European Journal of Pharmaceutical Sciences 159 (2021) 105691

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Exploring precipitation inhibitors to improve *in vivo* absorption of cinnarizine from supersaturated lipid-based drug delivery systems

Alexandra-Roxana Ilie [a,b], Brendan T. Griffin [b,*], Maria Vertzoni [c], Martin Kuentz [d], Ruzica Kolakovic [a], Anke Prudic-Paus [a], Ahmed Malash [a], Hugo Bohets [a], Jilly Herman [a], René Holm [a,e]

[a] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Beerse, Belgium
[b] School of Pharmacy, University College Cork, Cork, Ireland;
[c] Department of Pharmacy, National and Kapodistrian University of Athens, Zografou, Greece
[d] University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharma Technology, Muttenz, Switzerland
[e] Department of Science and Environment, Roskilde University, Roskilde, Denmark

ARTICLE INFO

Keywords:
Supersaturated lipid-based drug delivery systems
Precipitation inhibitors
High throughput screening
Bio-enabling formulations
Pharmacokinetic profiles

ABSTRACT

Supersaturated lipid-based drug delivery systems are increasingly being explored as a bio-enabling formulation approach, particularly in preclinical evaluation of poorlywater-soluble drugs. While increasing the drug load through thermally-induced supersaturation resulted in enhanced *in vivo* exposure for some drugs, for others, such as cinnarizine, supersaturated lipid-based systems have not been found beneficial to increase the *in vivo* bioavailability. We hypothesized that incorporation of precipitation inhibitors to reduce drug precipitation may address this limitation. Therefore, pharmacokinetic profiles of cinnarizine supersaturated lipid-based drug delivery systems with or without precipitation inhibitors were compared. Five precipitation inhibitors were selected for investigation based on a high throughput screening of twenty-one excipients. *In vivo* results showed that addition of 5% precipitation inhibitors to long chain monoglyceride (LCM) or medium chain monoglyceride (MCM) formulations showed a general trend of increases in cinnarizine bioavailability, albeit only statistically significantly increased for Poloxamer 407 + LCM system (i.e. 2.7-fold increase in $AUC_{0\text{-}24h}$ compared to LCM without precipitation inhibitors). It appeared that precipitation inhibitors mitigated the risk of *in vivo* precipitation of cinnarizine from sLBDDS and overall, bioavailability was comparable to that previously reported for cinnarizine after dosing of non-supersaturated lipid systems. In summary, for drugs which are prone to precipitation from supersaturated lipid-based drug delivery systems, such as cinnarizine, inclusion of precipitation inhibitors mitigates this risk and provides the opportunity to maximize exposure which is ideally suited in early efficacy and toxicology evaluation.

1. Introduction

Lipid-based drug delivery systems (LBDDS) have been intensively investigated as bio-enabling technologies and suggested as drug delivery solutions for poorly water-soluble drugs (PWSDs) based on their increased apparent solubilization capacity upon dilution in the gastrointestinal (GI) fluids (O'Driscoll and Griffin, 2008), (Feeney et al., 2016). More recently, improved or equivalent drug exposure was demonstrated when administered as supersaturated LBDDS (sLBDDS) compared to conventional non-supersaturated LBDDS for a number of drugs, including: venetoclax (Koehl et al., 2020), celecoxib (Ilie et al., 2020c), halofantrine (Thomas et al., 2012), (Michaelsen et al., 2016), simvastatin (Thomas et al., 2013), fenofibrate (Thomas et al., 2014), (Michaelsen et al., 2019) and R3040 (Siqueira Jørgensen et al., 2018). Such supersaturated systems, where the drug concentration in the lipid system exceeds the thermodynamic solubility, can be obtained by heating the drug and lipid excipients at 60 °C followed by cooling to ambient temperature (Ilie et al., 2020b). The benefit of increased drug load comes with the risk of physical instability based on the gap between the free energy of the supersaturated state and that of the equilibrium (Ilie et al., 2020b), (Price et al., 2019a), (Xu and Dai, 2013). As a result, the formulation-mediated enhanced solubility may not be achieved, as

* Corresponding author.
E-mail address: Brendan.griffin@ucc.ie (B.T. Griffin).

https://doi.org/10.1016/j.ejps.2020.105691
Received 30 June 2020; Received in revised form 23 November 2020; Accepted 21 December 2020
Available online 24 December 2020

this instability can potentially lead to precipitation of amorphous or crystalline material from the supersaturated system either upon long-term storage or upon dilution and dispersion in the GI tract (Ilie et al., 2020b), (Price et al., 2019a).

It is important to note the distinction between supersaturated drug delivery systems (i.e. drug is present at concentrations above thermodynamic solubility in the formulation) and the supersaturating drug delivery systems which are designed to generate drug supersaturation only after dispersion/dissolution in GI fluids. A supersaturating drug delivery system should ideally generate and maintain drug supersaturation in the GI fluids for at least a physiologically-relevant time (i.e. 2-4 h for a typical upper GI transit time) (Price et al., 2019a). This concept is commonly referred to as the "Spring and Parachute" model (Guzman et al., 2007). Another important distinction should be made between real and apparent supersaturation. Frank and co-workers used a dialysis procedure to quantify the molecularly dissolved PWSD, ABT-102 and compared this to apparent drug solubility in the same media, quantified through a centrifugation and filtration procedure. Such a distinction between real and apparent supersaturation is useful to clarify influence of solubilization enhancement on the permeation rate (Frank et al., 2012). A supersaturated solution or dispersion is typically generated from a higher energy form of the drug ("a spring") and is thermodynamically unstable, as previously outlined. Lipid-based drug delivery systems, co-solvent systems, amorphous solid dispersions, nanoparticles or co-crystals are considered as spring generators. Subsequently, polymers may sustain the drug temporarily in solution so they inhibit precipitation mostly kinetically, whereas surfactants and cyclodextrins may be considered thermodynamic precipitation inhibitors given that they increase the apparent solubility of the PWSD, thereby reducing supersaturation. Collectively, these excipients can be called precipitation inhibitors (PIs) if they prolong the duration of supersaturation or so called "parachute" effect (Xu and Dai, 2013), (Gao and Shi, 2012), (Price et al., 2019a). In the case of sLBDDS, which potentially generate high supersaturation on dispersion in the GI fluids, therefore presenting a risk of drug precipitation, it was hypothesized that addition of a precipitation inhibitor may mitigate such risk.

A large pool of excipients has been explored for their precipitation inhibition effects by either interference with nucleation and/or crystal growth or solubilization enhancement (Xu and Dai, 2013), (Warren et al., 2010), (Brouwers et al., 2009). Different surface active excipients, (i.e. Kolliphor® HS15, Kolliphor® RH40 or vitamin E TPGS) showed inhibitory effects on efflux transporters such as P-glycoprotein (P-gp), which is a mechanism for improved absorption of drugs that are P-gp substrates (Kuentz, 2012). Polymers such as hydroxypropyl methylcellulose (HPMC) or polyvinylpyrrolidone vinyl acetate (PVP/VA) are among the most studied PIs and it has been suggested that relatively low concentrations of 0.01 – 0.05% (w/w) can have a significant effect on drug precipitation inhibition by reducing drug nucleation and crystal growth rates through molecular interactions with the drug (i.e. hydrogen bonds, polar or dispersion forces) (Price et al., 2019a), (Warren et al., 2010), (Xu and Dai, 2013). Apart from these, excipients such as poloxamers (copolymers with a polyethylene oxide (PEO)-polypropylene oxide (PPO)-polyethylene oxide (PEO) structure), surfactants (D-α-Tocopherol polyethylene glycol 1000 succinate (vitamin E TPGS), Sodium Dodecyl Sulphate (SDS) and PEG-40 hydrogenated castor oil (Kolliphor® RH40)) or cyclodextrins have also been investigated as PIs (Xu and Dai, 2013). Several pharmacokinetic studies have assessed the impact of inclusion of PIs within LBBDS that generate supersaturation upon dispersion/digestion *in vivo* and are summarised in Table 1. In contrast, the work performed in the present study refers to inclusion of PIs in supersaturated LBDDS.

Nevertheless, one should bear in mind that these studies have investigated conventional non-supersaturated LBBDS that generate supersaturation upon dispersion in the GI tract (i.e. supersaturating drug delivery systems). Additionally, even though helpful in understanding the different effects of precipitation inhibition, these studies were performed with surfactant and co-solvent-based formulations with complex compositions or high precipitation risk upon *in vivo* dispersion and digestion. We have recently espoused the merits of simple, one or two-component lipid systems to improve *in vivo* performance, either as LBDDS (celecoxib, cinnarizine, JNJ-2A) (Ilie et al., 2020b) or sLBBDS (celecoxib) (Ilie et al., 2020c). While Bannow *et al.* has shown that the addition of PVP/VA 64 in 200 and 250% sLBDDS resulted in increased

Table 1
Overview of *in vivo* studies reported in the literature testing the potential benefits of PI-inclusion in LBDDS.

Drug (Reference)	PI (w/w %)	LBDDS/SEDDS composition	Effect *in vivo/in vitro*
Paclitaxel (Gao et al., 2003)	HPMC 5%	Kolliphor® EL, Glyceryl dioleate, polyethylene glycol (PEG) 400, ethanol	Rat study: 10-fold higher maximum plasma concentration (C_{max}) and 5-fold higher oral bioavailability
PNU-91325 (Gao et al., 2004)	HPMC (20%) Pluronic L44 (18%)	Kolliphor® EL, glycerol monooleate and dioleate (2:8), PEG 400, dimethyl acetamide	Dog study: bioavailability increased 6-fold relative to pure PEG 400 formulation,
AMG-517 (Gao et al., 2009)	HPMC 5%	Capmul® MCM, Tween® 80, PEG 400	Monkey study: ~30% higher mean C_{max} and comparable exposure (AUC) as compared to an aqueous suspension
Carbamazepine (Zhang et al., 2011)	PVP-K90 2%	Miglyol 812N (medium chain triglycerides), Kolliphor® EL, PEG 400	Dog study: bioavailability increased 5-fold relative to commercial tablet
Celecoxib (Shi et al., 2010) (Song et al., 2014)	PVP-12PF (~4%) + HPMC-E5 (~4%)	PEG 400, ethanol, Tween® 80, oleic acid, tromethamine	*In vitro* biphasic dissolution and correlation to human data, increase in AUC and C_{max} compared to Celebrex® and solution
	Soluplus® (4%)	Capryol® 90, Tween® 20, Tetraglycol®	Highest effective permeability coefficient and bioavailability increased 3.6-fold relative to aqueous suspension
Indirubin (Chen et al., 2012)	PVP K17 0.5%	Maisine® CC, Kolliphor® EL, Transcutol® P	Rat study: bioavailability increased 1.3-fold relative to PI-free SEDDS
Trans-resveratrol (Singh and Pai, 2016)	HPMC 5%	Lauroglycol FCC and Transcutol® P	Rat study: $AUC_{0\text{-}8h}$ increased by 1.3-fold versus PI-free SEDDS
Fenofibrate (Suys et al., 2018)	Eudragit® RL100 1%, PPGAE 1%, HPMC 5%	Kolliphor® EL, Transcutol® HP	Rat study: from all an increasing trend towards higher drug absorption, statistically significant for PPGAE (poly (propylene glycol) bis(2-aminopropyl ether)
Silymarin (Tung et al., 2019)	Poloxamer 407 10%	Labrafil M1944CS, Kolliphor® RH40 and Transcutol® P	Rabbit study: bioavailability increased 7.6-fold relative to commercial product (Legalon®)

drug concentration *in vitro* (in particular for the formulation containing 20% PI), however, to the best our knowledge no study had previously reported *in vivo* investigations on the use of PIs in sLBDDS (Bannow et al., 2020).

Previously Siqueira *et al.* have compared *in vivo* bioavailability of cinnarizine from a sLBDDS (at 200% S_{eq}), a LBDDS (at 80% S_{eq}) and a LBDDS suspension (at 200% S_{eq}) and reported that only the LBDDS system displayed a significantly increased bioavailability relative to a control aqueous suspension formulation (Siqueira et al., 2017). All tested (s)LBDDS did not contain PIs. While this may reflect the effect of the higher lipid dose load administered with the LBDDS formulation (as the drug dose was kept constant across all formulations), it was also interesting to note that bioavailability for the sLBDDS was similar to the aqueous suspension which suggested therefore that, in the case of cinnarizine, a sLBDDS approach was not an effective bio-enabling approach. It was hypothesized that inherent instability of cinnarizine upon dispersion of sLBDDS, leading to precipitation in the GI tract, may be a contributory factor (Siqueira et al., 2017). Alternatively, this may reflect the drug's fast crystallization tendency from sLBDDS as recently suggested (Ilie et al., 2020b). The aim of the present study was to explore in more detail the impact of inclusion of different PIs in sLBDDS, by applying a high throughput screening (HTS) approach to evaluate twenty-one different excipients for their precipitation inhibition effects *in vitro* and to investigate the potential *in vivo* benefits of adding 5% of selected PIs to long chain (LC) and medium chain (MC) sLBDDS containing cinnarizine. Single component LBDDS were chosen in combinations with PIs based on the better *in vivo* performance concluded after a pilot study, which compare single and two-component LBDDS containing lipids and a hydrophilic surfactant (i.e. Labrasol®). Addition of the surfactant was based on the hypothesis that an increase in the hydrophilic phase of the lipid system would result in better dispersibility and potentially an improved drug absorption as evaluated by Cuiné and co-workers (Cuiné et al., 2007).

2. Materials and Methods

2.1. Materials

Cinnarizine (weak base, 368.5 g/mol, logP=5.7) was obtained from Janssen Pharmaceutica (Beerse, Belgium). Analysis of plasma samples was done using flunarizine as an internal standard (Larsen et al., 2017) and was obtained from Janssen Pharmaceutica (Beerse, Belgium). Capmul® MCM C8 (medium chain mixed glycerides, MCM) was kindly donated by Abitec (Columbus, OH, USA). Maisine® CC (long chain mono-/di-glycerides, LCM) and Labrasol® ALF (Caprylocaproyl macrogol-8 glycerides, a hydrophilic surfactant, S) were kind gifts from Gattefossé (Lyon, France). Labrasol® consists of a small fraction of mono-, di- and triglycerides and mainly PEG-8 (Mw = 400) mono- and diesters of caprylic (C8) and capric (C10) acids. It has a critical micelle concentration (CMC, mg/L, 25°C) of 42 ±24 according to the manufacturer's datasheet. The different excipients used in the precipitation inhibition screening and the respective suppliers are listed in the Supporting information, Table S1. SIF powder was purchased from biorelevant.com (UK) and FaSSIF was prepared according to their instructions. N-Methyl-2-pyrrolidone (NMP) was purchased via VWR (Belgium).

3. Methods

3.1. Screening for precipitation inhibitors

In the precipitation inhibition screening, the precipitation behaviour of cinnarizine and the supersaturation stabilizing properties of a variety of excipients (i.e. polymers, surfactants and cyclodextrins) were investigated. A solvent shift method was applied to generate the supersaturated state, i.e. the drug was dissolved in a solvent (i.e. NMP) at high concentrations and added (small volume, target is < 1% (v/v)) to a biorelevant medium (i.e. FaSSIF), in which the drug had little or practically no solubility. The biorelevant medium consisted of FaSSIF or 1% (w/v) excipient dissolved in FaSSIF. After generation of apparent supersaturation (ratio of drug solubilized in aqueous colloidal phase and equilibrium solubility), the precipitation behaviour and the potential stabilizing effects of excipients were monitored by determination of the apparent concentration remaining in solution as a function of time. As such, the precipitation propensity of the drug was explored and excipients which could be used in the lipid drug delivery systems, to prevent or delay precipitation kinetics, were identified. The precipitation inhibition HTS assay was performed using the Hamilton Microlab STAR PLUS (Hamilton Germany GmbH – Robotics, Gräfelfing) and consisted of solubility measurements and PI screening.

3.1.1. Apparent solubility screening of cinnarizine in FaSSIF medium containing 1% PI

In a first step, apparent solubility (S*) of cinnarizine was studied in FaSSIF in the absence and presence of excipients at the concentration used during the actual precipitation inhibition test (1% w/v). Subsequently, in the PI screening, a solvent shift was applied to generate the apparent supersaturated state. Thereby, the effect of the solvent used during the solvent shift on the solubility at a concentration of 1% (v/v) was also assessed. An excess amount of cinnarizine (6-7 mg) was weighed into 10 mL glass vials where 6 mL of FaSSIF (± excipients) was added. A magnetic stirrer bar was added to each vial and the mixtures were stirred for 24 h at 37 °C. After 24 h, suspensions were filtered through a multi-well filter plate (0.45 µm GHP membrane filter) (Pall corporation, New York, USA) and the cinnarizine apparent concentration as drug solubilized in the aqueous colloidal phase was determined in the filtrate using a previously described method (Ilie et al., 2020b) with UPLC™-UV spectrometry. The calibration curve was confirmed linear between 0.0025 – 0.1 mg/mL.

3.1.2. *High throughput* *precipitation inhibition screening*

Preliminary evaluation of cinnarizine precipitation profiles indicated a sufficient time-resolution in the observed precipitation kinetics profile at an apparent cinnarizine concentration of 8-fold higher than the equilibrium solubility in FaSSIF. It was hypothesized that this would allow experimental discrimination between the precipitation inhibition capacity of the different excipients.

In the PI screening, two reference conditions (FaSSIF with/without 1% NMP) and the efficiency of twenty-one pharmaceutical excipients to maintain the supersaturated state were evaluated. Apparent supersaturation of cinnarizine was generated by dispersion of the highly concentrated cinnarizine solution in NMP. Samples were collected at 3, 13, 24, 34, 57, 67, 126, 246 minutes, filtered through multi-well filter plate (0.45 µm GHP membrane filter) and quantified with UPLC™-UV.

3.1.3. *Data handling* *and analysis*

The S* values determined in section 2.1.1 were used to calculate the $AUC_{solubility}$ considering that the value obtained at 24 h was the same as the one obtained at 246 minutes (last timepoint of the PI screening). The $AUC_{pp\ profiles}$ were calculated based on the trapezoidal rule from the concentration versus time curves from section 2.1.2 and included the contribution of $AUC_{solubility}$. Besides the general appearance of precipitation profiles, the decision to consider a certain excipient as a PI for formulations was also based on the values of $AUC_{pp\ profiles}$ and the calculation of the ratio $AUC_{pp\ profiles}/AUC_{solubility}$. The higher the values, the better chances of having a good precipitation inhibitor.

3.2. Preliminary testing of selected precipitation inhibitors

3.2.1. Compatibility of lipid-precipitation inhibitor and drug loading ability of resulting mixture

Based on the calculations mentioned above, excipients were selected

to be used as PIs in sLBDDS using either LCM or MCM as single lipid excipients. Therefore, the ability of the selected PIs to form single-phase systems with the lipids and to load cinnarizine in PI-LCM and PI-MCM were determined. Concentrations of 5, 20 and 30% of the selected PIs: Poloxamer 407 (P 407), Eudragit® L100-55 (Eu), Kolliphor® HS15 (K HS15), Kolliphor® RH40 (K RH40), vitamin E TPGS (vit E TPGS) and Soluplus® were tested in LCM or MCM at 60 °C. If the PI-sLBDDS mixtures were a clear single-phase, non-viscous solution, the different amounts of cinnarizine corresponding to 85% $S_{eq}^{60^0C}$ in LCM and MCM were loaded onto them. The 85% $S_{eq}^{60^0C}$ was previously determined and represents in the present study the drug concentration in the different sLBDDS to be tested *in vivo,* i.e. 73.6 mg/mL for LCM systems and 69.4 mg/mL for MCM systems (Ilie et al., 2020b). Equilibrium solubility determination at 60 °C (i.e. $S_{eq}^{60^0C}$) in LCM and MCM was also previously assessed (Ilie et al., 2020b).

3.2.2. Stability assessment of sLBDDS and sLBDDS-PI

The drug-free and cinnarizine-loaded sLBDDS and sLBDDS-PI were stored in sealed vials at 25 ± 1°C for up to 48 hours to assess their physical stability. The short time frame is deemed acceptable for dosing of animals in preclinical testing. The vials were visually analysed for possible macroscopic drug precipitation.

3.2.3. Assessing the effect of precipitation inhibitors on apparent solubility in lipid-based drug delivery systems at 60 °C

In order to determine the potential influence of adding PIs in LBDDS on the solubilization capacity of the whole system, apparent solubility of cinnarizine in the newly designed PI-lipid systems was assessed as previously described (Ilie et al., 2020b). In brief, excess amount of cinnarizine was weighed into glass vials containing a magnetic bar and 1 mL of PI-LBDDS solutions was added and stirred at 300 rpm in a climate chamber at 60 °C for 24 h, in triplicate. An one-way analysis of variance (ANOVA) followed by the Dunnett's test was used to test the statistical significance of differences in solubility determinations with Prism 8.4.2 from GraphPad Prism Software, LLC (San Diego, CA, USA). A statistical p-value < 0.05 was considered significant.

3.3. Pharmacokinetic evaluation of supersaturated lipid-based drug delivery systems

The protocol used for the *in vivo* pharmacokinetic evaluation of sLBDDS containing cinnarizine was approved by the institutional animal ethics committee in accordance with the Belgian law regulating animal use in experimental procedures. The study was in compliance with EC Directive 2010/63/EU and the NIH guidelines on animal welfare. Fasted male Sprague-Dawley rats weighting between 250-300 g, received 0.5 mL/kg sLBDDS with or without the PIs containing cinnarizine (n=4) by oral gavage. Lipid systems were stirred continuously the night before dosing at 60 °C, cooled to ambient temperature and dosed as clear solutions. Blood was collected after oral administration at defined timepoints: 0.5, 1, 2, 4, 6, 10, 24 h, where 200 µL blood was collected, 100 µL plasma was harvested after centrifugation and bioanalysis was performed on a Waters UPLC™ system with UV-detection. The corresponding dose for sLCM systems was 36.8 mg/kg and 34.7 for sMCM systems. The apparent degree of supersaturation (aDS) was calculated for sLCM and sMCM according to a method described before and was 133% and 143%, respectively (Ilie et al., 2020b). Additionally, together with PI-free sLCM and sMCM, the supersaturated sLCM+S and sMCM+S systems were tested using the same *in vivo* protocol in a pilot study. The corresponding doses were 28.2 mg/mL for sLCM+S and 27.4 mg/mL for sMCM+S and the aDS were 129% and 118%, respectively.

3.3.1. Quantitative analysis of plasma samples

Cinnarizine (CIN) in plasma after oral dosing of the sLBDDS was quantified using a reversed-phase UPLC (RP-UPLC™) method with flunarizine (FLU) as internal standard (Larsen et al., 2017). Flunarizine was solubilized in acetonitrile and 140 µL of this solution was added to 20 µL plasma sample containing cinnarizine. Plasma protein precipitation was successful after centrifugation for 20 min at 4 °C, 17500 rpm using an Eppendorf centrifuge 5430R (Eppendorf, Hamburg, Germany). With the suggested extraction method, recovery of cinnarizine was between 82-113%. The cinnarizine concentrations were determined by standard calibration curve analysis using linear fitting of a plot of CIN/FLU peak area ratios versus cinnarizine concentrations, respectively. The standard calibration curves were confirmed linear in the range 10 – 200 ng/mL for cinnarizine and the lowest limit of quantification was 10 ng/mL.

3.3.2. Pharmacokinetic and statistical analysis

The primary pharmacokinetic parameters: area under the plasma concentration-time curve (*AUC*), maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were obtained by non-compartmental analysis of the plasma data, using the linear trapezoidal method in Microsoft Excel (Office 365) with PK Solver add-in. Multiple sample comparison was performed on dose-normalized pharmacokinetic parameters, C_{max}, t_{max} and AUC_{0-24h}, by an ANOVA on ranks using SigmaPlot 12.5 from Systat Software, Inc. (Chicago, IL, USA). A statistical p-value < 0.05 was considered significant and for statistical contrast analysis, a Tukey post-hoc test was used. Additionally, the ratio of AUC_{0-4h}/AUC_{0-24h} was calculated to better evaluate the rate of drug absorption from the different sLBDDS. Results are expressed as mean ± SD for C_{max} and AUC_{0-24h} and median [min, max] for t_{max}.

4. Results

4.1. Selection of precipitation inhibitors

The selection of precipitation inhibitors for *in vivo* evaluation was guided by three general criteria: 1) ability to maintain apparent supersaturation during the testing time in the *in vitro* solvent shift method, 2) the higher ratio of $AUC_{pp\ profiles}/AUC_{solubility}$ and 3) the higher calculated value for $AUC_{pp\ profiles}$ relative to the reference (i.e. FaSSIF medium without excipients). The apparent solubility and the calculated *AUC* values for the twenty-one tested excipients are listed in Table 2 together with the two reference conditions. Addition of NMP (<1% v/v) in the FaSSIF medium did not significantly affect the solubility and precipitation behaviour of cinnarizine. The precipitation profiles of excipients that had a poor precipitation inhibition effect, presented as concentration versus time curves, are shown in Fig. 1. Profiles of SDS and Kolliphor® EL are depicted in Fig. 2 together with information on apparent solubility (S*) in FaSSIF medium containing 1% SDS or 1% Kolliphor® EL. In the case of SDS and Kolliphor® EL, while it may appear these two excipients are potentially suitable PIs based on high differences of *AUC* profiles relative to reference conditions, these differences were mainly based on increased drug solubilisation in media containing 1% of each excipient rather than the extent a precipitation inhibition effect (i.e. ratio $AUC_{pp\ profiles}/AUC_{solubility}$ were lower (SDS = 0.9) or comparable to FaSSIF (Kolliphor® EL = 1.77)). A plot of the selected excipients for further analysis is shown in Fig. 3. This initial set of excipients was selected based on overall combined highest ranked ratio of $AUC_{pp\ profiles}/AUC_{solubility}$ and $AUC_{pp\ profiles}$ values. Based on the $AUC_{pp\ profiles}$, the rank order of precipitation inhibition was: K HS15 > K RH40 > Vit E TPGS > Soluplus® > P 407 > Eu. However, if the AUC ratio was considered, the rank order was: Eu > Soluplus® > K RH40 > vit E TPGS = P 407 > K HS15.

4.2. Preliminary testing of selected precipitation inhibitors

4.2.1. Lipid-excipient compatibility and drug loading ability

Miscibility of an initial group of PIs with two lipid excipients (i.e. LCM, MCM) that were tested before *in vivo* as sLBDDS was first assessed

Table 2
Overview of calculated values for the tested excipients as PIs for cinnarizine. The selected PIs for further investigation are highlighted in light grey and in dark grey the reference conditions. $AUC_{pp\ profiles}$ = areas under the concentration time profiles obtained from the precipitation inhibition screen, calculated to rank order excipients based on their ability to maintain a higher cinnarizine concentration in solution; $AUC_{solubility}$ = area obtained in the same time interval for a saturated solution

Medium	Apparent solubility in medium (mg/mL)	$AUC_{solubility}$ (mg/mL·min)	$AUC_{pp\ profiles}$ (mg/mL·min)	Ratio $AUC_{pp\ profiles}$ / $AUC_{solubility}$
1% SDS in FaSSIF	1.0082	248.24	224.59	0.90
1% Kolliphor EL in FaSSIF	0.0891	21.93	38.78	1.77
1% Kolliphor HS15 in FaSSIF	0.0692	17.05	33.32	1.95
1% Kolliphor RH40 in FaSSIF	0.0589	14.50	31.31	2.16
1% Vitamin-E TPGS in FaSSIF	0.0583	14.36	28.35	1.97
1% Poloxamer 407 in FaSSIF	0.0380	9.36	18.44	1.97
1% Soluplus in FaSSIF	0.0201	4.94	19.06	3.86
1% Eudragit L100-55 in FaSSIF	0.0157	3.87	16.69	4.32
1% Tween 20 in FaSSIF	0.0340	8.37	10.19	1.22
1% Poloxamer 338 in FaSSIF	0.0181	4.45	7.46	1.68
1% SBEbCD in FaSSIF	0.0202	4.98	6.40	1.28
1% HPMC E5 in FaSSIF	0.0133	3.27	7.05	2.15
1% PVP VA64 in FaSSIF	0.0132	3.25	6.08	1.87
1% PVP K30 in FaSSIF	0.0129	3.17	5.16	1.63
FaSSIF	0.0124	3.06	5.23	1.71
1% NMP in FaSSIF	0.0124	3.06	5.06	1.65
1% PEG 6000 in FaSSIF	0.0131	3.22	4.83	1.50
1% Poloxamer 124 in FaSSIF	0.0117	2.88	5.13	1.78
1% HPβCD in FaSSIF	0.0141	3.47	4.44	1.28
1% Poloxamer 188 in FaSSIF	0.0128	3.15	4.40	1.40
1% Kollicoat IR in FaSSIF	0.0129	3.17	4.29	1.35
1% PEO 100K in FaSSIF	0.0049	1.21	3.67	3.02
1% HPMC AS LG in FaSSIF	0.0031	0.76	1.64	2.17

at a 5% concentration (w/w). While K HS15, K RH40 and Vit E TPGS readily formed single-phase solutions (<5 minutes), Soluplus® and P 407 produced clear single-phase solutions after 30 minutes of mixing, while Eu formed a two-phase system under the tested conditions. Therefore, Eudragit L100-55 was not tested at the 20 and 30% (w/w) concentrations. All remaining PIs formed clear single-phase solutions with the higher concentrations, that could be easily handled, except for Soluplus® which became very viscous and could not be accurately pipetted. Considering that this study intended to test the potential *in vivo* benefits of a number of different PIs, it was decided that the concentration of PI should remain constant across all systems. However, in order to assess if 5% PI impacts the drug loading ability of the sLBDDS, the cinnarizine loading capacity of the PI+sLBBDS was assessed. All five PIs investigated (P 407, K HS15, K RH40, Vit E TPGS, Soluplus®) at a 5% (w/w) concentration in LCM and MCM were able to dissolve the required amount of cinnarizine (73.6 mg/mL for LCMs and 69.4 mg/mL for MCMs). Accordingly, the inclusion of 5% PI did not adversely impact drug loading capacity and the five PI-sLBDDS were subsequently tested *in vivo*.

4.2.2. Physical stability assessment

Due to increased drug loading in sLBDDS the physical stability of the prepared lipid systems is of significant interest prior to *in vivo* oral dosing. Table 3 provides an overview of the physical stability at 25 ± 1°C, over 48 hours for drug-free and cinnarizine-loaded sLBDDS with or without PIs. The drug-free lipid-PI mixture remained stable over the investigated time frame whereas drug precipitate was observed in the cinnarizine-loaded sLBDDS ± PIs. All sLCM-PI systems were stable for longer time compared to sLCM, while in the case of sMCM systems, only the systems containing vitamin E TPGS and Soluplus® were stable for longer period compared to PI-free system (i.e. sMCM). This short study indicated the possibility to ensure a correct dosing of a homogeneous one-phase system, hence it should be considered as an "in-use" stability study.

4.2.3. Assessing the effect of precipitation inhibitors on apparent solubility in lipid-based drug delivery systems at 60 °C

In order to determine if addition of 5% (w/w) PIs in the lipid systems would significantly influence the solubilization capacity of the system, the apparent solubility of cinnarizine was determined at 60 °C and compared to previously determined $S_{eq}^{60^0C}$ for LCM and MCM. The results showed no statistically significant differences between cinnarizine solubility in PI-free lipid systems and in lipid excipient-PI mixtures (Table 4).

4.3. Pharmacokinetic evaluation of cinnarizine-loaded supersaturated lipid-based drug delivery systems

4.3.1. Assessing the effect of one and two-component lipid systems on in vivo drug absorption of supersaturated lipid-based drug delivery systems

While one-component systems may be preferable from a mechanistic and ease of preparation perspective, there was also a risk that single lipid excipients may display poor dispersibility. In order to assess if addition of a surfactant to the formulation was necessary to aid dispersibility *in vivo* and maximise bioavailability, a pilot *in vivo* study was performed to assess the effect of inclusion of the hydrophilic surfactant, Labrasol®. The calculated pharmacokinetic parameters for sLCM and sMCM and their respective surfactant (20% w/w) containing LBDDS (sLCM+S and sMCM+S) are shown in Table 5. Overall a higher drug exposure was observed after administration of sLCM and sLCM+S compared to their medium chain correspondents with a statistically significantly higher $AUC_{0\text{-}24h}$ for sLCM versus sMCM+S. The lower $AUC_{0\text{-}4h}/AUC_{0\text{-}24h}$ ratios for long chain LBDDS compared to medium chain systems may indicate that the absorption from these systems may have taken longer than 4 h and was rather prolonged after 4 hours. The key finding overall from this pilot study was that, the inclusion of Labrasol® did not improve the performance of either one-component sLBDDS and pharmacokinetic values were in general lower than those obtained after administration of

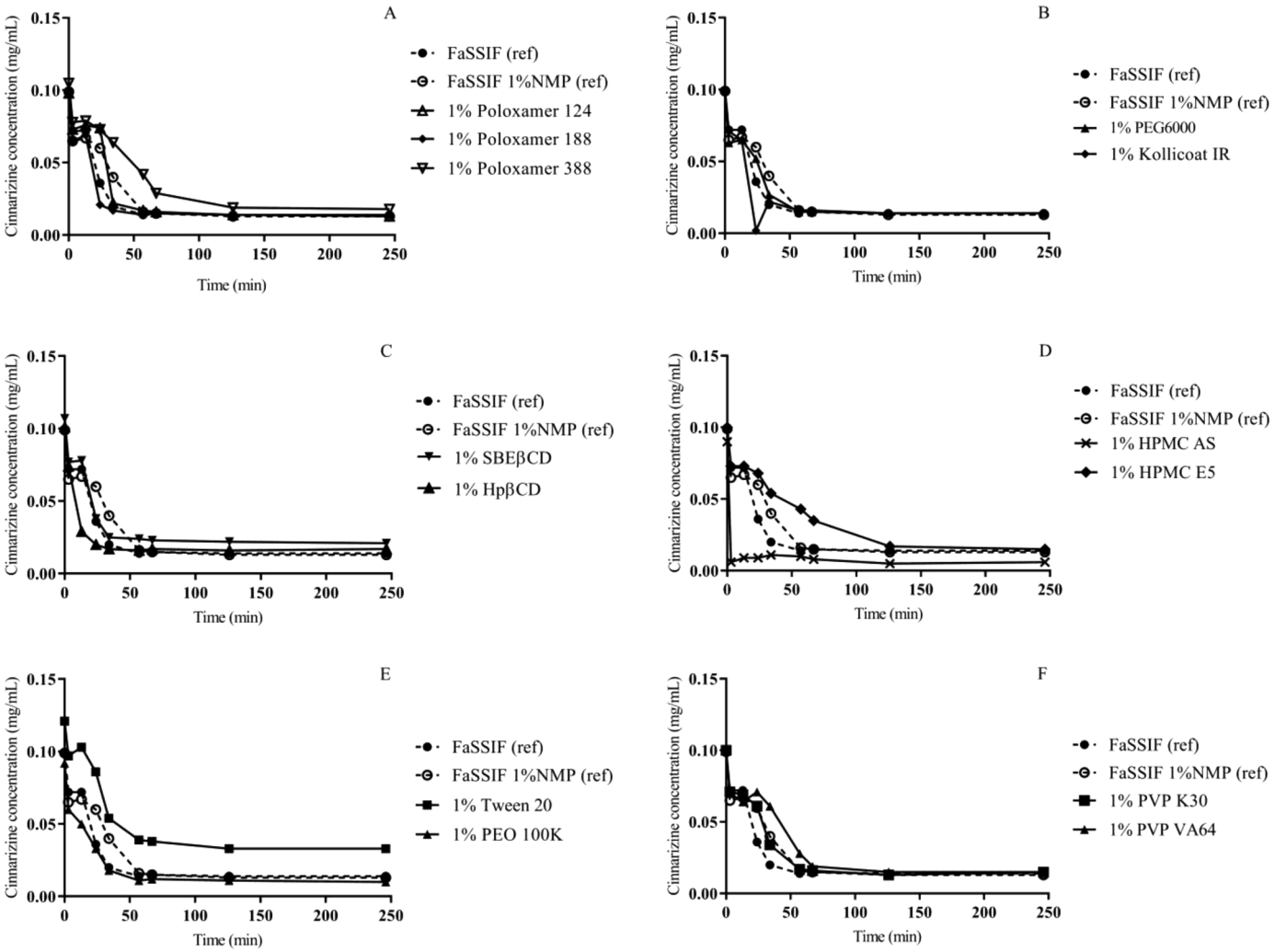

Fig. 1. Precipitation profiles (representation of cinnarizine apparent concentration as a function of time) of different excipients with poor precipitation inhibition effect based on appearance of precipitation profiles relative to reference conditions (FaSSIF without excipient); (A) Poloxamers 124, 188, 388; (B) PEG 6000 and Kollicoat® IR; (C) cyclodextrins - hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD); (D) HPMC AS and HPMC E5; (E) Tween® 20 and PEO 100K; (F) PVP K30 and PVP VA64.

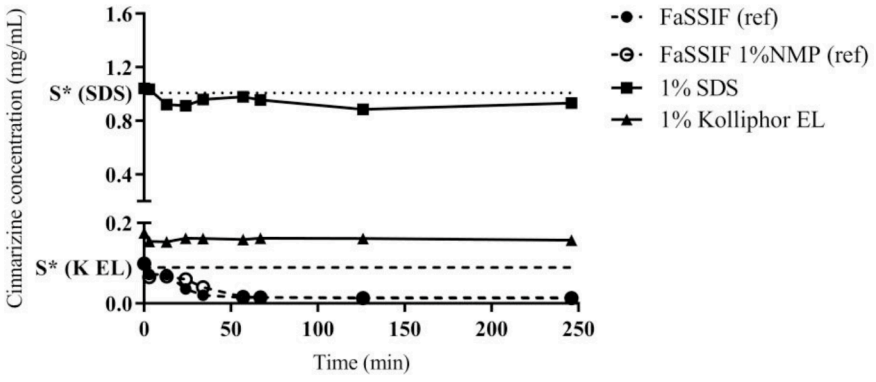

Fig. 2. Precipitation profiles for SDS and Kolliphor® EL together with values for cinnarizine apparent solubility (S*) in FaSSIF medium containing 1% SDS or 1% Kolliphor® EL.

single excipient formulations, sLCM and sMCM.

4.3.2. Evaluating in vivo benefits of supersaturated lipid-based drug delivery systems with precipitation inhibitors

Fig. 4 illustrates the dose-normalized plasma profiles of cinnarizine after administration of sLBDDS with or without PIs for either sLCMs or sMCMs. A depiction of the differences between drug exposure ($AUC_{0\text{-}24h}$) as compared to the reference sLCM and sMCM systems is shown in Fig. 5 and the corresponding pharmacokinetic parameters are shown in Table 6. A general trend of increased cinnarizine bioavailability was

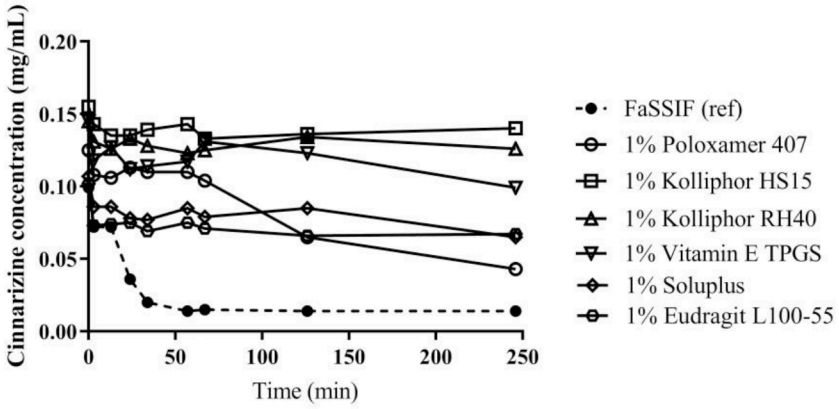

Fig. 3. Overview of precipitation profiles of PIs selected for further investigations after high throughput screening of twenty-one excipients.

Table 3
Physical stability on storage of drug-free and cinnarizine-loaded sLBDDS ± PI at 25 ± 1 °C.

sLBDDS		Physical stability	
		Drug-free	Drug-loaded
sLCMs	sLCM	> 48 hours	< 12 hours
	sLCM - Poloxamer 407	> 48 hours	> 48 hours
	sLCM - Kolliphor HS 15	> 48 hours	12 - 24 hours
	sLCM - Kolliphor RH40	> 48 hours	12 - 24 hours
	sLCM - Vitamin E TPGS	> 48 hours	12 - 24 hours
	sLCM - Soluplus	> 48 hours	12 - 24 hours
sMCMs	sMCM	> 48 hours	< 12 hours
	sMCM - Poloxamer 407	> 48 hours	< 12 hours
	sMCM - Kolliphor HS 15	> 48 hours	< 12 hours
	sMCM - Kolliphor RH40	> 48 hours	< 12 hours
	sMCM - Vitamin E TPGS	> 48 hours	> 48 hours
	sMCM - Soluplus	> 48 hours	> 48 hours

Table 4
Apparent solubility of cinnarizine at 60 °C in mixtures of PIs and lipid excipients (i.e. 5% w/w PI dissolved in lipid excipient). Results shown as mean ± SD in comparison to previously published equilibrium solubility data in LCM and MCM (Ilie et al., 2020b).

LBDDS	Apparent solubility (mg/mL)					
	No PI	+P 407	+K HS15	+K RH40	+Vit E TPGS	+Soluplus®
LCM	86.6 ± 6.5	76.5 ± 9.0	82.1 ± 7.4	80 ± 12	97.5 ± 7.5	95 ± 14
MCM	81.7 ± 4.0	66.8 ± 6.5	68.2 ± 6.9	82.2 ± 9.2	72.7 ± 1.5	77 ± 11

observed across all sLBDDS-PI relative to reference sLBDDS. This observation was further supported by the trend of increased $AUC_{0\text{-}24h}$, which was statistically significant for sLCM-P 407 relative to sLCM (2.7-fold increase). Addition of Poloxamer 407 also increased the $AUC_{0\text{-}24h}$ of the sMCM system (2.6-fold), albeit not statistically significant. Based on the drug exposure, the rank order of the different tested PIs in sLBDDS was: P 407 > K HS15 > Soluplus® > K RH40 > vit E TPGS for sLCM systems and P 407 > Soluplus® > K RH40 > vit E TPGS > K HS15 for sMCM systems, but were not statistically significant. Overall, non-statistically significant increases in C_{max} and prolongation of t_{max} were observed after oral administration of sLBDDS-PI with the same dose as the reference lipid systems, sLCM and sMCM. Regarding the ratios of $AUC_{0\text{-}4h}/AUC_{0\text{-}24h}$ it appeared that the calculated values were lower (not significant) compared to reference lipid systems indicating that the extent of absorption was lower in the first 4 hours and that it was more sustained and continued beyond this timepoint.

Table 5
Dose-normalized pharmacokinetic parameters obtained after administration of supersaturated sLCM and sMCM and their respective surfactant-containing correspondents. Results shown as mean ± SD for C_{max} and $AUC_{0\text{-}24h}$ and median [min, max] for t_{max} (n=4). LCM = long chain mono-/di-glycerides (Maisine® CC); MCM = medium chain mono-/di-glycerides (Capmul® MCM); S = surfactant; C_{max} = maximum plasma concentration; AUC = area under the plasma concentration-time curv; t_{max} = time to reach maximum plasma concentration

sLBDDS	sLCM	sLCM+S	sMCM	sMCM+S
C_{max} (ng/mL)	12.0 ± 2.5	13.4 ± 5.6	9.1 ± 1.1	8.1 ± 1.1
$AUC_{0\text{-}24h}$ (ng/mL·h)	96 ± 11[a]	80.0 ± 8.2	68 ± 20	54 ± 21[a]
$AUC_{0\text{-}4h}/AUC_{0\text{-}24h}$	0.37 ± 0.04	0.40 ± 0.07	0.43 ± 0.14	0.49 ± 0.12
t_{max} (h)	1.0 [0.5; 2.0]	2.0 [0.5; 4.0]	3.0 [0.5; 4.0]	2.0 [1.0; 4.0]

[a] $AUC_{0\text{-}24h}$: sLCM statistically significant relative to sMCM+S

5. Discussion

LBDDS were previously shown to enhance the absorption of PWSD through co-stimulation of solubilization and supersaturation upon dispersion and digestion in the GI fluids, therefore creating a "spring" of drug concentrations above equilibrium solubility, that in turn potentially boosts drug absorption (Williams et al., 2013). High apparent supersaturation ratios may be obtained upon dispersion and digestion of sLBDDS, which resulted in increased exposure of venetoclax, celecoxib, halofantrine, fenofibrate, simvastatin or R3040 in preclinical pharmacokinetic studies relative to conventional LBDDS or aqueous suspensions (Koehl et al., 2020), (Ilie et al., 2020c), (Thomas et al., 2012), (Michaelsen et al., 2016), (Thomas et al., 2013), (Siqueira Jørgensen et al., 2018). Nevertheless, high *in vivo* apparent supersaturation ratios may also determine drug precipitation and thus negatively impact drug exposure. Indeed, it has been suggested that a modest degree of supersaturation may be optimal for boosting drug absorption, while balancing the risk of drug precipitation which is higher at extremes of supersaturation (Kuentz, 2019). PIs can kinetically hinder precipitation by having a "parachute" effect and therefore prolong the supersaturation state and improve drug absorption. For cinnarizine the previously demonstrated inherent poor stability in sLBDDS may impact its exposure after oral administration and therefore, the *in vivo* investigation of this drug in sLBDDS with PIs was pursued in this study (Ilie et al., 2020b), (Siqueira et al., 2017).

In contrast to the previously outlined studies investigating PIs and LBDDS, the choice of promising PIs was based on results of an HTS assay of twenty-one excipients that may have precipitation inhibition effects. The screening was based on the solvent shift method, whereby the drug

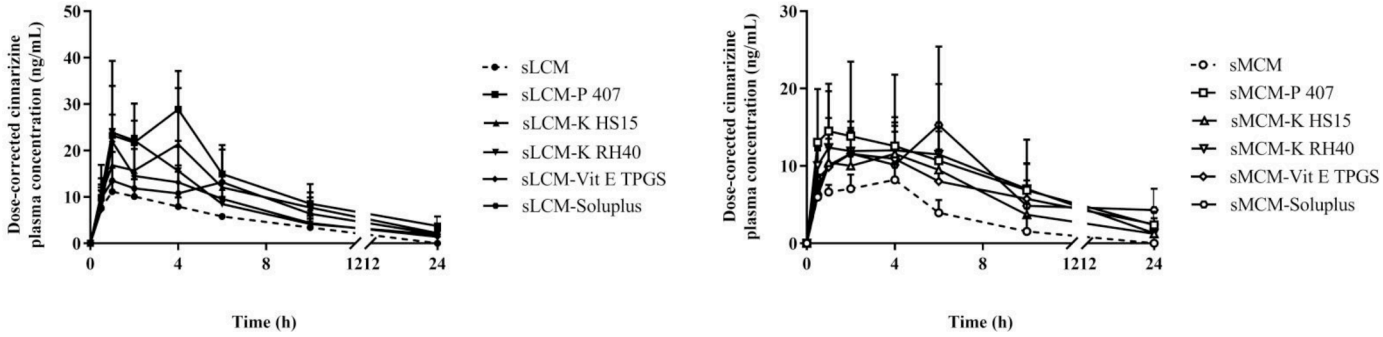

Fig. 4. Plasma profiles of supersaturated LBDDS without (interrupted lines) and with (continuous lines) precipitation inhibitors containing either LCM (full symbols) and MCM (empty symbols) lipid excipients (n=4). The investigated PIs were: Poloxamer 407 (P 407), Kolliphor® HS15 (K HS15). Kolliphor® RH40 (K RH40), vitamin E TPGS (vit E TPGS) and Soluplus®.

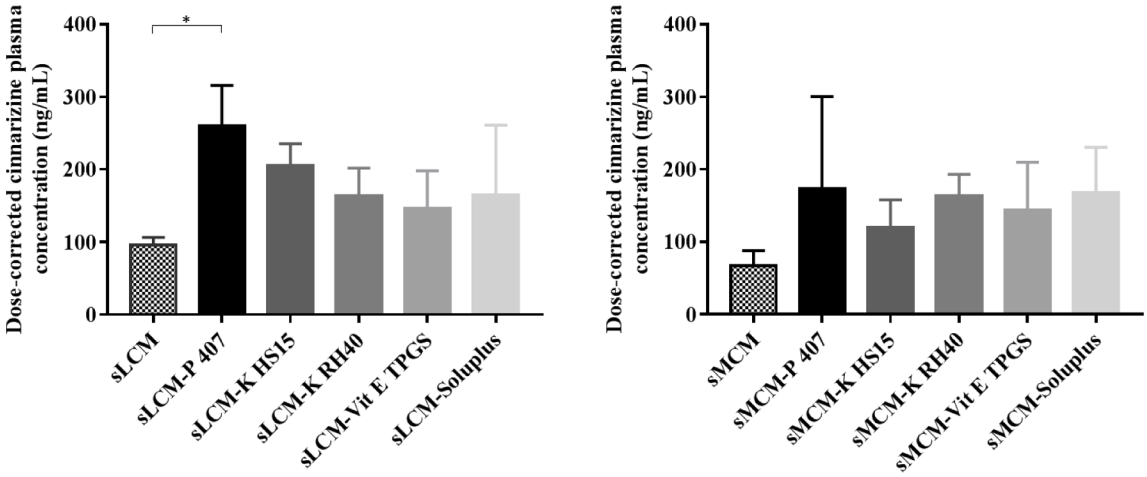

Fig. 5. Dose-normalized area under the concentration time curve for supersaturated LBDDS consisting of one lipid excipient (LCM or MCM) without or with precipitation inhibitors.

Table 6
Dose-normalized pharmacokinetic parameters obtained after oral administration of supersaturated LBDDS containing different precipitation inhibitors.

sLBDDS-PI	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng/mL·h)	$AUC_{0\text{-}4h}/AUC_{0\text{-}24h}$	t_{max} (h)
sLCM	12.0 ± 2.5	96 ± 11[a]	0.37 ± 0.04	1.0 [0.5; 2.0]
sLCM - Poloxamer 407	29.1 ± 8.2	260 ± 55[a]	0.32 ± 0.05	4.0 [1.0; 4.0]
sLCM - Kolliphor HS 15	27.0 ± 7.1	205 ± 30	0.31 ± 0.06	3.0 [1.0; 4.0]
sLCM - Kolliphor RH40	25.6 ± 8.7	164 ± 38	0.43 ± 0.08	1.5 [1.0; 2.0]
sLCM - Vitamin E TPGS	23 ± 16	146 ± 52	0.36 ± 0.11	1.0 [1.0; 4.0]
sLCM - Soluplus	19.6 ± 8.1	165 ± 96	0.29 ± 0.15	3.5 [1.0; 6.0]
sMCM	9.1 ± 1.1	68 ± 20	0.43 ± 0.14	3.0 [0.5; 4.0]
sMCM - Poloxamer 407	15.8 ± 8.5	174 ± 127	0.31 ± 0.03	1.0 [0.5; 2.0]
sMCM - Kolliphor HS 15	13.2 ± 3.8	120 ± 38	0.32 ± 0.09	5.0 [4.0; 6.0]
sMCM - Kolliphor RH40	15.5 ± 4.1	164 ± 30	0.28 ± 0.11	3.5 [1.0; 6.0]
sMCM - Vitamin E TPGS	13.7 ± 4.8	144 ± 66	0.30 ± 0.07	3.0 [2.0; 4.0]
sMCM - Soluplus	15.9 ± 9.6	168 ± 63	0.24 ± 0.03	4.0 [1.0; 6.0]

[a] $AUC_{0\text{-}24h}$: sLCM-Poloxamer 407 statistically significant relative to sLCM

was dissolved in a non-volatile solvent at high concentration and dispersed into FaSSIF medium that contained 1% PIs. Five excipients were finally selected based on the most optimal precipitation inhibition properties and on preliminary compatibility and solubility testing and were evaluated in a rat pharmacokinetic study using one-component supersaturated LBDDS, i.e. either long chain mono-di-glycerides (sLCM) or medium chain mixed glycerides (sMCM). The choice of one or two-component LBDDS was based on previous studies that showed that simple LBDDS, without co-solvent, can facilitate rapid preclinical testing in a pharmaceutical industry setting (Ilie et al., 2020b). In the present study, the supersaturated versions of these LBDDS which achieved the highest cinnarizine absorption (i.e. LCM and LCM+S) and their medium chain correspondents were tested. Surfactant addition was considered in order to reduce the risk of poor dispersibility upon *in vivo* dispersion of a pure lipid system. However, based on the findings of an *in vivo* pilot study presented here, including a surfactant was not beneficial for cinnarizine exposure, for either LCM nor MCM, thus only the one-component systems were chosen as test systems for the subsequent *in vivo* study with PIs.

Overall, inclusion of selected PIs resulted in a trend of increased cinnarizine absorption in the rats as compared to PI-free sLBDDS, albeit only statistically significant for sLCM-P 407 relative to sLCM. The findings here confirmed the benefits of a PI to maximise *in vivo* exposure of the precipitation-prone drug, cinnarizine, in lipid systems and are in line with studies by Gao *et al.* and Suys *et al.* on the merits of PI inclusion for fenofibrate and paclitaxel in LBDDS (Gao et al., 2003), (Suys et al., 2018). However, in these previous two reports, the studies involved formulation which were at high risk of precipitation on dispersion, due

to relatively high amount of surfactant (>50%) or presence of co-solvents (Gao et al., 2003), (Suys et al., 2018). In the present study with cinnarizine, no statistical differences were observed between the different tested PIs; however, Poloxamer 407 stood out as the optimal PI. To the best of our knowledge, this excipient has not previously been tested in sLBBDS. Poloxamer 407 is a PEGylated non-ionic surfactant used in a wide range of drug delivery applications as a thickening agent and gel former, as well as co-emulsifier and consistency enhancer in creams and liquid emulsions. Based on the equilibrium solubility data (Table 4) it was apparent that the mechanism of precipitation inhibition was not the increased solubilization capacity, but more complex effects such as adsorption on the surface of oil droplets formed upon dispersion in the GI tract and hence possible inhibition of lipolysis-triggered drug precipitation are possible. Feeney *et al.* indicated that adsorption of PEGylated non-ionic surfactants on surface of droplets formed upon dispersion of LBDDS in GI fluids, reduced the lipolysis-driven drug precipitation and thus improved danazol solubilisation in *in vitro* lipolysis and drug absorption *in vivo* (Feeney et al., 2014). Similar droplet adherence effects could also explain the better performance of Poloxamer 407 in the case of sLBDDS containing cinnarizine in the present study. Rao *et al.* reported design of a Pluronic-functionalized silica−lipid hybrid (Plu-SLH) microparticle system for oral delivery of cinnarizine using Pluronic® F127 (i.e. Poloxamer 407) as PI and Labrasol® as lipid phase. This system showed a 2-fold increase in rat bioavailability relative to administration of aqueous suspension of cinnarizine (Rao et al., 2015). It was suggested by the authors that the presence of poloxamer at the lipid-water interface reduced the endogenous lipid digestion and the synergistic poloxamer-Labrasol® effects created poloxamer-stabilized emulsion droplets and thus minimized the risk of drug precipitation (Rao et al., 2015).

In the present study with sLBDDS, addition of Soluplus®, resulted in a trend towards increased drug exposure after oral administration of both sLCM and sMCM when compared to the PI-free lipid systems; however, this was not statistically significant.. Considering its non-ionizable nature, its solubility was not expected to change greatly throughout the GI tract (Shamma and Basha, 2013). Based on its relatively low surface activity, Soluplus® has more options to mechanistically act as a precipitation inhibitor compared to amphiphilic polymers. An important aspect could have been the excipient adsorption on droplet surfaces during dispersion and digestion that could lead to precipitation inhibition in the GI tract as in the case of Poloxamer 407, which is in line with findings previously reported in the literature (Song et al., 2014). Addition of two classical solubilizers, Kolliphor® RH40 and vitamin E TPGS, resulted in the poorest *in vivo* performance of sLCMs (increases of 1.7-fold and 1.5-fold, respectively, relative to sLCM without PI) and third and fourth poorest for sMCMs (increases of 2.4 and 2.1-fold, respectively, relative to sMCM without PI). The observed better effect on drug absorption from combinations with sMCM may be a consequence of a high hydrophilic-lipophilic balance (HLB) of Kolliphor® RH40 and vitamin E TPGS (i.e. 14-16 and 13.2), which may be better mixed with more hydrophilic lipid excipients, such as Capmul® MCM, HLB = 4, rather than lipophilic excipients, such as Maisine® CC, HLB = 1 (Zhang et al., 2012), (Tran et al., 2018). For Kolliphor® RH40, it was shown that combination with LC lipid excipients results in a higher particle size (i.e. 50 nm) upon dispersion relative to combinations with MC lipid excipients (i.e. 20 nm), which may have an influence on the better effect of cinnarizine exposure after administration of sMCM-K RH40 relative to sLCM-K RH40 (Tran et al., 2018). Addition of Kolliphor® HS15, a non-ionic hydrophilic solubilizer and emulsifying agent, soluble in lipids, increased (not significant) the cinnarizine absorption after administration of sLCM relative to sLCM without PI (2.1-fold) and to a lesser extent after administration of sMCM-K HS15 relative to sMCM (1.8-fold). This effect may be a consequence of a previously reported permeability increase of Kolliphor® HS15 through membrane disruption or an increased passive permeability (Shaukat and Kolter, 2019), (Pinjari et al., 2017).

While cinnarizine is a frequently used model drug for research of LBDDS, it was previously indicated that cinnarizine did not benefit from formulation as sLBDDS (55% Soybean oil + Maisine® CC, 35% Kolliphor® RH40 and 10% ethanol) relative to an aqueous suspension after administration to rats (Siqueira et al., 2017). This relatively low exposure could have been explained by the drug's poor physical stability from supersaturated lipid systems with respect to precipitation and crystallization (Siqueira et al., 2017), (Ilie et al., 2020b). In this study, changing the composition of sLBDDS to a single lipid excipient (i.e. Maisine® CC) achieved similar drug exposure in rats (i.e. $AUC_{0\text{-}24h}$ = 95.8 ± 10.6 ng/mL·h) as the sLBDDS formulation investigated by Siqueira *et al.* (i.e. $AUC_{0\text{-}24h}$ = 112 ng/mL·h after dose normalization) (Siqueira et al., 2017). Therefore, it appears that exposure of cinnarizine is low after administration of sLBDDS independent of formulation composition. Furthermore, comparing the bioavailability observed in the current study with sLBDDS, to compositionally equivalent non-supersaturated LBDDS (at 85% $S_{eq}^{37^{\circ}C}$), confirms that cinnarizine bioavailability is significantly lower for supersaturated systems. Dose normalised $AUC_{0\text{-}24h}$ of cinnarizine following oral dosing of a sLCM to rats was significantly lower at 96 ± 11 ng/mL·h compared to 285 ± 76 ng/mL·h for LCM (at 85% $S_{eq}^{37^{\circ}C}$) (Ilie et al, 2020b). A key distinction here, relative to other studies reported in the literature, is that the dose of lipid was constant in all tested rat groups. Hence, the administered lipid systems are precisely equivalent and therefore confirm that the reduced exposure of cinnarizine in a sLBDDS relative to non-supersaturated LBDDS is a drug-specific effect rather than formulation-dependent.

It may be hypothesized that the lower cinnarizine absorption from sLBDDS is due to the poor glass forming ability of cinnarizine, i.e. a class II drug based on its glass forming ability, with high precipitation propensity from supersaturated lipid systems (Ilie et al., 2020b). The poor physical stability of cinnarizine was identified also in non-supersaturated LBDDS and thus indicated an inherent drug instability in lipid systems (Ilie et al., 2020b). Therefore, in this study the physical stability of sLBDDS containing PIs was investigated in comparison to PI-free sLBDDS. Results showed that the physical stability upon storage at 25 ± 1°C of all sLCM systems containing PIs was improved compared to sLCM to longer than 12 hours. In the case of sMCM systems, only sMCM-vit E TPGS and sMCM-Soluplus had an improved physical stability of longer than 48 hours relative to sMCM (12 hours). While overall the enhancements in storage stability are relatively modest, the improved durations of stabilization are considered sufficient for formulations prepared *ad hoc* in preclinical development, which require particularly high exposure for example in toxicological animal studies.

Automated HTS is being used in drug discovery and development to an increasing extent, either for lead optimization or formulation design and development of bio-enabling formulations for PWSD (Dai et al., 2007), (Ratanabanangkoon et al., 2008), (Sakai et al., 2010). In the present study, an HTS approach was used to determine the precipitation inhibition effect of twenty-one excipients following dispersion of highly concentrated cinnarizine solution in 1% excipient in FaSSIF medium. Based on increased solubilization capacity as a 1% (w/v) solution in FaSSIF, optimal precipitation profiles, miscibility with lipids and dose-loading ability, five excipients were finally tested in a rat pharmacokinetic study. Using the HTS it was possible to quickly (< 1 week) discriminate between twenty-one excipients and to select only a few PIs for further testing and thus to reduce the animal testing burden. Such HTS approaches are particularly attractive for efficient use of resources and reducing the overall number of *in vivo* studies. In addition to HTS assays, *in silico* tools such as Design of Experiments or Conductor like Screening Model for Real Solvents (COSMO-RS) are considered promising tools for computational screenings of PIs (Price et al., 2019b). Furthermore, a valuable follow-up study could be represented by the evaluation of drug permeation through biomimetic membranes of

supersaturated LBDDS with or without PIs using either the static side-by-side diffusion model, the dynamic PermeaLoop™ model or the 96-Well Two-Compartment Microplate which is suitable for HTS (Ilie et al., 2020a), (Jacobsen et al., 2020), (Jacobsen et al., 2019).

The present study demonstrated the proof-of-concept of incorporation of PIs in sLBDDS for improvement of cinnarizine absorption relative to PI-free sLBDDS. Nevertheless, sLBDDS+PIs were broadly equivalent to conventional non-supersaturated LBDDS without a PI. For example, in our previous studies we observed an $AUC_{0\text{-}24h}$ of 285 ± 76 ng/mL·h for an LCM system (at 85% $S_{eq}^{37^{\circ}C}$) (Ilie et al, 2020b). This is within the range of *AUC*s observed in the current study where PIs were included in the sLCM systems with *AUC*s between 165 ± 96 and 260 ± 55 ng/mL·h. Therefore, even though a good "parachute" effect was observed for the tested PIs, the potentially high transient drug concentration created by the increase in dose loading did not make the sLBDDS-PI perform better than the non-supersaturated LBDDS. The prerequisite for successful formulation of supersaturating drug delivery systems is to identify the optimal combination of "spring" and "parachute". Thus, a high degree of supersaturation or excessive "spring" effect could be difficult to maintain by any excipient in order to provide a sufficient "parachute" effect given the high driving force towards precipitation. Thus, in terms of simplicity of the formulation, at least in the case of a drug such as cinnarizine, a conventional LBDDS may be favourable for optimal drug exposure. More studies investigating sLBDDS-PIs with different PWSD are needed to further understand if PI addition can only match what can be achieved with conventional lipid systems or if they have the potential to go beyond the potential of classical non-supersaturated LBDDS.

6. Conclusions

The novel use of precipitation inhibitors in supersaturated lipid-based drug delivery systems was successful in achieving enhanced oral absorption of the poorly-soluble drug cinnarizine compared to PI-free supersaturated lipid systems. The selection of suitable precipitation inhibitors was based on a high throughput screening method, which tested the precipitation inhibition effects of twenty-one excipients with different physico-chemical properties. Five excipients were chosen and evaluated in a rat pharmacokinetic study. Overall, administration of the combination sLCM-Poloxamer 407 was found to significantly increase drug exposure of cinnarizine compared to a PI-free sLCM system. The study has shown that for drugs prone to precipitation in sLBBDS, such as cinnarizine, inclusion of a PI can be used to mitigate the impact and is therefore suitable to include in preclinical formulation strategies to maximise *in vivo* exposure of such drug types.

CRediT authorship contribution statement

Alexandra-Roxana Ilie: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing - original draft, Writing - review & editing. **Brendan T. Griffin:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing - review & editing. **Maria Vertzoni:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing - review & editing. **Martin Kuentz:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing - review & editing. **Ruzica Kolakovic:** Conceptualization, Supervision, Writing - review & editing. **Anke Prudic-Paus:** Conceptualization, Methodology, Writing - review & editing. **Ahmed Malash:** Methodology, Formal analysis, Software, Writing - review & editing. **Hugo Bohets:** Conceptualization, Methodology, Writing - review & editing. **Jilly Herman:** Methodology, Formal analysis, Resources, Writing - review & editing. **René Holm:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing - review & editing.

Acknowledgements

All authors are part of the PEARRL European Training network, which has received funding from the Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks programme under grant agreement No. 674909. The personnel in the animal facility at Janssen Pharmaceutica (Beerse, Belgium) are highly acknowledged for their skilful handling of these *in vivo* studies.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105691.

References

Bannow, J., Yorulmaz, Y., Löbmann, K., Müllertz, A., Rades, T., 2020. Improving the drug load and in vitro performance of supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS) using polymeric precipitation inhibitors. Int. J. Pharm. 575, 118960 https://doi.org/10.1016/j.ijpharm.2019.118960.

Brouwers, J., Brewster, M.E., Augustijns, P., 2009. Supersaturating Drug Delivery Systems : The Answer to Solubility-Limited Oral Bioavailability ?, 98, 10.1002/jps.

Chen, Z.Q., Liu, Y., Zhao, J.H., Wang, L., Feng, N.P., 2012. Improved oral bioavailability of poorly water-soluble indirubin by a supersaturatable self-microemulsifying drug delivery system. Int. J. Nanomedicine 7, 1115–1125. https://doi.org/10.2147/IJN.S28761.

Cuiné, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J.H., 2007. Increasing the proportional content of surfactant (Cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. Pharm. Res. 24, 748–757. https://doi.org/10.1007/s11095-006-9194-z.

Dai, W.G., Dong, L.C., Li, S., Pollock-Dove, C., Chen, J., Mansky, P., Eichenbaum, G., 2007. Parallel screening approach to identify solubility-enhancing formulations for improved bioavailability of a poorly water-soluble compound using milligram quantities of material. Int. J. Pharm. 336, 1–11. https://doi.org/10.1016/j.ijpharm.2006.11.034.

Feeney, O.M., Crum, M.F., McEvoy, C.L., Trevaskis, N.L., Williams, H.D., Pouton, C.W., Charman, W.N., Bergström, C.a.S., Porter, C.J.H., 2016. 50 years of oral lipid-based formulations: Provenance, progress and future perspectives. Adv. Drug Deliv. Rev. 101, 167–194. https://doi.org/10.1016/j.addr.2016.04.007.

Feeney, O.M., Williams, H.D., Pouton, C.W., Porter, C.J.H., 2014. ' Stealth ' lipid-based formulations : Poly (ethylene glycol) -mediated digestion inhibition improves oral bioavailability of a model poorly water soluble drug. J. Control. Release 192, 219–227. https://doi.org/10.1016/j.jconrel.2014.07.037.

Frank, K.J., Westedt, U., Rosenblatt, K.M., Hölig, P., Rosenberg, J., Mägerlein, M., Brandl, M., Fricker, G., 2012. Impact of FaSSIF on the solubility and dissolution-/permeation rate of a poorly water-soluble compound. Eur. J. Pharm. Sci. 47, 16–20. https://doi.org/10.1016/j.ejps.2012.04.015.

Gao, P., Akrami, A., Alvarez, F., Hu, J., Li, L.A.N., Ma, C., Surapaneni, S., 2009. Characterization and Optimization of AMG 517 Supersaturatable Self-Emulsifying Drug Delivery System (S-SEDDS) for Improved Oral Absorption 98, 516–528. 10.1002/jps.

Gao, P., Guyton, M.E., Huang, T., Bauer, J.M., Kevin, J., Lu, Q., Gao, P., Guyton, M.E., Huang, T., Bauer, J.M., 2004. Enhanced Oral Bioavailability of a Poorly Water Soluble Drug PNU - 91325 by Supersaturatable Formulations Enhanced Oral Bioavailability of a Poorly Water Soluble Drug PNU-91325 by Supersaturatable Formulations 9045. 10.1081/DDC-120028718.

Gao, P., Rush, B.D., Pfund, W.P., Huang, T., Bauer, J.M., Morozowich, W., Kuo, M., Hageman, M.J., 2003. Development of a Supersaturable SEDDS (S-SEDDS) Formulation of Paclitaxel with Improved Oral Bioavailability 92, 2386–2398.

Gao, P., Shi, Y., 2012. Characterization of Supersaturatable Formulations for Improved Absorption of Poorly Soluble Drugs. AAPS J 14, 703–713. https://doi.org/10.1208/s12248-012-9389-7.

Guzman, H.R., Tawa, M., Zhang, Z., Ratanabanangkoon, P., Shaw, P., Gardner, C.R., Chen, H., Moreau, J.-P., Almarsoon, O., Remenar, julius F., 2007. Combined Use of Crystalline Salt Forms and Precipitation Inhibitors to Improve Oral Absorption of Celecoxib from Solid Oral Formulations. J. Pharm. Sci. 96, 2686–2702. https://doi.org/10.1002/jps.20906.

Ilie, A.-R., Griffin, B.T., Brandl, M., Bauer-Brandl, A., Jacobsen, A.-C., Vertzoni, M., Kuentz, M., Kolakovic, R., Holm, R., 2020a. Exploring impact of supersaturated lipid-based drug delivery systems of celecoxib on in vitro permeation across Permeapad® membrane and in vivo absorption. Eur. J. Pharm. Sci. https://doi.org/10.1016/j.ejps.2020.105452.

Ilie, A.-R., Griffin, B.T., Kolakovic, R., Vertzoni, M., Kuentz, M., Holm, R., 2020b. Supersaturated lipid-based drug delivery systems–exploring impact of lipid composition type and drug properties on supersaturability and physical stability. Drug Dev. Ind. Pharm. 46, 356–364. https://doi.org/10.1080/03639045.2020.1721526.

Ilie, A.R., Griffin, B.T., Vertzoni, M., Kuentz, M., Cuyckens, F., Wuyts, K., Kolakovic, R., Holm, R., 2020c. Toward simplified oral lipid-based drug delivery using mono-/di-

glycerides as single component excipients. Drug Dev. Ind. Pharm. 46, 2051–2060. https://doi.org/10.1080/03639045.2020.1843475.
Jacobsen, A.-C., Nielsen, S., Brandl, M., Bauer-Brandl, A., 2020. Drug Permeability Profiling Using the Novel Permeapad® 96-Well Plate. Pharm. Res. 37, 93. https://doi.org/10.1007/s11095-020-02807-x.
Jacobsen, A.C., Krupa, A., Brandl, M., Bauer-Brandl, A., 2019. High-throughput dissolution/permeation screening — A 96-well two-compartment microplate approach. Pharmaceutics 11, 1–15. https://doi.org/10.3390/pharmaceutics11050227.
Koehl, N.J., Henze, L.J., Kuentz, M., Holm, R., Griffin, B.T., 2020. Supersaturated Lipid-Based Formulations to Enhance the Oral Bioavailability of Venetoclax. Pharmaceutics 12, 1–20. https://doi.org/10.3390/pharmaceutics12060564.
Kuentz, M., 2019. Drug supersaturation during formulation digestion, including real-time analytical approaches. Adv. Drug Deliv. Rev. 142, 50–61. https://doi.org/10.1016/j.addr.2018.11.003.
Kuentz, M., 2012. Lipid-based formulations for oral delivery of lipophilic drugs. Drug Discov. Today Technol. 9, 1–8. https://doi.org/10.1016/j.ddtec.2012.03.002.
Larsen, A.T., Holm, R., Müllertz, A., 2017. 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. 117, 308–314. https://doi.org/10.1016/j.ejpb.2017.04.023.
Michaelsen, M., Siqueira, S.D., Mahad, I., Wasan, K.M., Rades, T., Müllertz, A., 2019. European Journal of Pharmaceutics and Biopharmaceutics Feno fi brate oral absorption from SNEDDS and super-SNEDDS is not signi fi cantly a ff ected by lipase inhibition in rats 142, 258–264. 10.1016/j.ejpb.2019.07.002.
Michaelsen, M.H., Wasan, K.M., Sivak, O., Müllertz, A., Rades, T., 2016. The Effect of Digestion and Drug Load on Halofantrine Absorption from Self-nanoemulsifying Drug Delivery System (SNEDDS). AAPS J 18, 180–186. https://doi.org/10.1208/s12248-015-9832-7.
O'Driscoll, C.M., Griffin, B.T., 2008. Biopharmaceutical challenges associated with drugs with low aqueous solubility-The potential impact of lipid-based formulations. Adv. Drug Deliv. Rev. 60, 617–624. https://doi.org/10.1016/j.addr.2007.10.012.
Pinjari, M.J.S.K., Somani, R.S., Gilhotra, R.M., 2017. Investigation of different formulation approaches to enhance oral bioavailability of paromomycin. Indian J. Pharm. Sci. 79, 568–575. https://doi.org/10.4172/pharmaceutical-sciences.1000264.
Price, D.J., Ditzinger, F., Koehl, N.J., Jankovic, S., Tsakiridou, G., Nair, A., Holm, R., Kuentz, M., Dressman, J.B., Saal, C., 2019a. Approaches to increase mechanistic understanding and aid in the selection of precipitation inhibitors for supersaturating formulations – a PEARRL review. J. Pharm. Pharmacol. https://doi.org/10.1111/jphp.12927.
Price, D.J., Nair, A., Kuentz, M., Dressman, J., Saal, C., 2019b. Calculation of drug-polymer mixing enthalpy as a new screening method of precipitation inhibitors for supersaturating pharmaceutical formulations. Eur. J. Pharm. Sci. 132, 142–156. https://doi.org/10.1016/j.ejps.2019.03.006.
Rao, S., Richter, K., Nguyen, T.H., Boyd, B.J., Porter, C.J.H., Tan, A., Prestidge, C.A., 2015. Pluronic-Functionalized Silica-Lipid Hybrid Microparticles: Improving the Oral Delivery of Poorly Water-Soluble Weak Bases. Mol. Pharm. 12, 4424–4433. https://doi.org/10.1021/acs.molpharmaceut.5b00622.
Ratanabanangkoon, P., Guzman, H., Almarsson, O., Berkovitz, D., Tokarcyzk, S., Straughn, A.B., Chen, H., 2008. A high-throughput approach towards a novel formulation of fenofibrate in omega-3 oil. Eur. J. Pharm. Sci. 33, 351–360. https://doi.org/10.1016/j.ejps.2008.01.005.
Sakai, K., Yoshimori, T., Obata, K., Maeda, H., 2010. Design of self-microemulsifying drug delivery systems using a high-throughput formulation screening system. Drug Dev. Ind. Pharm. 36, 1245–1252. https://doi.org/10.3109/03639041003710169.
Shamma, R.N., Basha, M., 2013. Soluplus®: A novel polymeric solubilizer for optimization of Carvedilol solid dispersions: Formulation design and effect of method of preparation. Powder Technol 237, 406–414. https://doi.org/10.1016/j.powtec.2012.12.038.
Shaukat, A., Kolter, K., 2019. American Pharmaceutical Review [WWW Document]. Am. Pharm. Rev. URL. https://www.americanpharmaceuticalreview.com/Featured-Articles/358749-Kolliphor-HS-15-An-Enabler-for-Parenteral-and-Oral-Formulations/.
Shi, Y., Gao, P., Gong, Y., Ping, H., 2010. Application of a biphasic test for characterization of in vitro drug release of immediate release formulations of celecoxib and its relevance to in vivo absorption. Mol. Pharm. 7, 1458–1465. https://doi.org/10.1021/mp100114a.
Singh, G., Pai, R.S., 2016. In vitro and in vivo performance of supersaturable self-nanoemulsifying system of trans-resveratrol. Artif. Cells. Nanomedicine Biotechnol 44, 510–516. https://doi.org/10.3109/21691401.2014.966192.
Siqueira Jørgensen, S.D., Al Sawaf, M., Graeser, K., Mu, H., Müllertz, A., Rades, T., 2018. 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. 124, 116–124. https://doi.org/10.1016/j.ejpb.2017.12.014.
Siqueira, S.D.V.S., Müllertz, A., Gräeser, K., Kasten, G., Mu, H., Rades, T., 2017. Influence of drug load and physical form of cinnarizine in new SNEDDS dosing regimens: in vivo and in vitro evaluations. AAPS J 19. https://doi.org/10.1208/s12248-016-0038-4.
Song, W.H., Yeom, D.W., Lee, D.H., Lee, K.M., Yoo, H.J., Chae, B.R., Song, S.H., Choi, Y. W., 2014. In situ intestinal permeability and in vivo oral bioavailability of celecoxib in supersaturating self-emulsifying drug delivery system. Arch. Pharm. Res. 37, 626–635. https://doi.org/10.1007/s12272-013-0202-7.
Suys, E.J.A., Chalmers, D.K., Pouton, C.W., Porter, C.J.H., 2018. Polymeric Precipitation Inhibitors Promote Fenofibrate Supersaturation and Enhance Drug Absorption from a Type IV Lipid-Based Formulation. Mol. Pharm. 15, 2355–2371. https://doi.org/10.1021/acs.molpharmaceut.8b00206.
Thomas, N., Holm, R., Garmer, M., Karlsson, J.J., Müllertz, A., Rades, T., 2013. Supersaturated self-nanoemulsifying drug delivery systems (Super-SNEDDS) enhance the bioavailability of the poorly water-soluble drug simvastatin in dogs. AAPS J 15, 219–227. https://doi.org/10.1208/s12248-012-9433-7.
Thomas, N., Holm, R., Mullertz, A., Rades, T., 2012. In vitro and in vivo performance of novel supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS). J. Control. Release 160, 25–32. https://doi.org/10.1016/j.jconrel.2012.02.027.
Thomas, N., Richter, K., Pedersen, T.B., Holm, R., Müllertz, A., Rades, T., 2014. In vitro lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery systems containing fenofibrate. AAPS J 16, 539–549. https://doi.org/10.1208/s12248-014-9589-4.
Tran, T., Rades, T., Müllertz, A., 2018. Formulation of self-nanoemulsifying drug delivery systems containing monoacyl phosphatidylcholine and Kolliphor® RH40 using experimental design. Asian J. Pharm. Sci. 13, 536–545. https://doi.org/10.1016/j.ajps.2017.09.006.
Tung, N.T., Tran, C.S., Nguyen, H.A., Nguyen, T.D., Chi, S.C., Pham, D.V., Bui, Q.D., Ho, X.H., 2019. Formulation and biopharmaceutical evaluation of supersaturatable self-nanoemulsifying drug delivery systems containing silymarin. Int. J. Pharm. 555, 63–76. https://doi.org/10.1016/j.ijpharm.2018.11.036.
Warren, D.B., Benameur, H., Porter, C.J.H., Pouton, C.W., 2010. Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility. J. Drug Target. 18, 704–731. https://doi.org/10.3109/1061186X.2010.525652.
Williams, H.D., Trevaskis, N.L., Yeap, Y.Y., Anby, M.U., Pouton, C.W., Porter, C.J.H., 2013. Lipid-based formulations and drug supersaturation: Harnessing the unique benefits of the lipid digestion/absorption pathway. Pharm. Res. https://doi.org/10.1007/s11095-013-1126-0.
Xu, S., Dai, W.G., 2013. Drug precipitation inhibitors in supersaturable formulations. Int. J. Pharm. 453, 36–43. https://doi.org/10.1016/j.ijpharm.2013.05.013.
Zhang, N., Zhang, W., Jin, Y., Quan, D.Q., 2011. Studies on preparation of carbamazepine (CBZ) supersaturatable self-microemulsifying (S-SMEDDS) formulation and relative bioavailability in beagle dogs. Pharm. Dev. Technol. 16, 415–421. https://doi.org/10.3109/10837451003774419.
Zhang, Z., Tan, S., Feng, S.S., 2012. Vitamin E TPGS as a molecular biomaterial for drug delivery. Biomaterials 33, 4889–4906. https://doi.org/10.1016/j.biomaterials.2012.03.046.