compared to the solid and aqueous phase. The amount of venetoclax decreased from 66.5 ± 7.3% upon dispersion to 8.3 ± 2.3% during digestion, which correlated with the amount recovered in the solid phase (Figure 5-2 C). This indicates that the venetoclax crystals in the Peceol® suspension are entrapped in the lipid excipient, an observation which is similar to that previously demonstrated for lipid nilotinib suspensions [121]. Overall, a comparison between the formulations suggested that the sLBF has the ability to maintain higher concentrations in the lipid and aqueous phase relative to the suspensions, and hence may offer benefits *in vivo*.

Venetoclax recovery in the solid phase is presented in Figure 5-2 C. The drug particles recovered in the solid phase need to undergo dissolution prior to absorption [75]. High amounts indicate that the formulation performance *in vivo* might be hampered by the poor dissolution and solubility of venetoclax in the aqueous media. The lowest amount of solid was recovered for the sLBF throughout dispersion and digestion. During dispersion, no solid phase was present. After initiation of digestion, drug precipitation occurred with up to approximately 9% of the theoretical dose recovered in the solid phase. Thus, the amount of drug that needed to undergo redissolution was the lowest for the sLBF suggesting a promising formulation performance *in vivo*. The highest amount of drug in the solid phase was recovered in the case of the aqueous suspension. Above 98% was recovered throughout dispersion and digestion in the solid phase, which was 11-fold higher compared to sLBF at the end of digestion. Upon dispersion, 33.3 ± 7.3% of the theoretical venetoclax dose was recovered in the solid phase for the Peceol® suspension, which further increased to 89.4 ± 4.1% upon initiation of digestion,

which was 10-fold higher compared to the sLBF. The results indicated that the suspended particles in the Peceol® suspension were residing/entrapped in the lipid vehicle.

### 5.4.4 *In vivo* bioavailability of venetoclax

The *in vivo* performance of venetoclax was evaluated in male landrace pigs. Each pig was administered 100 mg venetoclax as either a Peceol® sLBF (50 mg/mL (5% w/v)), Peceol® suspension (5% w/v) or as a pure powder in a capsule. Absolute bioavailability was calculated using in-house data [170]. The plasma concentration-time profiles and the absolute bioavailability are shown in Figure 5-4 and Figure 5-3, respectively, and the pharmacokinetic parameters in Table 5-5.

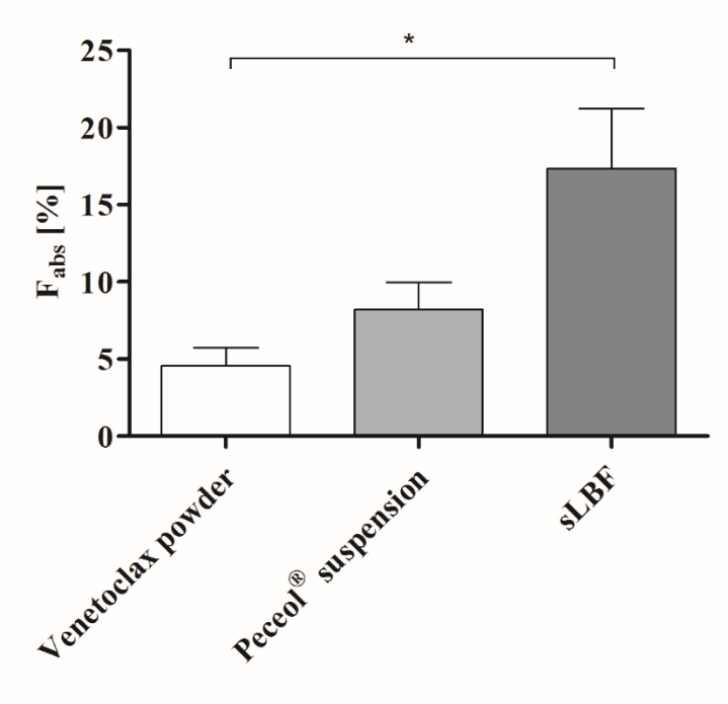



Figure 5-3 Absolute bioavailability ($F_{abs}$) of 100 mg venetoclax as venetoclax powder capsule, Peceol® suspension, supersaturated Peceol® solution (sLBF) in male landrace pigs. All data is presented as mean ± SD, where n = 3 (except for sLBF, where n = 2).

All lipid formulations showed a higher bioavailability when compared to the venetoclax powder capsule. The highest exposure was observed for sLBF with a relative bioavailability of 429.5 ± 151.1% compared to the venetoclax powder (Table 5-5, Figure 5-3, Figure 5-4). While there was a trend towards a higher exposure of sLBF compared to the Peceol® suspension, sLBF was only significantly different from the venetoclax powder ($p < 0.05$). These results indicate that the formulation success depended on the lipid excipient and the solubilisation of the drug in the sLBF.

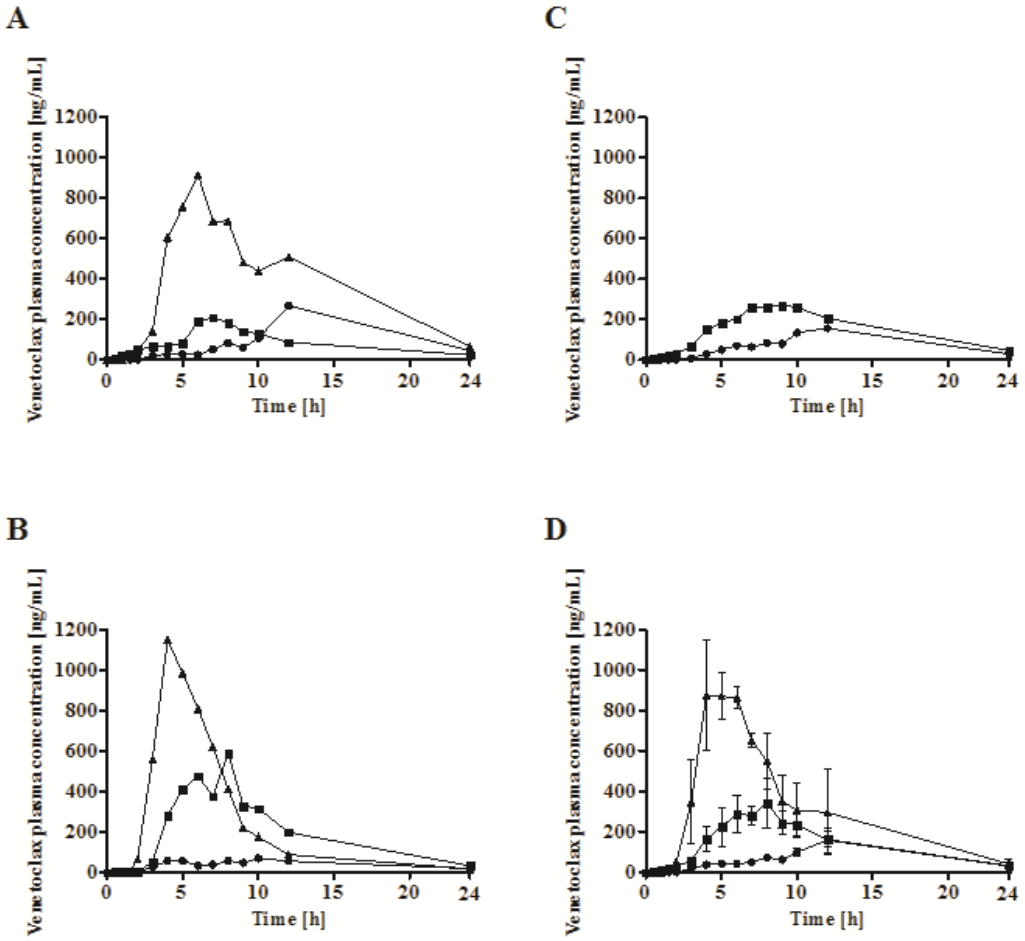



Figure 5-4 Venetoclax plasma concentration versus time profiles from 0 – 24 h for 100 mg venetoclax in male landrace pigs. Venetoclax powder capsule (●), Peceol® suspension (5% w/v) (■), sLBF (5% w/v) (▲). A-C: Individual plasma concentration versus time profiles D: Mean plasma concentration versus time profiles.

The *in vivo* study also demonstrated a faster absorption of venetoclax from sLBF. Relative to the powder capsule, sLBF demonstrated a statistically significant $t_{max}$, MRT and MAT. Approximately 6 h shorter residence and absorption times were observed for the sLBF in comparison to the powder capsule, which was statistically significant ($p < 0.05$). Relative to the Peceol® suspension, sLBF showed a trend towards a quicker absorption and shorter MRT and $t_{max}$. While this trend was not statistically significant, it indicated that the fast absorption, and hence less risk of precipitation, may be a factor in explaining the improved bioavailability for sLBF.

Table 5-5 Pharmacokinetic parameters of oral administration of 100 mg/pig venetoclax in male landrace pigs. Venetoclax was administered as a powder capsule, Peceol® suspension, sLBF (n = 3, except sLBF, where n = 2). $t_{max}$ and MRT are given as median (range), all other parameters as mean ± SD.

| | Powder capsule | Peceol® suspension | sLBF |
|---|---|---|---|
| $c_{max}$ [ng/mL] | 162.67 ±99.24 | 355.17 ± 205.40 | 1032.52 ± 165.03 |
| $t_{max}$ [h] (range) | 12 (10-12) | 8 (7-9) | 5 (4-6) |
| AUC 0 h – inf. [µg*h/mL] | 2.00 ± 0.89 | 3.59 ± 1.35 | 7.59 ± 2.40 |
| MRT [h] (range) | 14.46 (13.67 – 15.53) | 11.90 (10.21 – 12.43) | 8.77 (7.13 – 10.41) |
| MAT | 10.58 (9.79 – 11.65) | 8.02 (6.33 – 8.55) | 4.89 (3.25 – 6.53) |
| $F_{rel}$ [%] ǂ | 100.00 | 233.24 ± 183.80 | 429.46 ± 151.06 |
| $F_{abs}$ [%] | 4.56 ± 2.03 | 8.19 ± 3.08 | 17.35 ± 5.48 |

ǂ Relative to the powder capsule

## 5.5 Discussion

LBFs represent a formulation approach for enhancing oral absorption of poorly water-soluble drugs [102]. The biopharmaceutical benefits of LBFs such as avoidance of a dissolution step and improved solubilisation *in vivo* upon dispersion and digestion of the formulation [15]. Further advantages may come from improved intestinal permeability and promotion of lymphatic transport. Many of these benefits have been demonstrated for highly lipophilic 'grease ball' drugs, which are able to form a LBF solution. However, the drive of discovery technologies and methodologies like high throughput screening, the addition of lipophilic substituents during lead optimization or the shift in therapeutic target identification increasingly produces more hydrophobic as well as lipophilic candidates with a trend of increased molecular weight [3]. A consequence of these particular hydrophobic drugs is an overall lower aqueous and lipid solubility, which may come with lower permeability due to molecular bulkiness and therefore, the applicability of the classical LBF solutions is limited. As a consequence, strategies like lipophilic salts/ionic liquids [38; 40; 43], supersaturated lipid solutions [31-33; 66; 34] or hybrid systems [171; 172] were proposed that focus on increasing the drug load in LBFs. Nevertheless, it remains unclear, whether LBFs offer biopharmaceutical benefits to enhance bioavailability of these beyond Rule-of-Five drugs which display high lipophilicity, molecular weight and moderate hydrophobicity.

Thermally induced supersaturation of venetoclax was observed in all lipid vehicles, with concentrations increasing between 26% to 6690% over the experimentally determined apparent solubility at 37 ºC. In effect, heating the drug in lipid excipients, therefore, potentially increases drug loadings in lipid vehicles between 126% to 6790%. The % increases in drug loading in lipids observed for venetoclax was higher compared to typical dose loadings reported for other drugs in sLBFs [31-33; 66]. For example in the case of halofantrine [31] and fenofibrate [33] dose

loading increased to 150% compared to the solubility at 37 ºC, whereas for simvastatin [32] and R4040 [66] dose loading was increased to 200% compared to solubility at 37 ºC. This indicates that many model drugs already show a reasonable high solubility in the lipid vehicles or a lower therapeutic dose compared to venetoclax. In fact, the choice of lipid vehicle is important, as indicated by the better ability to obtain and maintain higher supersaturated venetoclax concentrations in monoglycerides when compared to triglycerides. While venetoclax displayed an ability to supersaturate in all lipid vehicles, the final choice of lipid vehicle will be influenced by factors such as the overall required dose loading and the stability of the formulation over time. In our study, appropriate short-term stability was observed for the Peceol® sLBF, which concentration was maintained above the target of 50 mg/mL over a 2-week observational period with no macroscopic signs of instability.

In the *in vivo* study, the highest absolute oral bioavailability was observed for sLBF with 17.4% ± 5.5%. sLBF showed a 2.1-fold increased bioavailability relative to the Peceol® suspension and a 3.8-fold increase when compared to the venetoclax powder capsule, which in the latter case was statistically significantly different ($p < 0.05$). Additionally, sLBF showed a faster absorption and shorter residence time in pigs. The *in vivo* study results were in line with observations from the *in vitro* lipolysis, which suggested the combined effect of maintaining drug within the absorbable lipid phase and the increased venetoclax concentration observed in aqueous digestive phase. In a previously study with fenofibrate in minipigs, a sLBF with 150% drug load (compared to equilibrium solubility) was compare to a LBF suspension with the same drug load (100% dissolved and 50% suspended) [33]. The bioavailability and $c_{max}$ of both sLBF and LBF suspension was similar [33]. In a separate study, the drug R3040 was studied as a sLBF with 200% drug load and a LBF suspension with the same drug load (100% dissolved and 100% suspended) in rats [66]. A similar bioavailability was observed for both formulations,

while the $c_{max}$ tended to be higher in the case of the sLBF [66]. The results of the present study with the more atypical molecular characteristics and the higher degree of supersaturation of venetoclax, therefore, extended the range of application of the supersaturated formulation approach in lipid vehicles.

The *in vitro* lipolysis showed a higher venetoclax concentration in the aqueous phase for the sLBF compared to a Peceol® suspension and aqueous suspension after 60 min of digestion. After 60 min, venetoclax concentrations of 20.4 ± 1.2 µg/mL in fasted state simulating digestion buffer were measured. This was in the range of previously reported amorphous solubility of venetoclax under simulated intestinal fluid conditions in the fasted state of approximately 21 µg/mL - 34 µg/mL depending on the pH [99]. Therefore, venetoclax concentrations near the maximum reported aqueous concentrations were reached with the developed sLBF. Such high venetoclax concentrations in this study agree with previous reports, which have shown that venetoclax can achieve high supersaturation in aqueous media [99]. In addition, venetoclax is a class III glass former (Figure II-3, Figure II-4, Table 5-1) with a high glass forming ability suggesting a reasonable stable supersaturated formulation [173]. Interestingly, in our study a distinctly yellow 'fourth' phase, presumed to be a venetoclax rich phase, was evident after ultracentrifugation at the bottom of the centrifugation tube (Figure II-1). Given that previous studies have reported that concentrations above the amorphous solubility lead to a glass-liquid phase separation (GLPS) [99], this fourth phase after centrifugation may potentially represent a venetoclax rich phase that has formed from high drug concentrations achieved during digestion in the aqueous phase. While this drug rich phase is observed under non-sink conditions after ultracentrifugation, the likely impact of such elevated concentrations *in vivo* is to drive the absorptive flux which is especially important for BCS class IV drugs like venetoclax.

In addition, the lowest amount of solid was recovered throughout dispersion and digestion for sLBF. In fact, despite the increased kinetic stress due to dispersion and digestion, most venetoclax was recovered in the lipid phase for sLBF, which maintained drug supersaturation within the 60 min. While in previous studies supersaturated drug concentrations in *in vitro* lipolysis media have been reported [32], such high concentrations of drug within the lipid phase during digestion of a LBF have not been reported previously for a sLBF. The high venetoclax concentration in the lipid phase may be critical for maintaining bio-enhancing effects *in vivo*, as the high aqueous concentrations may be maintained by venetoclax partitioning from the lipid into the aqueous phase.

The developed supersaturation protocol in the present study was an efficient method to supersaturate lipid vehicles within 30 - 60 min including heating and cooling. Previously published supersaturation protocols required ultra-sonification, a heating period of about 3 - 5 h at 60 ºC as well as cooling overnight at 37 ºC [31-33]. Therefore, previous reports to induce supersaturation in lipids involved processing between 13 h and 15 h [31-33]. The newly proposed protocol employed in this study therefore represents a more streamlined approach to increase dose loading in LBFs, which can be readily applied in a pre-clinical drug development setting, especially for instable *ad hoc* prepared formulations.

While this study demonstrated a proof of concept for a novel sLBF approach to enhance *in vivo* exposure, the study is not without limitations. Firstly, it was not possible to complete a full 3x3 way cross-over due to a failure in dosing one of the experimental units the sLBF formulation. However, at an individual level and overall the higher bioavailability of the sLBF was significant. In addition, while the solubility in biorelevant media was consistent, we have also reported venetoclax specific variability in the estimate of venetoclax solubility in lipid

excipients. It was not readily apparent why venetoclax showed the variable results, nonetheless it is an important point to note, as in early stages of drug development variability, especially between batches, is likely to be a major consideration given relatively limited experience with the drug. Finally, we acknowledge that the findings presented herein are more suited to a pre-clinical environment, where a focus on maximising exposure for safety assessment is a key priority. The approach outlined allows increasing dose loading in lipid excipients without any needed advanced processing which is normally involved for a technological approach that routinely requires a large amount of drug substance to produce formulations with an appropriate short-term stability. Clearly more work is required to explore long-term stability as well as the possible improvement of supersaturation stability considering the incorporation of stability and performance enhancing excipients such as polymeric stabilisers (PIs) to support stabilisation of the supersaturated state in the formulation and upon dispersion and digestion.

## 5.6 Conclusion

The present study investigated the potential of a sLBF approach to increase the oral bioavailability of the poorly water- and lipid-soluble compound venetoclax. A rapid and efficient supersaturation protocol was developed and sLBFs were successfully created leading to increased dose loadings of between 1.2-fold and 67.9-fold. A higher degree of supersaturation was observed in monoglycerides compared to triglycerides. While the supersaturated concentrations were high, a therapeutically relevant dose was only achieved in the long chain monoglyceride Peceol®. A Peceol® sLBF was a viable approach for dosing beyond Rule-of-Five drugs in a pre-clinical setting leading to an increased *in vivo* exposure of approximately 4-fold compared to a venetoclax powder capsule. Compared to the corresponding LBF suspension an approximately 2-fold increase was demonstrated for sLBF. The *in vitro* lipolysis provided a mechanistic basis for explaining the sLBF performance

relative to a control LBF and aqueous suspension in terms of higher solubility in the aqueous digest phase and the ability to maintain supersaturated drug concentrations in the lipid phase even after 60 min of digestion. Consequently, the present study demonstrated that a sLBF approach is a viable approach for high molecular weight, hydrophobic and/or lipophilic beyond Rule-of-Five drugs in a pre-clinical setting.

# 6 Chapter 6: *In silico, in vitro* and *in vivo* evaluation of precipitation inhibitors in supersaturated lipid-based formulations of venetoclax

**Niklas J. Koehl[1], Laura J. Henze[1], Harriet Bennett-Lenane[1], Daniel J. Price[2,3], René Holm[4,5], Martin Kuentz[6], Brendan T. Griffin[1]**

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

[2] Merck KGaA, Darmstadt, Germany

[3] Goethe University Frankfurt, Frankfurt am Main, Germany

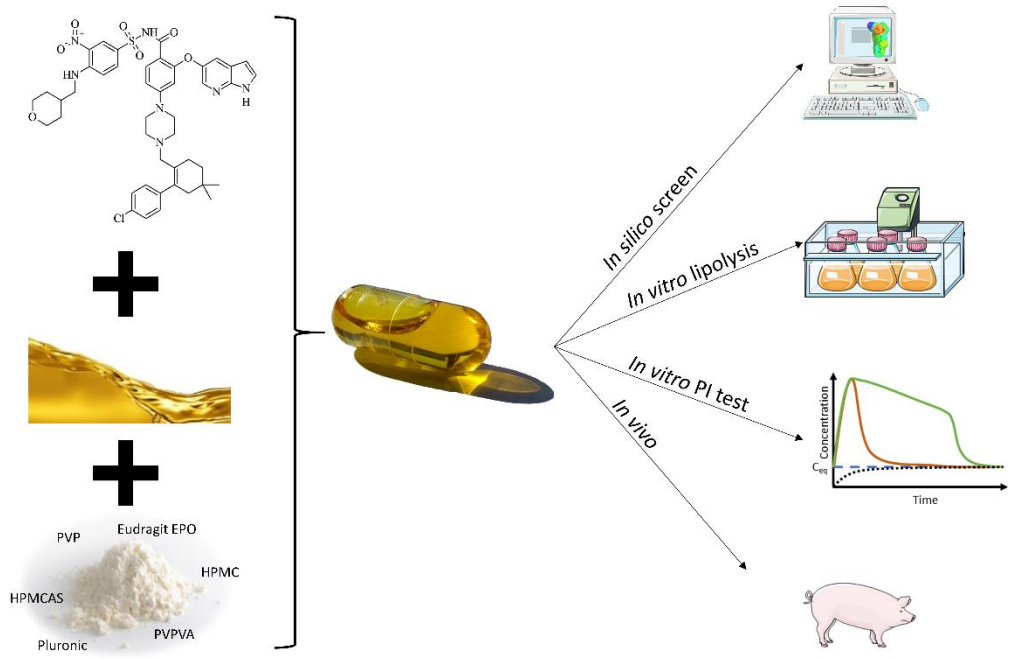
[4] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

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

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

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## 6.1 Abstract




Graphical abstract 6-1 Evaluation of PIs in sLBFs using *in silico*, *in vitro* and *in vivo* tools.

**Purpose** The concept of using PIs to sustain supersaturation is well established for amorphous formulations, but less in the case of LBFs. This study applied a systematic *in silico-in vitro-in vivo* approach to assess the merits of incorporating PIs in sLBF using the model drug venetoclax.

**Methods** sLBFs containing HPMC, HPMCAS, PVP, PVP/VA, Pluronic® F108 and Eudragit® EPO were assessed *in silico* calculating a drug-excipient mixing enthalpy, *in vitro* using a PI solvent shift test and finally bioavailability was assessed *in vivo* in landrace pigs.

**Results** The estimation of pure interaction enthalpies of drug and excipient were deemed useful in determining the most promising PIs for venetoclax. The sLBF alone (i.e. no PI present) displayed high initial concentration in the aqueous phase during *in vitro* screening. sLBF with Pluronic® F108 displayed the highest venetoclax concentration in the aqueous phase and sLBF with Eudragit® EPO the lowest. *In vivo*, the sLBF alone showed the highest bioavailability of

26.3 ± 14.2%. Interestingly, a trend towards a decreasing bioavailability was observed for sLBF containing PIs, with PVP/VA being significantly lower compared to sLBF alone.

**Conclusion** In conclusion, the ability of a sLBF to generate supersaturated concentrations of venetoclax *in vitro* was translated to increased absorption *in vivo*. While *in silico* and *in vitro* PI screening suggested benefits in terms of prolonged supersaturation, addition of a PI did not increase *in vivo* bioavailability. The findings of this study are of particular relevance to pre-clinical drug development, where the high *in vivo* exposure of venetoclax was achieved using a sLBF approach and despite a perceived risk of drug precipitation from a sLBF, including of a PI may not be merited in all cases.

## 6.2 Introduction

Favourable solubility in the gastrointestinal fluids and intestinal permeability is a prerequisite for a high oral bioavailability of any drug. However, drug discovery approaches such as high throughput screenings, modifications during lead optimization as well as the noticeable therapeutic target shift towards intracellular targets deliver more drugs displaying low aqueous solubility and beyond Rule-of-Five properties [3; 6; 167]. These drug candidates display sub-optimal biopharmaceutical properties and create a need for bio-enabling formulation approaches. The design of such formulations includes strategies to generate and maintain high concentrations or supersaturation in intraluminal fluids. Prominent examples of such supersaturating formulations are amorphous solid dispersions and the use of LBFs [18; 19]. In particular, drugs with a low aqueous solubility, a high lipophilicity and/or bioavailability that is increased by co-ingestion of fatty meals, LBFs can offer particular formulation advantages [18; 19; 102]. The most convenient and conventional LBFs are lipid solutions, where the drug is dissolved in the lipid vehicle, and hence most widely applicable for drugs that show high lipid solubility. As the drug is dissolved in the LBF solution, this circumvents the

dissolution process and provides an enhanced intestinal solubilisation for lipophilic drugs in the gastrointestinal environment [61]. Upon digestion of the lipid excipient, the changing nature of the intraluminal colloids potentially generates supersaturated drug concentrations in the gastrointestinal fluids effectively promoting absorption [61]. Alternatively, for drugs which display low solubility in lipid vehicles, strategies to increase dose loading in the lipid vehicles may be required such as lipid suspensions [44; 121; 174; 175], sLBF [31; 32; 34; 176], lipophilic salts [38; 40; 41], or lipid-hybrid systems [46; 47; 172].

sLBFs can be beneficial in delivering drug candidates of this type and interest in their application has increased over the past number of years. The most common method to generate drug supersaturation in lipids is by heating excess drug in the lipids, followed by cooling [34], whereupon the amount of dissolved drug in the lipid excipients may exceed the thermodynamic solubility (at lower temperatures) and the LBF becomes supersaturated. A supersaturated formulation is considered kinetically stable, and sLBFs have successfully been applied to improve oral bioavailability of a number of poorly water-soluble drugs such as cinnarizine, simvastatin and halofantrine [31-33; 65; 66]. An sLBF formulation approach offers a number of advantages particularly in early stages of development. These advantages include the ease of preparation, suitability for ease of dosing in pre-clinical models and the ability to prepare prototype formulations on a small scale while keeping the development costs low at times where the attrition rate is usually high. Thomas *et al.* developed a sLBF of simvastatin at 150% of the saturation solubility and assessed bioavailability in dogs [32]. Oral bioavailability of the sLBF was 1.8-fold higher when compared to the same dose of a LBF solution at 75% of the saturation solubility [32]. Recently, our group has reported that a sLBF of venetoclax (containing venetoclax at 350% of the apparent saturation solubility) increased oral bioavailability 2.1-fold when compared to a control LBF (i.e. lipid suspension) and 3.8-fold when compared to the

crude drug powder (Chapter 5) [176]. Additionally, the venetoclax study demonstrated during *in vitro* lipolysis testing of the sLBF that the drug concentration of venetoclax in the aqueous digest phase were in the range of the reported amorphous solubility in FaSSIF (chapter 5) [176]. With such elevated aqueous phase concentrations reached by a sLBF, the risk of precipitation is deemed high and hence it was hypothesized that incorporation of a PI may be beneficial.

While the incorporation of PIs in a supersaturated drug delivery system has been widely explored for solid solutions, to the best of our knowledge, to date no study has reported the incorporation of PIs in sLBFs testing *in vivo*. A limited number of studies have explored the use of PIs to reduce the risk of drug precipitation upon dispersion and digestion of classical LBFs [177-180]. Gao and co-workers developed an undersaturated self-emulsifying LBF for paclitaxel with hydroxypropyl methylcellulose (HPMC) as PI [177]. The HPMC concentration was 50 mg/mL and a 10-fold higher oral bioavailability was observed by incorporating HPMC compared to a HPMC-free formulation in rats [177]. Gao and colleagues described such formulations as 'supersaturable' referring primarily to the ability of many LBFs to generate supersaturated drug concentrations on dispersion/digestion in intestinal fluids. However, it is worth noting that supersaturable LBFs explored by Gao *et al.* contain drug below saturation solubility and are distinct from sLBFs even though this nomenclature is not consistently used throughout the literature. A subsequent study examined the effect of incorporating HPMC in LBFs for the investigational drug PNU-91325. The study confirmed that the use of HPMC as a PI resulted in a higher aqueous phase concentration *in vitro* as well as increased bioavailability in dogs [178]. The LBFs explored in these studies contained 70 – 100% (w/w) co-solvents and surfactants (i.e. type IV of the LFCS [12; 13]), which in general present a greater risk of drug precipitation on dispersion. Similarly, Suys *et al.* recently reported that the utility of PIs to prolong supersaturation was more evident for a type IV (50% co-solvent) and

type IIIB (25% co-solvent and 25% surfactants) formulation, whereas for a type IIIA formulation (no co-solvent, 35% surfactant), the studied PIs had no impact on prolonging supersaturation during *in vitro* digestion [180]. Collectively, these studies demonstrate the merits of incorporating PIs in a LBF where there is a perceived high risk of precipitation on dispersion/digestion due to for example a rapid co-solvent depletion. However, to date no study has explored the utility of PIs to maintain supersaturation in sLBFs, which the present study aimed to investigate.

Venetoclax is a highly lipophilic drug (log*P* of 5.5 [96]) with a high molecular mass of 868.44 g/mol [96] and a $T_m$ of 139 ºC (Table III-1) represents a recently licenced drug with properties in the beyond Rule-of-Five space. The drug is classified as a BCS class IV drug based on low aqueous solubility and permeability. The commercial formulation Venclyxto® displays a pronounced food dependent oral bioavailability with a 3.4-fold increase in oral bioavailability after a low-fat meal and a 5-fold increase after a high fat meal compared to the fasted state [96].

This study explored the merits of PIs on the oral bioavailability of an oil-based sLBF of venetoclax. A range of promising PIs have been identified based on calculated excess enthalpy of mixing (COSMOquick software), which served to estimate molecular excipient interaction in the more complex aqueous dispersions. The ability of the selected PIs to maintain supersaturation of venetoclax in biorelevant media was tested *in vitro* and the impact of incorporating PIs into the sLBF in comparison to the separate addition (pre-dissolved) of the PIs in the simulated intestinal fluids was evaluated. A subsequent *in vivo* study examined the impact of incorporated PIs in sLBFs on the oral bioavailability of venetoclax in landrace pigs.

## 6.3 Materials and methods

### 6.3.1 Chemicals and materials

Venetoclax was purchased from Kemprotec Ltd. (UK) (Batch # 1810004). Olive oil, highly refined and low acidity, taurodeoxycholic acid (NaTDC) and pancreatic lipase (8 x USP) were ordered from Sigma-Aldrich (Ireland). Lipoid® E PC S was obtained from Lipoid GmbH (Germany) and Eudragit® EPO was obtained from Evonik (Germany). Hydroxypropyl methylcellulose acetate succinate (HPMCAS) (AQOAT (HPMCAS-MF)) was purchased from ShinEtsu (Japan) and Pluronic® F108, hydroxypropyl methylcellulose (HPMC), Polyvinylpyrrolidone (PVP) were purchased from MilliporeSigma (St Louis, MO, USA). Kollidon® VA 64 (PVP/VA) was kindly donated by BASF (Germany). Capmul MCM® and Captex® 1000 were kindly donated by Abitec corporation. A sample of Peceol® was kindly donated by Gattefossé (France) and FaSSIF/FeSSIF powder Version 1 was kindly donated by biorelevant.com (UK). Water was purified by a MilliQ® water system. All other chemicals and solvents were of analytical or HPLC grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 6.3.2 Apparent solubility

Apparent solubility was determined in olive oil, Captex® 1000, Peceol® and Capmul MCM®. In brief, an excess of venetoclax was added to 2 mL of the excipients and stirred at 200 rpm (25% power) (Mixdrive 15, 2MAG, Germany) at 37 °C. Solid excipients were melted at 50 ºC and cooled to 37 ºC prior to venetoclax addition. Samples were taken after 24 h, 48 h, 72 h and centrifuged at 21,380*g* (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again using identical conditions. To solubilise the oily excipient, the supernatant was diluted in

acetonitrile:ethyl acetate (1:3 v/v). Followed by further 1:10 (v/v) dilution with acetonitrile:ethyl acetate (3:1 v/v). The obtained samples were diluted appropriately with mobile phase before analysis by reverse phase HPLC as described below. All samples were run in triplicates.

### 6.3.3 Biorelevant solubility

FaSSIF and FeSSIF were prepared according to the instructions by biorelevant.com. FeSSIF was used directly, whereas FaSSIF was left at room temperature for 2 h prior to usage. Excess venetoclax was added to 2 mL of biorelevant media and placed in a water bath shaker at 200 shakes/min (GLS400, Grant Instruments, UK) and 37 °C. Samples were taken after 3 h, 6 h and 24 h and centrifuged at 21,380*g* (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again under identical conditions. Subsequently, the supernatant was diluted with a mobile phase before analysis by HPLC.

The samples were analysed using an Agilent 1200 series HPLC system (Agilent Technology Inc., US) that comprised a binary pump, degasser, autosampler and variable wavelength detector. Data were analysed using the software EZChrom Elite® version 3.2. Venetoclax was separated from the sample matrix 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 40 °C. The mobile phase consisted of (a) acetonitrile with 0.5% TFA and (b) water with 0.5% TFA at a ratio of 53:47 (a:b v/v) and was used at a flow rate of 1 mL/min. The injection volume was 20 µL and the detection wavelength was set to 316 nm. The LOD was 20 ng/mL and the LOQ 65 ng/mL determined using the standard error of y-intercept according to ICH Q2 guideline [122].

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

In the *in vivo* study and *in vitro* PI screen, the supersaturated lipid solution (sLBF) was prepared as previously reported with a lower temperature to reduce the thermal impact on the drug and excipient (chapter 5) [176]. In brief, 300 mg venetoclax were added to 6 mL Peceol® and dispersed at 600 rpm (Stuart CD162 heat-stir, Cole-Parmer, UK) and sealed with parafilm. A continuous nitrogen stream into the vial removed oxygen throughout the preparation. After suspending the drug particles, the obtained suspension was slowly heated to 55 °C (Stuart CD 162 heat-stir, Cole-Parmer, UK). The mixture was kept at 55 °C for 10 min and cooled to 25 °C while continuously stirring at 600 rpm. Subsequently, the mixture was heated a second time under the same conditions as stated above and cooled to room temperature to obtain the final sLBF. The absence of crystals was confirmed using cross-polarized light microscopy. For the *in vivo* study, sLBF was administered in hard gelatine capsules size 00EL (Licaps®, Capsugel, Lonza Group Ltd.) with 1 mL/capsule.

For the preparation of the sLBF with PI, HPMC, HPMCAS, Pluronic® F108, Eudragit® EPO, PVP and PVP/VA were added to the sLBF at a drug:PI ratio of 1:1 (w/w). At the given PI concentration (50 mg/mL) Pluronic® F108, Eudragit® EPO, PVP and PVP/VA were soluble in Peceol® at 37 ºC, while HPMC and HPMCAS resulted in a suspension. In the case of the soluble PIs, the Peceol®-PI-solution was used to prepare the PI containing sLBF using the method described above. In the case of HPMC and HPMCAS, the sLBF was prepared as described above and HPMC and HPMCAS were added and dispersed *ad hoc* into the sLBF before *in vitro* and *in vivo* experiments.

### 6.3.5 Cross-polarised light microscopy

The absence of crystalline material in the supersaturated solutions was confirmed by means of cross-polarised light microscopy using an Olympus BX51 with an Olympus SC100 camera operated by Olympus Stream essentials 2.3.3. The light was polarized using the polarizer U-POT and analysed with the analyser U-ANT. The absence of crystals was assumed, if no birefringence was observed.

### 6.3.6 *In vitro* evaluation: drug solubilization during formulation dispersion and digestion

*In vitro* lipolysis was performed using a pH-stat apparatus (Metrohm AG, Herisau, Switzerland) comprising a Titrando® 907 stirrer, 804 Ti-stand, a pH electrode (Metrohm AG, 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 used as previously reported (chapter 4) [175]. 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 (digestion buffer) and stirred for 12 h before further usage. The pancreatin extract was prepared freshly by adding 5 mL of 5 °C digestion 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* (Rotina 380 R, Andreas Hettich GmbH & Co. KG, Germany) and 4 mL of supernatant was recovered and stored at 2 – 8 °C before further usage.

For the *in vitro* lipolysis experiment 1.075 g of lipid formulation was dispersed into 39 mL of digestion buffer for 10 min at a stirring speed of 450 rpm, which was maintained throughout

the experiment. 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. To the remaining 36 mL (1.0 g lipid formulation) dispersion 4 mL of pancreatin extract was added to initialize digestion. After 60 min the released non-ionized free fatty acids were determined by a pH increase of the buffer to pH 9.

Samples of 1.0 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 mixed. This sample was used to quantify the total drug recovery, which allowed adjustment of inhomogeneous samples. All samples were centrifuged at 37 °C and 21,000*g* for 30 min using a benchtop centrifuge of the type Hettich Micro 200 R (Andreas Hettich GmbH & Co. KG, Germany).

### 6.3.7 *In silico* precipitation inhibitor screening: COSMO-RS calculations

The excess enthalpy of mixing between venetoclax and the polymeric PI was calculated using the COSMOquick software (COSMOlogic, Germany, Version 1.6). The software is based on the Conductor like Screening Model for Real Solvents(COSMO-RS) [181; 182] that combined quantum chemical surface charge calculations with statistical thermodynamics. The COSMOquick approach in particular allows for a fast estimation of these surface charge densities for further calculations [183]. Venetoclax and the polymers were entered in smiles notation. As the quantum chemical calculations cannot capture the full complexity of the polymers, the polymer structures were in this study approximated as trimers. The drug:PI ratio was set at a stoichiometric ratio of 1:1 to represent the formulations used *in vitro* and *in vivo*

and the temperature was set to 37 °C. In line with previous applications of COSMOquick for co-former screening in co-crystal selection [184], drug solubility estimations in glycerides [185] and polymer screening for supersaturated formulations [186], a more negative value of calculated excess enthalpy ranks the strength of molecular drug-excipient interaction. This is just an approximation, as the presence of an aqueous phase is not considered in the calculations nor the complexity of any biorelevant medium. Therefore, the results should be understood as a first *in silico* estimation of relative excipient comparison. This has previously been referred to as a higher or lower 'COSMO-Rank' [186].

### 6.3.8 *In vitro* precipitation inhibitor testing

The *in vitro* PI testing for venetoclax was done by means of a solvent shift. The effect of a fully hydrated PI (dissolved in FaSSIF) was compared against the PI in the formulation. Thus, the employed PIs HPMC, HPMCAS, Eudragit® EPO, PVP, PVP/VA and Pluronic® F108 were dissolved in either FaSSIF (FaSSIF-PI) or either suspended or dissolved in the supersaturated lipid solution (sLBF-PI). For the hydrated PI test, 5 mg PI was dissolved in 5 mL FaSSIF (FaSSIF-PI) (prepared according to biorelevant.com) and 5 mg of venetoclax dissolved in either DMSO (100 mg/mL) or Peceol® (sLBF 50 mg/mL) was added. For the evaluation of the PI in the lipid formulation, 100 µL of the sLBF-PI (50 mg/mL venetoclax and 50 mg/mL PI) was added to 5 mL FaSSIF. The vials were sealed and placed in a water bath shaker at 200 shakes/min and 37 °C (GLS400, Grant Instruments, UK). After 2, 5, 10, 15, 30, 60, 120, 180 min 250 µL samples were taken. The samples were filtered with a 0.2 µm syringe filter (Whatman® Spartan 13/0.2) and samples that contained lipids were additionally centrifuged for 30 min at 21,380*g* and 37 °C (Mikro 200 R, Hettich GmbH, Germany). The aqueous phase was

collected, and one part was diluted 1:10 (v/v) with acetonitrile (including 0.5% (v/v) TFA). A pure (non-diluted) and diluted sample was analysed by HPLC as described above.

Additionally, the solubility of venetoclax was determined in FaSSIF with dissolved PI (FaSSIF-PI). Excess venetoclax was added to 5 mL FaSSIF with 5 mg PI. 250 µL samples were taken at 3 h, 6 h, 24 h and filtered through a 0.2 µm syringe filter (Whatman® Spartan 13/0.2), diluted with acetonitrile (containing 0.5% (v/v) TFA) and analysed by HPLC as described above.

### 6.3.9 *In vivo* study

All experiments were approved and conducted with licences issued by the Health Product Regulatory Authority, Ireland (project licence AE19130/P058) as directed by the EU Statutory instruments of the EU directive 2010/63/EU (Protection of Animals used for Scientific Purposes). Local ethical approval was granted by University College Cork Animal Experimentation Ethics Committee (AEEC). In order to test all PIs two bioavailability studies had to be conducted. The first study was with 5 pigs and a 6-way crossover and the second study with 3 pigs and a 4-way crossover. Both studies were randomised and conducted in male landrace pigs (15 – 17 kg) and each pig received a single dose of 100 mg venetoclax. Pigs were fed approximately 175 g of standard weanling pig pellet feed twice daily. In the fasted study legs the final feed of 175 g was given 24 h prior to dosing. As part of the study design any remaining food was removed 16 h before dosing, however, no food remained at this point in any of the groups. On day 1, an indwelling intravenous catheter was inserted from the ear vein into the jugular vein under general anaesthesia. In the first study, on day 3, following an overnight fast of 16 h, pigs were administered either sLBF or sLBF with PI (sLBF-PI), respectively. The tested PIs included HPMC, HPMCAS, PVP, PVP/VA, Pluronic® F108. In the second study, on day 3, following an overnight fast of 16 h, pigs were administered either

a reference capsule with venetoclax powder, a venetoclax Peceol® suspension, a sLBF or a sLBF including Eudragit® EPO, respectively. The results of the venetoclax powder, Peceol® suspension and sLBF have previously been reported (chapter 5) [176] and are not further described in the current study. In both studies all formulations were administered with the aid of a dosing gun followed by 50 mL of water via a syringe. In order to control the water intake with the dosage forms, the water availability was restricted for 3 h post-dosing. At all other times water was available *ad libitum*. To facilitate handling during the oral administration, an intramuscular dose of ketamine (5mg/kg) and xylazine (1mg/kg) was administered in both studies. Blood samples were collected after 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24 h in heparinised tubes. Upon collection, blood samples were immediately centrifuged at 3000*g*, 4 ºC for 5.5 min (Eppendorf 5702 R, Rotor A-4-38, Eppendorf Ltd., UK). The supernatant plasma was harvested and stored at -20 ºC until further analysis. A 6-day washout period was maintained between the study legs.

### 6.3.10 Bioanalysis

The plasma concentrations of venetoclax were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., US) comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was operated, and the data analysed with EZChrom Elite® version 3.3.2. 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 for the separation of venetoclax. The mobile phase consisted of water and acetonitrile with 0.5% (v/v) TFA at a ratio of 47:53 (v/v) and was used at a flow rate of 1.0 mL/min. The sample and column temperature were set at 5 °C and 40 °C, respectively, and the detection wavelength was set to 250, 290 and 316 nm. Venetoclax was extracted from the plasma samples by liquid-liquid extraction as reported previously

(chapter 5) [176]. In brief, venetoclax was extracted from 500 µL plasma using acetonitrile and ethyl acetate. Vemurafenib was used as internal standard. The extraction solvents were dried under a nitrogen stream at 60 °C and the residues were reconstituted in 100 µL mobile phase (excluding TFA) followed by centrifugation at 25 °C, 11,500*g* for 5 min (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany). The injection volume used for HPLC analysis of the supernatant was 50 µL.

### 6.3.11 Data Analysis

Prior to statistical analysis the Bartlett's test was used to check for equal variances. A one-way ANOVA was performed for the lipolysis data using a Tukey post-hoc test to compare the different formulation performances. While the concentration time profiles of the *in vitro* PI test were analysed using a two-way ANOVA with the Bonferroni post-test, the AUC was analysed using a one-way ANOVA with a Tukey post-hoc test. The pharmacokinetic parameters were calculated using Microsoft® Excel® by means of the trapezoidal rule. The plasma concentration profiles were analysed by non-compartmental analysis. The statistical analysis for the *in vivo* parameters of the formulations within the same crossover study was performed using a one-way ANOVA after using the Bartlett's test to check for equal variances. The pairwise comparison of the groups was done using Tukey's multiple comparison test. All statistical analyses were carried out using GraphPad Prism® 5 (GraphPad Software, USA).

## 6.4 Results

### 6.4.1 Apparent solubility of venetoclax in lipid excipients

Solubility screening in pure lipid excipient indicated that venetoclax showed a higher apparent solubility in long chain than medium chain-based excipients (Figure 6-1 A). Within the long chain and medium chain excipients, a higher apparent solubility was observed for the monoglycerides compared to the triglycerides. Subsequently, biorelevant solubility of venetoclax was determined (Figure 6-1 B). Solubility in FaSSIF was 6.8 ± 1.8 µg/mL translating to only 1.7% of a 100 mg dose venetoclax in 250 mL of gastrointestinal fluid. In the fed state the solubility increased 3.9-fold to 6.7% of the 100 mg venetoclax dose, which in general is in agreement with the observation in increased bioavailability for venetoclax reported from clinical studies [96].

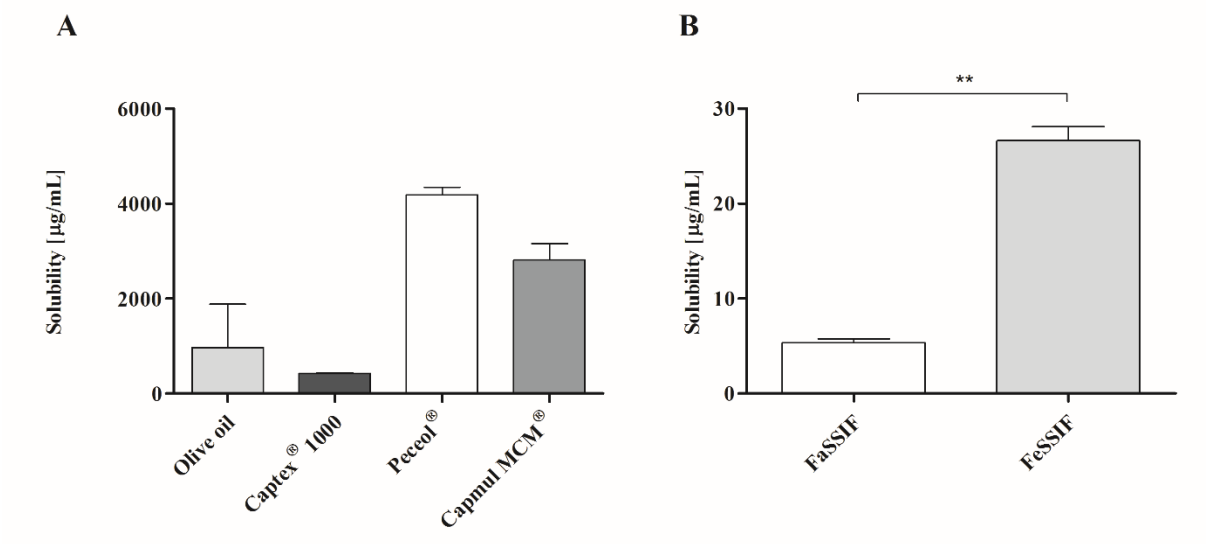



Figure 6-1 A: Solubility in the lipid excipients at 37 °C, B: Solubility in fasted state simulating intestinal fluids (FaSSIF) and fed state simulating intestinal fluids (FeSSIF) at 37 °C (n = 3, mean ± SD).

### 6.4.2 sLBF achieves high aqueous drug concentration during *in vitro* dispersion and digestion.

Previous studies by our group developed a venetoclax sLBF using a preparation method at 70 ºC (chapter 5) [176]. Process optimisation in this study focused on improving the thermally induced supersaturation process by lowering the overall energy input while maintaining the desired level of supersaturation. sLBFs were prepared at the target dose load of 50 mg/mL by heating a LBF suspension (50 mg/mL) to 55 ºC. The LBF was kept at 55 ºC for 10 min to dissolve venetoclax before cooling the solution to room temperature. In this study, the obtained sLBF had a 11.9-fold (1193.7%) higher drug load compared to the apparent saturation solubility. The venetoclax concentrations in the aqueous phase during dispersion and digestion are shown in Table 6-1 and Figure III-1.

Table 6-1 Venetoclax concentration in the *in vitro* lipolysis experiment after 10 min of dispersion and after 5 min and 60 min of digestion. Venetoclax was formulated as sLBF (50 mg/mL), Peceol® suspension (50 mg/mL) and aqueous suspension (50 mg/mL). All experiments were run with n = 3 (mean ± SD).

| | Venetoclax concentration (µg/mL) in aqueous phase (AP) during LBF dispersion and digestion | | |
|---|---|---|---|
| Formulation | $AP_{Dispersion}$ (10 min) | $AP_{Digestion}$ (5 min) | $AP_{Digestion}$ (60 min) |
| sLBF [a)] | 18.7 ± 0.0 | 37.5 ± 3.2 | 73.8 ± 6.4 |
| LBF suspension [b,c)] | 1.0 ± 0.1 | 1.7 ± 0.3 | 2.9 ± 0.5 |
| aqueous suspension [c)] | 3.0 ± 0.1 | 9.1 ± 0.2 | 9.4 ± 0.3 |

[a)] sLBF drug loading was 1194% of determined apparent solubility
[b)] LBF drug loading was 100% solubilised + 158% suspended of determined apparent solubility
[c)] Data as previously reported (chapter 5) [176].

As the drug needs to be dissolved prior to absorption by the body, the solubilised drug in the aqueous phase of the *in vitro* lipolysis test is often used as a guide to formulation performance *in vivo*. The sLBF displayed relatively high concentrations in the aqueous phase throughout

*in vitro* lipolysis, with an average venetoclax concentration of 18.6 ± 1.3 µg/mL during dispersion and 73.8 ± 6.4 µg/mL after 60 min of digestion. Interestingly, the final concentration at the end of the digestion, was equivalent to 6.4 ± 0.5% of the venetoclax dose used in the experiment and was 2.8-fold higher when compared to the apparent solubility of venetoclax in FeSSIF. Additionally, the measured concentration was higher than the reported amorphous solubility in FeSSIF of 26.4 – 54.6 µg/mL [99]. Most of venetoclax was recovered in the lipid phase with an average of 98.5 ± 2.8% of the venetoclax dose during dispersion and 88.6 ± 0.2% after 60 min of digestion (Figure III-1). The lipid phase can act as a reservoir for the drug, which gradually diffuses into the aqueous phase during the transit through the gastrointestinal tract. Additionally, the lipid phase is digested during the transit releasing venetoclax. In the case of the sLBF the lowest amount of venetoclax was recovered in the solid phase. sLBF showed no precipitation during dispersion and after 60 min of digestion only 6.2 ± 0.6% of the venetoclax dose was precipitated. These results are in line with previous studies showing a superior performance (high aqueous phase concentration, low solid phase concentration) of the sLBF *in vitro* relative to a lipid-based suspension and aqueous suspension (chapter 5) [176]. The optimised processing method, therefore, yielded comparable formulation performance.

### 6.4.3 COSMO-RS as a preliminary *in silico* screening tool for PI selection

To identify the most promising PIs, reduce the extent of *in vitro* testing and to evaluate the utility of this computational tool in the formulation design of sLBFs, the interactions between venetoclax and various PIs were evaluated *in silico*. Based on the COSMO-RS theory [181; 187] that combines quantum chemistry and statistical thermodynamics, the COSMOquick software was utilized to predict the PI-drug interactions similarly to previous reports where this screening technique of PI selection was utilised for silica formulations [186]. The COSMOquick software predicts the interactions of two molecules based on equilibrium thermodynamics and

the predicted chemical potential in liquids. The stronger the interaction between venetoclax and the PI, the more negative the calculated excess enthalpy of interaction. A simplified view assigns to a PI that has a more negative excess enthalpy, a higher 'COMSO-Rank' with respect to its suitability as PI. While this tool may be beneficial to reduce *in vitro* testing by identifying the most promising PI-drug interactions as shown for other formulation approaches [186], it must be acknowledged that the *in silico* calculations did not consider the presence of water nor other formulation excipients, potentially limiting the applicability of this tool for the more complex bio-enabling formulation approach of LBFs. In the present study, the excess enthalpy of mixing for a variety of PIs was calculated, and PIs with varying negative enthalpy of mixing were selected. The chosen PIs had the following 'COSMO-Rank': Eudragit® EPO > Pluronic® > HPMCAS ≥ PVP ≥ PVP/VA > HPMC as shown in Figure 6-2.

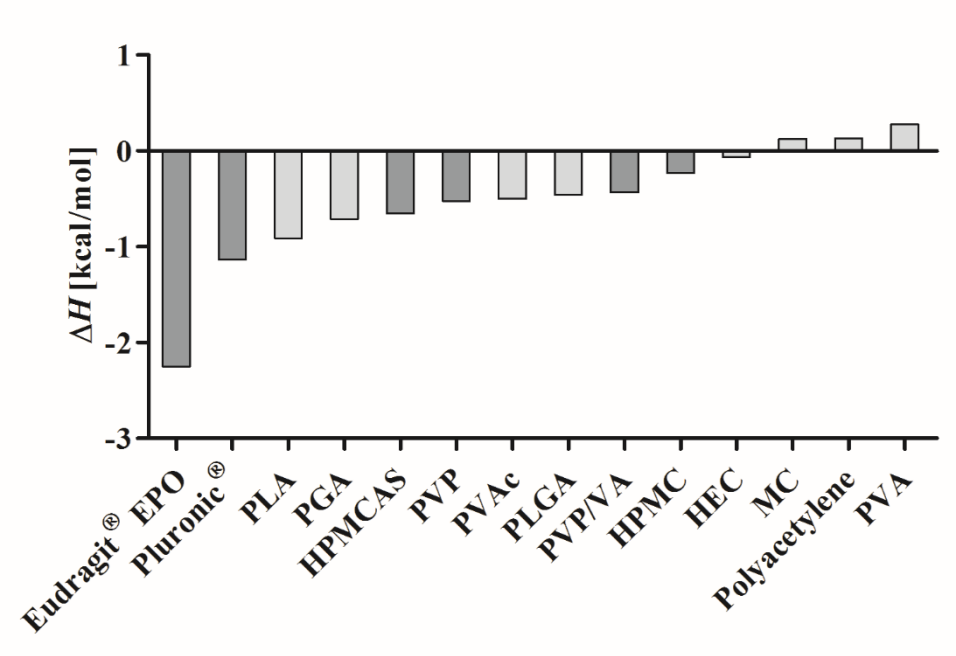



Figure 6-2 COSMO-RS screen. Calculated excess enthalpy of interaction between venetoclax and the PIs. The dark grey PIs were selected for further *in vitro* and *in vivo* evaluation. The calculation was based on a 1:1 ratio of drug to PI. PLA: Polylactic acid, PGA: Polyglycolic acid, HPMCAS: Hydroxypropyl methylcellulose acetate succinate, PVP: Polyvinylpyrrolidone, PVAc: Polyvenyl acetate, PLGA: Poly lactic-co-glycolic acid, PVP/VA: Polyvinylpyrrolidone-co-vinyl acetate, HPMC: Hydroxypropyl methylcellulose, HEC: Hydroxypropyl cellulose, MC: Methylcellulose, PVA: Polyvinyl alcohol.

### 6.4.4 *In vitro* testing for PIs for prolonging supersaturation

The selected PIs from the *in silico* screening were further evaluated experimentally *in vitro* to assess the PI performance (aqueous phase concentration) under simulated conditions and with LBFs. The *in vitro* PI test was performed by means of solvent shift by dispersing the formulation in (i) FaSSIF or (ii) FaSSIF with pre-dissolved PI (FaSSIF-PI). In FaSSIF-PI was dispersed (a) venetoclax dissolved in DMSO (100 mg/mL), as a positive control and (b) sLBF (sLBF-aqPI). In FaSSIF was dispersed (c) sLBF without PI (sLBF-noPI) and (d) sLBF with incorporated PI (sLBF-PI). Polymers that were soluble at the tested concentration of 50 mg/mL were dissolved in sLBF (PVP, PVP/VA, Pluronic® F108, Eudragit® EPO) and those that were

not soluble in the sLBF were therefore *ad hoc* suspended in the sLBF (HPMC, HPMCAS). Additionally, the solubility of venetoclax in FaSSIF-PI was measured. The results of the PI testing are shown in Figure 6-3 and Table 6-2.

In all cases, the venetoclax solubility in FaSSIF-PI was higher compared to the solubility in FaSSIF with an increase in solubility from 1.3-fold to 3.4-fold. In order of decreasing solubility in FaSSIF-PI the ranking was Eudragit® EPO, HPMC, Pluronic® F108, PVP/VA, HPMCAS and PVP. In general, upon dispersion of the DMSO solution in FaSSIF-PI HPMC, Pluronic® F108, PVP/VA, HPMCAS and PVP venetoclax concentrations reached FaSSIF-PI solubility, which was maintained throughout the experiment. In the case of Eudragit® EPO a decrease below the FaSSIF-PI solubility was observed.

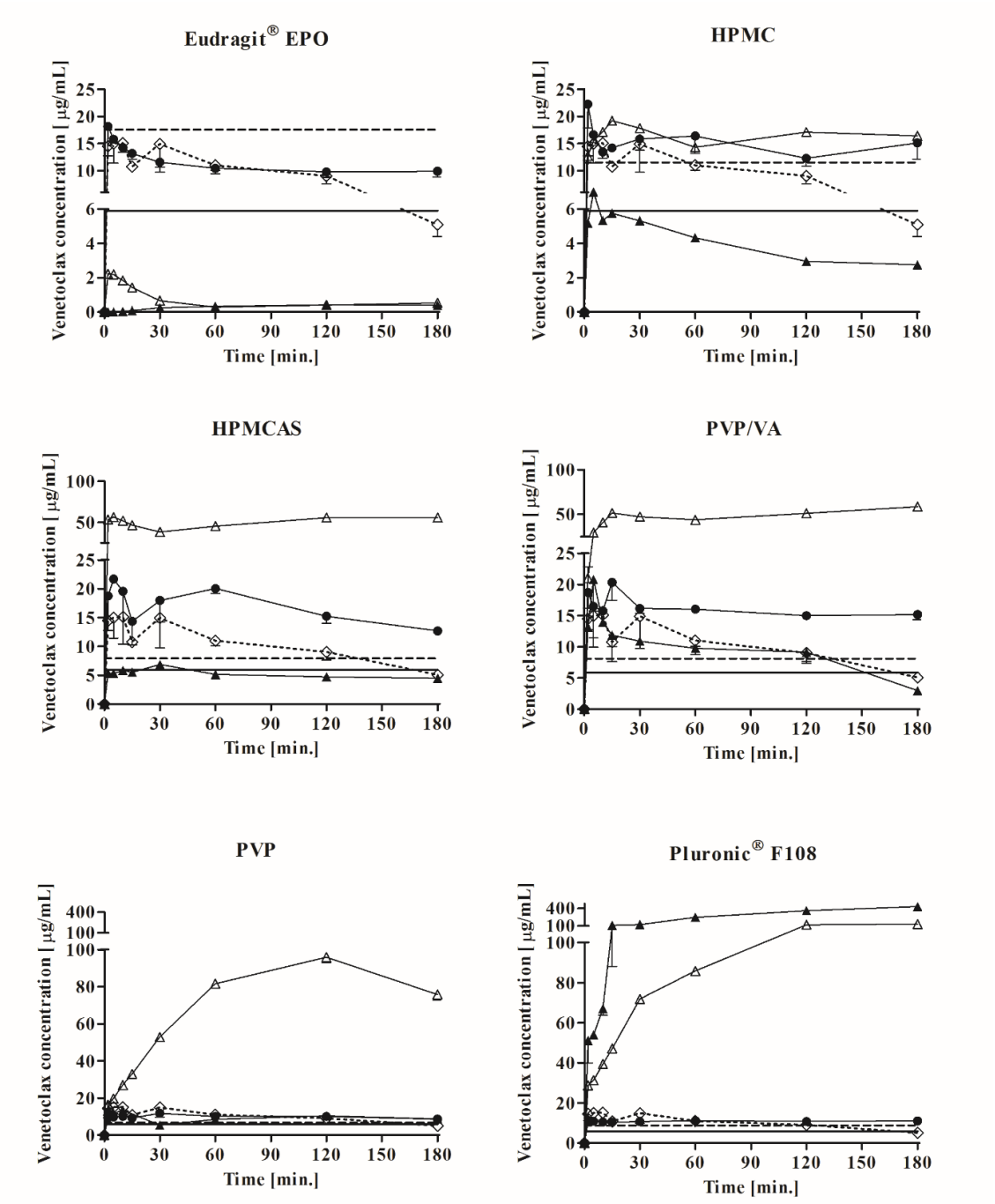



Figure 6-3 *In vitro* evaluation of venetoclax concentration profile during dispersion of sLBFs in FaSSIF (mean ± SD with n = 3). The dashed line represents the apparent venetoclax solubility in FaSSIF with pre-dissolved PI (FaSSIF-PI) and the solid line represents FaSSIF solubility. The dotted line (◊) represents the sLBF alone dispersed in FaSSIF (sLBF-noPI). Venetoclax dissolved in DMSO dispersed in FaSSIF-PI (●), sLBF dispersed in FaSSIF-PI (sLBF-aqPI) (▲), sLBF with PI (sLBF-PI) dispersed in FaSSIF (Δ).

In the case of the sLBF-noPI, a high initial concentration was observed (14.5 ± 1.7 µg/mL), which was above the FaSSIF solubility (5.2 ± 0.4 µg/mL). In addition, the initial venetoclax concentrations of sLBF-noPI was above FaSSIF-PI in the case of HPMC, PVP/VA, HPMCAS, PVP and Pluronic® F108 indicating that the investigated PI-free sLBF-noPI was able to generate supersaturation, i.e. a spring effect upon dispersion. The observed initial venetoclax concentrations, i.e. spring effect, of the sLBF-noPI were also similar to all the initial aqueous phase concentrations of the positive control with DMSO, which had the PIs pre-dissolved in the media. Relative to the FaSSIF solubility, an apparent supersaturation ratio of 2.2 ± 0.5 after 5 min of dispersion was observed for the sLBF-noPI (Table 6-2). The degree of supersaturation from the sLBF-noPI gradually decreased to 1.3 ± 0.2 after 2 h, whereas at 3 h the drug concentration had dropped to FaSSIF solubility. While the sLBF-noPI was not able to maintain supersaturated concentrations throughout the experiment, the use of PIs in the positive control maintained supersaturation ratios > 1 for up to 3 h. As the function of the PIs was intended as a 'parachute' to prolong supersaturation, the results indicated a beneficial precipitation inhibitory effect of PIs on the formulation performance.

Interstingly, the combination of PIs in sLBF (sLBF-PI) yielded a higher spring concentration in the case of HPMCAS, PVP, PVP/VA and Pluronic® F108 when compared to sLBF-noPI and the positive control with DMSO. While the sLBF-PI with HPMC showed an initial venetoclax concentration similar to sLBF-noPI, sLBF-PI with Eudragit® EPO resulted in a statistically significant lower venetoclax concentration in the media when compared to sLBF-noPI and DMSO ($p < 0.05$). In fact, the venetoclax concentration was below FaSSIF and FaSSIF-PI solubility when Eudragit® EPO and lipid were present simultaneously. In the case of dispersing sLBF into FaSSIF-PI (sLBF-aqPI), an increased initial venetoclax concentration

was only evident for Pluronic® F108 when compared to sLBF-noPI. All other PIs resulted in a similar or decreased initial concentration.

The impact of polymer-sLBF combinations on the ability to solubilise venetoclax and prolong supersaturation across six different PIs is summarised in Table 6-2 and Figure 6-3. In the case of HPMC, HPMCAS and Eudragit® EPO, the sLBF-aqPI resulted in a lower amount of solubilised venetoclax throughout the experiment compared to sLBF-noPI. Similarly, in comparison to DMSO controls the sLBF-aqPI resulted in a lower AUC in the case of Eudragit® EPO, HPMC, HPMCAS and PVP/VA. As an example, the amount of the solubilised venetoclax dose was 0.033 ± 0.002% for Eudragit® EPO in the case of sLBF-aqPI. In addition, sLBF-aqPI resulted in the case of Eudragit® EPO, HPMC, HPMCAS, PVP and PVP/VA in a similar or lower apparent supersaturation ratio compared to sLBF-noPI and the respective DMSO controls. In the case of Pluronic® F108, the amount of venetoclax solubilised and the apparent supersaturation ratio was increased compared to the sLBF-noPI and DMSO control. Nevertheless, overall the results of the sLBF-aqPI suggested that a pre-dissolved PI in FaSSIF was less effective at prolonging venetoclax supersaturation, indicating that concomitant administration (i.e. chase dosing) of a PI with a sLBF would not potentially offer benefits in terms of sustained high concentrations for absorption *in vivo*.

Table 6-2 Area under the curve (AUC) of the *in vitro* PI testing for sLBF dispersed in FaSSIF-PI (sLBF-aqPI), sLBF with PI incorporated in the formulation (sLBF-PI) and dispersed in FaSSIF, venetoclax dissolved in DMSO dispersed in FaSSIF-PI (DMSO) and sLBF alone dispersed in FaSSIF (sLBF-noPI), % solubilised venetoclax and apparent supersaturation ratio for sLBF-aqPI and sLBF-PI as well as FaSSIF-PI solubility (mean ± SD, n = 3).

| PI | AUC [mg*min/mL] | | | Apparent FaSSIF-PI solubility [µg/mL] | solubilised venetoclax [%] [a] | | apparent supersaturation ratio [b] | |
|---|---|---|---|---|---|---|---|---|
| | DMSO | sLBF-aqPI | sLBF-PI | | sLBF-aqPI | sLBF-PI | sLBF-aqPI | sLBF-PI |
| Eudragit® EPO | 1.9 ± 0.3 | 0.1 ± 0.004 | 0.1 ±0.02 | 17.6 ± 0.8 | 0.03 ± 0.002 | 0.1 ± 0.01 | < LOQ | 0.3 ± 0.03 |
| HPMC | 2.6 ± 0.5 | 0.7 ± 0.1 | 2.9 ±0.3 | 11.5 ± 2.4 | 0.4 ± 0.04 | 1.6 ± 0.2 | 0.9 ±0.2 | 2.3 ± 0.2 |
| HPMCAS | 3.0 ± 0.3 | 0.9 ±0.03 | 9.1 ± 0.4 | 7.9 ± 1.6 | 0.5 ± 0.02 | 5.1 ± 0.2 | 0.8 ± 0.1 | 8.3 ± 0.3 |
| PVP | 1.8 ± 0.3 | 1.5 ± 0.2 | 13.5 ± 0.1 | 6.7 ± 0.2 | 0.9 ± 0.1 | 7.5 ± 0.04 | 1.7 ± 0.2 | 2.9 ± 0.4 |
| PVP/VA | 2.8 ± 0.2 | 1.6 ± 0.2 | 8.8 ± 0.3 | 8.1 ± 1.4 | 0.9 ± 0.1 | 4.9 ± 0.2 | 3.0 ± 1.6 | 4.3 ± 0.3 |
| Pluronic® F108 | 2.0 ± 0.4 | 50.4 ± 2.4 | 17.5 ± 0.6 | 8.9 ± 0.2 | 28.1 ± 1.3 | 9.8 ± 0.3 | 7.9 ± 0.2 | 4.6 ± 0.2 |
| sLBF-noPI | | 1.8 ± 0.3 | | | 1.0 ± 0.2 | | 2.2 ± 0.5 | |

[a] % solubilised was calculated by dividing the AUC of the concentration versus time profiles by the maximum AUC, i.e. representing 100% solubilised, over the same period of time.

[b] Apparent supersaturation ratio of venetoclax after 5 min dispersion (determined venetoclax concentration relative to FaSSIF solubility)

In the case of incorporating HPMC, HPMCAS, PVP, PVP/VA and Pluronic® F108 within the sLBF higher amount of venetoclax was solubilised compared to sLBF-noPI. Furthermore, the sLBF-PIs resulted in a higher amount of solubilised venetoclax in comparison to the sLBF-aqPI. In addition, higher apparent supersaturation ratios (after 5 min of dispersion) were observed for sLBF-PI relative to sLBF-noPI. In the case of HPMC, HPMCAS, PVP and PVP/VA the apparent supersaturation ratios were higher compared to the sLBF-aqPI indicating that these sLBF-PI combinations achieved a balance between generating and maintaining supersaturated concentrations. However, Pluronic® F108 and Eudragit® EPO did not follow this trend. Pluronic® F108 showed a higher supersaturation ratio and % solubilised venetoclax for the sLBF with pre-dissolved Pluronic® F108 (sLBF-aqPI), compared to the incorporation into the sLBF (sLBF-PI). Such high venetoclax concentrations and supersaturation ratios in the presence of Pluronic® F108 may be attributed to the surfactant nature of the polymer, which can increase the solubilisation capacity of the test media, especially when pre-dissolved. In the case of Eudragit® EPO, a significantly decreased amount of solubilised venetoclax and supersaturation ratios < 1 in all cases of lipid excipient-PI combinations were observed.

Based on the amount of venetoclax solubilised in the *in vitro* PI test, the general performance ranking was Pluronic® F108 > PVP > HPMCAS ≥ PVP/VA > HPMC > Eudragit® EPO in the case of sLBF-PI and Pluronic® F108 > PVP/VA ≥ PVP ≥ HPMCAS ≥ HPMC > Eudragit® EPO in the case of sLBF-aqPI. While this performance ranking was not in line with the *in silico* calculated COSMO-Rank, it should be acknowledged that Eudragit® EPO was not able to generate supersaturated venetoclax concentrations in the presence of the lipid excipient. Therefore, this particular PI, may not be suitable to enhance the sLBF performance. Subsequently, the relationship between the *in vitro* determined solubilised venetoclax and the *in silico* calculated COSMO-Rank was reassessed without Eudragit® EPO. The results for

sLBF-PI and sLBF-aqPI are summarised in Figure 6-4. It was evident that there is a trend that a higher COSMO-Rank, i.e. higher interaction between venetoclax and the PIs resulted in a higher supersaturation ratio in the aqueous phase of the *in vitro* test in the case of sLBF-PI. From the *in silico* screen and *in vitro* experiments a relationship between COSMO-Rank and venetoclax solubilisation and supersaturation was less evident for sLBF-aqPI (no relationship was observed after 180 min). The results of both approaches (*in silico* and *in vitro*) indicated that for lipid systems an overly strong interaction between the drug and the PI may be less favourable and in the case of Eudragit® EPO resulted in a depletion of aqueous venetoclax concentrations.

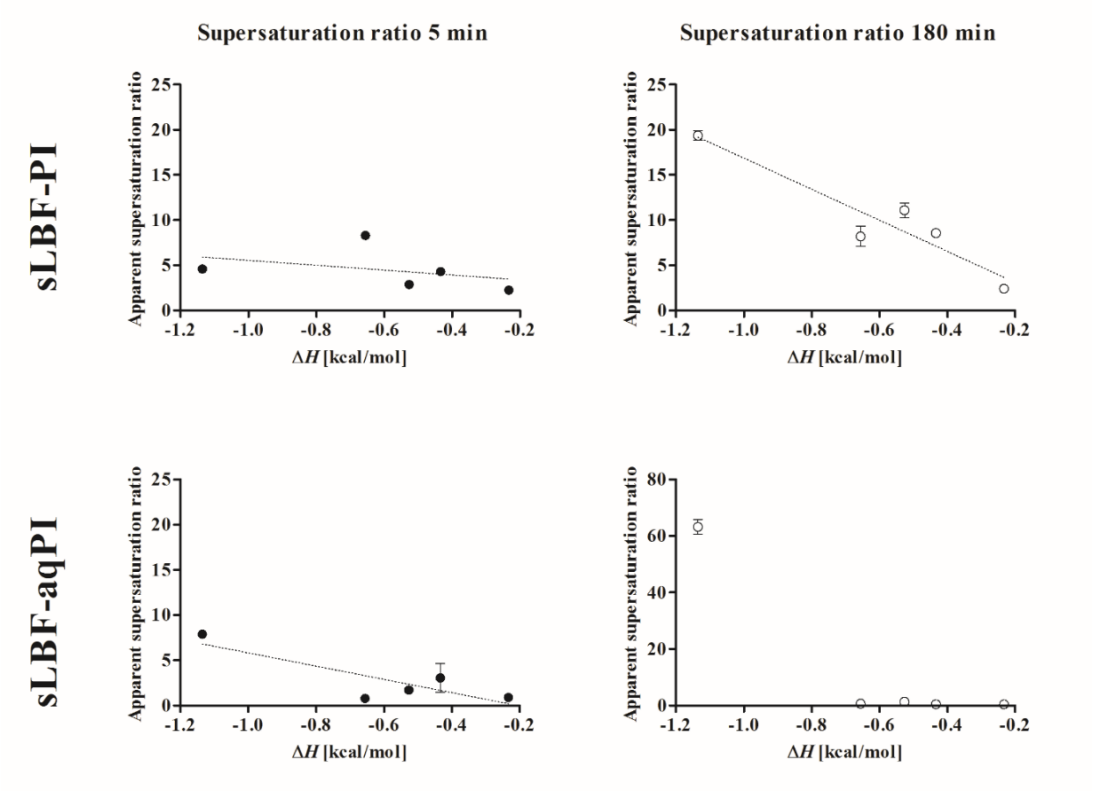



Figure 6-4 Relationship between excess enthalpy of mixing calculating *in silico* using COSMOquick and the *in vitro* apparent supersaturation ratio after 5 min and 180 min, respectively for sLBF with incorporated PI added to FaSSIF (sLBF-PI) and sLBF that was added to FaSSIF-PI (sLBF-aqPI). Eudragit® EPO was excluded from the dataset due to the inability to generate supersaturation (mean ± SD, n = 3).

### 6.4.5 Impact of PIs on *in vivo* bioavailability of venetoclax from sLBFs

The aim of the *in vivo* study was to explore whether the *in vitro* observations would translate to *in vivo* using sLBF-PI across six different PIs. Venetoclax formulations (50 mg/mL) were prepared as a supersaturated Peceol® solution alone (sLBF-noPI) or in combination with HPMC, HPMCAS, PVP, PVP/VA, Pluronic® F108, Eudragit® EPO at a ratio of 1:1, respectively. PVP, PVP/VA, Pluronic® F108 and Eudragit® EPO were readily soluble in the sLBF, whereas HPMC and HPMCAS were not soluble in the sLBF and were therefore mixed in the sLBF prior to oral administration. An oral dose of 100 mg venetoclax was used. Absolute bioavailability was determined with reference to intravenous data that has been previously reported for landrace pigs [170]. The absolute bioavailability as a function of each formulation is shown in Figure 6-5 and the associated pharmacokinetic parameters presented in Table 6-3. Individual plasma profiles for all tested formulations are shown in Figure III-2 and Figure III-3 in the appendix. While it was only feasible to conduct a full six by six way cross-over study in this experiment facility, a seventh study leg using Eudragit® EPO was performed in a separate group of three pigs. In this case only mean comparisons were possible, whereas for all other groups formulation differences within each pig were performed.

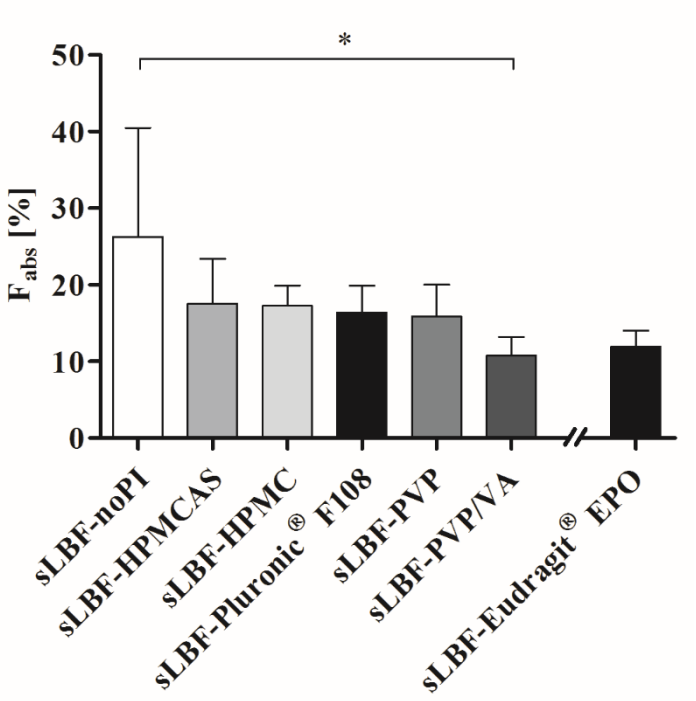



Figure 6-5 Absolute bioavailability ($F_{abs}$) in landrace pigs for 100 mg venetoclax as a 6-way crossover with sLBF-noPI, sLBF-HPMC, sLBF-HPMCAS, sLBF-PVP, sLBF-PVP/VA, sLBF-Pluronic® F108 and an additional study including sLBF-Eudragit® EPO. All data is presented as mean ± SD, where n = 5 (except sLBF- Eudragit® EPO, where n = 3).

The sLBF-noPI showed the highest mean bioavailability (26.3 ± 14.2%) compared to all other tested lipid formulations. An increased bioavailability was not observed by incorporation of PIs but rather a trend towards a decreased oral bioavailability, albeit only the sLBF-PVP/VA formulation displayed statistically significant lower bioavailability relative to the sLBF-noPI ($p < 0.05$). The rank order of the oral bioavailability was sLBF-noPI ≥ sLBF-HPMCAS ≥ sLBF-HPMC ≥ sLBF-Pluronic® F108 ≥ aLBF-PVP ≥ sLBF-Eudragit® EPO ≥ sLBF-PVP/VA. The observed bioavailability for sLBF-noPI is in agreement with previously reported venetoclax bioavailability in fasted pigs (17.3 ± 5.5%). The $t_{max}$, MRT and MAT tended to increase with lower oral bioavailability, however, this observation is not statistically significant. Overall the results showed that the use of PIs did not enhance the sLBF formulation performance *in vivo*.

Table 6-3 Pharmacokinetic parameters of venetoclax after oral administration of 100 mg in landrace pigs. Venetoclax was administered in a 6-way crossover as a supersaturated Peceol® solution (sLBF-noPI), and as sLBF with HPMC (sLBF-HPMC), HPMCAS (sLBF-HPMCAS), PVP (sLBF-PVP), PVP/VA (sLBF-PVP/VA), Pluronic® F108 (sLBF- Pluronic® F108) and in a 2-way crossover including Eudragit® EPO (sLBF- Eudragit® EPO), respectively. $t_{max}$, MAT and MRT are given as median (range), all other parameters as mean ± SD (n = 5, except sLBF-Eudragit® EPO, where n = 3).

| **Pharmacokinetic parameters** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | sLBF-noPI | sLBF-HPMC | sLBF-HPMCAS | sLBF-PVP | sLBF-PVP/VA | sLBF-Pluronic® F108 | sLBF-Eudragit® EPO [b] |
| $c_{max}$ [µg/mL] | 1.38 ± 0.84 | 0.92 ± 0.24 | 0.83 ± 0.20 | 0.81 ± 0.19 | 0.48 ± 0.67 | 0.80 ± 0.20 | 0.51 ± 0.28 |
| $t_{max}$ [h] (range) | 6 (2 – 10) | 6 (6-8) | 8 (7-10) | 8 (6-8) | 9 (8-10) | 9 (6-10) | 10 (7-10) |
| AUC 0 h – inf. [µg*h/mL] | 11.40 ± 6.15 | 7.39 ± 1.40 | 7.69 ± 2.53 | 6.90 ± 1.83 | 4.73 ±1.08 | 7.19 ± 1.50 | 5.23 ± 1.60 |
| MRT [h] (range) | 8.64 (7.41 – 14.20) | 10.17 (9.43 – 15.23) | 10.96 (9.69 – 12.03) | 10.44 (9.49 – 11.71) | 11.48 (8.67 – 11.73) | 10.62 (8.54 – 12.58) | 13.67 (10.82 - 15.62) |
| MAT [h] (range) | 4.76 (3.53 – 10.32) | 6.29 (5.55 – 11.35) | 7.08 (5.81 – 8.15) | 6.56 (5.62 – 7.83) | 7.61 (4.79 – 7.86) | 6.74 (4.66 – 8.70) | 9.79 (6.94 – 11.74) |
| $F_{rel}$ [%] [a] | 100 | 76.62 ± 32.34 | 78.86 ± 37.81 | 72.22 ± 30.56 | 52.95 ±29.23 | 80.24 ± 42.16 | 68.87 ± 21.01 [c] |
| $F_{abs}$ [%] | 26.28 ± 14.20 | 17.25 ± 2.63 | 17.57 ± 5.80 | 15.84 ± 4.18 | 10.76 ± 2.44 | 16.39 ± 3.47 | 11.95 ± 3.64 |

[a] Relative to sLBF.
[b] sLBF-Eudragit® EPO originated from a second *in vivo* study. sLBF for the second *in vivo* study has previously been reported (chapter 5) [176].
[c] Relative to a previously reported sLBF (chapter 5) [176].

## 6.5 Discussion

Bio-enabling formulations that enhance the extent of oral drug absorption are increasingly required to meet the challenge of poor water-soluble properties of drugs emerging from discovery pipelines. Drug candidates which fail to meet Lipinski's Rule-of-Five criteria are a common target for such bio-enabling formulation strategies [167] due to poor biopharmaceutical properties, resulting from low aqueous solubility and/or poor permeability. Ideally, a formulation design that rapidly generates elevated drug concentrations in the gastrointestinal fluids, i.e. that generates the so-called 'spring effect' to increase the concentration above the saturation solubility in gastrointestinal fluids and therein promote absorption. In order to maintain such high drug concentrations, PIs have been used to delay the onset of precipitation, i.e. the so-called parachute [188; 189]. This fundamental advantage of prolonged supersaturated drug concentrations in the intraluminal environment is assumed to be beneficial for absorption *in vivo* [190; 191; 189]. However, the choice of suitable PIs can be complicated [179] as the inhibitory effect is reported to be drug specific and there are gaps in our understanding which PIs are suited for a particular drug type [180; 189]. While various *in vitro* screening tests have been reported, to date the PI selection is mostly empirical and there is a lack of comprehensive studies comparing across all the various PI types [189]. Attempts have been made to increase the mechanistic understanding to aid with the selection of PIs to streamline formulation development [189], however, it is often not known how effective an *in vitro* tool is in estimating the impact on *in vivo* absorption, especially for rather complex bio-enabling formulations. In addition, given the complexity of polymer-drug interactions, there is also a need for more computational tools to guide excipient selection.

In recent years, LBF approaches have been mechanistically described as 'supersaturable' drug delivery systems, on the basis of generating supersaturation following dispersion/digestion in

the intraluminal environment. Indeed, it is widely recognised that dispersion/digestion can present a key risk to prolonged supersaturation, where there is a lower solubilisation capacity of the post-digestive milieu. In particular, LBFs that contain high % of co-solvents (such as LFCS type IV systems) are considered to be at greatest risk of drug precipitation in the gastrointestinal tract. It has been shown for such type IIIB/IV LBF systems that the addition of an excipient to prolong supersaturation can lead to enhanced bioavailability [177-180]. The present study, therefore, aimed to explore the utility of PIs to enhance performance for supersaturated lipid solutions (sLBFs). Such systems are being explored particularly for poorly water-soluble drugs when a high drug load and exposure is needed for example in pre-clinical development studies. Our group and others have previously shown that sLBFs enhance oral bioavailability of poorly water-soluble drugs, albeit there is a perceived risk of drug precipitation due to the systems being considered merely kinetically stable. To address this, in the current study, the utility of several PIs to enhance formulation performance of a sLBF of venetoclax has been evaluated *in vitro* and *in vivo*. We further calculated the excess enthalpy of mixing of the drug and various polymers to approximate the interaction between venetoclax and the PIs in a more complex aqueous environment. This approach facilitated a rationalisation of *in vitro* testing, which was based on a solvent shift to assess the precipitation inhibitory effect.

The *in vitro* lipolysis confirmed the ability of sLBF to generate supersaturated aqueous phase concentrations of venetoclax during dispersion and digestion, which exceeded the experimentally determined apparent solubility in FaSSIF (Figure 6-1) and the reported values for the experimentally determined amorphous solubility in FaSSIF (20.7 ± 0.5 µg/mL, pH 5.3; 33.7 ± 13.5 µg/mL, pH6.9) and FeSSIF (26.4 ± 0.2 µg/mL, pH 5.3; 54.6 ± 2.0 µg/mL, pH 6.9) [99]. Such elevated concentrations were not observed for an aqueous and lipid-based suspension and therefore confirm the ability of sLBF to generate supersaturated drug

concentrations on dispersion/digestion in intestinal fluids. Additionally, as a class III glass former (chapter 5) [176] and due to the high molecular weight, venetoclax tends to crystallise more slowly. Moreover, venetoclax has been reported to undergo liquid-liquid-phase separation at concentrations above the amorphous solubility and it was assumed that supersaturation is maintained for a duration that is physiologically promising (24 h) [99]. While in the case of the sLBF the physical state of the drug in such a separated drug-rich phase is unknown it can potentially serve as a reservoir of absorbable drug [99]. The co-existence of a drug rich phase and an aqueous phase at amorphous solubility (which were not separated) may explain the amount of venetoclax measured above the amorphous solubility in this test setup.

The *in vitro* PI screening method revealed that the incorporation of the PI into the sLBF resulted in higher venetoclax concentration in the aqueous phase compared to the addition of sLBF to the media containing pre-dissolved PI. Interestingly, the sLBF formulation demonstrated an initial supersaturated venetoclax concentration, which was maintained for up to 2 h even in the absence of a PI (i.e. sLBF-noPI). These findings are in line with the observations during *in vitro* lipolysis and confirm the ability of sLBF approaches to generate supersaturation, i.e. to act as a spring. In the case of PVP/VA, HPMCAS, PVP, Pluronic® F108 and HPMC, the incorporation of the PIs into the sLBF (sLBF-PI) proved beneficial for venetoclax resulting in prolonged supersaturation *in vitro* (apparent supersaturation ratio > 2.4 for all polymers after 180 min of dispersion). However, the incorporation of Eudragit® EPO resulted in a decrease in venetoclax concentration in the aqueous phase, to below FaSSIF solubility (0.08-fold reduction relative to FaSSIF solubility). It was also noticeable that the sLBF-Eudragit® EPO was poorly dispersible in FaSSIF, forming a two-phase system with drug rich agglomerates dispersed in buffer (Figure III-4). In contrast, all the sLBF-noPI and sLBFs containing PIs dispersed consistently in FaSSIF to form a homogenous dispersion on mixing. One possible explanation

for the significant lower venetoclax concentrations observed for the sLBF-Eudragit® EPO system may reflect the poor dispersion in FaSSIF. This observation suggested that venetoclax may have remained within the lipid-rich agglomerates and was not released from the formulation into the aqueous phase. Additionally, Eudragit® EPO (in the unionised form) and venetoclax are relatively lipophilic and interact strongly with each other, as indicated by the calculated negative excess enthalpy of interaction with the *in silico* tool, which may have further promoted the drug retention in the lipid phase. Another aspect is that the unionised Eudragit® EPO was not expected to swell in aqueous medium and such a swelling of a more hydrophilic polymer is likely to contribute to the performance of a PI. This complexity in aqueous medium was not captured by the simple mixing enthalpy calculations of binary drug-polymer systems.

The *in vivo* study demonstrated that the highest oral bioavailability was obtained with the sLBF-noPI. The sLBF-PI formulations showed a trend towards a decreased oral bioavailability, when compared to sLBF-noPI. While the overall bioavailability of 26.3 ± 14.2% is in line with previous reports of venetoclax bioavailability in large animal models [98; 176], the results of the present study were unexpected since previously published studies exploring the inclusion of PIs with LBF resulted in an increased bioavailability [177-179]. However, in the previous reported studies LFCS type IIIB/IV systems were used, which contained high amounts of co-solvents and exhibit a high risk of precipitation due to the dilution effect upon dispersion [180]. Therefore, this confirmed that the ability of an oil-only sLBF to generate supersaturated concentrations of venetoclax *in vitro* was translated to increased absorption *in vivo* and that the duration of supersaturation for the sLBF was sufficient to obviate the inclusion of a PI. This observation that a PI was not required may be specific for venetoclax, given that the drug as a class III glass former has a low tendency to crystallise [99]. Hence, for other drugs such as poor glass formers

as well as sLBF containing higher proportions of co-solvents further studies are required to assess whether sLBF approaches with PIs are needed to maximise absorption and mitigate a perceived risk of precipitation *in vivo*.

The median MAT for the sLBF-PI formulations was higher among all PI containing formulations relative to sLBF-noPI (Table 6-3). Overall, given the variability in absorption rate of venetoclax in each group, no statistically significant differences were observed. However, it would appear that the inclusion of a PI may present a risk of a delayed absorption that may reflect a delay of drug release from the formulation. An explanation for this observation might be a combined effect of the extent of drug-polymer interaction and a reduced polymer swelling. The incorporation of the PI into the sLBF may have reduced polymer swelling (which normally happened in the aqueous media) and caused trapping of the drug. In addition, the interaction between the PIs and the drug reduces diffusion of both PI and drug and hence the partitioning of venetoclax and PIs from the sLBF to the aqueous phase. The partitioning may be further reduced due to an increased viscosity of the sLBF-PI formulations, which further decreases diffusion, but also dispersibility and subsequently digestibility. A less dispersed LBF exhibits a lower surface area, which potentially leads to less drug released from the sLBF. However, further studies are needed to explore the effect of viscosity on sLBF performance *in vivo*.

From the *in vivo* data it appears that the solubility of the polymer in the sLBF may have impacted the bioavailability. In the cases of PIs being soluble in the sLBF, i.e. Pluronic® F108, PVP, PVP/VA and Eudragit® EPO dissolved completely in the sLBF, a lower bioavailability was observed when compared to the PIs that showed a lower solubility in the sLBF, i.e. HPMC and HPMCAS formed suspensions in the sLBF. One explanation for this observation may be that a PI that is soluble in the lipid vehicle can, in combination with a high drug affinity of the

PI, lead to a drug retention in the vehicle instead of showing a more favourable PI functionality of reducing precipitation once the sLBF has dispersed in the intraluminal fluids. On the other hand, a more hydrophilic polymer that is suspended in the sLBF may lead to polymer swelling upon aqueous dispersion to allow for drug release and the intended PI functionality. Further studies exploring polymer solubility in sLBF and its effect on drug release may therefore be merited to fully predict the impact of PIs on *in vivo* performance.

Overall, a relationship between the *in silico* calculated excess enthalpy of mixing and the *in vitro* determined supersaturation ratios and amounts of venetoclax solubilised was established in the case of PIs that generated supersaturated concentrations. This study showed that with increasing 'COSMO-Rank' (i.e. a more negative excess enthalpy of mixing), higher apparent supersaturation ratios and higher amounts of solubilised venetoclax were obtained. Since Eudragit® EPO resulted in undersaturated aqueous solutions (venetoclax concentration below FaSSIF saturation solubility) due to a lower release from the formulation, the PI was not considered in the analysis of the relationship between *in silico* and *in vitro*. However, the result of Eudragit® EPO showed a simple consideration of high negative excess enthalpy for a more lipophilic polymer in sLBF should be interpreted with care regarding PI performance.

The deviation of the *in vivo* results from the *in silico* calculations by the COSMO-quick software may reflect an oversimplification of calculated parameters. The calculations considered the interactions between the drug and the PI, but the interaction between the drug or the PI with formulation excipients, water or the components of gastrointestinal fluids such as bile salts and phospholipids were not taken into account. While this might have not been crucial for an *in vitro* dispersion experiment, the digestion of the lipid excipients *in vivo* further increased the complexity of the gastrointestinal fluids, by releasing fatty acids and other

digestion products in the gastrointestinal environment. It is, hence, unclear whether a drastic simplification to solely the drug and PI by the selected COSMOquick approach is applicable for LBFs to define PIs. Nevertheless, the current *in silico* approach may be useful for type IV LBFs/sLBFs that contain less digestible excipients, i.e. co-solvents, and may be a quick screening tool to reduce the initial PI choice to a reasonable number, which subsequently can be tested *in vitro* and *in vivo*. Furthermore, the complementing *in vitro* and *in silico* techniques used in the present study may be helpful in understanding the formulation behaviour of sLBFs *in vivo*.

A lack of PI impact on increasing bioavailability may also reflect that the *in vitro* model was poorly predictive of the *in vivo* situation. The reasons for the *in vitro* test not being predictive of a reduce overall drug absorption in the presence of PIs can be manifold such as (a) more complex intestinal conditions *in vivo* [77], (b) a lack of absorptive sink *in vitro* [192] or (c) a venetoclax specific effect. The employed *in vitro* test exhibited a high drug and lipid load as well as higher hydrodynamics compared to *in vivo*. In addition, a solvent shift may meet the industry need for a fast screening tool, however, in the case of LBFs it is not physiologically relevant. A combined dispersion-digestion setup [180] or a dispersion-digestion setup with absorptive sink [83; 87; 84] may provide more mechanistic, albeit lower throughput, results. A further limitation of the study may have been the incorporation of the PI into the sLBF. While this decision was guided by *in vitro* data, in light of the *in vivo* results it is unclear whether separating the PI and sLBF may have conferred advantages *in vivo*.

## 6.6 Conclusion

The formulation approach of using PIs to prolong supersaturation is well recognised for amorphous formulations, but less well explored for sLBFs. The present study, applied *in silico*,

*in vitro* and *in vivo* models to extend the concept of PIs in an oil-only sLBF. An *in silico* tool was successfully used for an initial PI selection and aided in explaining the low free venetoclax concentration *in vitro* in case of the sLBF with Eudragit® EPO. It was found that the strong predicted interaction between the drug and the polymer may have led to an overall reduction in drug release from the formulation. In addition, the *in vitro* PI screening tool showed that the incorporation of the PI into the sLBF yielded higher free drug concentrations compared to the separate addition of the PI. While the *in vitro* screening showed prolonged supersaturated concentrations in the presence of PIs in five out of six cases, *in vivo* a trend towards a lower overall bioavailability was observed for PI containing formulations, indicating that incorporating a PI into the sLBF was not necessary. The oral bioavailability of venetoclax was highest for the PI free sLBF-noPI, which suggests that reduced oral absorption due to precipitation from an oil-only sLBF was low.

# 7 Chapter 7: Lipophilic salts and lipid-based formulations for bridging the food effect gap of venetoclax

**Niklas J. Koehl[1], Laura J. Henze[1], René Holm[2,3], Martin Kuentz[4], John J. Keating[1], Thomas De Vijlder[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

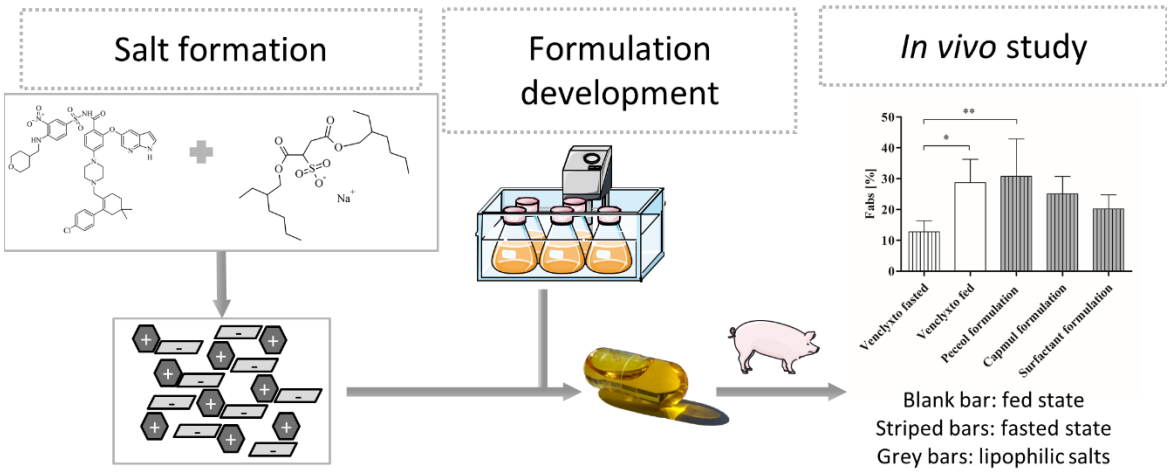
[3] Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark

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

[5] Small Molecule Pharmaceutical Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

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## 7.1 Abstract




Graphical abstract 7-1 Lipophilic salt preparation to overcome drug loading limitations of venetoclax in LBFs and to enhance bioavailability in order to overcome food dependent exposure of venetoclax.

**Purpose** LBFs have shown to overcome food dependent bioavailability for some poorly water-soluble drugs. However, the utility of LBFs can be limited by low dose loading due to a low drug solubility in LBF vehicles. This study investigated the solubility and drug loading increases in LBFs using lipophilic counterions to form lipophilic salts of venetoclax.

**Methods** Venetoclax docusate was formed from venetoclax free base and verified by $^{1}$H NMR. Formation of stable venetoclax-fatty acid associations with either oleic acid or decanoic acid were attempted, however the molecular associations were less consistent based on $^{1}$H NMR.

**Results** Venetoclax docusate displayed a up to 6.2-fold higher solubility in SEDDS when compared to the venetoclax free base solubility resulting in a higher dose loading. A subsequent bioavailability study in landrace pigs demonstrated for the lipophilic salt containing long chain SEDDS a 2.5-fold higher bioavailability compared to the commercially available solid dispersion Venclyxto® in the fasted state. The bioavailability of all lipophilic salt SEDDS in the fasted state was similar to Venclyxto® in the fed state.

**Conclusion** This study confirmed that lipophilic drug salts increase the dose loading in LBFs and showed that lipophilic salt-SEDDS combinations are able to overcome bioavailability limitations of drugs with low inherent dose loading in lipid vehicles. Furthermore, the present study demonstrated the utility of a LBF approach, in combination with lipophilic salts, to overcome food dependent variable oral bioavailability of drugs.

## 7.2 Introduction

Current drug discovery programs increasingly result in poorly water-soluble drugs as a consequence of high throughput screening technology and a targeting of more lipophilic receptor types [3]. In addition, many poorly water-soluble drugs fall outside the classical Lipinski Rule-of-Five drug discovery space [193; 167], which poses a particular challenge during drug development to reach sufficient drug exposure upon oral administration. High oral bioavailability is especially critical for high doses of pre-clinical studies, such as required during early pre-clinical toxicity evaluation. A key requirement for poorly water-soluble drugs is the need for bio-enabling formulation approaches to maximise *in vivo* bioavailability. LBFs are one such approach that have been shown to increase drug solubilisation *in vivo* and enhanced oral absorption [102]. Interestingly, it was also demonstrated that LBFs reduce the food dependent variable bioavailability of various drugs [116] such as cyclosporin (Neoral®, Novartis) [194] or isotretinoin (Absorica®, Sun Pharma) [195]. The presence of lipids in the gastrointestinal tract leads to enhancement of drug solubility due to the alteration of gastrointestinal fluid composition (i.e. bile salt and phospholipid secretion), recruitment of lymphatic transport as well as the interaction with drug transport processes [18]. However, the merits of LBFs are limited to drugs with sufficient lipid solubility to dissolve the entire dose in the lipid vehicle. In general, ‘grease ball’ type drugs are favoured for LBFs, due to high lipophilic characteristics, to facilitate favourable solvation in lipids and low $T_m$ to ensure that

energy for disruption of the crystal lattice is not limiting [52]. However, for drugs which display high crystal lattice energy, i.e. high $T_m$ drugs, dose loading in LBFs will be limited and strategies to increase dose loading in LBF approaches such as sLBFs [31; 32; 34] or lipophilic salts/ionic liquids [60; 35-38; 40-42] have been proposed.

The combination of a lipophilic drug salt and lipid SEDDS as a bio-enabling approach is promising as it was shown that both drug dose loading and oral bioavailability can be increased [37; 38; 40; 41]. Lipophilic salts take advantage of a lipophilic and bulky counterion that decreases the crystal packing due to stereochemical hinderance and increases the solubility of the salt in lipid excipients [43]. In general, the decreased crystal energy is reflected by a lower $T_m$ of the lipophilic salt compared to the free base. Recent work suggested a possible advantage, in terms of higher lipid solubility, of longer chain length and chain branching of alkyl sulfate counterions for basic drugs [196]. Lipophilic salts with a $T_m < 100$ ºC are so-called ionic liquids, which are considered favourable in LBFs as the lower the hydrophobic burden from the crystal lattice, the potentially higher is the dose loading in a LBF. For example, in the case of itraconazole, the solubility of the lipophilic drug salts in a medium and long chain-based SEDDS was increased with decreasing $T_m$ [37]. The formulation strategy of lipophilic salts have been applied to a variety of drugs (18 in total [43]) belonging to all classes of the BCS such as ceritinib [40], amlodipine [41], metformin [41] and itraconazole [37]. The *in vivo* performance of lipophilic salt/ionic liquid-SEDDS combinations are drug-dependent, but in general have shown to result in a higher oral bioavailability in pre-clinical studies when compared to an aqueous or lipid suspension (Table 1-2). For example, an itraconazole docusate SEDDS solution resulted in a 2.5-fold higher oral bioavailability compared to the commercially available, itraconazole free base containing Sporanox® (amorphous solid dispersion with HPMC, spray dried on a sugar spheres, Janssen-Cilag Ltd) in rats [37]. In fact, the oral

bioavailability of itraconazole was 21.6-fold higher compared to a physical mixture (suspension) of the drug and docusate (counterion) in the same SEDDS formulation, thereby demonstrating that significant bioavailability increases can be achieved when formulating the salt form/ionic liquid in a SEDDS solution [37]. In the same study, a cinnarizine decylsulfate SEDDS solution was evaluated *in vivo* in rats at high (equivalent to 125 mg/kg cinnarizine) and low dose (equivalent to 35 mg/kg cinnarizine) and the oral bioavailability was compared to a cinnarizine free base SEDDS suspension at high dose (125 mg/kg) and a SEDDS solution at low dose (35 mg/kg). While the ionic liquid-SEDDS solution showed a 1.8-fold increase in oral bioavailability relative to a SEDDS suspension at high dose, the bioavailability was similar in the case of the ionic liquid-SEDDS solution and the SEDDS solution at low dose [37]. This showed that lipophilic salts are a viable approach to increase dose loading in a LBF/SEDDS, however, the salt form itself is not necessarily improving the bioavailability of the drug. In another study by the same group, the oral bioavailability of ionic liquid drug salts of tolfenamic acid (using a variety of ammonium counterions such as octadecylammonium) was even decreased compared to a tolfenamic free acid aqueous suspension [39]. It was concluded from this study that the partitioning of tolfenamic acid from the SEDDS excipients into the aqueous phase was reduced, resulting in decreased oral bioavailability. These studies demonstrated that lipophilic salts are able to increase lipophilicity and decrease hydrophobicity (originating from the crystal lattice of the free drug form) of a drug substance resulting in higher dose loadings, but this may come with a risk of incomplete release due to retaining of the drug within the lipid matrix.

In the current study, the BCS class IV drug venetoclax [99], a selective B-cell lymphoma-2 inhibitor licenced for the treatment of leukaemia in 2016 [96], was used as a model compound. Venetoclax displays a high lipophilicity (log*P* 5.5) [96], a mid-range $T_m$ (138 ºC onset) [97] and

relatively high molecular weight (868.44 g/mol) [96]. The solubility of venetoclax in aqueous buffer was reported to be < 0.0042 μg/mL at pH 7.4 [96] and in various medium and long chain triglycerides < 1 mg/mL (chapter 5 and 6). With a single dosage form containing 100 mg (final daily dose of 400 mg), the drug therefore is insufficiently soluble in lipid vehicles and it would therefore benefit from a combined lipophilic salt/LBF approach. The commercialised venetoclax free base formulation, Venclyxto® (AbbVie Deutschland GmbH & Co. KG), is an orally administered tablet which shows a profound food effect with a 3.4-fold increase in oral bioavailability after a low-fat meal and a 5-fold increase after a high fat meal compared to the fasted state [96]. Venclyxto® is a solid dispersion of venetoclax free base in copovidone [97] and must be taken with food to achieve an adequate bioavailability, which suggested that lipids may be advantageous for enhancing oral absorption of venetoclax. Our rational is, therefore, to develop a LBF strategy to overcome food dependent bioavailability of venetoclax. In addition, as a bulky, high molecular weight drug that exhibit high lipophilicity but low lipid excipient solubility, the ability to produce lipophilic salts or co-crystals of venetoclax to increase dose loading was evaluated. Finally, the potential of a lipophilic salt-SEDDS combination as a food-independent formulation approach was evaluated *in vivo* in a porcine food effect study.

## 7.3 Materials and methods

### 7.3.1 Chemicals and materials

Venetoclax was purchased from Kemprotec Ltd. (UK) (Batch # 180620). Venclyxto® (100 mg, tablet, AbbVie Deutschland GmbH & Co. KG) were sourced through a local pharmacy. A sample of Peceol® was kindly donated by Gattefossé (France). Kolliphor® RH40, Tween® 85, oleic acid, *n*-decanoic acid, sodium docusate, sodium sulfate, were obtained from Sigma

Aldrich (Ireland) and used without further purification. Sodium taurocholate hydrate was obtained from Thermo Fisher Scientific (UK) and Lipoid® E PC S was obtained from Lipoid GmbH (Germany). All other chemicals and solvents were of analytical or HPLC grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 7.3.2 Preparation of FaSSIF

FaSSIF version 1 was prepared according to previously published instructions [197; 198]. In brief, 1.65 g sodium taurocholate was dissolved in 200 mL FaSSIF buffer without any bile components. To this was added, 5.9 mL of a solution containing 100 mg/mL lecithin in methylene chloride to form an emulsion, and the methylene chloride was removed under vacuum at 40 ºC until the solution was clear. The volume was adjusted to 1 L with FaSSIF buffer.

### 7.3.3 Formulation development

SEDDS formulations for the *in vivo* pig study were developed using a design of experiment (DoE) approach employing the software package MODDE Pro® 12.1. All formulation development was based on the free base of venetoclax instead of the lipophilic salt. The formulations contained Peceol®, Cremophor® RH40 and Tween® 85 as factors. The three factors were varied from 0.1 – 0.5 (w/w) for Peceol® and 0.2 - 0.7 (w/w) for Cremophor® RH40 and Tween® 85. A total of 12 different runs and 3 centre points were tested for three responses, i.e. dispersibility time, micelles/droplet size and solubility of venetoclax free base in a dispersion of the formulation containing 1 mL formulation in 150 mL FaSSIF. The optimiser tool was used to find the optimum formulation with a short dispersion time and a high solubility upon dispersion utilising a partial least square (PLS) regression model. Finally, the lipid

excipient in the prototype formulation was varied from long chain (Peceol®) to medium chain (Capmul MCM®) and no lipid in order to maintain the surfactant to lipid and surfactant to surfactant ratio (w/w). Solubility of venetoclax free base and venetoclax docusate was determined in the formulation and the drug load was fixed at 25 mg/mL.

### 7.3.4 Solubility studies in lipid excipients

Venetoclax and venetoclax docusate solubilities were determined in various excipients. An excess of venetoclax free base or venetoclax docusate was added to 2 mL of excipients/formulations or media and stirred at 200 rpm (25% power) (Mixdrive 15, 2MAG, Germany) at 37 °C. Solid excipients were melted at 50 ºC and cooled to 37 ºC prior to venetoclax addition. Samples were taken after 24 h, 48 h, 72 h and centrifuged at 21,380*g* (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again under identical conditions. To solubilise the excipients, the supernatant was diluted in a mixture of acetonitrile and ethyl acetate (1:3, v/v), followed by further 1:10 (v/v) dilution with a mixture of acetonitrile and ethyl acetate (3:1, v/v). The obtained samples were further diluted with mobile phase before analysis by reverse phase HPLC. All samples were run in triplicates.

### 7.3.5 Solubility upon dispersion

A paddle dissolution apparatus (USP type II) with 300 mL FaSSIF was used for dispersion of the lipid formulations. The media was stirred at 100 rpm and kept at 37 °C throughout the experiments. To FaSSIF 2 mL lipid formulation was added and the time that was needed to visually disperse the formulation was measured. Samples of 5 mL of the dispersions were used for the solubility experiments. Excess venetoclax free base was added to the dispersions and

placed in a water bath shaker (200 shakes/min) (GLS400, Grant Instruments, UK) at 37 °C. After 2 h, 4 h, 5 h and 24 h, 500 µL samples were taken and centrifuged at 21,380*g* (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany) for 15 min. The supernatant was transferred to a new sample tube and centrifuged again under identical conditions. Subsequently, the supernatant was diluted appropriately with a mixture of acetonitrile and ethyl acetate (70:30, v/v) before analysis by HPLC.

Samples were analysed as described previously (chapter 5) [176]. In brief, an Agilent 1200 series HPLC system (Agilent Technology Inc., US) comprising a binary pump, degasser, autosampler and variable wavelength detector was used for the analysis. Data analysis was done with EZChrom Elite® version 3.2. Venetoclax was separated from the sample matrix 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 40 °C. The mobile phase consisted of (a) acetonitrile with 0.5% (v/v) TFA and (b) water with 0.5% TFA at a ratio of 53:47 (v/v) and was used at a flow rate of 1 mL/min. The sample injection volume was 20 µL and the detection wavelength was set to 316 nm.

### 7.3.6 Dynamic light scattering

The micelles/droplet size was measured by means of dynamic light scattering using a Malvern Nano-Zetasizer® (Malvern Panalytical Ltd., UK). A 1 mL sample of the dispersions (2 mL formulation in 300 mL FaSSIF) were measured undiluted using a disposable ZEN0040 cuvette and the micelles/droplet size was recorded according to the intensity-based size distribution. Measurements were done at 37 ºC, using 3 measurements with 12 runs of 10 sec each. The measurement angle was 173º. The dispersant medium was customised as FaSSIF buffer containing the buffer salts and sodium chloride (according to the concentrations in

FaSSIF V1 [198]) with a refractive index of 1.332 and a viscosity of 0.7252 cP. The material absorption was set to 0.01 and the material refractive index to 1.44.

### 7.3.7 Venetoclax docusate formation

Venetoclax docusate was prepared by initially converting sodium docusate to docusic acid. Sodium docusate (2.048 g) was dissolved in THF (300 mL) and methanolic hydrochloride solution (1.25 M, 3.69 mL) was added while constantly stirring, forming a white precipitate. The suspension was cooled to - 20 ºC and filtered through a 0.45 µm filter (Durapore®, Merck KGaA, Germany) to obtain a clear supernatant. The resulting docusic acid solution in THF was added to a solution of venetoclax free base (2.000 g) in THF (300 mL) at a molar ratio of 1:1 (venetoclax free base/docusic acid) to obtain a clear yellow solution. The solution was dried *in vacuo* at 40 ºC to obtain venetoclax docusate as a yellow solid. Venetoclax docusate was purified by dissolving the material in dichloromethane (40 mL) and the resulting solution was filtered under vacuum through a 0.45 µm filter (Durapore®, Merck KGaA, Germany). The solution was subsequently washed with water (3 x 20 mL), dried (anhydrous sodium sulfate) and filtered. The resulting solution was reduced to dryness *in vacuo* at 40 ºC and the resulting residue recrystallised from methanol. The resulting venetoclax docusate was dried using vacuum for 48 h. The overall isolated yield of venetoclax docusate was 90%.

### 7.3.8 Molecular association between venetoclax and free fatty acids

The formation of molecular associations between venetoclax free base and *n*-decanoic acid or oleic acid, respectively, as co-crystals or salts were also attempted using the evaporation method [199]. In brief, equivalent molar amounts of venetoclax free base (1.0 g, 1.15 mmol) and *n*-decanoic acid (0.198 g, 1.15 mmol) or oleic acid (0.325 g, 0.15 mmol), respectively, were

dissolved in THF (150 mL) and stirred for 3 h at 40 ºC. The solvent was removed *in vacuo* at 40 ºC, affording a yellow solid, which was further dried under vacuum for 48 h.

### 7.3.9 Nuclear magnetic resonance (NMR) analysis

$^{1}$H NMR spectra of venetoclax docusate were recorded at 500 MHz and $^{13}$C NMR spectra were recorded at 125.8 MHz on a Bruker Avance 500 NMR spectrometer using a Quattro Nucleus Probe (QNP) at 300 K. $^{1}$H NMR spectra of venetoclax co-crystals were recorded at 500 MHz on a Bruker Avance III 500 NMR spectrometer using a Tripe Resonance cryoprobe (TCI) at 298 K.

In addition, variable temperature $^{1}$H NMR spectra were recorded to evaluate conformational isomers (rotamers) of venetoclax in the presence of the free fatty acids. $^{1}$H NMR spectra at 298 K, 333.15 K and 363.15 K for venetoclax co-crystals were acquired at 400 MHz on a Bruker Avance II equipped with a Broad Band cryoprobe (BBO). All spectra were processed using Topspin 3.2 software. Spectra were acquired in deuterated dimethylsulfoxide (DMSO-*d6*) and referenced to the residual solvent signal at 2.50 ppm ($^{1}$H NMR) or 39.5 ppm ($^{13}$C NMR). Chemical shifts (δH and δC) are expressed as parts per million (ppm), positive shift being downfield relative to tetramethylsilane (TMS). Coupling constants (J) are expressed in hertz (Hz). Splitting patterns in $^{1}$H NMR spectra are designated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sext (sextet), sept (septet) and m (multiplet).

### 7.3.10 Elemental analysis

The carbon, hydrogen and nitrogen content in the salts/co-crystals was analysed by combustion analysis using a CE-440 elemental analyser (Exeter Analytical, UK). Venetoclax lipophilic salt

and co-crystals samples of 1.5 – 3.0 mg were weight into disposable tin capsules and inserted into a high temperature furnace held at 1200 ºC. Samples were combusted in pure oxygen. Each sample was run in triplicate and results expressed as % (w/w) of the total mass. In addition, a blank (empty tin capsule) and three standards (acetanilide, benzoic acid and benzylthiuronium chloride) were measured.

### 7.3.11 Liquid chromatography – Mass spectrometry (LC-MS)

Venetoclax and its different salts/co-crystals were dissolved to a concentration of approximately 0.3 mg/mL in a methanol/acetonitrile mixture of 2:1 (v/v). Samples were thoroughly vortexed and briefly sonicated to ensure complete dissolution and a volume of 1 mL of the solution was transferred to a HPLC vial.

Ultra-High Performance Liquid Chromatography – Mass Spectrometry (UHPLC-MS) analyses were conducted to detect the presence of impurities in the selected samples. Analysis were performed using a H-Class UHPLC system equipped with a diode array detector (DAD-detector), coupled to a Synapt G2 HDMI QTOF mass spectrometer (Waters Inc). For the analyses, an injection volume of 2 µL was used. Chromatographic separations were performed using a 150 mm x 2.1 mm ID Waters Acquity BEH $C_{18}$ 1.7 µm column. The column heater was kept at 45 ºC and the flow rate was 0.35 mL/min. Eluent A consisted of 10 mM ammonium acetate in water, eluent B was acetonitrile. The gradient elution consisted of a linear gradient from 5% to 100% of eluent B in 12 min and this plateau of 100% eluent B was kept for 3 min for rinsing. Afterwards, an equilibration of 5 min was applied at the starting conditions.

The effluent of the column was directed to the diode array detector, acquiring spectra between 200 and 500 nm, and then sequentially to the mass spectrometer. High resolution, accurate

mass data were acquired in both positive and negative ion mode using an electrospray ionization source. In positive ion mode, this source was operated at a capillary voltage of 3 kV and a cone voltage of 30 V and desolvation temperatures were set at 120 °C and 350 °C, respectively. Ions were recorded in a precursor ion scan with a mass range from *m/z* 80 to *m/z* 1200. In negative ion mode, the electrospray source was operated at – 2 kV with the other parameters as described above. All ion fragmentation was performed in all analyses operating at a linear collision energy ramp in the trap collision cell ranging from 20 to 50 eV over the mass range. The precursor ion spectra were used to confirm the elemental formula of the proposed compound (with a mass error tolerance of 5 ppm). The product ion spectra obtained in the all ion fragmentation scan were used to confirm the proposed structures by the fragmentation pattern of the compounds.

### 7.3.12 FT-Infrared spectroscopy

Measurements were performed using a Fourier Transform Infrared (FTIR) spectrophotometer (Spectrum Two, Perkin Elmer, UK), equipped with a universal attenuated total reflection (UATR) unit. FTIR spectra were obtained in transmission mode between 400 $cm^{-1}$ and 4500 $cm^{-1}$. The resolution was 1 $cm^{-1}$ for the samples and background. Perkin Elmer Spectrum version 10.4 was used for data acquisition and analysis.

### 7.3.13 Water content analysis by Karl Fisher

Residual water content in venetoclax docusate and co-crystals were determined using a Karl Fischer CA-31 moisture meter (Mitsubishi chemical analytech, Japan). Approximately 50 mg samples were automatically titrated, and the results given as % (w/w) of the added sample mass.

### 7.3.14 Purity analysis

Approximately 0.3 mg/mL of venetoclax docusate and co-crystals were dissolved in a methanol/acetonitrile mixture of 2/1 (v/v) and the concentration of venetoclax free base was measured using the LC-MS method described above. Purity was determined as the % of the area of venetoclax free base relative to the detected total area at 283 nm.

### 7.3.15 Measurement of venetoclax docusate composition by HPLC

The composition of the venetoclax docusate salt was determined by measuring the amount of venetoclax free base in a given mass of venetoclax docusate. A solution of 3.7 mg venetoclax docusate (equivalent to 2.5 mg venetoclax free base) in 100 mL acetonitrile was prepared and diluted with mobile phase to obtain 12 different concentrations ranging from 50 ng/mL to 12.5 µg/mL of venetoclax free base equivalents. All concentrations were measured in triplicates. The HPLC method was identical to the described methodology above.

### 7.3.16 Modulated differential scanning calorimetry (mDSC)

The melting peak temperature, enthalpy and entropy of fusion of venetoclax docusate and co-crystals were studied using a TA Q1000 with a TA Refrigerated Cooling system 90 (TA Instruments, New Castle, DE). The cell was purged with nitrogen at 50 mL/min. 5 mg samples were weighed into a t-zero pan and heated from 20 ºC to 200 ºC at 3 ºC/min. The modulation amplitude was set to ± 1.0 ºC and the modulation to 60 sec.

### 7.3.17 *In vivo* study

All experiments were approved and conducted with licences issued by the Health Product Regulatory Authority, Ireland (project licence AE19130/P058) as directed by the EU Statutory instruments of the EU directive 2010/63/EU (Protection of Animals used for Scientific Purpose). Local ethical approval was granted by University College Cork Animal Experimentation Ethics Committee (AEEC). The study was randomised and conducted in five male landrace pigs (15 – 17 kg) using a 6-way crossover. Pigs were fed approximately 175 g of standard weanling pig pellet feed twice daily. In the fasted study legs the final feed of 175 g was given 24 h prior to dosing. As part of the study design any remaining food was removed 16 h before dosing, however, no food remained at this point in any of the groups. In the fed study leg, half a portion of a standard high-caloric, high-fat FDA breakfast (444 kcal, 315 g, one slice of bacon, one slice buttered toast, one fried egg, 118 mL whole milk, 60 g hashed brown potatoes) [200; 201] was fed 30 min prior to oral dosing.

On day one, an indwelling intravenous catheter was inserted from the ear vein into the jugular vein under general anaesthesia, which was used for blood sample collection throughout the study. The pigs were recovered for two days and the first oral dosing commenced on day three. Each pig was administered a single dose of 100 mg venetoclax free base (or equivalent venetoclax docusate) with the aid of a dosing gun followed by 50 mL of water via a syringe. In order to control the water intake with the dosage forms, the water intake was restricted for 3 h post-dosing. At all other times water was available *ad libitum*. To facilitate animal handling during the oral administration, an intramuscular dose of ketamine (5 mg/kg) and xylazine (1 mg/kg) was administered. Blood samples were collected after 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h in heparinised tubes. Upon collection, blood samples were immediately centrifuged at 3000*g*, 4 ºC for 5.5 min (Eppendorf 5702 R, Rotor A-4-38, Eppendorf Ltd., UK).

The supernatant plasma was harvested and stored at -20 ºC until further analysis. A 6-day washout period was maintained between the study arms. The following treatment groups were conducted:

1. Venclyxto® (fasted state)
2. Venclyxto® (fed state)
3. Long chain SEDDS (venetoclax docusate, fasted state)
4. Medium chain SEDDS (venetoclax docusate, fasted state)
5. Surfactant-only SEDDS (venetoclax docusate, fasted state)
6. Intravenous formulation

The results of the food effect study with Venclyxto® in the fasted and fed state as well as the intravenous formulation have previously been published by Henze and co-workers [170]. A single dose of the long chain (Peceol®), medium chain (Capmul®) and surfactant-only formulation were administered as four hard gelatine capsules (00EL Licaps®, Capsugel, Lonza Group Ltd.) containing 1 mL LBF, respectively. All four capsules contained venetoclax docusate, equivalent to 100 mg venetoclax free base (25 mg/mL).

### 7.3.18 Bioanalysis

The plasma concentrations of venetoclax were determined by reversed phase HPLC. The Agilent 1260 series HPLC system (Agilent Technology Inc., US) comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was operated, and the data analysed with EZChrom Elite® version 3.3.2. 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 for the separation of venetoclax from the sample matrix. The mobile phase consisted of water and acetonitrile with 0.5% (v/v) TFA at a ratio of 47:53 (v/v) and was used at a flow rate of 1.0 mL/min. The sample and column temperature

were set at 5 °C and 40 °C, respectively, and the detection wavelength was set to 250, 290 and 316 nm. Venetoclax was extracted from the plasma samples by liquid-liquid extraction from 500 µL plasma using vemurafenib as internal standard as described previously (chapter 5) [176]. In brief, venetoclax was extracted from 500 µL plasma using 450 µL acetonitrile and 2 x 1 mL ethyl acetate. Vemurafenib was used as internal standard. The extraction solvents were dried under a nitrogen stream at 60 °C and the residues were reconstituted in 100 µL mobile phase (excluding TFA) followed by centrifugation at 25 °C, 11,500*g* for 5 min (Mikro 200 R, Andreas Hettich GmbH & Co. KG, Germany). The injection volume used for HPLC analysis of the supernatant was 50 µL.

### 7.3.19 Data Analysis

Pharmacokinetic parameters were calculated using Microsoft® Excel® by means of the trapezoidal rule. Absolute bioavailability was calculated using pig individual intravenous data, which has previously been reported [170]. Venetoclax plasma concentration profiles were analysed by non-compartmental analysis. Statistical analyses for all *in vivo* parameters were performed using a one-way ANOVA after using the Bartlett's test to check for equal variance. The subsequent pairwise comparison of the groups was done using Tukey's multiple comparison test. All statistical analyses were carried out using GraphPad Prism® 5 (GraphPad Software, USA).

## 7.4 Results

### 7.4.1 Characterisation of venetoclax docusate

The reaction of venetoclax free base with docusic acid yielded the corresponding salt form, i.e. venetoclax docusate (Figure 7-1). The formation of the lipophilic salt was expected, as the pKa of venetoclax was more than 2 units higher compared to docusic acid (Table 7-2). The resultant venetoclax docusate was isolated by evaporation to dryness, recrystallised from hot methanol and dried under vacuum for 48 h. The bright yellow solid was characterised for its solid state characteristics using DSC, FTIR and NMR as well as purity using elemental analysis, HPLC and LC-MS. The results are presented in Table 7-1 as well as in the appendix Figure IV-1, Figure IV-3, Figure IV-4, Figure IV-7, Figure IV-14 and Figure IV-16.

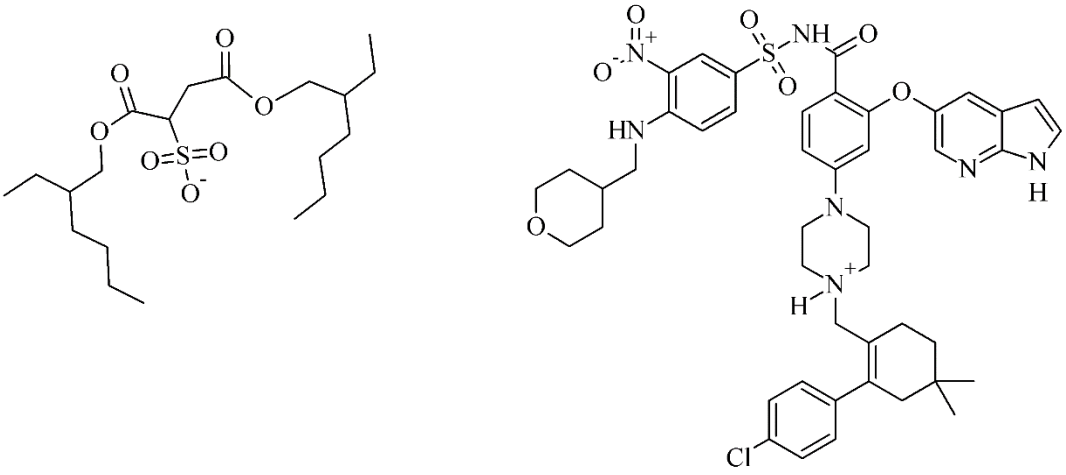


Figure 7-1 Chemical structure of venetoclax docusate.

The obtained venetoclax docusate demonstrated a purity of 99.8% by UPLC (Figure IV-1). Further HPLC analysis showed that the measured venetoclax free base equivalent of venetoclax docusate was consistent with the theoretical value of a 1:1 molar ratio of venetoclax and docusate (Table 7-1). This was confirmed by elemental analysis, where the theoretical carbon, hydrogen and nitrogen content of a 1:1 molar ratio of venetoclax and docusate was in

agreement with the measured values (Table 7-1). In addition, $\delta^1$H NMR spectroscopy was performed and confirmed the salt formation and ratio (Figure IV-3, Figure IV-4, Figure IV-7). For the ratio identification of docusate in $^1$H NMR spectra, protons at the four methyl groups (12 H) were used, which had a chemical shift of 0.85 ppm and were clearly distinguished from other protons in the venetoclax docusate spectra. Additionally, the protons at the β-carbon of the sulfonate, with a chemical shift of 2.9 ppm were utilised for the lipophilic salt ratio confirmation. In the case of venetoclax, the methine proton of the tetrahydropyran ring was used, which had a chemical shift of 1.9 ppm, as well as the geminal dimethyl proton signal at 0.90 ppm.

The mDSC data on venetoclax docusate suggested that the salt was crystalline due to the presence of an endothermic melting peak with absence of an exothermic recrystallisation peak (Table 7-1, Figure IV-16). A melting range of 143 ºC – 160 ºC with a middle point of 151.94 ± 0.13 ºC was measured, which was approximately 12 ºC higher compared to the $T_m$ of venetoclax free base (Table 7-2).

Table 7-1 Venetoclax docusate thermal properties: melting point ($T_m$), enthalpy of fusion ($\Delta H_{fus}$), entropy of fusion ($\Delta S_{fus}$) as well as venetoclax free base content in 1 g venetoclax docusate and elemental analysis of venetoclax docusate (mean ± SD, n = 3).

| | Venetoclax docusate |
|---|---|
| $T_m$ [ºC] (range) | 151.9 ± 0.1 (143.0 ± 0.2 – 159.9 ± 0.6) |
| $\Delta H_{fus}$ [kJ/mol] | 42.4 ± 0.4 |
| $\Delta S_{fus}$ x$10^{-2}$ [kJ/mol] | 10.0 ± 0.1 |
| Theoretical venetoclax content (mgA/g) [a)] | 672.7 |
| Measured venetoclax content (mgA/g) | 672.5 ± 7.0 |
| Theoretical C/H/N content [% w/w] [a)] | 60.1 / 6.8 / 7.6 |
| Measured C/H/N content [% w/w] [c)] | 60.1 ± 0.04 / 6.8 ± 0.1 / 7.3 ± 0.1 |

[a)] Corrected for water content of 0.6% (w/w).
[b)] mgA/g: mg of the active venetoclax per 1 g venetoclax docusate.
[c)] A deviation of 0.3% (w/w) is considered acceptable due to instrument specifications.

### 7.4.2 Characterisation of the molecular venetoclax-free fatty acid associations

In the case of oleic acid and *n*-decanoic acid, a proton transfer to form a salt with venetoclax was not expected as the pKa differences between both acids and venetoclax (pKa (conjugate acid of base) – pKa (acid)) is < 1 [202] (Table 7-2). Based on this rather simplified approach in the FDA guideline on 'Regulatory Classification of Pharmaceutical Co-Crystals' [202], the venetoclax-free fatty acid entities can be classified as co-crystals. Fatty acids have shown to improve oral bioavailability of drugs [18; 203], which is the reason for the attempt to produce venetoclax-fatty acid co-crystals in a 1:1 molar ratio. The results of DSC, FTIR, $^1$H NMR, HPLC and LC-MS analyses are presented in the appendix Figure IV-1, Figure IV-2, Figure IV-9 to Figure IV-13, Figure IV-15, Figure IV-17, Figure IV-18 and Table IV-1.

UPLC analysis of the obtained co-crystals revealed only a purity of 78.6% (area/area) for the *n*-decanoic acid and 83.8% (area/area) for the oleic acid co-crystal (Figure IV-1). The major impurity present in both co-crystals was identified by LC-MS analysis as venetoclax *N*-oxide (Figure IV-2). In addition, elemental analysis in the case of the *n*-decanoic acid co-crystal showed a difference of > 1% between the theoretical and measured carbon content (Table IV-1) indicating a significant amount of impurities. Also, inconsistencies were observed for $^{1}$H NMR analysis in both co-crystals (Figure IV-9, Figure IV-11). Minor signals, which were adjacent to, and appeared to replicate the multiplicity of many of the major signals, were visible in the $^{1}$H NMR spectra for both co-crystals. Performing variable temperature $^{1}$H NMR studies at 60 ºC and 90 ºC (Figure IV-10, Figure IV-12) resulted in various upfield and downfield shift of signals. For example, in the case of venetoclax/*n*-decanoic acid co-crystal, the four overlapping signals at 8.40 – 8.59 ppm undergo increasing separation into pairs of analogous signals as the temperature increases (Figure IV-12). The shifting of other minor and major proton signals was also observed for both venetoclax co-crystal samples, as illustrated in Figure IV-10 and Figure IV-12. These observations led to the hypothesis that venetoclax may exist as rotamers in the presence of *n*-decanoic acid and oleic acid. An in-depth investigation of this potential phenomenon is beyond the scope of the current study. Due to the inconsistencies of the solid state analysis and the impurities of the co-crystals, the development of both oleic acid and *n*-decanoic acid co-crystals was not further pursued for this study.

Table 7-2 Physicochemical characteristics of venetoclax, docusic acid, oleic acid and *n*-decanoic acid.

| | Venetoclax | Docusic acid | Oleic acid | *n*-decanoic acid |
|---|---|---|---|---|
| Molecular weight [g/mol] | 868.4 | 444.6 | 282.5 | 172.3 |
| clog*P* | 5.5 [a)] | 4.31 [c)] | 7.21 [c)] | 3.89 [c)] |
| pKa | 3.4 and 10.3 [b)] | 0.21 [c)] | 5.08 [c)] | 4.97 [c)] |
| $T_m$ [ºC] | 140.2 ± 0.1 [a)] | n/a | 13.4 [c)] | 31.5 [d)] |

[a)] Chapter 5 [176].
[b)] Reference [96].
[c)] Calculated with ADMET predictor® 9.5.0.16.
[d)] Reference [204].

### 7.4.3 Development of SEDDS formulations

The formulation development of a lipid-based solution is usually based on the solubility of the drug substance in formulation excipients [205]. However, upon dispersion or digestion of a SEDDS, precipitation of the drug may occur, hampering the formulation performance and leading to a reduced bioavailability of the drug [206; 207; 75]. In the case of lipophilic salts, it has been reported that the precipitate from solution can be the free base of the drug rather than the lipophilic salt itself [38; 40]. Therefore, in this study, the formulation development was based on venetoclax free base in an approach to mitigate the risk of formulation under-performance upon addition into a biorelevant surrounding. An additional benefit of this method was that the available amount of venetoclax docusate had only to be used in solubility studies of the final prototype formulations streamlining the development process.

The development of a SEDDS was performed by applying a DoE approach. Initially, three excipients were chosen: a long chain oil (Peceol®) and two surfactants (Tween® 85 and Cremophor® RH40). Based on varying the proportions of these three excipients, three response attributes were determined namely: drug solubility upon dispersion, time of dispersibility and micelles/droplet size on dispersion in FaSSIF. The excipients were chosen based on previous

*in vivo* reports as well as measured solubility of venetoclax free base. A supersaturated Peceol® formulation with venetoclax free base has previously shown promising results in landrace pigs by displaying a 4-fold higher bioavailability compared to venetoclax free base powder (chapter 5). Additionally, venetoclax free base showed a high solubility in Peceol® relative to other oily excipients. Venetoclax free base also showed a high solubility in both Tween® 85 and Cremophor® RH40 relative to other surfactants, which were initially screened. The responses were chosen to target the highest drug concentration on dispersion, in the shortest dispersion time and the smallest droplet size of the emulsified formulation as an indicator of a more stable micro-emulsion that potentially shows a good *in vivo* performance, regardless of the solubility of venetoclax free base or venetoclax docusate in the lipid excipients. The most important response was the solubility upon dispersion of venetoclax free base, which represents the solubilisation capacity of 2 mL SEDDS in 300 mL FaSSIF. A higher solubility may reduce the apparent supersaturation ratio upon dispersion (drug concentration in the colloidal aqueous phase/solubility of the drug in the colloidal aqueous phase) and thereby reduce the risk of drug precipitation [208; 76]. Dispersibility time was the time needed for 2 mL of the SEDDS to generate a homogenous lipid dispersion in 300 mL FaSSIF. Droplet or colloidal size was used as a surrogate parameter for a more stable system. The response contour plots are illustrated in Figure 7-2.

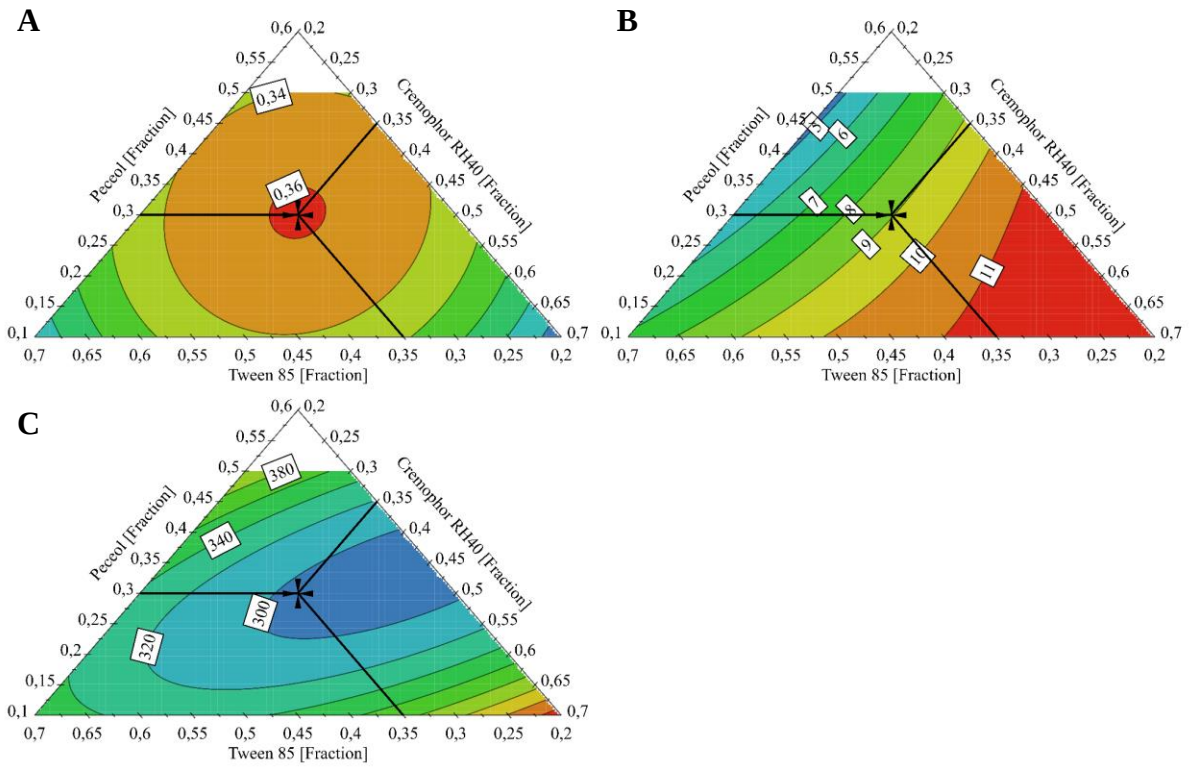



Figure 7-2 Contour ternary diagrams of A: solubility of free venetoclax base in SEDDS dispersions, B: dispersibility time, C: micelles/droplet size of dispersion.

Using a PLS model, the optimised long chain SEDDS was calculated having the highest possible solubility upon dispersion and a minimum dispersibility time. As the differences in micelles/droplet size were only minor and the model validity for this particular response low, the micelles/droplet size of the lipidic dispersions was not considered in the optimisation process. The final long chain SEDDS consisted of 30% (w/w) Peceol® and 35% (w/w) of Tween® 85 and Cremophor® RH40, respectively, which was the centre point of the DoE design space.

Having optimised the SEDDS design using a long chain glycerides as oil-phase, a further two SEDDS were prepared using similar % compositions to explore (a) the impact of medium chain-based SEDDS versus long chain-based SEDDS by exchanging the glyceride

fraction with a medium chain monoglyceride (Capmul MCM®) and (b) the impact of a glyceride-free (i.e. surfactant only) formulation by removing the glyceride fraction and keeping the surfactant ratio the same (i.e. 1:1 (w/w) Tween® 85:Crempophr® RH40). This allowed a direct comparison of a long chain-based type IIIA SEDDS versus a medium chain-based type IIIA SEDDS versus a surfactant only type IV SEDDS on *in vivo* performance. The final formulations evaluated *in vivo* are presented in Table 7-3. In addition, the solubility of venetoclax free base and venetoclax docusate in the prototype formulations are also listed. A higher solubility of venetoclax docusate was confirmed relative to venetoclax free base with an increase between 2.6- and 6.2-fold in the selected SEDDS formulations (Table 7-3).

Table 7-3 Formulation composition and solubility of venetoclax free base and venetoclax docusate in prototype SEDDS.

| | | **Solubility [mg/mL]** | |
|---|---|---|---|
| | Composition [a)] | Venetoclax free base | Venetoclax docusate |
| Long chain SEDDS | Peceol® 30%<br>RH40® 35%<br>Tween® 85 35% | 5.17 ± 0.48 | 27.09 ± 3.39 |
| Medium chain SEDDS | Capmul® 30%<br>RH40® 35%<br>Tween® 85 35% | 7.76 ± 0.97 | 48.42 ± 7.44 |
| Surfactant-only SEDDS | RH40® 50%<br>Tween® 85 50% | 12.15 ± 1.27 | 31.87 ± 3.97 |

[a)] RH40: Cremophor® RH40; Capmul: Capmul MCM®.

## 7.4.4 *In vivo* bioavailability of venetoclax

The *in vivo* performance of venetoclax docusate was evaluated in a full 6-way crossover study, covering the three test formulations and two comparator study legs for the commercial formulation Venclyxto®, under fasted and fed state conditions as well as an intravenous

formulation. Each pig was administered 100 mg venetoclax in 4 mL (25 mg/mL) SEDDS/pig as either long chain SEDDS, medium chain SEDDS or surfactant-only SEDDS in the fasted state. Additionally, for the two comparator study legs, each pig received 100 mg Venclyxto® under both fasted and fed state conditions. Pigs were fed with standard high fat FDA-style breakfast meal [200] following the recent reported protocol for a fed state porcine model [201] 30 min prior oral administration. The absolute bioavailability is shown in Figure 7-3, the mean plasma concentration versus time profiles in Figure 7-4 and the pharmacokinetic parameters in Table 7-4.

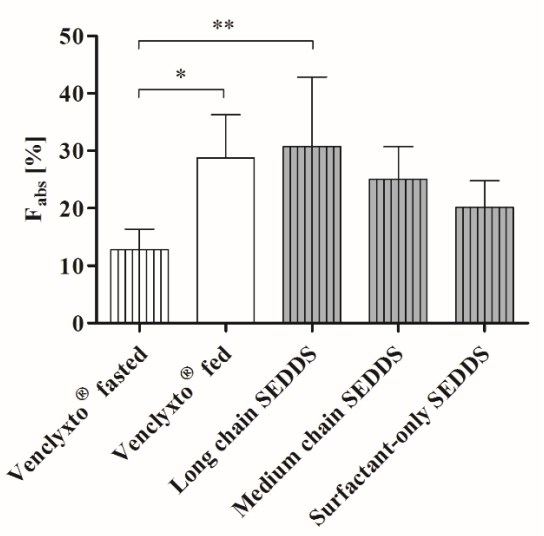



Figure 7-3 *In vivo* bioavailability of 100 mg venetoclax in male landrace pigs (mean ± SD, n = 5). Venclyxto® is the commercialised product and the data has previously been reported [170]. SEDDS contained venetoclax docusate at an equivalent dose of 100 mg venetoclax free base. Formulations were administered in the fasted state (striped bars) and fed state (blank bar).

Among the SEDDSs, the highest bioavailability was observed for the long chain SEDDS with 30.8 ± 12% followed by the medium chain SEDDS with a bioavailability of 25.1 ± 5.7%. While the surfactant-only SEDDS showed the lowest bioavailability of 20.2 ± 4.6% relative to the

other SEDDSs, all SEDDSs were not statistically significantly different. These results showed that long chain lipids resulted in a higher bioavailability compared to medium chain lipids.

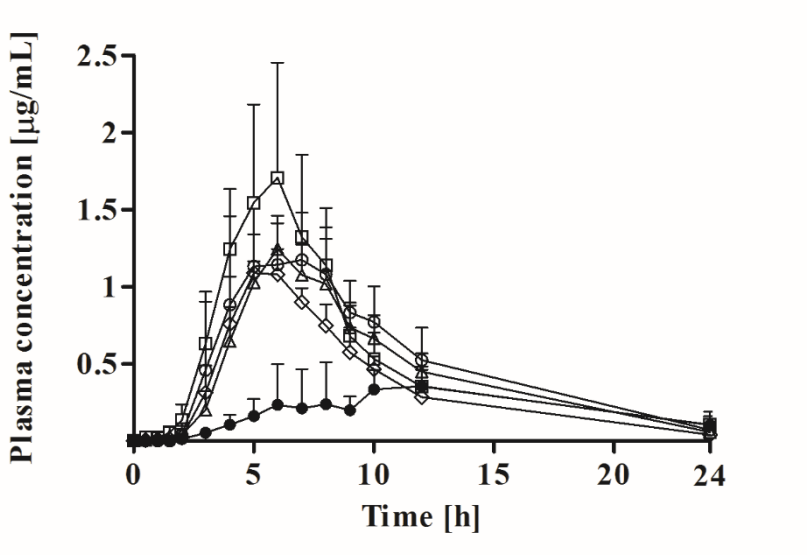



Figure 7-4 Mean plasma concentration versus time profiles for 100 mg venetoclax in male landrace pigs (mean ± SD, n = 5). Pigs were administered the commercially available product Venclyxto® in the fasted state (●) and in the fed state (○) (FDA standard meal) as previously reported [170]. Venetoclax docusate (equivalent to 100 mg venetoclax free base) was administered in a long chain SEDDS (□), medium chain SEDDS (Δ) and surfactant SEDDS (◊).

Compared to Venclyxto® in the fasted state, all venetoclax docusate SEDDS formulations showed a higher bioavailability. While only the long chain SEDDS was statistically significant different ($p < 0.05$), there was a clear trend towards a higher bioavailability in the presence of lipids and/or surfactants indicating that lipid excipients improve the bioavailability of venetoclax when compared to lipid free systems.

The commercialised product, Venclyxto®, showed a profound food effect with a statistically significant 2.3-fold higher bioavailability in the fed state (28.7 ± 7.5%) when compared to the fasted state (12.8 ± 3.6%) [170]. Compared to Venclyxto® in the fed state, the long chain SEDDS

showed a higher mean bioavailability, albeit not statistically significant different. In fact, all SEDDSs showed a similar bioavailability compared to Venclyxto® in the fed state, mimicking the food effect.

Table 7-4 Pharmacokinetic parameters of orally administered SEDDS containing 100 mg venetoclax as venetoclax docusate in male landrace pigs. The time to reach maximum plasma concentrations ($t_{max}$), mean residence time (MRT) and mean absorption time (MAT) are given as median with range, all other parameters are given as mean ± SD, where n = 5.

| **Pharmacokinetic parameters** | | | | | |
|---|---|---|---|---|---|
| | Long chain SEDDS | Medium chain SEDDS | Surfactant-only SEDDS | Venclyxto® (fasted) [b] | Venclyxto® (fed) [b] |
| $c_{max}$ [µg/mL] | 1.7 ± 0.7 | 1.3 ± 0.2 | 1.2 ± 0.2 | 0.4 ± 0.2 | 1.2 ± 0.3 |
| $t_{max}$ [h] (range) | 6 (5 – 7) | 6 (6 – 8) | 5 (4 – 7) | 12 (8 – 12) | 6 (4 – 8) |
| AUC 0 h – inf. [µg*h/mL][a] | 13.0 ± 3.4 | 10.9 ± 2.2 | 8.7 ± 1.2 | 5.5 ± 1.2 | 12.5 ± 3.2 |
| MRT [h] (range) | 9.2 (7.2 – 12.3) | 9.7 (9.0 – 10.3) | 8.8 (8.5 – 10.2) | 14.4 (11.0 – 19.3) | 9.4 (8.6 – 11.3) |
| MAT [h] (range) [a] | 5.7 (3.6 – 8.3) | 5.7 (5.4 – 6.1) | 5.0 (4.9 – 5.6) | 10.0 (7.4 – 15.3) | 6.0 (4.7 – 7.4) |
| $F_{rel}$ [%] | 258.9 ± 88.6 | 198.2 ± 34.3 | 159.3 ± 16.2 | 100 | 227.4 ± 54.4 |
| $F_{abs}$ [%] [a] | 30.8 ± 12.1 | 25.1 ± 5.7 | 20.2 ± 4.6 | 12.8 ± 3.6 | 28.7 ± 7.5 |

[a] Calculated using intravenous data as previously reported [170].
[b] As previously reported by Henze and co-workers [170].

## 7.5 Discussion

Drugs displaying a low aqueous solubility, high lipophilicity and poor permeability are common outputs of modern drug discovery programs, posing challenges to the development of drug products. Most notable is the reduced exposure upon oral administration of these drugs, especially during the early development stages. While LBFs have shown to improve oral bioavailability, the bio-enabling formulation approach is mostly limited to drugs showing sufficient high solubility in lipid vehicles. Several advanced LBF approaches have been proposed for drugs that show dose loading limitations such as lipid suspensions [44; 121], sLBFs [31; 32; 34], solid LBFs (including hybrid systems) [46; 47] or lipophilic salts [36-38; 40; 43]. As a thermodynamic stable lipid drug solution is preferred [61], the approach of lipophilic salts, which have demonstrated to increase lipid excipient solubility, offers particular advantages. However, it remains unclear whether drugs that show low lipid solubility due to a large, bulky structure and high molecular weight, would benefit from the approach of lipophilic salts in terms of higher lipid solubility and an increased bioavailability, as this formulation strategy further increases size and molecular weight of the drug-counterion molecular association.

Venetoclax docusate was prepared and isolated as a yellow crystalline substance, which exhibited up to 6.2-fold higher solubility in the prototype LBFs compared to the venetoclax free base. This was in line with observations previous reported for drugs with lower molecular weight, where substantial solubility increases for the docusate salts of drugs such as erlotinib, gefitinib, and ceritinib were reported to be at least 5-fold higher compared to the solubility obtained with their corresponding free bases [40]. Interestingly, venetoclax docusate has a higher $T_m$ (151.9 ± 0.1 ºC) relative to venetoclax free base (140.2 ± 0.1 ºC). This was unexpected, since docusate as a bulky counterion, likely hinders the crystal packing [43]. A lowering of $T_m$ is generally favourable due to the lower crystal energy as shown for example for the above

mentioned kinase inhibitors [40], lumefantrine [42], atazanavir [38] and itraconazole [37]. While the higher $T_m$ of venetoclax docusate may suggest a higher crystal energy compared to venetoclax free base, venetoclax docusate showed a pronounced (up to 6.2-fold) increased solubility in lipid excipients. While it cannot be discounted that including of docusate in the formulation may contribute to improved solubility [40] of venetoclax free base solubility, this up to 620% increase in solubility clearly indicated that more favourable solvation characteristics of the venetoclax docusate salt lead to the solubility gains. Therefore, despite the higher $T_m$, docusate as a counterion shows great potential to increase dose loading in SEDDS.

Venetoclax docusate SEDDS showed a statistically significant higher oral bioavailability relative to the commercially available product Venclyxto® in the fasted state. The exposure of venetoclax docusate from a SEDDS in the fasted state ranged from 20.2 ± 4.6% in a surfactant only SEDDS to 30.8 ± 12.1% in a long chain-based SEDDS, which was 1.6 to 2.4-fold higher when compared to Venclyxto® in the fasted state. Similar observations have been reported for itraconazole in rats, where itraconazole docusate increased the oral bioavailability 2.5-fold compared to the commercially available product Sporanox®, which contains the itraconazole free base (amorphous solid dispersion, Janssen-Cilag Ltd) [37]. In another study, cinnarizine decylsulfate increased the bioavailability in a SEDDS solution 1.2-fold compared to a cinnarizine free base SEDDS suspension. However, a cinnarizine free base SEDDS solution showed similar bioavailability compared to the cinnarizine decylsulfate SEDDS solution. While lipophilic salts of drugs increase the drug loading in SEDDS and make the administration of a SEDDS solution feasible, as in the case of venetoclax in this study, the lipophilic salt itself does not necessarily increase the oral bioavailability. If a SEDDS solution of the drug's free form can be generated it may demonstrate a similar increase in bioavailability compared to a SEDDS solution containing a lipophilic salt (at the same dose). This study

demonstrated that the bioavailability of venetoclax docusate SEDDS formulations (20.2 – 30.8%) was similar to the bioavailability observed when dosing Venclyxto® in the fed state (28.7 ± 7.5%). With venetoclax being able to dissolve in the lipid excipients the full potential of LBF excipients was harnessed and therefore bridging the gap between fasted and fed state dosing [116].

Among the venetoclax docusate SEDDSs, the highest mean bioavailability was observed in the case of the long chain-based SEDDS. A possible explanation for this is that Peceol®, which mainly consists of glycerol monooleate, may have increased the lymphatic transport of venetoclax. For a 100 mg venetoclax (free base) dose in the fed state it was demonstrated that the bioavailability in dogs of 38.8 ± 7.2% decreased to 20.2 ± 2.1% in thoracic duct-cannulated dogs [98]. Therefore, 43 ± 8% of the venetoclax was absorbed through the lymphatic system [98]. Since the lymphatic transport is increased by administration of long chain-based lipid excipients [209; 210], Peceol® may have been a major contributor to the high observed bioavailability when compared to the other SEDDS. The second highest mean bioavailability was observed for the medium chain SEDDS, with the lowest overall mean observed for the surfactant-only SEDDS. While the differences between these three SEDDS formulations were not statistically significant, it would appear that glyceride-based (oily) excipients in the formulation are favourable to improve the bioavailability of venetoclax when compared to increasing surfactant composition in the formulation.

While this study demonstrated that formulating a high molecular weight, lipophilic drug such as venetoclax as a lipophilic salt in SEDDS is a viable option to increase the bioavailability of venetoclax *in vivo*, this work is not without its limitations. The co-crystal formation of venetoclax with *n*-decanoic acid and oleic acid did not result in a drug substance with adequate

purity. Further $^{1}$H NMR analyses hypothesised that venetoclax exhibits rotameric behaviour in the presence of fatty acids. While additional examination and in-depth analysis could have confirmed the conformational isomers, this was considered out of scope in the present study. It must also be acknowledged that further formulation optimisation and solubility screening using venetoclax docusate instead of venetoclax free base could increase the drug loading in a SEDDS further. Nevertheless, the developed SEDDS successfully demonstrated that a streamlined approach in the case of only small available amounts of drug substance can yield a formulation with superior *in vivo* performance and this proof-of-concept study confirms the utility of a lipid-based approach to overcome the food effect. Finally, with a venetoclax docusate dose that is equivalent to 100 mg venetoclax free base approximately 49 mg docusic acid would be administered, it should be acknowledged that docusate is used as a mild laxative at a dose of 100 mg (Docusol® adult 50 mg/5 mL solution, Typharm Ltd.). While a laxative effect was not evident in this single dose *in vivo* study, it is unknown whether effects after chronic administration of a docusate salt would have an impact or whether an alternative non-active counterion would be required.

## 7.6 Conclusion

This study demonstrated the potential of a combined lipophilic salt-LBF approach to bridge the food effect gap and overcome food dependent bioavailability observed for the commercially available amorphous solid dispersion formulation Venclyxto®. Up to 6-fold higher solubility in prototype SEDDS was obtained using venetoclax docusate compared to the free base. Following oral dosing in pigs, the venetoclax docusate containing SEDDS showed an increase in oral bioavailability of up to 2.4-fold compared to the commercial amorphous solid dispersion in the fasted state. Furthermore, the venetoclax docusate SEDDS formulations displayed a similar bioavailability compared to Venclyxto® in the fed state. This study

illustrated that LBF formulations have the potential to improve the bioavailability of venetoclax, a highly lipophilic and high molecular weight BCS class IV compound. The impact of this study is that a food independent formulation strategy can be achieved by harnessing the biopharmaceutical benefits of lipid excipients, including drugs that fall outside the classical Lipinski Rule-of-Five properties.

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Köhl, N. J. 2020. Exploring the utility of lipid-based formulation technology to enhance oral bioavailability of BCS class IV molecules. PhD Thesis, University College Cork.

Please note that Chapter 8 (pp. 233-263) is unavailable due to a restriction requested by the author.

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# 9 Overall conclusion and future perspectives

The thesis has, firstly, demonstrated the ability of true LBF suspensions to improve oral bioavailability of the poorly lipid- and water-soluble BCS class IV drugs nilotinib and venetoclax. The beneficial lipid effect, however, was only evident in the case of more dispersible lipid suspension such as monoglycerides or surfactant suspensions, while low dispersible suspension such as triglycerides resulted in a particle entrapment causing a significant decrease in oral bioavailability. If these phenomena are generalisable, poorly dispersible oil only suspensions should be avoided for high lipophilic poorly water- and lipid-soluble compounds. However, further studies with a diverse set of poorly lipid-soluble compounds are needed to elucidate the relationship between lipophilicity, hydrophobicity, drug solubility in the LBF and the impact on the *in vivo* bioavailability.

Secondly, the bio-enabling effect of poorly dispersible excipients was shown by utilizing the chase dosing approach as an alternative to the lipid suspensions. The concomitant administration of a drug aqueous suspension and the drug-free lipid excipients circumvented the particle entrapment and revealed the potential bioavailability improvement of triglycerides for the 'brick dust' drug nilotinib. In this thesis it was demonstrated for the first time that this administration/formulation approach has been proven useful to overcome formulation issues, rather than its applicability to, for example, improve stability or dose uniformity. To build on this work, further uses of chase dosing could be explored such as the concomitant administration of PIs with sLBFs to overcome viscosity increasing and dispersibility/digestibility decreasing effects. In general, further studies to reveal the potential of this approach are merited and especially studies with compounds that are highly solubility limited in LBFs with a variety of chemical characteristics would be beneficial.

Thirdly, a higher oral bioavailability for the 'brick dust' drug nilotinib was demonstrated for more digestible surfactant suspensions. Additionally, *in vitro* digestibility was essential for achieving high aqueous phase concentrations of an oil-only sLBF containing venetoclax. Digestibility of the LBF for poorly water- and lipid-soluble compounds was crucial for the formulation performance *in vivo*. The use of 'brick dust' molecules in this thesis revealed unexpected formulation behaviour, as previously published studies on lipid solutions [146] showed that digestion is triggering precipitation and might result in lower oral bioavailability. To build on this work, further studies involving drugs with unfavourable LBF characteristics are needed to evaluate the applicability of these findings to a wider chemical space and to evaluate the feasibility to administer highly digestible LBFs to improve bioavailability of poorly water- and lipid-soluble drugs.

In addition, for venetoclax a lipophilic salt was successfully developed and an improved bioavailability in pigs was evident. The administration of the lipophilic salt containing LBF would avoid the administration of the drug with food, which is currently employed for the commercially available product Venclyxto®. For the first time, the work in this thesis, evaluated a lipophilic salt in a larger pre-clinical species. Further studies are now needed to address the feasibility and toxicology of the employed counterions of delivering lipophilic salts in human subjects. In order to fully clarify the utility of this promising approach, clinical validation will be required.

Finally, a Lipid Formulation Developability Guide (LFDG) was proposed based on the findings in this thesis as well as previous reports in the literature. The thesis demonstrated that LBFs are a suitable formulation approach for a diverse array of drug candidates, including lipophilic 'brick dust' molecules exhibiting low dose loading in lipid vehicles, to improve oral

bioavailability in the early pre-clinical phases right through to the clinical and commercial stages of development. The use of one bio-enabling strategy throughout the development process is beneficial as opposed to switches between different bio-enabling formulation approaches as generated formulation knowledge can be utilised throughout the development process. The use of LBFs from very early phases during drug development offer the advantage of early knowledge generation that can be utilised at later stages to streamline the development of a commercially viable LBF. Going forward, it would be of particular importance to re-evaluate the commonly used model compounds in contemporary LBF research to initiate a switch to compounds that represent the current pipeline molecules in order to address the unmet need of further strategic bio-enabling formulation guidelines in the pharmaceutical industry.

Appendix I  Chapter 4

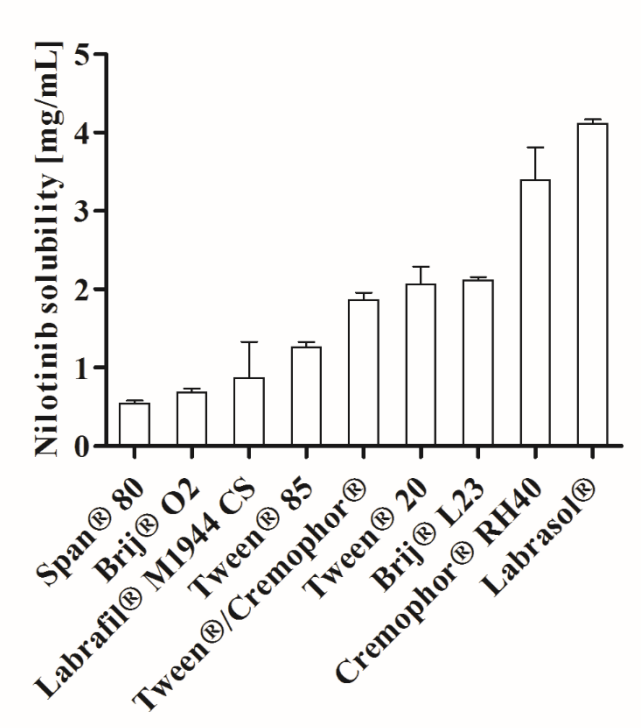



Figure I-1 Solubility of nilotinib in the selected excipients at 37 °C. Tween®/Cremophor® is a mixture of Tween® 85 and Cremophor® RH40 (67:33 w/w). Solubility was measured up to 72 h (mean ± SD, n = 3).

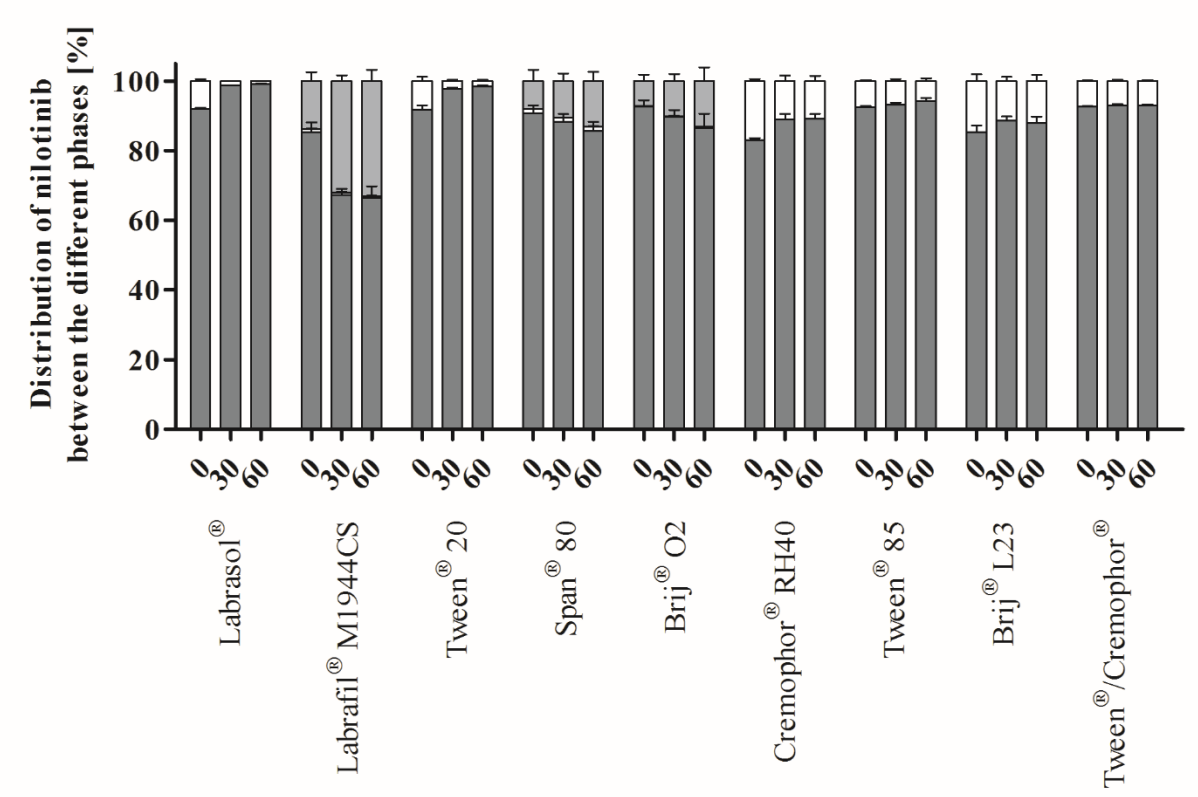



Figure I-2 Distribution of nilotinib into the solid (dark grey bars), aqueous (white bars) and lipid phase (light grey bars) just before initiation of digestion (0) and after 30 and 60 min of digestion (mean ± SD, n = 3). Tween® /Cremophor® is a mixture of Tween® 85 and Cremophor® RH40 (67:33 w/w).

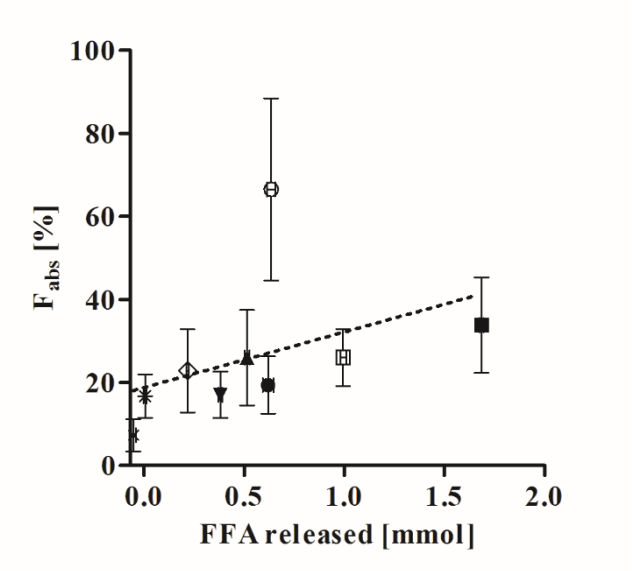



Figure I-3 Relationship of absolute bioavailability ($F_{abs}$) and free fatty acids (FFA) released during *in vitro* digestion as a surrogate parameter for digestibility of the excipient. Cremophor® RH40 (◊), Brij® L23 (*), Tween® 20 (○), Span® 80 (▲), Labrasol® (■), Labrafil® M1944 CS (□), Tween® 85 (●), Brij® O2 (×), Tween® 85/Cremophor® RH40 mixture (67:33 w/w) (▼) (mean ± SD, $F_{abs}$ n = 5, FFA released n = 3).

# Appendix II Chapter 5

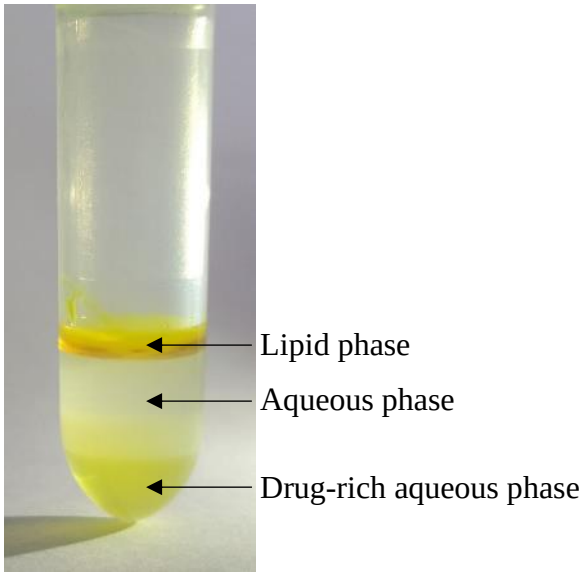



Figure II-1 *In vitro* lipolysis sample of sLBF after ultracentrifugation. Four phases were present: Lipid phase, aqueous phase, solid phase (not shown) and a fourth drug-rich aqueous phase.

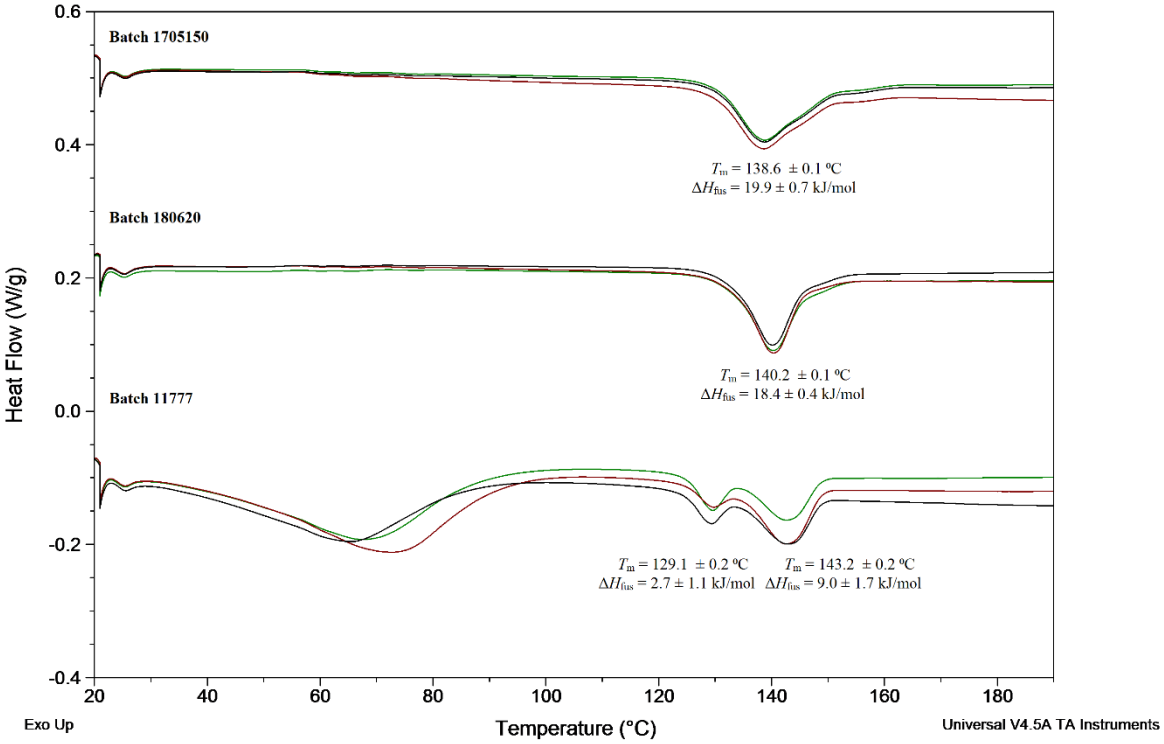



Figure II-2 DSC of venetoclax batches. Given are the melting temperatures and enthalpy of fusions (mean ± SD, n = 3).

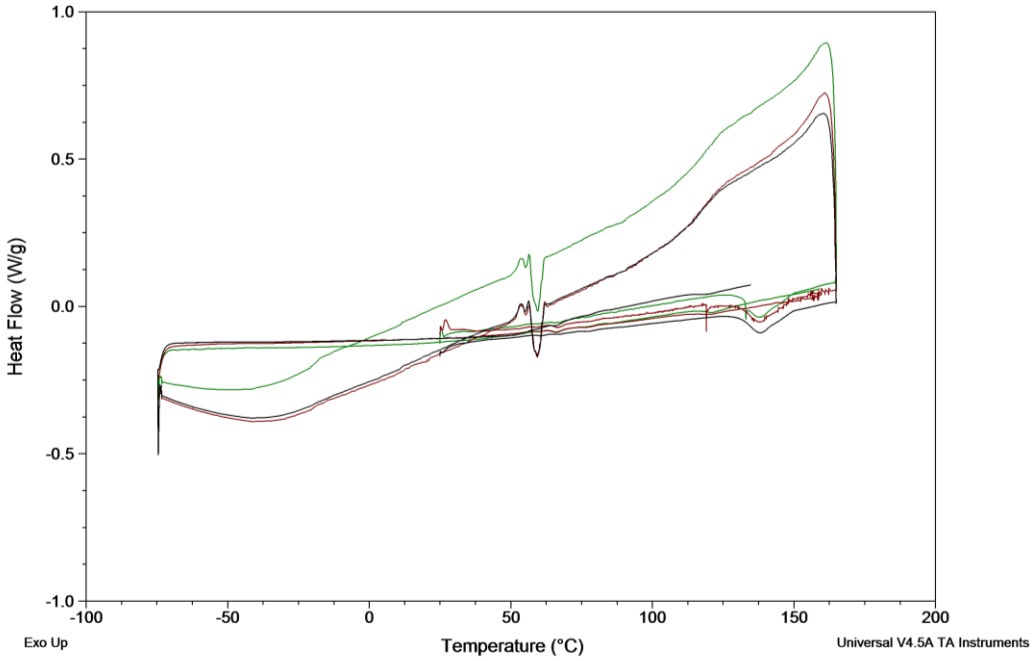



Figure II-3 DSC of venetoclax batch 1 (1705150). A heat-cool-heat protocol was used to determine the glass forming ability (n = 3).

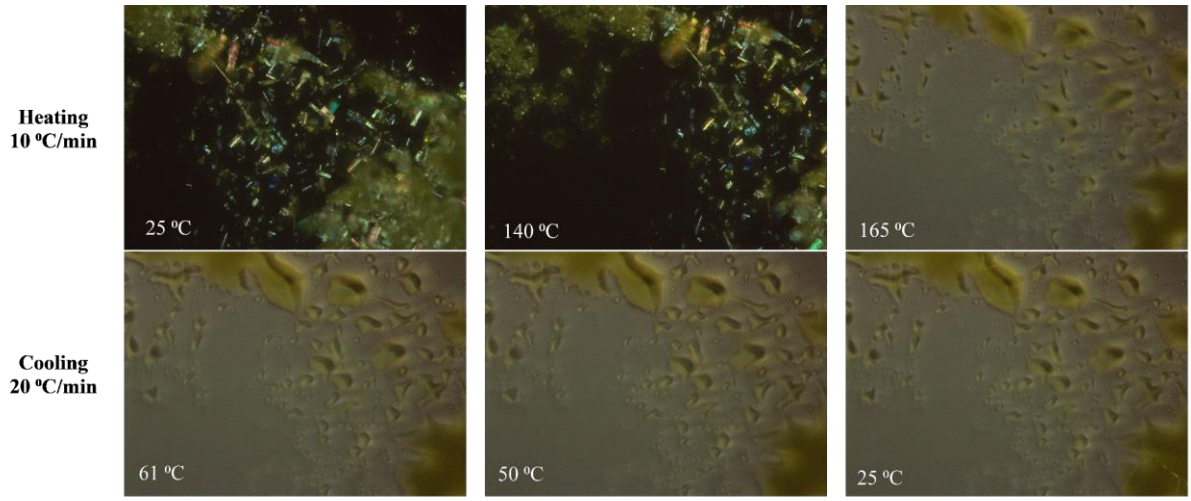



Figure II-4 Hot stage microscopy of venetoclax batch 1 under cross-polarised light. Pictures were taken at various temperatures during heating from room temperature to 165 ºC (10 ºC/min) and during cooling to 25 ºC (20 ºC/min).

Table II-1 Thermal properties of venetoclax. Enthalpy of fusion ($\Delta H_{fus}$), melting point ($T_m$) and entropy of fusion ($\Delta S_{fus}$).

| Batch | $\Delta H_{fus}$ (kJ/mol) | $T_m$ (ºC) | $\Delta S_{fus}$ x $10^{-2}$ (kJ/mol/K) |
|---|---|---|---|
| 1705150 | 19.9 ± 0.7 | 138.6 ± 0.1 | 4.8 ± 0.2 |
| 180620 | 18.4 ± 0.4 | 140.2 ± 0.1 | 4.5 ±0.1 |
| 11777 main peak | 9.0 ± 1.7 | 143.2 ± 0.2 | 2.2 ±0.4 |
| 11777 minor peak | 2.7 ± 1.1 | 129.1 ± 0.2 | 0.7 ±0.3 |

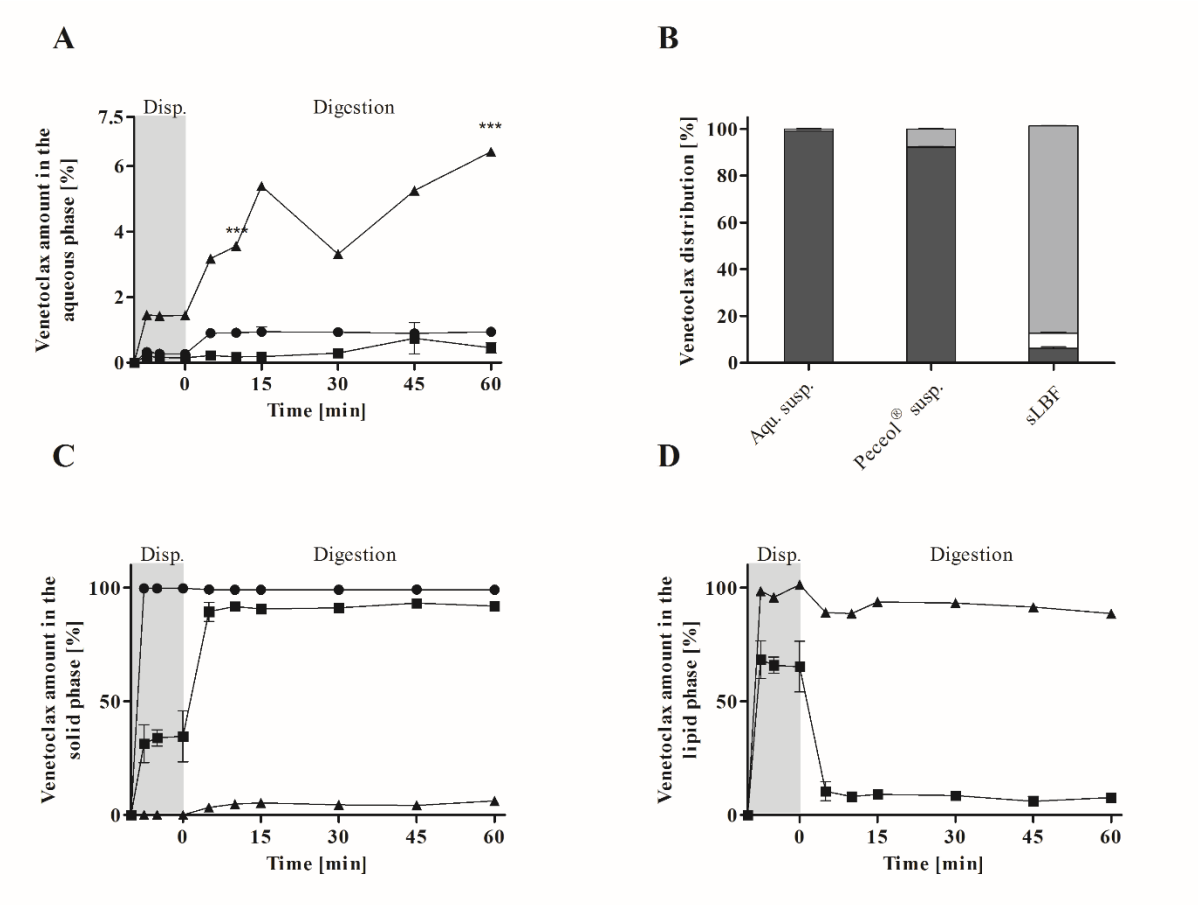



Figure III-1 *In vitro* lipolysis of venetoclax formulated as supersaturated Peceol® solution (sLBF) (▲), as well as previously reported aqueous suspension (●), Peceol® suspension (■) [176]. A: % of venetoclax in the aqueous phase. Statistical significance is compared to Peceol® and aqueous suspension at the same time points, B: Distribution of venetoclax into the different phases after 60 min of lipolysis, C: % of venetoclax in the solid phase, D: % of the venetoclax in the lipid phase. All experiments were run with n = 3 and results are shown as mean ± SD.

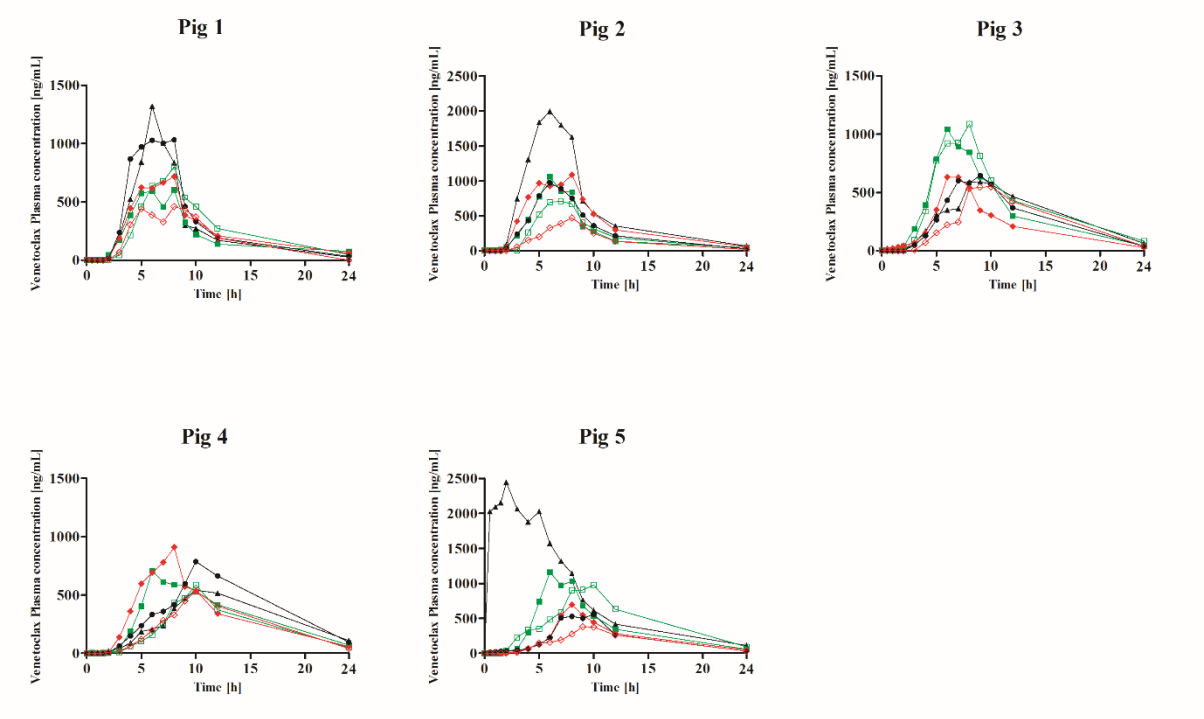



Figure III-2 Individual plasma concentration versus time profiles from 0 – 24 h of 100 mg venetoclax in landrace pigs for the tested formulations. sLBF-noPI (▲), sLBF-HPMC (■ - green), sLBF-HPMCAS (□ - green), sLBF-PVP (♦ - red), sLBF-PVP/VA (◊ - red), sLBF-Pluronic® F108 (●).

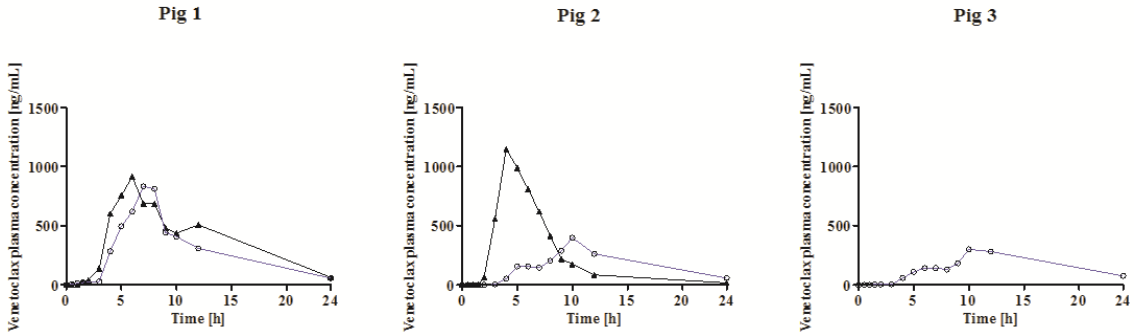



Figure III-3 Individual plasma concentration versus time profiles from 0 - 24 h of 100 mg venetoclax in landrace pigs for the tested formulations. sLBF-noPI (▲), sLBF-Eudragit® EPO (○ - blue). Due to an administration error, the sLBF-noPI of pig 3 was not obtained. sLBF-noPI has previously been reported [176].

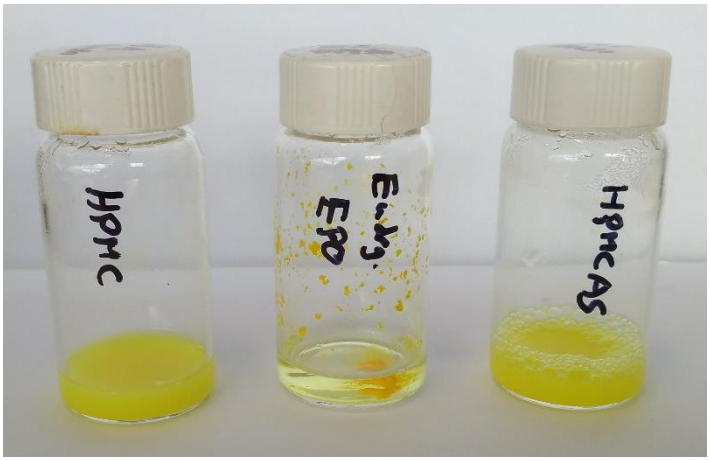
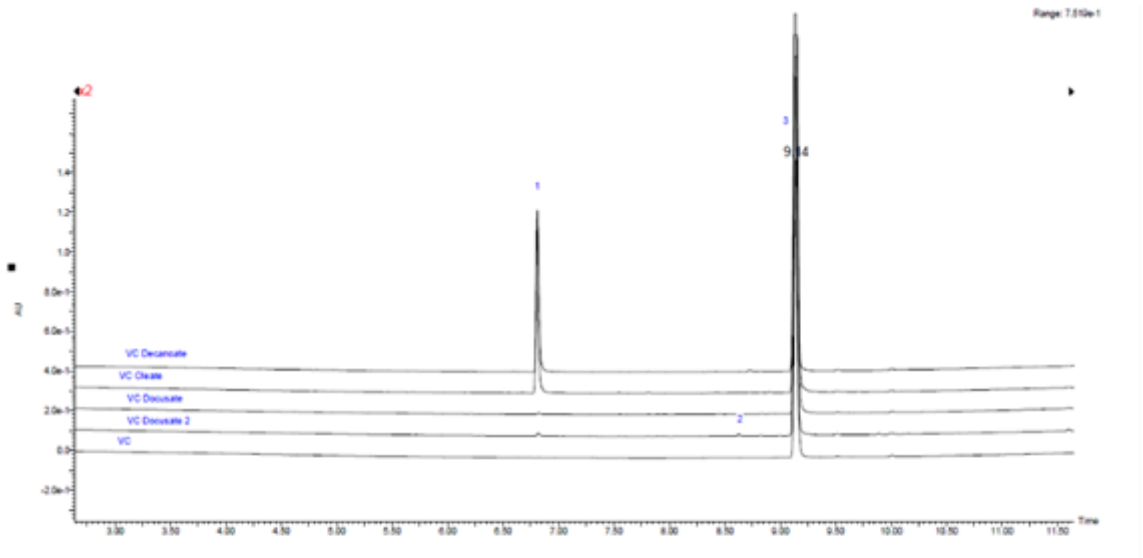



Figure III-4 PI test samples of sLBF-PI with HPMC, Eudragit® EPO and HPMCAS after 180 min in FaSSIF. Agglomeration of Eudragit® EPO can be observed.

Table III-1 Thermal properties of venetoclax. Melting point ($T_m$), Enthalpy of fusion ($\Delta H_{fus}$), Entropy of fusion ($\Delta S_{fus}$).

| | $T_m$ [ºC] | $\Delta H_{fus}$ [kJ/mol] | $\Delta S_{fus}$ x$10^{-2}$ [kJ/mol/K] |
|---|---|---|---|
| Venetoclax batch 1810004 | 139.1 ± 0.03 | 19.2 ± 0.03 | 4.7 ± 0.01 |

Appendix IV Chapter 7



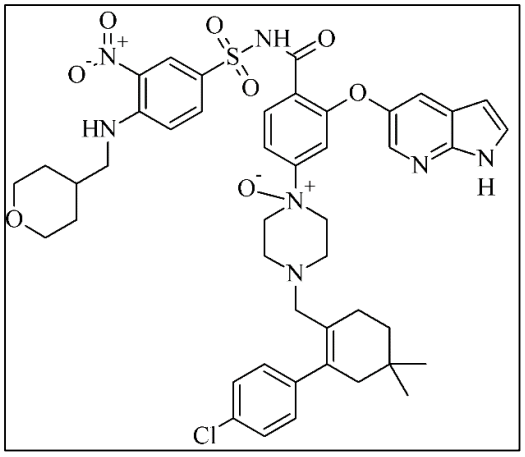

Purity according to relative area by UV at 283 nm:

- Venetoclax docusate batch 1: 98.7 %
- Venetoclax docusate batch 2: 99.8 %
- Venetoclax/oleic acid: 83.8 %
- Venetoclax/ *n*-decanoic acid: 78.6 %

Figure IV-1 Purity of venetoclax docusate and venetoclax co-crystals by UPLC according to the relative area by UV at 283 nm.

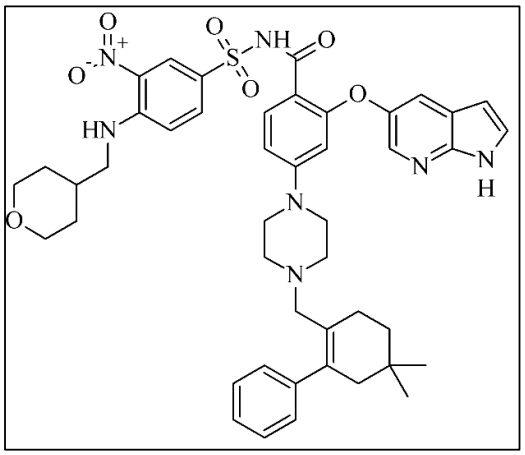
**Major compounds identified in both venetoclax co-crystals**

**1.**



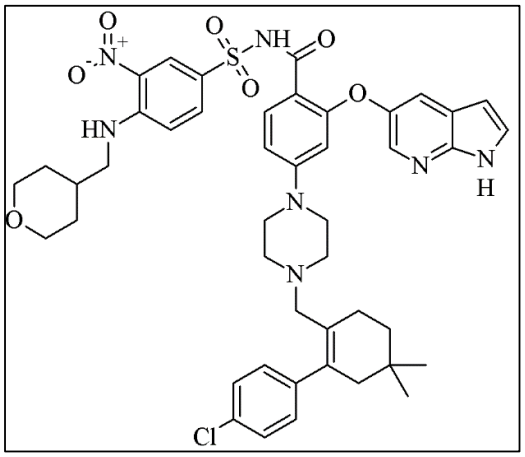
| | |
|---|---|
| Retention time: | 6.81 min |
| Empirical formula: | $C_{45}H_{50}ClN_7O_8S$ |
| Mw: | 884.44 g/mol |
| Mw (exact mass): | 883.3130 g/mol |
| Remarks: | *N*-oxide could be on either side of the piperazine ring) location based on Liu *et al.*) |

**2.**



| | |
|---|---|
| Retention time: | 8.60 min |
| Empirical formula: | $C_{45}H_{51}N_7O_7S$ |
| Mw: | 833.99 g/mol |
| Mw (exact mass): | 833.3571 g/mol |
| Remarks: | n/a |

**3.**



| | |
|---|---|
| Retention time: | 9.14 min |
| Empirical formula: | $C_{45}H_{50}ClN_7O_7S$ |
| Mw: | 868.44 g/mol |
| Mw (exact mass): | 867.3181 g/mol |
| Remarks: | Venetoclax |

Figure IV-2 Major compounds identified in venetoclax/oleic acid and venetoclax/ n-decanoic acid co-crystals as analysed by LC-MS. The impurities are indicated by numbers in Figure IV-1 at the given retention times. Liu *et al.* [223].

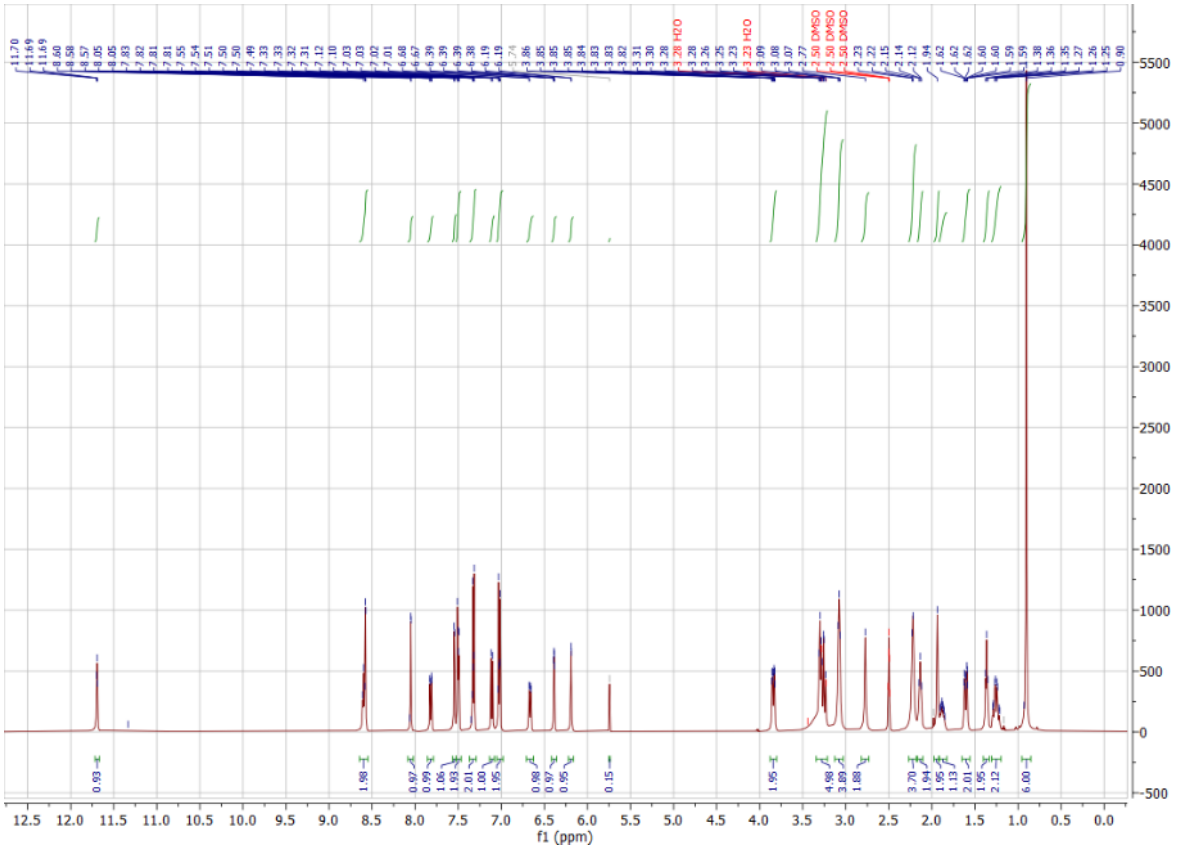



Figure IV-3 Venetoclax free base $^{1}$H-NMR in DMSO- $d_6$ at 300 K, 500 MHz: δ 11.69 (t, J = 2.3 Hz, 1H), 8.55 – 8.64 (m, 2H), 8.05 (d, J = 2.6 Hz, 1H), 7.82 (dd, J = 2.3, 9.2 Hz, 1H), 7.55 (d, J = 2.6 Hz, 1H), 7.47 – 7.53 (m, 2H), 7.28 – 7.38 (m, 2H), 7.11 (d, J = 9.4 Hz, 1H), 6.99 – 7.06 (m, 2H), 6.67 (dd, J = 2.3, 9.1 Hz, 1H), 6.39 (dd, J = 1.9, 3.4 Hz, 1H), 6.19 (d, J = 2.3 Hz, 1H), 3.88 (ddd, j = 1.9, 4.5, 11.4 Hz, 2H), 3.21 – 3.34 (m, 5H), 3.08 (t, J = 5.1 Hz, 4H), 2.77 (s, 2H), 2.22 (d, J = 6.1 Hz, 4H), 2.14 (d, J = 6.5 Hz, 2H), 1.94 (s, 2H), 1.88 (ddt, J = 3.9, 7.8, 11.3 Hz, 1H), 1.61 (ddd, J = 2.0, 4.1, 12.8 Hz, 2H), 1.36 (t, J = 6.5 Hz, 2H), 1.25 (qd, J = 4.5, 12.1 Hz, 2H), 0.92 (s, 6H).

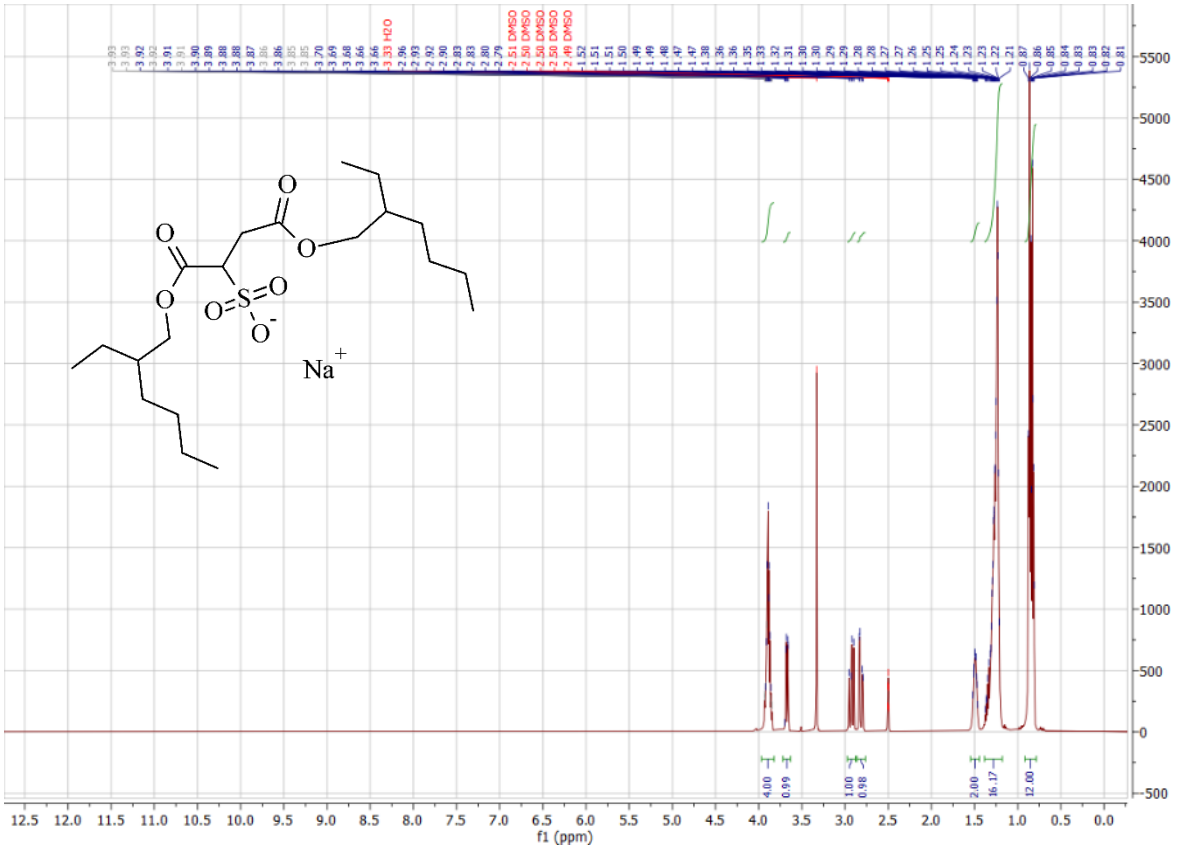



Figure IV-4 Sodium docusate $^{1}$H-NMR in DMSO- $d_6$ at 300 K, 500 MHz: δ 3.83 – 3.98 (m, 4H), 3.67 (dd, J = 3.6, 11.6 Hz, 1H), 2.93 (dd, J = 11.6, 17.2 Hz, 1H), 2.81 (dd, J = 3.6, 17.8 Hz, 1H), 1.43 – 1.56 (m, 2H), 1.18 – 1.40 (m, 16 H), 0.78 -0.92 (m, 12 H).

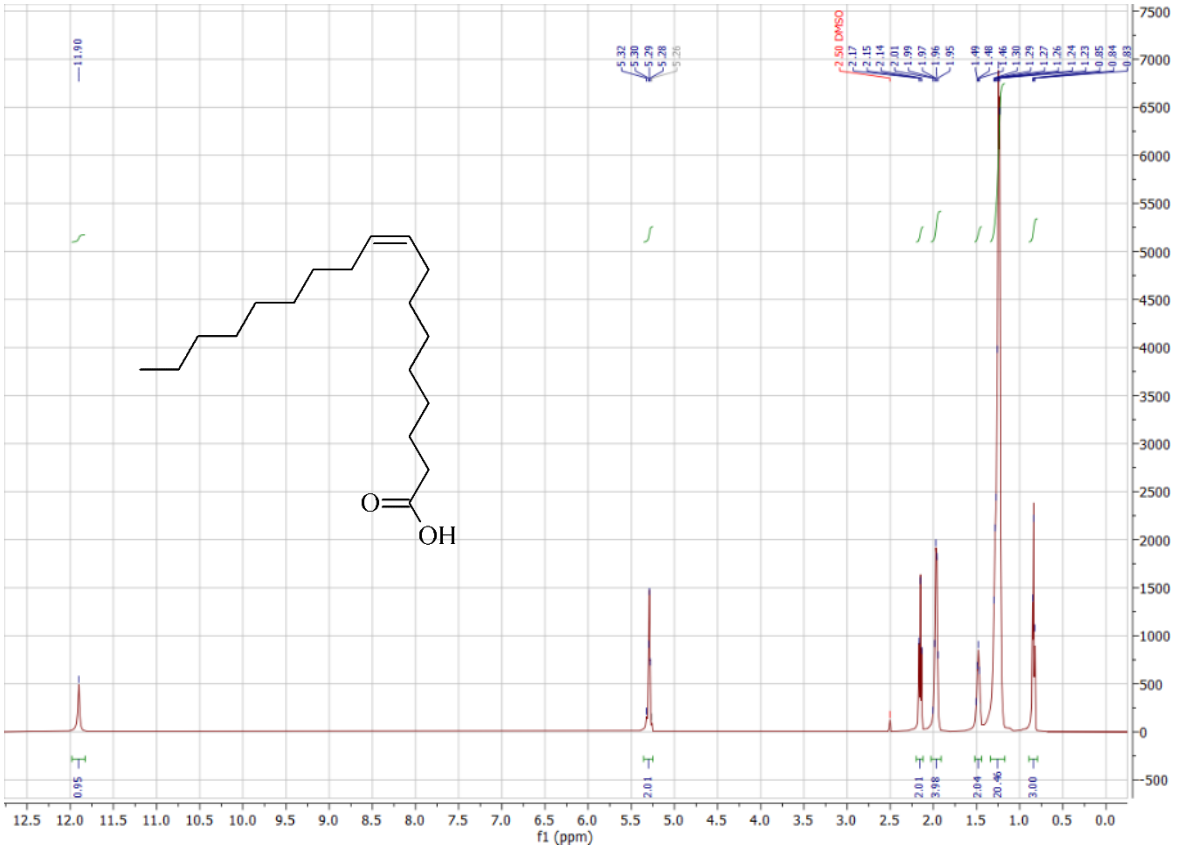



Figure IV-5 Oleic acid $^{1}$H-NMR in DMSO- $d_6$ at 300 K, 500 MHz: δ 11.90 (s, 1H), 5.29 (t, J = 5.0 Hz, 2H), 2.15 (t, J = 7.2 Hz, 2H), 1.97 (q, J = 6.4 Hz, 4H), 1.49 (q, J = 7.1 Hz, 2H), 1.20 – 1.33 (m, 20H), 0.84 (t, J = 6.4 Hz, 3H).

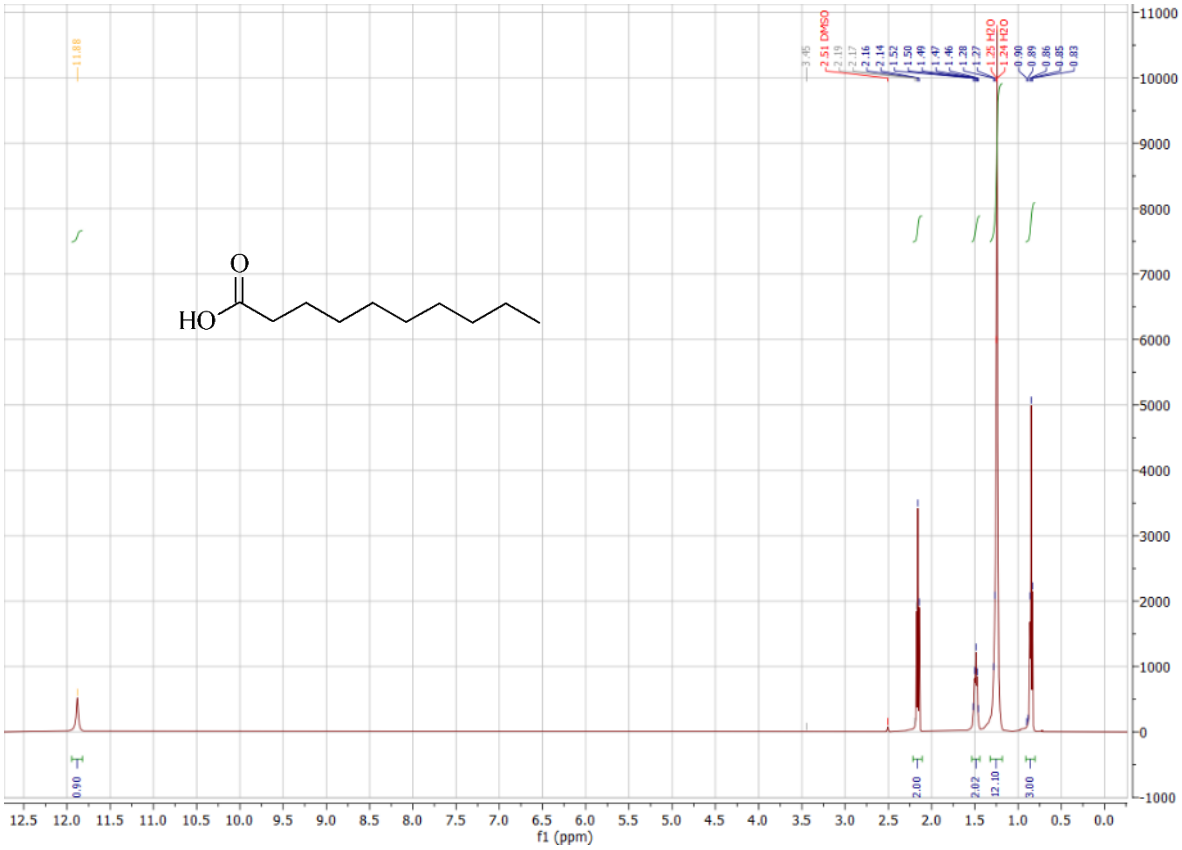



Figure IV-6 *n*-decanoic acid $^{1}$H-NMR in DMSO- $d_6$ at 300 K, 500 MHz: δ 11.88 (s, 1H), 2.15 (d, J = 7.4 Hz, 2H), 1.49 (quin, J = 7.0 Hz, 2H), 1.28 (d, J = 7.3 Hz, 12H), 0.85 (t, J = 6.8 Hz, 3H).

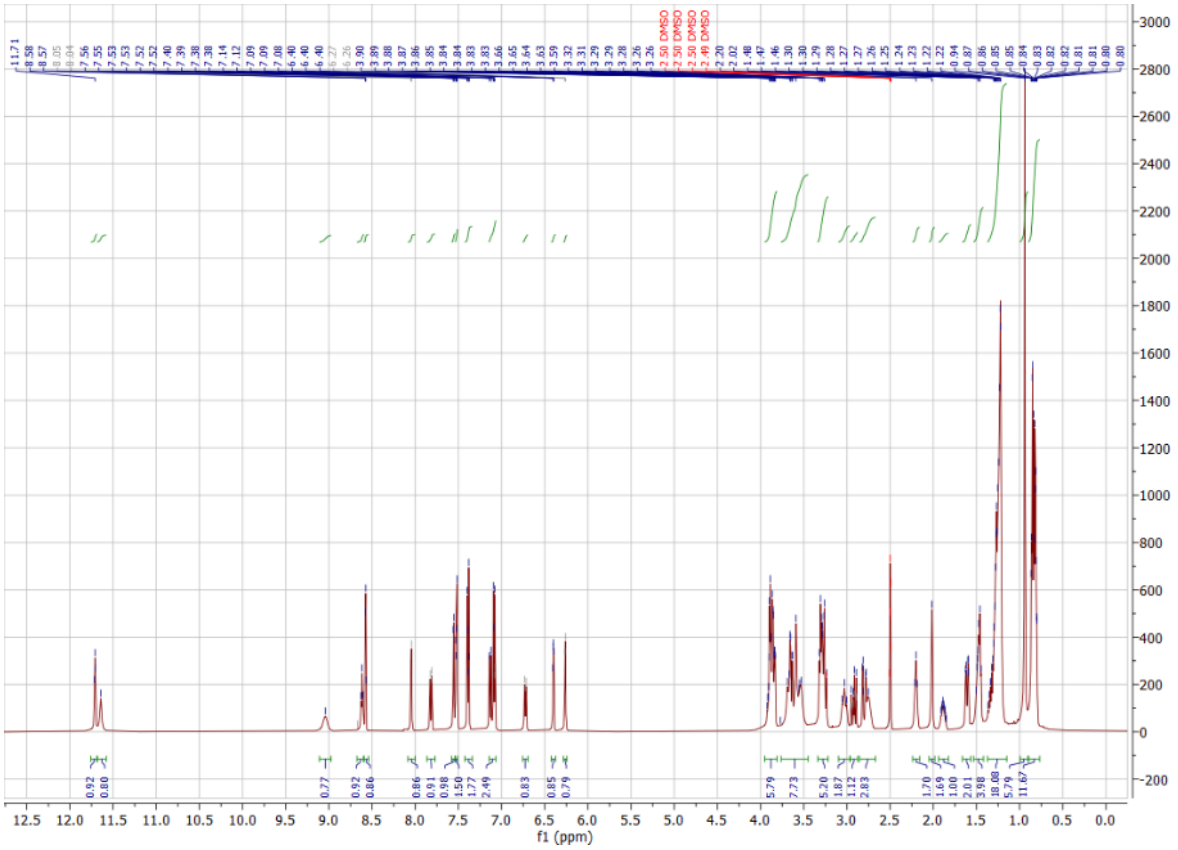



Figure IV-7 Venetoclax docusate $^{1}$H-NMR in DMSO-$d_6$ at 300 K, 500 MHz: δ 11.71 (s, 1H), 11.64 (s, 1H), 9.04 (s, 1H), 8.62 (t, J = 6.0 Hz, 1H), 8.58 (d, J = 2.2 Hz, 1H), 8.05 (d, J = 1.9 Hz, 1H), 7.82 (dd, J = 1.7, 9.6 Hz, 1H), 7.56 (d, J = 2.5 Hz, 1H), 7.49 – 7.56 (m, 1H), 7.43 – 7.53 (m, 2H), 7.11 (dd, J = 8.9, 22.3 Hz, 2H), 6.72 (dd, J = 2.5, 8.4 Hz, 1H), 6.40 (dt, J = 3.7, 1.8 Hz, 1H), 6.26 (d, J = 2.1 Hz, 1H), 3.80 – 3.95 (m, 6H), 3.50 – 3.72 (m, 8H), 3.21 – 3.35 (m, 5H), 3.03 (t, J = 12.6 Hz, 2H), 2.92 (dd, J = 17.0, 11.8 Hz, 1H), 2.77 (overlap of dd and s, 3H), 2.20 (t, J = 6.3 Hz, 2H), 2.02 (s, 2H), 1.89 (dqd, J = 3.3, 7.3, 11.1 Hz, 1H), 1.61 (d, J = 12.6 Hz, 2H), 1.47 (dt, J = 6.3, 12.8 Hz, 4H), 1.19 – 1.39 (m, 18H), 0.94 (s, 6H), 0.77 – 0.89 (m, 12H).

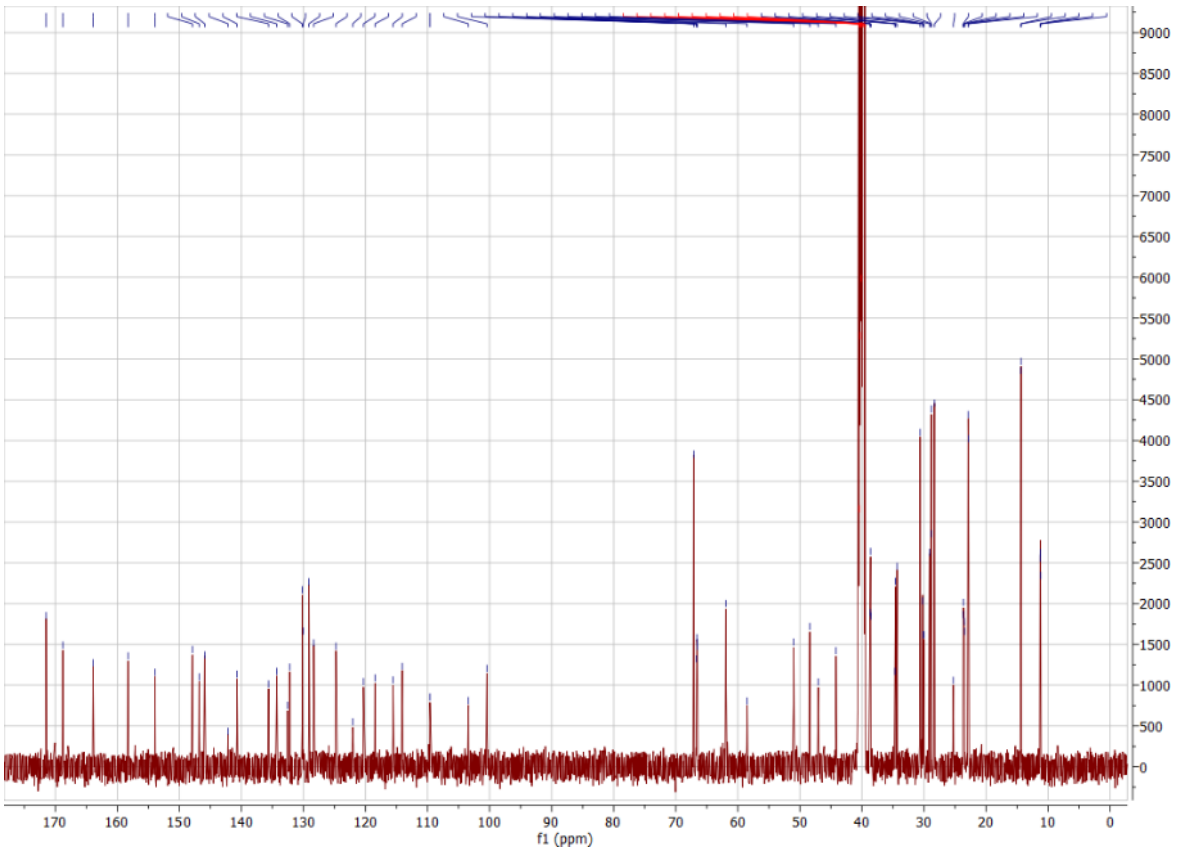



Figure IV-8Venetoclax docusate $^{13}$C-NMR in DMSO-$d_6$ at 300 K, 126 MHz: δ 171.49, 168.79, 163.93, 158.26, 153.93, 147.90, 146.81, 145.92, 142.15, 140.74, 135.63, 134.32, 132.58, 132.23, 130.20, 130.06, 129.13, 128.35, 124.77, 122.06, 120.33, 118.47, 115.58, 114.07, 109.61, 103.42, 100.45, 67.09, 66.66, 66.59, 66.56, 66.52, 61.92, 58.49, 51.02, 48.40, 47.02, 44.18, 40.01 (dp, J = 41.8, 20.9 Hz), 38.64, 38.60, 38.57, 34.68, 34.58, 34.33, 30.62, 30.21, 30.09, 30.03, 29.10, 28.82, 28.80, 28.30, 25.27, 23.67, 23.64, 23.48, 23.46, 22.88, 22.85, 14.37, 14.34, 11.28, 11.24, 11.20.

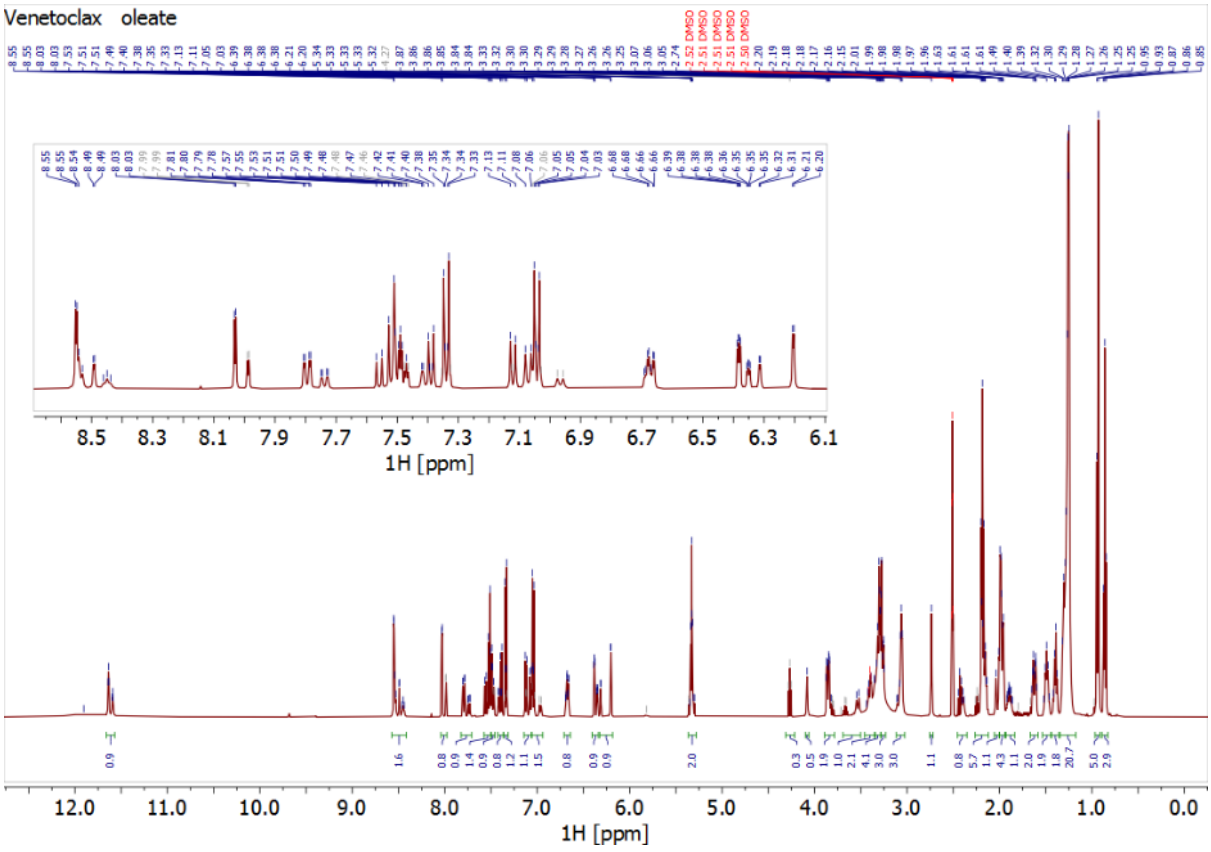



Figure IV-9 Venetoclax/oleic acid $^{1}$H-NMR in DMSO-$d_6$ at 298 K, 500 MHz: δ 11.61 (d, J = 22.6 Hz, 1H), 8.42-8.56 (ddd, J = 29.2, 2.3 Hz, 2H), 8.03/7.98 (dd, J = 21.3, 2.5 Hz ,1H), 7.71 – 7.83 (ddd, J = 28.7, 9.1, 2.3 Hz, 1H), 7.50 – 7.59 (dd, J = 8.8, 20.2 Hz 1H), 7.46 – 7.50 (dt, J = 2.9, 9.9 Hz, 1H), 7.37 – 7.43 (dd, J = 1.9, 6.5 Hz, 1H), 7.31 – 7.37 (dt, J = 1.9, 6.4 Hz, 1H), 7.06 – 7.14 (ddd, J = 2.2, 6.5, 24.5 Hz, 1H), 6.94 – 7.06 (ddt, J = 2.0, 8.4, 38.0 Hz, 2H), 6.64 – 6.71 (ddd, J = 2.6, 5.0, 8.8 Hz, 1H), 6.34 – 6.41 (ddd, J = 1.8, 3.3, 15.6 Hz, 1H), 6.18 – 6.32 (dd, J = 2.3, 54.9 Hz, 1H), 5.33 (tt, J = 1.2, 4.7, 9.6 Hz, 2H), 3.83 – 3.87 (m, 2H), 3.50 – 3.69 (m, 1H), 3.22 – 3.46 (m, 9H), 3.02 – 3.12 (dt, J = 5.0, 18.8 Hz, 3H), 2.74 (s, 1H), 2.35 – 2.45 (m, 1H), 2.12 – 2.26 (m, 6H), 1.94 – 2.06 (sext, J = 5.7, 6.5, 21.9 Hz, 5H), 1.83 – 1.93 (m, 1H), 1.58 – 1.67 (m, 2H), 1.44 – 1.54 (quin, J = 7.2, 14.4 Hz, 2H), 1.36 – 1.44 (dd, J = 7.4, 14.2 Hz, 2H), 1.18 – 1.35 (m, 21H), 0.92 – 0.97 (d, J = 9.3 Hz, 5H), 0.82 – 0.89 (t, J = 7.0 Hz, 3H).

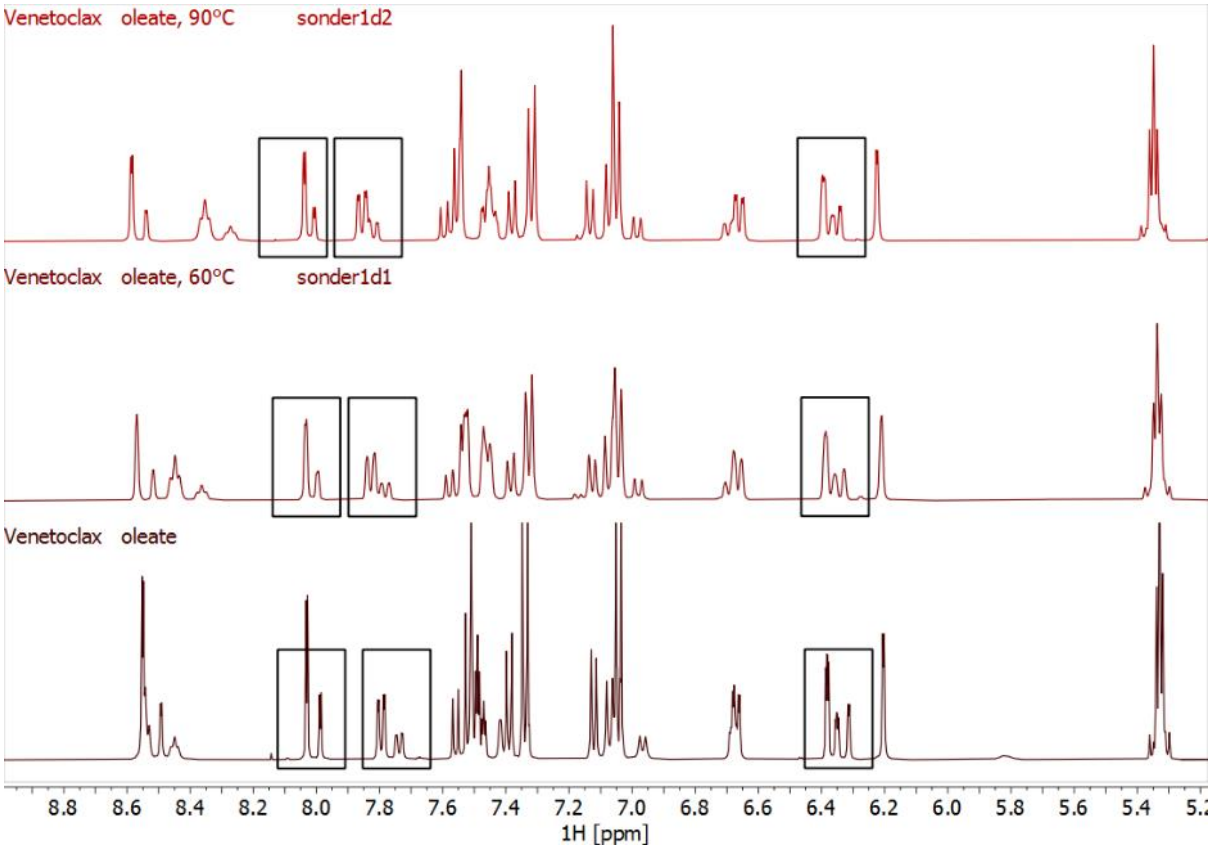



Figure IV-10 Venetoclax/oleic acid $^{1}$H-NMR in DMSO-$d_6$ at 298 K, 333.15 K (60 ºC), 363.15 K (90 ºC), 400 MHz. Displayed is the aromatic region of the spectra. Shifts and merging of the minor and major peaks can be observed.

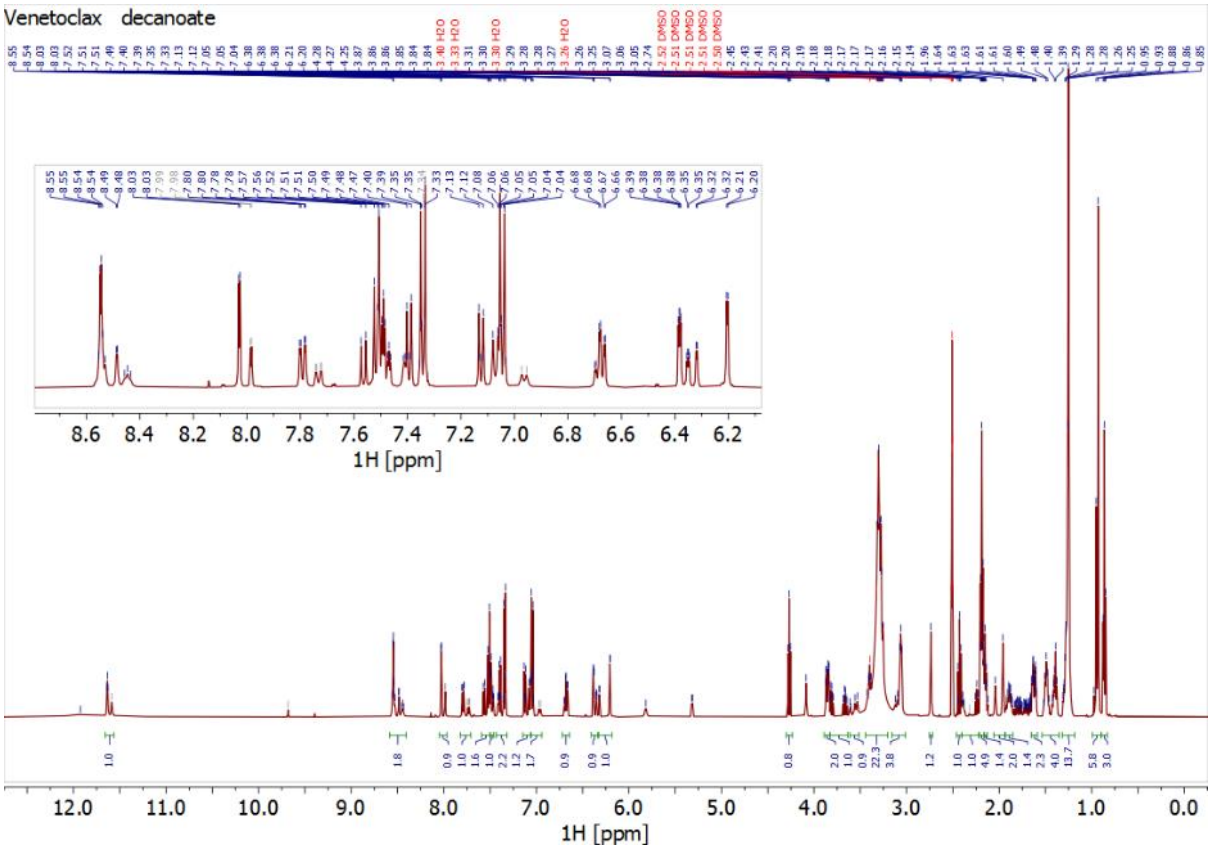



Figure IV-11 Venetoclax/ *n*-decanoic acid $^{1}$H-NMR in DMSO-$d_6$ at 300 K, 500 MHz: δ 11.63 (d, J = 23.7 Hz, 1H), 8.40 – 8.59 (overlap dd and dt, 2H), 8.01 – 8.06 (dd, 2.6, 22.1 Hz, 1H), 7.71 – 7.82 (ddd, J = 2.4, 9.1, 29.9 Hz, 1H), 7.50 – 7.59 (dd, J = 8.6, 24.6 Hz, 2H), 7.46 – 7.50 (dt, J = 3.1, 10.6 Hz, 1H), 7.032 – 7.43 (ddt, J = 1.9, 8.4, 25.9 Hz, 2H), 7.06 – 7.16 (ddd, J = 2.1, 6.4, 29.2 Hz, 1H), 6.94 – 7.06 (dd, J = 2.0, 6.5 Hz, 2H), 6.64 – 6.72 (td, J = 2.4, 8.4 Hz, 1H), 6.33 – 6.41 (dq, J = 1.9, 16.0 Hz, 1H), 6.18 – 6.33 (dd, J = 2.1, 57.0 Hz, 1H), 4.23 – 4.30 (t, J = 7.0 Hz, 1H), 3.83 – 3.89 (m, 2H), 3.64 – 3.83 (dtd, J = 6.0, 7.7, 75.2 Hz, 1H), 3.51 – 3.61 (dd, J = 13.4, 26.2 Hz, 1H), 3.20 – 3.45 (m, 22H), 3.01 – 3.16 (overlap of d and t, 4H), 2.74 (s, 1H), 2.40 – 2.46 (dd, J = 3.1, 8.0 Hz, 1H), 2.22 – 2.41 (dt, J = 7.6, 74.7 Hz, 1H), 2.16 – 2.21 (m, 5H), 2.13 – 2.16 (m, 1H), 1.94 – 2.06 (d, J = 41.3 Hz, 2H), 1.85 – 1.93 (m, 1H), 1.59 – 1.65 (m, 2H), 1.35 – 1.54 (dquin, J = 7.1, 44.5 Hz, 4H), 1.18 – 1.32 (overlap of s and m, 14H), 0.90 – 0.99 (overlapping 3x s, 6H), 0.83 – 0.89 (t, J = 6.8 Hz, 3H).

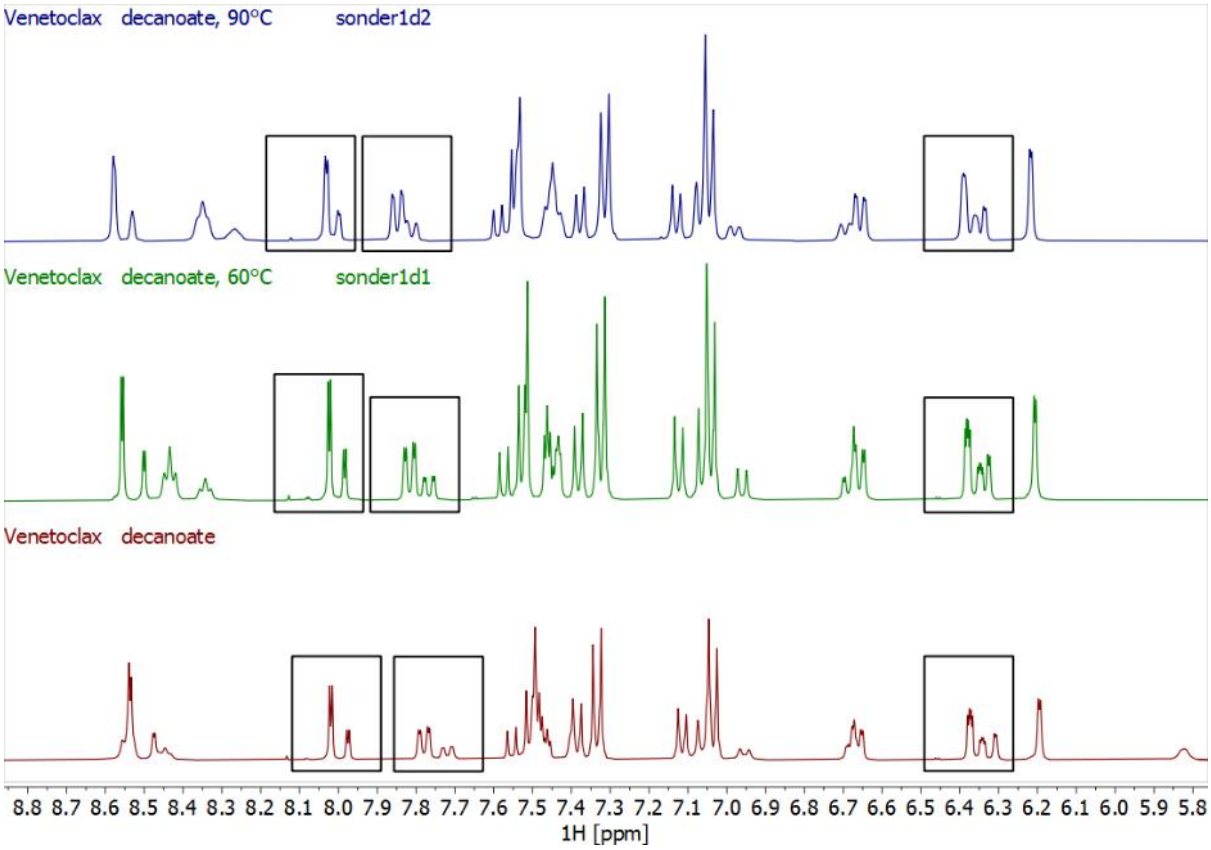



Figure IV-12 Venetoclax/ *n*-decanoic acid $^{1}$H-NMR in DMSO-$d_6$ at 298 K, 333.15 K (60 ºC), 363.15 K (90 ºC), 400 MHz. Displayed is the aromatic region of the spectra. Shifts and merging of the minor and major peaks can be observed.

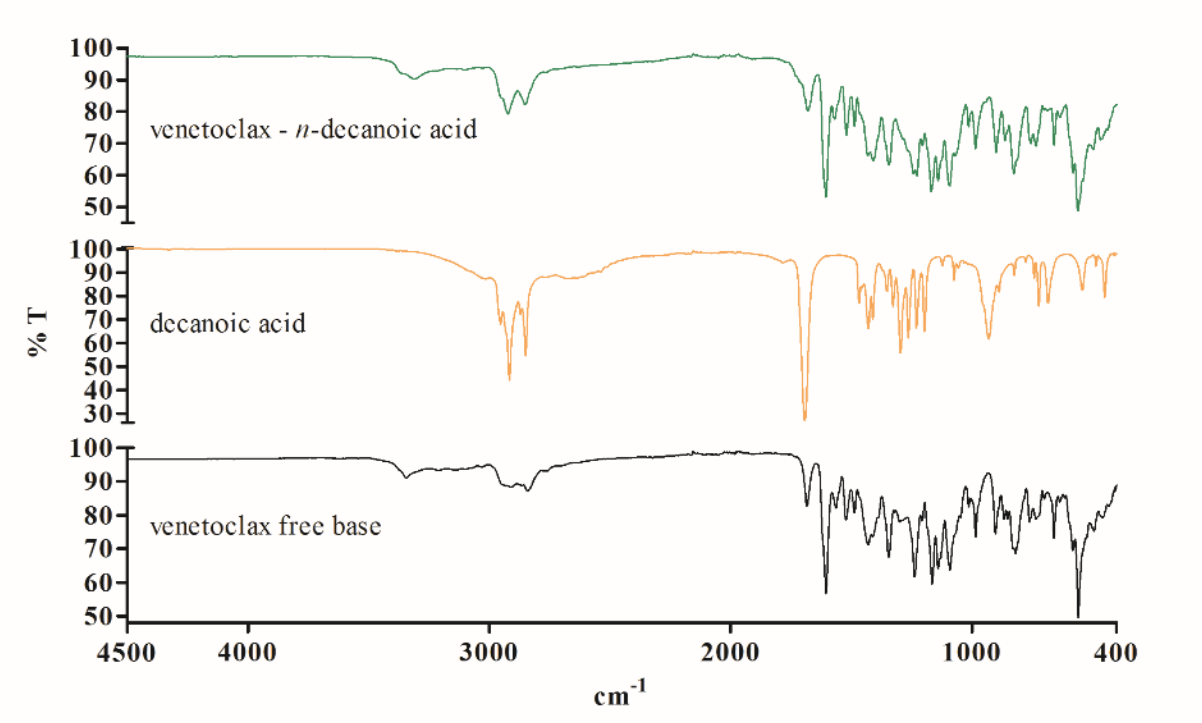



Figure IV-13 FT-IR overlay for venetoclax/*n*-decanoic acid co-crystal.

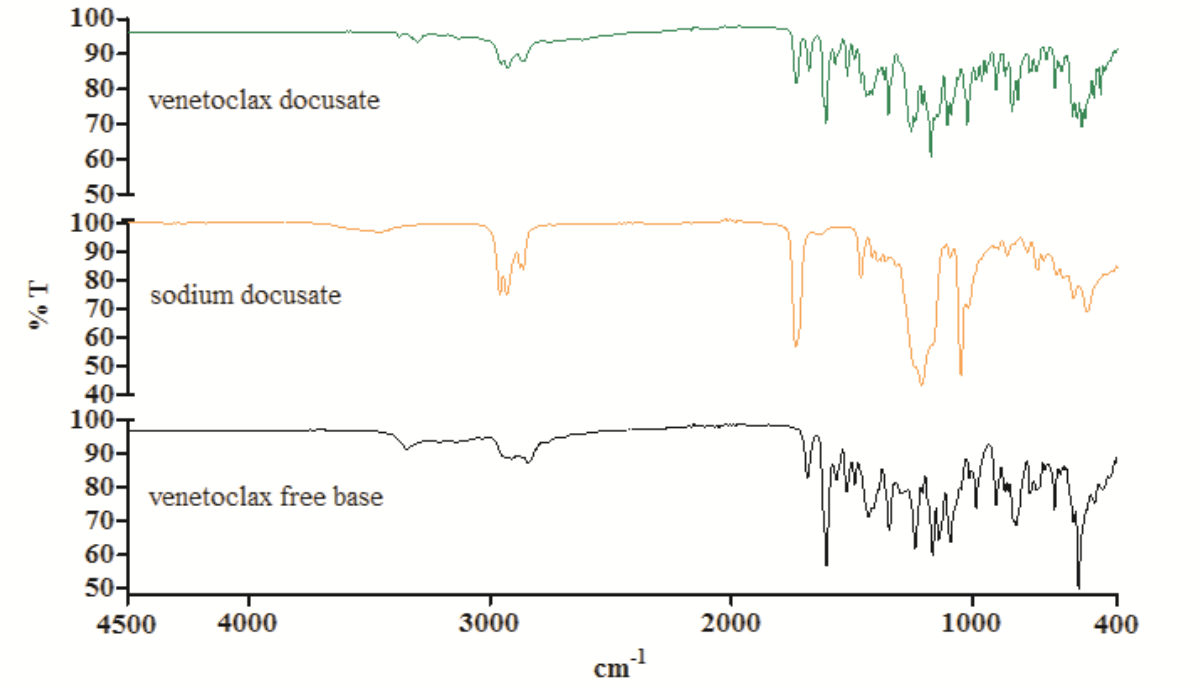



Figure IV-14 FT-IR overlay for venetoclax docusate.

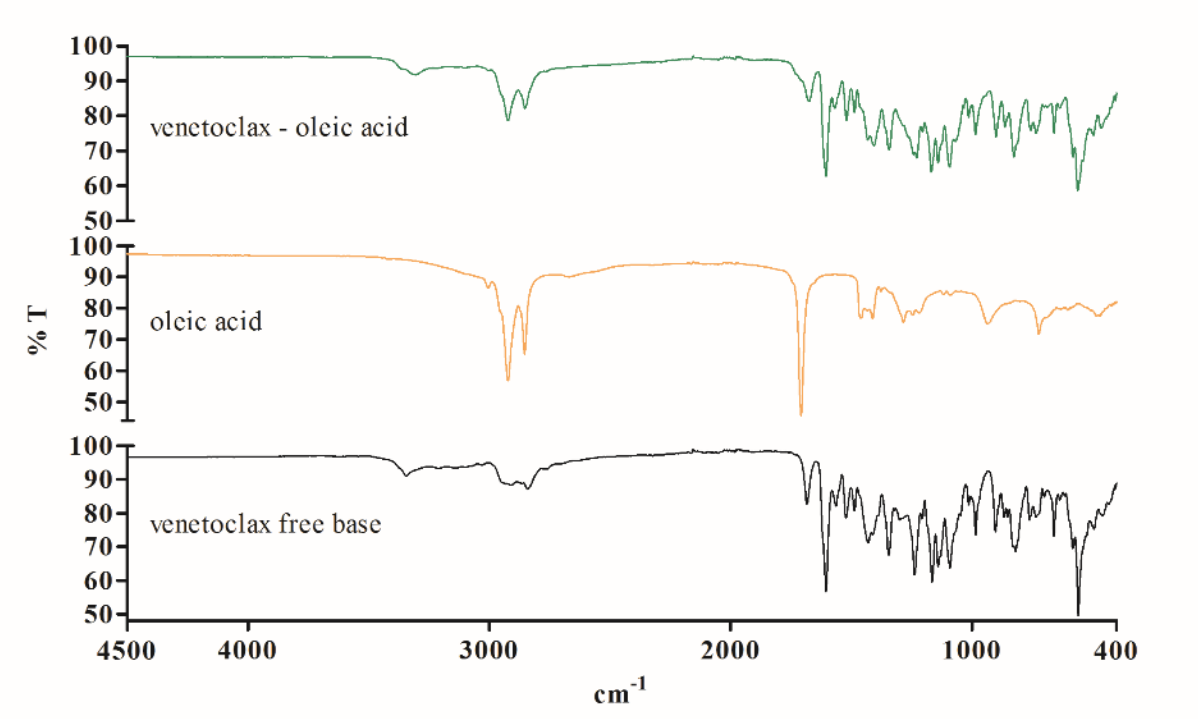



Figure IV-15 FT-IR overlay for venetoclax/oleic acid co-crystal.

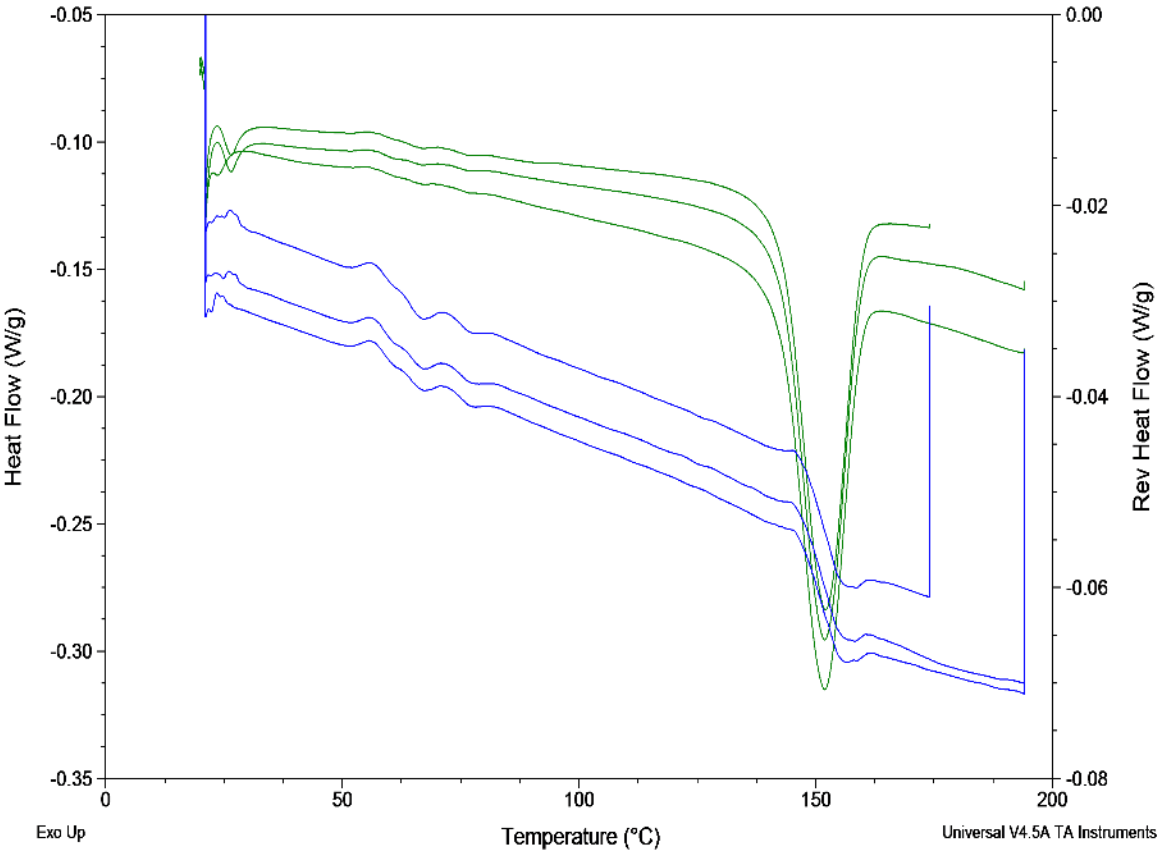



Figure IV-16 Modulated DSC of venetoclax docusate. Heat flow (green) and reversed heat flow (blue) are shown. Exothermic events are illustrated by going up (n = 3).

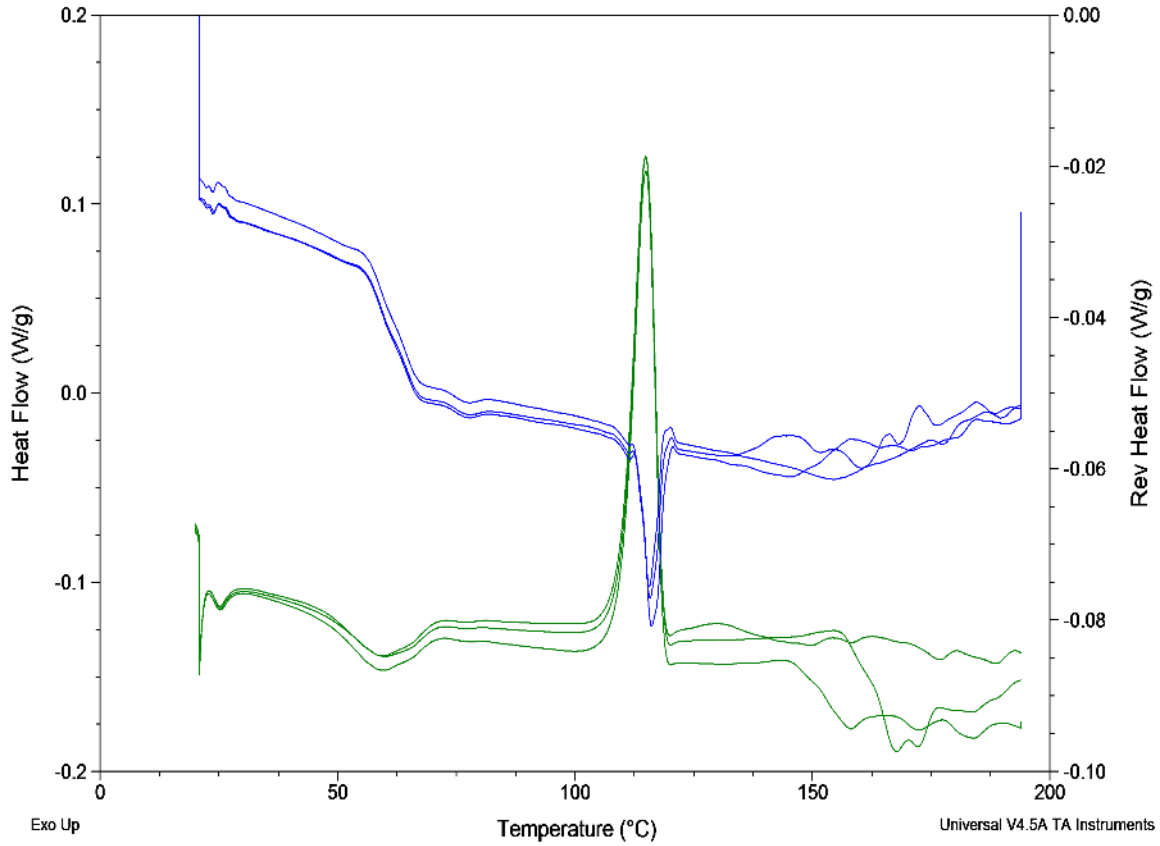



Figure IV-17 Modulated DSC of venetoclax/*n*-decanoic acid co-crystal. Heat flow (green) and reversed heat flow (blue) are illustrated with exothermic events going up (n = 3).

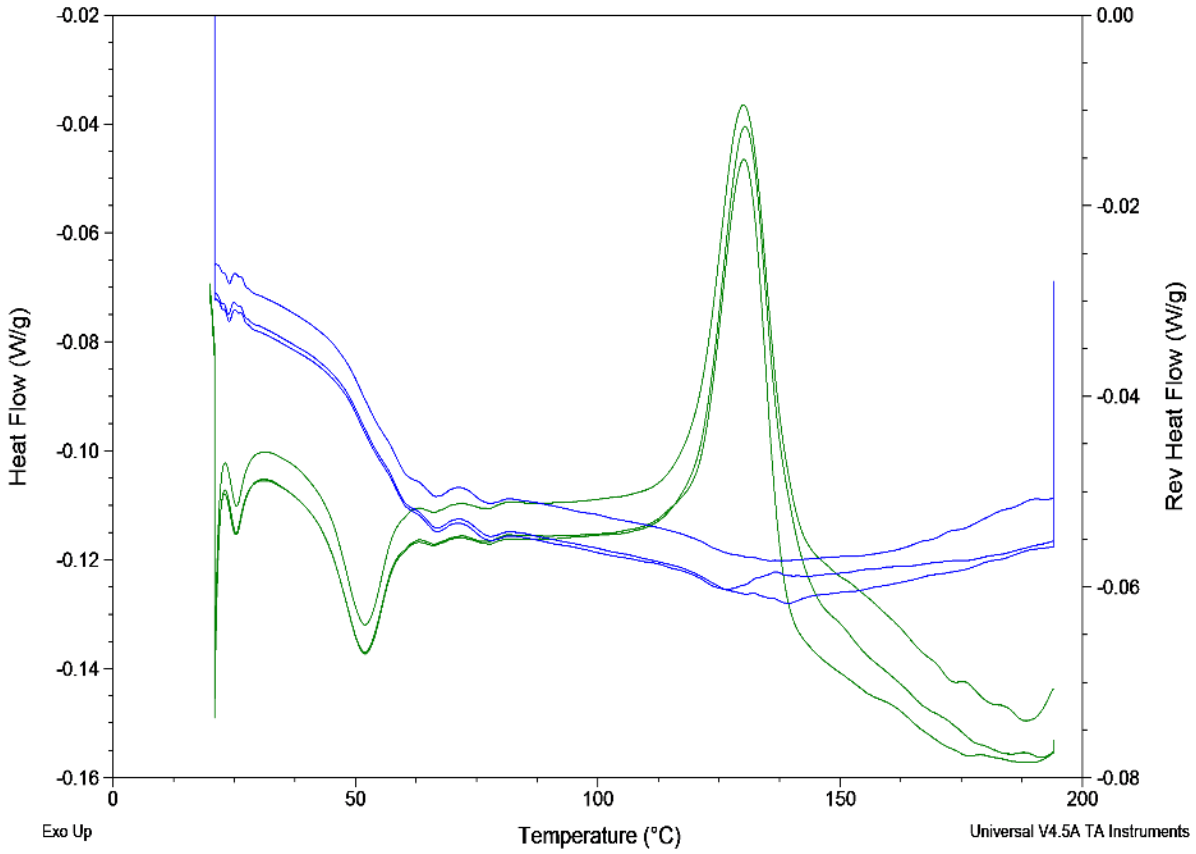



Figure IV-18 Modulated DSC of venetoclax/oleic acid co-crystal. Heat flow (green) and reversed heat flow (blue) are illustrated with exothermic events going up (n = 3).

Table IV-1 Venetoclax co-crystal thermal properties: glass transition temperature ($T_g$), Recrystallisation temperature ($T_{cryst}$), enthalpy of recrystallisation ($\Delta H_{cryst}$), entropy of recrystallisation ($\Delta S_{cryst}$), as well as elemental analysis results (mean ± SD, n = 3).

| | Venetoclax/oleic acid | Venetoclax/ *n*-decanoic acid |
|---|---|---|
| $T_g$ [ºC] | 53.4 ± 4.2 | 59.0 ± 0.3 |
| $T_g$ range [ºC] | 49.2 ± 1.9 – 58.3 ± 1.7 | 57.0 ± 0.1 – 65.3 ± 0.7 |
| $T_{cryst}$ [ºC] | 130.5 ± 0.2 | 114.9 ± 0.04 |
| Onset $T_{cryst}$ [ºC] | 120.3 ± 0.5 | 109.8 ± 0.1 |
| $\Delta H_{cryst}$ [kJ/mol] | 21.7 ± 1.7 | 29.0 ± 0.3 |
| $\Delta S_{cryst}$ x$10^{-2}$ [kJ/mol/K] | 5.4 ± 0.4 | 7.5 ± 0.1 |
| Theoretical C/H/N content [% w/w] [a)] | 65.2 / 7.3 / 8.5 | 62.5 / 6.7 / 9.3 |
| Measured C/H/N content [% w/w] [b)] | 64.9 ± 0.1 / 7.2 ± 0.2 / 8.07 ± 0.04 | 61.3 ± 0.1 / 6.8 ± 0.03 / 8.5 ± 0.1 |

[a)] Corrected for water content. Venetoclax/oleic acid co-crystal: 0.9% (w/w), Venetoclax/ *n*-decanoic acid co-crystal: 1.5% (w/w)

[b)] A deviation of 0.3% (w/w) is considered acceptable as per instrument specifications.

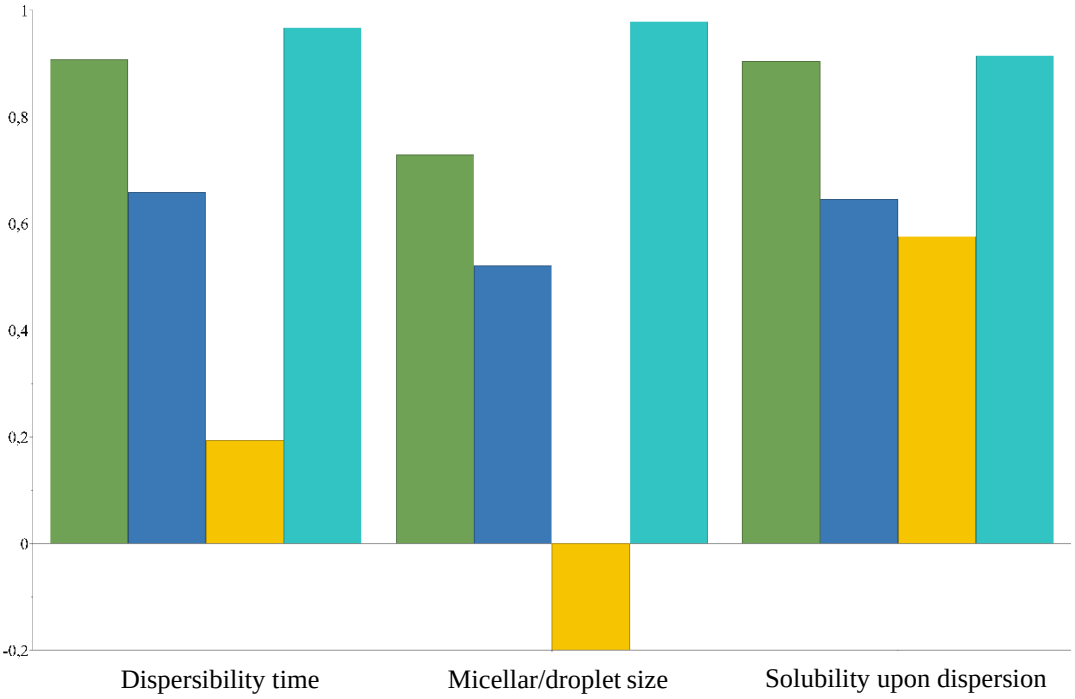



Figure IV-19 Summary of fit for the responses (dispersibility time, micelles/droplet size and solubility upon dispersion) for the PLS model used in the design of experiments approach. Green bars: $R^2$, Blue bars: $Q^2$, yellow bars: model validity and turquoise bars: reproducibility. Dispersibility: N = 15, DF = 9, $R^2$ = 0.91; micelles/droplet size: N = 15, DF = 9, $R^2$ = 0.73; solubility upon dispersion: N = 14, DF = 8, $R^2$ = 0.90.

# Abbreviation list

| | |
|---|---|
| **ADME** | Absorption, distribution, metabolism, elimination |
| **aLFDG** | Advanced lipid formulation developability guide |
| **ANOVA** | Analysis of variance |
| **AUC** | Area under the curve |
| **BCS** | Biopharmaceutical classification system |
| $c_{max}$ | Peak plasma concentration |
| **CMC** | Critical micelle concentration |
| **CYP** | Cytochromes P450 |
| **DCS** | Developability classification system |
| **DMF** | Dimethylformamide |
| **DMSO** | Dimethyl sulfoxide |
| **DoE** | Design of experiment |
| **EC** | European commission |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **FaSSIF** | Fasted state simulated intestinal fluid |
| **FDA** | Food and Drug Administration |
| **FeSSIF** | Fed state simulated intestinal fluid |
| **FFA** | Free fatty acid |
| **FHNW** | University of Applied Sciences Northwestern Switzerland |
| **HLB** | Hydrophilic-lipophilic balance |
| **HPLC** | High-performance liquid chromatography |
| **ICH** | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| **LBF** | Lipid-based formulation |
| **LFCS** | Lipid formulation classification system |
| **LFDG** | Lipid formulation developability guide |
| **LOD** | Limit of detection |
| **log*P*** | Octanol/water partition coefficient |
| **LOQ** | Limit of quantification |
| **MAT** | Mean absorption time |
| **MRT** | Mean residence time |
| **NaTDC** | Sodium taurodeoxycholate |
| **NIH** | National institute of health |

| | |
|---|---|
| **PC** | Phosphocholine |
| **PEARRL** | Pharmaceutical Education And Research with Regulatory Links |
| **P-gp** | P-glycoprotein |
| **PI** | Precipitation inhibitor |
| **PLS** | Partial least squares regression |
| **SD** | Standard deviation |
| **SEDDS** | Self-emulsifying drug delivery system |
| **SEM** | Standard error of the mean |
| **SLAD** | Solubility limited absorbable dose |
| **sLBF** | supersaturated lipid-based formulation |
| **SNEDDS** | Self-nanoemulsifying drug delivery system |
| **TFA** | Trifluoroacetic acid |
| **THF** | Tetrahydrofuran |
| $T_m$ | Melting point |
| $t_{max}$ | Time to reach peak plasma concentrations |
| **UK** | United Kingdom |
| **USA** | United states of America |
| **USP** | United States pharmacopeia |

# References


1. YU LX et al. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharm Res* 2002; 19(7): 921-925.
2. CUSTODIO JM et al. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Adv Drug Deliv Rev* 2008; 60(6): 717-733.
3. DI L et al. Bridging solubility between drug discovery and development. *Drug Discov Today* 2012; 17(9-10): 486-495.
4. LIPP R. The innovator pipeline: Bioavailability challenges and advanced oral drug delivery opportunities. *American Pharmaceutical Review* 2013; 16(3).
5. RANE SS, ANDERSON BD. What determines drug solubility in lipid vehicles: Is it predictable? *Adv Drug Deliver Rev* 2008; 60(6): 638-656.
6. BERGSTROM CA et al. Computational prediction of formulation strategies for beyond-rule-of-5 compounds. *Adv Drug Deliv Rev* 2016; 101: 6-21.
7. MAO F et al. Melting Point Distribution Analysis of Globally Approved and Discontinued Drugs: A Research for Improving the Chance of Success of Drug Design and Discovery. *ChemistryOpen* 2016; 5(4): 357-368.
8. WILLIAMS HD et al. Strategies to address low drug solubility in discovery and development. *Pharmacol Rev* 2013; 65(1): 315-499.
9. AYAD MH. Rational formulation strategy from drug discovery profiling to human proof of concept. *Drug Deliv* 2015; 22(6): 877-884.
10. BUTLER JM, DRESSMAN JB. The developability classification system: application of biopharmaceutics concepts to formulation development. *J Pharm Sci* 2010; 99(12): 4940-4954.

11. SAVLA R et al. Review and analysis of FDA approved drugs using lipid-based formulations. *Drug Dev Ind Pharm* 2017: 1-16.

12. POUTON CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur J Pharm Sci* 2000; 11(Suppl 2): 93-98.

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

14. MU HL et al. Lipid-based formulations for oral administration of poorly water-soluble drugs. *Int J Pharmaceut* 2013; 453(1): 215-224.

15. O' DRISCOLL CM, GRIFFIN BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility--the potential impact of lipid-based formulations. *Adv Drug Deliv Rev* 2008; 60(6): 617-624.

16. HAUSS DJ. *Oral lipid-based formulations : enhancing the bioavailability of poorly water-soluable drugs*, Drugs and the pharmaceutical sciences, New York: Informa Healthcare, 2007.

17. MUELLERTZ A et al. New perspectives on lipid and surfactant based drug delivery systems for oral delivery of poorly soluble drugs. *J Pharm Pharmacol* 2010; 62(11): 1622-1636.

18. PORTER CJH et al. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat Rev Drug Discov* 2007; 6(3): 231-248.

19. PORTER CJH et al. Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Adv Drug Deliver Rev* 2008; 60(6): 673-691.

20. GAUTSCHI N et al. Rapid determination of drug solubilization versus supersaturation in natural and digested lipids. *Int J Pharm* 2016; 513(1-2): 164-174.

21. GOOLE J et al. The effects of excipients on transporter mediated absorption. *Int J Pharm* 2010; 393(1-2): 17-31.

22. WASAN KM. *Role of lipid excipients in modifying oral and parenteral drug delivery : basic principles and biological examples*, Hoboken, N.J.: Wiley-Interscience, 2007.

23. AL-ALI AAA et al. 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.

24. LINDMARK T et al. Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers. *J Pharmacol Exp Ther* 1995; 275(2): 958-964.

25. LINDMARK T et al. 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): 362-369.

26. LINDMARK T et al. 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): 215-223.

27. ASPENSTROM-FAGERLUND B et al. 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.

28. HEERKLOTZ H. Interactions of surfactants with lipid membranes. *Q Rev Biophys* 2008; 41(3-4): 205-264.

29. CHARMAN WNA, STELLA VJ. Estimating the Maximal Potential for Intestinal Lymphatic Transport of Lipophilic Drug Molecules. *Int J Pharmaceut* 1986; 34(1-2): 175-178.

30. LAWLESS E et al. Exploring the impact of drug properties on the extent of intestinal lymphatic transport - in vitro and in vivo studies. *Pharm Res* 2015; 32(5): 1817-1829.

31. THOMAS N et al. In vitro and in vivo performance of novel supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS). *J Control Release* 2012; 160(1): 25-32.

32. THOMAS N et al. Supersaturated self-nanoemulsifying drug delivery systems (Super-SNEDDS) enhance the bioavailability of the poorly water-soluble drug simvastatin in dogs. *Aaps J* 2013; 15(1): 219-227.

33. THOMAS N et al. In vitro lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery systems containing fenofibrate. *Aaps J* 2014; 16(3): 539-549.

34. ILIE AR et al. Supersaturated lipid-based drug delivery systems - exploring impact of lipid composition type and drug properties on supersaturability and physical stability. *Drug Dev Ind Pharm* 2020: 1-9.

35. FERRAZ R et al. Ionic liquids as active pharmaceutical ingredients. *ChemMedChem* 2011; 6(6): 975-985.

36. PORTER C et al. Compositions and preparation methods of low melting ionic salts of poorly-water soluble drugs. US: MW Encap Ltd, 2014.

37. SAHBAZ Y et al. Transformation of poorly water-soluble drugs into lipophilic ionic liquids enhances oral drug exposure from lipid based formulations. *Mol Pharm* 2015; 12(6): 1980-1991.

38. MORGEN M et al. Lipophilic salts of poorly soluble compounds to enable high-dose lipidic SEDDS formulations in drug discovery. *Eur J Pharm Biopharm* 2017; 117: 212-223.

39. SAHBAZ Y et al. Ionic Liquid Forms of Weakly Acidic Drugs in Oral Lipid Formulations: Preparation, Characterization, in Vitro Digestion, and in Vivo Absorption Studies. *Mol Pharm* 2017; 14(11): 3669-3683.

40. WILLIAMS HD et al. Enhancing the Oral Absorption of Kinase Inhibitors Using Lipophilic Salts and Lipid-Based Formulations. *Mol Pharm* 2018; 15(12): 5678–5696.

41. WILLIAMS HD et al. Transformation of Biopharmaceutical Classification System Class I and III Drugs Into Ionic Liquids and Lipophilic Salts for Enhanced Developability Using Lipid Formulations. *J Pharm Sci* 2018; 107(1): 203-216.

42. TAY E et al. Ionic Liquid Forms of the Antimalarial Lumefantrine in Combination with LFCS Type IIIB Lipid-Based Formulations Preferentially Increase Lipid Solubility, In Vitro Solubilization Behavior and In Vivo Exposure. *Pharmaceutics* 2019; 12(1).

43. WILLIAMS HD et al. Unlocking the full potential of lipid-based formulations using lipophilic salt/ionic liquid forms. *Adv Drug Deliv Rev* 2019; 142: 75-90.

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

45. LARSEN A et al. Lipid-based Formulations for Danazol Containing a Digestible Surfactant, Labrafil M2125CS: In Vivo Bioavailability and Dynamic In Vitro Lipolysis. *Pharm Res-Dordr* 2008; 25(12): 2769-2777.

46. SIMOVIC S et al. Dry hybrid lipid-silica microcapsules engineered from submicron lipid droplets and nanoparticles as a novel delivery system for poorly soluble drugs. *Mol Pharm* 2009; 6(3): 861-872.

47. TAN A et al. Silica-lipid hybrid (SLH) microcapsules: a novel oral delivery system for poorly soluble drugs. *J Control Release* 2009; 134(1): 62-70.

48. SCHULTZ HB et al. Supersaturated silica-lipid hybrids (super-SLH): An improved solid-state lipid-based oral drug delivery system with enhanced drug loading. *Eur J Pharm Biopharm* 2018; 125: 13-20.

49. JOYCE P et al. Solidification to improve the biopharmaceutical performance of SEDDS: Opportunities and challenges. *Adv Drug Deliv Rev* 2019; 142: 102-117.

50. SCHULTZ HB et al. Supersaturated Silica-Lipid Hybrid Oral Drug Delivery Systems: Balancing Drug Loading and In Vivo Performance. *J Pharmacol Exp Ther* 2019; 370(3): 742-750.

51. SCHULTZ HB et al. Supersaturated-Silica Lipid Hybrids Improve in Vitro Solubilization of Abiraterone Acetate. *Pharm Res* 2020; 37(4): 77.

52. DITZINGER F et al. Lipophilicity and hydrophobicity considerations in bio-enabling oral formulations approaches - a PEARRL review. *J Pharm Pharmacol* 2018.

53. ALSKAR LC et al. Tools for Early Prediction of Drug Loading in Lipid-Based Formulations. *Mol Pharm* 2016; 13(1): 251-261.

54. PERSSON LC et al. Computational prediction of drug solubility in lipid based formulation excipients. *Pharm Res* 2013; 30(12): 3225-3237.

55. CALIPH SM et al. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J Pharm Sci-Us* 2000; 89(8): 1073-1084.

56. THI TD et al. Formulate-ability of ten compounds with different physicochemical profiles in SMEDDS. *Eur J Pharm Sci* 2009; 38(5): 479-488.

57. DUMANLI I et al. Mechanistica studies to elucidate the role of lipid vehicles on solubility, formulation and bioavailability of poorly soluble compounds. Open Access Dissertations: University of Rhode Island, 2002 (dissertation).

58. BIRRU WA et al. Computational Models of the Gastrointestinal Environment. 1. The Effect of Digestion on the Phase Behavior of Intestinal Fluids. *Mol Pharm* 2017; 14(3): 566-579.

59. JANKOVIC S et al. Application of the solubility parameter concept to assist with oral delivery of poorly water-soluble drugs - a PEARRL review. *J Pharm Pharmacol* 2018.

60. HOUGH WL, ROGERS RD. Ionic liquids then and now: From solvents to materials to active pharmaceutical ingredients. *B Chem Soc Jpn* 2007; 80(12): 2262-2269.

61. FEENEY OM et al. 50years of oral lipid-based formulations: Provenance, progress and future perspectives. *Adv Drug Deliv Rev* 2016; 101: 167-194.

62. BALA V et al. Lipophilic Prodrugs of SN38: Synthesis and in Vitro Characterization toward Oral Chemotherapy. *Mol Pharm* 2016; 13(1): 287-294.

63. HAN S et al. Targeted delivery of a model immunomodulator to the lymphatic system: comparison of alkyl ester versus triglyceride mimetic lipid prodrug strategies. *J Control Release* 2014; 177: 1-10.

64. MICHAELSEN MH et al. The Effect of Digestion and Drug Load on Halofantrine Absorption from Self-nanoemulsifying Drug Delivery System (SNEDDS). *Aaps J* 2016; 18(1): 180-186.

65. SIQUEIRA SD et al. Influence of drug load and physical form of cinnarizine in new SNEDDS dosing regimens: in vivo and in vitro evaluations. *Aaps J* 2017; 19(2): 587-594.

66. SIQUEIRA JORGENSEN SD et al. The ability of two in vitro lipolysis models reflecting the human and rat gastro-intestinal conditions to predict the in vivo performance of SNEDDS dosing regimens. *Eur J Pharm Biopharm* 2018; 124: 116-124.

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

68. CHAKRABARTI S, BELPAIRE FM. Biovailability of phenytoin in lipid containing dosage forms in rats. *J Pharm Pharmacol* 1978; 30(5): 330-331.

69. EL-LAITHY HM et al. Novel self-nanoemulsifying self-nanosuspension (SNESNS) for enhancing oral bioavailability of diacerein: Simultaneous portal blood absorption and lymphatic delivery. *Int J Pharm* 2015; 490(1-2): 146-154.

70. LARSEN AT et al. Solution or suspension - Does it matter for lipid based systems? In vivo studies of chase dosing lipid vehicles with aqueous suspensions of a poorly soluble drug. *Eur J Pharm Biopharm* 2017; 117: 308-314.

71. PORTER CJH et al. Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: Studies with halofantrine. *J Pharm Sci-Us* 2004; 93(5): 1110-1121.

72. ROLAN PE et al. Examination of some factors responsible for a food-induced increase in absorption of atovaquone. *Br J Clin Pharmacol* 1994; 37(1): 13-20.

73. JANNIN V et al. Approaches for the development of solid and semi-solid lipid-based formulations. *Adv Drug Deliv Rev* 2008; 60(6): 734-746.

74. O'SHEA JP et al. Lipidic dispersion to reduce food dependent oral bioavailability of fenofibrate: In vitro, in vivo and in silico assessments. *Eur J Pharm Biopharm* 2015; 96: 207-216.

75. LARSEN AT et al. In vitro lipolysis models as a tool for the characterization of oral lipid and surfactant based drug delivery systems. *Int J Pharmaceut* 2011; 417(1-2): 245-255.

76. WILLIAMS HD et al. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 3: understanding supersaturation versus precipitation potential

during the in vitro digestion of type I, II, IIIA, IIIB and IV lipid-based formulations. *Pharm Res* 2013; 30(12): 3059-3076.

77. GRIFFIN BT et al. Comparison of in vitro tests at various levels of complexity for the prediction of in vivo performance of lipid-based formulations: Case studies with fenofibrate. *Eur J Pharm Biopharm* 2014; 86(3): 427-437.

78. FERNANDEZ S et al. 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): 1901-1910.

79. BAKALA-N'GOMA JC et al. 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): 1279-1287.

80. CHRISTOPHERSEN PC et al. 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: 232-239.

81. CHRISTIANSEN ML et al. Cinnarizine food-effects in beagle dogs can be avoided by administration in a Self Nano Emulsifying Drug Delivery System (SNEDDS). *Eur J Pharm Sci* 2014; 57: 164-172.

82. BIBI HA et al. Simultaneous lipolysis/permeation in vitro model, for the estimation of bioavailability of lipid based drug delivery systems. *Eur J Pharm Biopharm* 2017; 117: 300-307.

83. KEEMINK J et al. Lipolysis-Permeation Setup for Simultaneous Study of Digestion and Absorption in Vitro. *Mol Pharm* 2019; 16(3): 921-930.

84. O'DWYER PJ et al. Novel Biphasic Lipolysis Method To Predict in Vivo Performance of Lipid-Based Formulations. *Mol Pharmaceut* 2020; 17(9): 3342-3352.

85. DI CAGNO M et al. New biomimetic barrier Permeapad for efficient investigation of passive permeability of drugs. *Eur J Pharm Sci* 2015; 73: 29-34.

86. BERBEN P et al. Drug permeability profiling using cell-free permeation tools: Overview and applications. *Eur J Pharm Sci* 2018; 119: 219-233.

87. ALVEBRATT C et al. An in vitro dissolution-digestion-permeation assay for the study of advanced drug delivery systems. *Eur J Pharm Biopharm* 2020; 149: 21-29.

88. CARRIERE F. Impact of gastrointestinal lipolysis on oral lipid-based formulations and bioavailability of lipophilic drugs. *Biochimie* 2016; 125: 297-305.

89. EMEA. Tasigna (Nilotinib) European public assessment report. *Tasigna (Nilotinib) European public assessment report.* https://www.ema.europa.eu/en/documents/scientific-discussion/tasigna-epar-scientific-discussion_en.pdf: EMEA, 2007.

90. FDA. Nilotinib (Tasigna) - Clinical Pharmacology and Biopharmaceutics Review. *Tasigna (Nilotinib) Oral Capsules Drug Approval Package.* https://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068toc.cfm: U.S. Department of Health and Human Services, 2008.

91. MAHON FX et al. Evidence that resistance to nilotinib may be due to BCR-ABL, Pgp, or Src kinase overexpression. *Cancer Res* 2008; 68(23): 9809-9816.

92. YAMAKAWA Y et al. Distinct interaction of nilotinib and imatinib with P-Glycoprotein in intracellular accumulation and cytotoxicity in CML Cell Line K562 cells. *Biol Pharm Bull* 2014; 37(8): 1330-1335.

93. YIN OQ et al. Effect of grapefruit juice on the pharmacokinetics of nilotinib in healthy participants. *J Clin Pharmacol* 2010; 50(2): 188-194.

94. EMEA. Annex I Summary of product characteristics. *Annex I Summary of product characteristics.* https://www.ema.europa.eu/en/documents/product-information/tasigna-epar-product-information_en.pdf: EMEA, 2007.

95. TIAN X et al. Clinical Pharmacokinetic and Pharmacodynamic Overview of Nilotinib, a Selective Tyrosine Kinase Inhibitor. *J Clin Pharmacol* 2018; 58(12): 1533-1540.

96. FDA. Venclexta (Venetoclax) Clinical pharmacology and biopharmaceutics review(s) application number: 208573Orig1s000. In: review, O. o. c. p. ed. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208573Orig1s000ClinPharmR.pdf: U.S. Department of Health and Human Services, 2015.

97. EMEA. Assessment report Venclyxto. *Committee for Medicinal Products for Human Use (CHMP).* https://www.ema.europa.eu/en/documents/assessment-report/venclyxto-epar-public-assessment-report_en.pdf, 2016.

98. CHOO EF et al. The role of lymphatic transport on the systemic bioavailability of the Bcl-2 protein family inhibitors navitoclax (ABT-263) and ABT-199. *Drug Metab Dispos* 2014; 42(2): 207-212.

99. EMAMI RIEDMAIER A et al. Mechanistic Physiologically Based Pharmacokinetic Modeling of the Dissolution and Food Effect of a Biopharmaceutics Classification System IV Compound-The Venetoclax Story. *J Pharm Sci* 2018; 107(1): 495-502.

100. LIPINSKI CA. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* 2000; 44(1): 235-249.

101. BERGSTROM CA, YAZDANIAN M. Lipophilicity in Drug Development: Too Much or Not Enough? *Aaps J* 2016; 18(5): 1095-1100.

102. KUENTZ M. Lipid-based formulations for oral delivery of lipophilic drugs. *Drug Discov Today Technol* 2012; 9(2): e71-e174.

103. SHAH SM et al. Preclinical formulations: insight, strategies, and practical considerations. *Aaps Pharmscitech* 2014; 15(5): 1307-1323.

104. O'NEIL MJ. *The Merck index : an encyclopedia of chemicals, drugs, and biologicals*, 13th edn. Whitehouse Station, N.J.: Merck, 2001.

105. GUIDECHEM. Nilotinib. 2019.

106. WILLIAMS HD et al. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 1: method parameterization and comparison of in vitro digestion profiles across a range of representative formulations. *J Pharm Sci* 2012; 101(9): 3360-3380.

107. MUSTER TH, PRESTIDGE CA. Water adsorption kinetics and contact angles of pharmaceutical powders. *J Pharm Sci* 2005; 94(4): 861-872.

108. PIRRO E et al. A New HPLC-UV Validated Method for Therapeutic Drug Monitoring of Tyrosine Kinase Inhibitors in Leukemic Patients. *Journal of Chromatographic Science* 2011; 49(10): 753-757.

109. EUROPEAN PHARMACOPOEIA COMMISSION., COUNCIL OF EUROPE. *European pharmacopoeia 9.0, General notices*, 9.0 edn., 2016.

110. SHUKLA S et al. Synthesis and characterization of a BODIPY conjugate of the BCR-ABL kinase inhibitor Tasigna (nilotinib): evidence for transport of Tasigna and its fluorescent derivative by ABC drug transporters. *Mol Pharm* 2011; 8(4): 1292-1302.

111. XIA B et al. Nilotinib preclinical pharmacokinetics and practical application toward clinical projections of oral absorption and systemic availability. *Biopharm Drug Dispos* 2012; 33(9): 536-549.

112. STILLHART C, KUENTZ M. Trends in the Assessment of Drug Supersaturation and Precipitation In Vitro Using Lipid-Based Delivery Systems. *J Pharm Sci* 2016; 105(9): 2468-2476.

113. SMITH BT. *Physical pharmacy*, Remington education, London: Pharmaceutical Press, 2016.

114. LENTZ KA. Current methods for predicting human food effect. *Aaps J* 2008; 10(2): 282-288.

115. HOLM R et al. Can Human Food Effects Be Predicted In A Rat Model? *Proceedings of the Conference Can Human Food Effects Be Predicted In A Rat Model?* : AAPS, 2017.

116. O'SHEA JP et al. Food for thought: formulating away the food effect - a PEARRL review. *J Pharm Pharmacol* 2018.

117. HUMBERSTONE AJ, CHARMAN WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv Drug Deliver Rev* 1997; 25(1): 103-128.

118. CHRISTIANSEN ML et al. Effect of food intake and co-administration of placebo self-nanoemulsifying drug delivery systems on the absorption of cinnarizine in healthy human volunteers. *Eur J Pharm Sci* 2016; 84: 77-82.

119. LARSEN AT et al. Solution or suspension - does it matter for lipid based systems? In vivo studies of chase dosing lipid vehicles with aqueous suspensions of a poorly soluble drug. *Eur J Pharm Biopharm* 2017; 117: 308 - 314.

120. ALSKAR LC et al. Effect of lipids on absorption of carvedilol in dogs: Is coadministration of lipids as efficient as a lipid-based formulation? *Journal of Controlled Release* 2019; 304: 90-100.

121. KOEHL NJ et al. New Insights into Using Lipid Based Suspensions for "Brick Dust' Molecules: Case Study of Nilotinib. *Pharm Res-Dordr* 2019; 36(4).

122. ICH GUIDELINE. Validation of analytical procedures: text and methodology Q2 (R1). *International conference on harmonization, Geneva, Switzerland.* ICH harmonised tripartite guideline, 2005.

123. LANGENBUCHER F, MYSICKA J. Invitro and Invivo Deconvolution Assessment of Drug Release Kinetics from Oxprenolol Oros Preparations. *Brit J Clin Pharmaco* 1985; 19: S151-S162.

124. HENZE LJ et al. Exploring gastric emptying rate in minipigs: Effect of food type and pre-dosing of metoclopramide. *Eur J Pharm Sci* 2018; 118: 183-190.

125. MCLAUGHLIN JT et al. Fatty acids stimulate cholecystokinin secretion via an acyl chain length-specific, Ca2+-dependent mechanism in the enteroendocrine cell line STC-1. *J Physiol* 1998; 513 ( Pt 1): 11-18.

126. MCLAUGHLIN J et al. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology* 1999; 116(1): 46-53.

127. KAUKONEN AM et al. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm Res-Dordr* 2004; 21(2): 254-260.

128. FEINLE C et al. Modulation of gastric distension-induced sensations by small intestinal receptors. *Am J Physiol Gastrointest Liver Physiol* 2001; 280(1): G51-57.

129. QUALLS-CREEKMORE E et al. Gastric emptying of enterally administered liquid meal in conscious rats and during sustained anaesthesia. *Neurogastroenterol Motil* 2010; 22(2): 181-185.

130. BOYD BJ et al. 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.

131. MOOTER GVD. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug Discov Today Technol* 2012; 9(2): e71-e174.

132. SINGH A, VAN DEN MOOTER G. Spray drying formulation of amorphous solid dispersions. *Adv Drug Deliv Rev* 2016; 100: 27-50.

133. MERISKO-LIVERSIDGE E et al. Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur J Pharm Sci* 2003; 18(2): 113-120.

134. CHEN J et al. Impact of surfactants on the crystallization of aqueous suspensions of celecoxib amorphous solid dispersion spray dried particles. *Mol Pharm* 2015; 12(2): 533-541.

135. INDULKAR AS et al. Impact of Micellar Surfactant on Supersaturation and Insight into Solubilization Mechanisms in Supersaturated Solutions of Atazanavir. *Pharm Res* 2017; 34(6): 1276-1295.

136. VINAROV Z et al. Micellar solubilization of poorly water-soluble drugs: effect of surfactant and solubilizate molecular structure. *Drug Dev Ind Pharm* 2018; 44(4): 677-686.

137. WU L et al. Physical and chemical stability of drug nanoparticles. *Adv Drug Deliv Rev* 2011; 63(6): 456-469.

138. PARK SH, CHOI HK. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. *Int J Pharm* 2006; 321(1-2): 35-41.

139. CORNAIRE G et al. Effect of polyoxyl 35 castor oil and Polysorbate 80 on the intestinal absorption of digoxin in vitro. *Arzneimittelforschung* 2000; 50(6): 576-579.

140. MA L et al. Effects of Pluronic F68 and Labrasol on the intestinal absorption and pharmacokinetics of rifampicin in rats. *Arch Pharm Res* 2011; 34(11): 1939-1943.

141. MYERS D. *Surfactant science and technology*, 3rd edn. Hoboken, N.J.: J. Wiley, 2006.

142. VINAROV Z et al. Effect of Surfactant-Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media. *Mol Pharm* 2018; 15(12): 5741-5753.

143. TADROS TF. *Applied Surfactants: Principles and Applications*: Wiley, 2006.

144. XAVIER-JUNIOR FH et al. 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.

145. REN X et al. Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity in vitro and in vivo. *Eur J Pharm Sci* 2009; 36(4-5): 401-411.

146. ANBY MU et al. 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): 2063-2079.

147. ANDERBERG EK et al. Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. *Pharm Res* 1993; 10(6): 857-864.

148. AUNGST BJ. Intestinal permeation enhancers. *J Pharm Sci* 2000; 89(4): 429-442.

149. HIGAKI K et al. Estimation of absorption enhancement by medium-chain fatty acids in rat large intestine. *Res Commun Mol Pathol Pharmacol* 2001; 109(3-4): 231-240.

150. KAMP F et al. Rapid flip-flop of oleic acid across the plasma membrane of adipocytes. *J Biol Chem* 2003; 278(10): 7988-7995.

151. CEREZO J et al. Atomistic molecular dynamics simulations of the interactions of oleic and 2-hydroxyoleic acids with phosphatidylcholine bilayers. *J Phys Chem B* 2011; 115(40): 11727-11738.

152. IBARGUREN M et al. The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochim Biophys Acta* 2014; 1838(6): 1518-1528.

153. PABLA D et al. Intestinal permeability enhancement of levothyroxine sodium by straight chain fatty acids studied in MDCK epithelial cell line. *Eur J Pharm Sci* 2010; 40(5): 466-472.

154. SEK L et al. Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with in situ densitometric analysis. *J Pharm Biomed Anal* 2001; 25(3-4): 651-661.

155. GANDHI NN. Applications of lipase. *Journal of the American Oil Chemists' Society* 1997; 74(6): 621-634.

156. CUINE JF et al. Evaluation of the impact of surfactant digestion on the bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to dogs. *J Pharm Sci* 2008; 97(2): 995-1012.

157. BERTHELSEN R et al. Kolliphor surfactants affect solubilization and bioavailability of fenofibrate. Studies of in vitro digestion and absorption in rats. *Mol Pharm* 2015; 12(4): 1062-1071.

158. FERNANDEZ S et al. Comparative study on digestive lipase activities on the self emulsifying excipient Labrasol, medium chain glycerides and PEG esters. *Biochim Biophys Acta* 2007; 1771(5): 633-640.

159. CHRISTIANSEN A et al. 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): 376-382.

160. LI Y, MCCLEMENTS DJ. 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): 423-431.

161. FEENEY OM et al. '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: 219-227.

162. VITHANI K et al. 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.

163. WACHER VJ et al. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* 1998; 87(11): 1322-1330.

164. TOMITA M et al. Absorption-enhancing mechanism of sodium caprate and decanoylcarnitine in Caco-2 cells. *J Pharmacol Exp Ther* 1995; 272(2): 739-743.

165. KATAOKA M et al. Impact of dietary intake of medium-chain triacylglycerides on the intestinal absorption of poorly permeable compounds. *Mol Pharm* 2019.

166. BLAY JY, VON MEHREN M. Nilotinib: a novel, selective tyrosine kinase inhibitor. *Semin Oncol* 2011; 38 Suppl 1: S3-9.

167. DEGOEY DA et al. Beyond the Rule of 5: Lessons Learned from AbbVie's Drugs and Compound Collection. *J Med Chem* 2018; 61(7): 2636-2651.

168. BODE G et al. The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol Methods* 2010; 62(3): 196-220.

169. BAIRD JA et al. A classification system to assess the crystallization tendency of organic molecules from undercooled melts. *J Pharm Sci* 2010; 99(9): 3787-3806.

170. HENZE LJ et al. Combining species specific in vitro & in silico models to predict in vivo food effect in a preclinical stage - case study of Venetoclax. *Eur J Pharm Sci* 2020; under review.

171. DENING TJ et al. Montmorillonite-lipid hybrid carriers for ionizable and neutral poorly water-soluble drugs: Formulation, characterization and in vitro lipolysis studies. *Int J Pharm* 2017; 526(1-2): 95-105.

172. MAGHREBI S et al. An update on polymer-lipid hybrid systems for improving oral drug delivery. *Expert Opin Drug Deliv* 2019; 16(5): 507-524.

173. BLAABJERG LI et al. Influence of Glass Forming Ability on the Physical Stability of Supersaturated Amorphous Solid Dispersions. *J Pharm Sci* 2019; 108(8): 2561-2569.

174. KOEHL NJ et al. Chase Dosing of Lipid Formulations to Enhance Oral Bioavailability of Nilotinib in Rats. *Pharm Res-Dordr* 2020; 37(7): 124.

175. KOEHL NJ et al. Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib. *Mol Pharm* 2020.

176. KOEHL NJ et al. Supersaturated Lipid-Based Formulations to Enhance the Oral Bioavailability of Venetoclax. *Pharmaceutics* 2020; 12(6).

177. GAO P et al. Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. *J Pharm Sci* 2003; 92(12): 2386-2398.

178. GAO P et al. Enhanced oral bioavailability of a poorly water soluble drug PNU-91325 by supersaturatable formulations. *Drug Dev Ind Pharm* 2004; 30(2): 221-229.

179. GAO P et al. Characterization and Optimization of AMG 517 Supersaturatable Self-Emulsifying Drug Delivery System (S-SEDDS) for Improved Oral Absorption. *J Pharm Sci-Us* 2009; 98(2): 516-528.

180. SUYS EJA et al. Polymeric Precipitation Inhibitors Promote Fenofibrate Supersaturation and Enhance Drug Absorption from a Type IV Lipid-Based Formulation. *Mol Pharm* 2018; 15(6): 2355-2371.

181. KLAMT A, ECKERT F. COSMO-RS: a novel and efficient method for the a priori prediction of thermophysical data of liquids. *Fluid Phase Equilibria* 2000; 172(1): 43-72.

182. HORNIG M, KLAMT A. COSMOfrag: a novel tool for high-throughput ADME property prediction and similarity screening based on quantum chemistry. *J Chem Inf Model* 2005; 45(5): 1169-1177.

183. LOSCHEN C, KLAMT A. COSMOquick: A Novel Interface for Fast sigma-Profile Composition and Its Application to COSMO-RS Solvent Screening Using Multiple Reference Solvents. *Ind Eng Chem Res* 2012; 51(43): 14303-14308.

184. ABRAMOV YA et al. Rational coformer or solvent selection for pharmaceutical cocrystallization or desolvation. *J Pharm Sci* 2012; 101(10): 3687-3697.

185. ALSENZ J, KUENTZ M. From Quantum Chemistry to Prediction of Drug Solubility in Glycerides. *Mol Pharm* 2019; 16(11): 4661-4669.

186. PRICE DJ et al. Calculation of drug-polymer mixing enthalpy as a new screening method of precipitation inhibitors for supersaturating pharmaceutical formulations. *Eur J Pharm Sci* 2019; 132: 142-156.

187. KLAMT A. The COSMO and COSMO-RS solvation models. *WIREs Computational Molecular Science* 2011; 1(5): 699-709.

188. XU S, DAI WG. Drug precipitation inhibitors in supersaturable formulations. *Int J Pharm* 2013; 453(1): 36-43.

189. PRICE DJ et al. Approaches to increase mechanistic understanding and aid in the selection of precipitation inhibitors for supersaturating formulations - a PEARRL review. *J Pharm Pharmacol* 2019; 71(4): 483-509.

190. BROUWERS J et al. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? *J Pharm Sci* 2009; 98(8): 2549-2572.

191. GAO P, SHI Y. Characterization of supersaturatable formulations for improved absorption of poorly soluble drugs. *Aaps J* 2012; 14(4): 703-713.

192. STILLHART C et al. Biopharmaceutical Modeling of Drug Supersaturation During Lipid-Based Formulation Digestion Considering an Absorption Sink. *Pharm Res-Dordr* 2014; 31(12): 3426-3444.

193. BERGSTROM CAS, PORTER CJH. Understanding the Challenge of Beyond-Rule-of-5 Compounds. *Adv Drug Deliv Rev* 2016; 101: 1-5.

194. MUELLER EA et al. Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation. *Pharm Res* 1994; 11(1): 151-155.

195. WEBSTER GF et al. Comparative pharmacokinetic profiles of a novel isotretinoin formulation (isotretinoin-Lidose) and the innovator isotretinoin formulation: a randomized, 4-treatment, crossover study. *J Am Acad Dermatol* 2013; 69(5): 762-767.

196. FORD L et al. API ionic liquids: probing the effect of counterion structure on physical form and lipid solubility. *Rsc Adv* 2020; 10(22): 12788-12799.

197. MARQUES M. Dissolution media simulating fasted and fed states. *Dissolution Technologies* 2004; 11(2): 16-19.

198. KLEIN S. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *Aaps J* 2010; 12(3): 397-406.

199. CHOW SF et al. Kinetic entrapment of a hidden curcumin cocrystal with phloroglucinol. *Cryst Growth Des* 2014; 14(10): 5079-5089.

200. FDA. Guidance for Industry Food-Effect Bioavailability and Fed Bioequivalence Studies. In: Services, U. S. D. o. H. a. H., et al eds. http://www.fda.gov/cder/guidance/index.htm, 2002.

201. HENZE LJ et al. Toward the establishment of a standardized pre-clinical porcine model to predict food effects - Case studies on fenofibrate and paracetamol. *Int J Pharm X* 2019; 1: 100017.

202. FDA. Regulatory Calssification of Pharamceutical Co-Crystals Guidance for Industry. In: Services, U. S. D. o. H. a. H., Administration, F. a. D. eds., 2018.

203. HACKETT MJ et al. Fatty acids as therapeutic auxiliaries for oral and parenteral formulations. *Adv Drug Deliv Rev* 2013; 65(10): 1331-1339.

204. SONG S et al. Stearic–capric acid eutectic/activated-attapulgiate composite as form-stable phase change material for thermal energy storage. *Energy conversion and management* 2014; 81: 306-311.

205. HOLM R. Bridging the gaps between academic research and industrial product developments of lipid-based formulations. *Adv Drug Deliv Rev* 2019; 142: 118-127.

206. DAI WG et al. Evaluation of drug precipitation of solubility-enhancing liquid formulations using milligram quantities of a new molecular entity (NME). *J Pharm Sci* 2007; 96(11): 2957-2969.

207. MOHSIN K et al. Design of Lipid-Based Formulations for Oral Administration of Poorly Water-Soluble Drugs: Precipitation of Drug after Dispersion of Formulations in Aqueous Solution. *J Pharm Sci-Us* 2009; 98(10): 3582-3595.

208. WILLIAMS HD et al. Toward the establishment of standardized in vitro tests for lipid-based formulations. 2. The effect of bile salt concentration and drug loading on the performance of type I, II, IIIA, IIIB, and IV formulations during in vitro digestion. *Mol Pharm* 2012; 9(11): 3286-3300.

209. O'DRISCOLL CM. Lipid-based formulations for intestinal lymphatic delivery. *Eur J Pharm Sci* 2002; 15(5): 405-415.

210. FAISAL W et al. Bioavailability of lycopene in the rat: the role of intestinal lymphatic transport. *J Pharm Pharmacol* 2010; 62(3): 323-331.

211. ZANE P et al. In vivo models and decision trees for formulation development in early drug development: A review of current practices and recommendations for biopharmaceutical development. *Eur J Pharm Biopharm* 2019; 142: 222-231.

212. ANDREAS CJ et al. Introduction to the OrBiTo decision tree to select the most appropriate in vitro methodology for release testing of solid oral dosage forms during development. *Eur J Pharm Biopharm* 2018; 130: 207-213.

213. ANANTHULA HK et al. Preclinical pharmacokinetic evaluation to facilitate repurposing of tyrosine kinase inhibitors nilotinib and imatinib as antiviral agents. *BMC Pharmacol Toxicol* 2018; 19(1): 80.

214. GU CH et al. Predicting effect of food on extent of drug absorption based on physicochemical properties. *Pharm Res* 2007; 24(6): 1118-1130.

215. TEMPLETON AC. *Discovering and developing molecules with optimal drug-like properties,* AAPS advances in the pharmaceutical sciences series,, New York: AAPSPress, Springer, 2015.

216. MATHIAS N et al. Food Effect in Humans: Predicting the Risk Through In Vitro Dissolution and In Vivo Pharmacokinetic Models. *Aaps J* 2015; 17(4): 988-998.

217. FDA. Guidance for industry on Statistical Approaches to Establishing Bioequivalence. In: Services, U. S. D. o. H. a. H., et al eds., 2001.

218. BLAABJERG LI et al. Is there a correlation between the glass forming ability of a drug and its supersaturation propensity? *Int J Pharm* 2018; 538(1-2): 243-249.

219. EMEA. Assessment report Ofev *Committee for Medicinal Products for Human Use (CHMP)*. 2014.

220. WEI Y et al. Enhanced oral bioavailability of silybin by a supersaturatable self-emulsifying drug delivery system (S-SEDDS). *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2012; 396: 22-28.

221. SCHULTZ HB et al. Enhancement of Abiraterone Acetate Oral Bioavailability by Supersaturated-Silica Lipid Hybrids. *Int J Pharm* 2020: 119264.

222. DITZINGER F et al. Opportunities for Successful Stabilization of Poor Glass-Forming Drugs: A Stability-Based Comparison of Mesoporous Silica Versus Hot Melt Extrusion Technologies. *Pharmaceutics* 2019; 11(11).

223. LIU H et al. Metabolism and Disposition of a Novel B-Cell Lymphoma-2 Inhibitor Venetoclax in Humans and Characterization of Its Unusual Metabolites. *Drug Metab Dispos* 2017; 45(3): 294-305.