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Supersaturated lipid-based drug delivery systems – exploring impact of lipid composition type and drug properties on supersaturability and physical stability

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Supersaturated lipid-based drug delivery systems – exploring impact of lipid composition type and drug properties on supersaturability and physical stability

Objective

The objective of the current study was to systematically investigate the impact of lipid composition on the ability to design supersaturated lipid-based drug delivery systems (sLBDDS) using three model drugs with different physico-chemical properties.

Significance

This study expands the list of investigated sLBDDS by using alternative vehicle compositions relative to current literature.

Methods & Results

Drug supersaturation was thermally-induced based on previously reported methods and was successfully achieved for celecoxib and cinnarizine. For the novel drug, JNJ-2A, a lower supersaturation potential was observed for the tested LBDDS. For celecoxib and cinnarizine, crystalline precipitate was observed for some sLBDDS upon storage at 25°C/65%RH, particularly for medium chain sLBDDS (celecoxib) and long chain sLBDDS (cinnarizine). The greater risk of precipitation observed for celecoxib and cinnarizine, particularly at higher apparent degree of supersaturation (aDS) may be related to their higher crystallization tendency as determined by differential scanning calorimetry.

Conclusions

In conclusion, the potential for supersaturation in LBDDS, and the risk of precipitation, was found to be highly drug dependent. The apparent degree of supersaturation was considered a major factor impacting the ability to maintain drug supersaturation upon storage.

Keywords; Supersaturated lipid-based drug delivery systems, Pre-formulation, Solubility screening, Formulation development, Physical stability

1. Introduction

Poorly water-soluble drug (PWSD) candidates typically exhibit biopharmaceutical challenges when formulated as conventional oral dosage forms, leading to erratic or incomplete absorption, low bioavailability, high pharmacokinetic variability and food dependent uptake [1-3]. With the increasing trend of PWSD emerging from drug discovery programmes [4], there is a need to develop novel bio-enabling formulation technologies to address these biopharmaceutical limitations [5,6]. Several bio-enabling approaches based on physical modifications of drugs have been investigated and include (1) particle size reduction (micronization or nanonization), (2) modification of crystal form (co-crystals, polymorphs), (3) complexation/solubilisation (inclusion in cyclodextrins or lipid vehicles) or (4) drug dispersion in carriers (solid dispersions, solid solutions or eutectic mixtures) [7-9].

As a bio-enabling strategy, lipid-based drug delivery systems (LBDDS) offer the advantage to present the PWSD pre-solubilised to gastro-intestinal (GI) tract [4,8,9]. LBDDS may be formulated with a wide range of lipid excipients, generally including either triglycerides, partially digested triglycerides (i.e. mono- and di-glycerides), surfactants or co-solvents. While various classification schemes have attempted to guide industry on the choice of excipient type, the final choice of excipient is highly influenced by the drug type, as well empirically driven, based on in-house experience and prior regulatory acceptability [2]. Lipid excipients are predominantly obtained from natural sources (i.e. vegetable oils) and their function in LBDDS is not only to solubilize and improve the dissolution of PWSDs, but may also serve to reduce food-dependent drug absorption and enhance bioavailability by stimulation of lymphatic transport [9]. While there are numerous examples of commercially successful LBDDS [9-11], a key limitation for more widespread application of LBDDS is that the maximum dose loading of drug within the LBDDS is limited by the inherent solubility of the drug in the lipid vehicle. This is particularly the case for PWSDs displaying both solid-state limited solubility (e.g. high hydrophobicity) and solvation limited solubility (e.g. low to

medium lipophilicity), where the dose exceeds the drug equilibrium solubility in lipid excipients [4]. Formulation strategies to improve maximum dose loading in LBDDS, such as the use of co-solvents, are therefore commonly explored [12]. More recently, the potential to generate supersaturated drug solutions in LBDDS, i.e. supersaturated LBDDS (sLBDDS), have been reported as an approach to enhance drug dose loading in lipid vehicles [13-16].

From a drug development perspective, formulation scientists are increasingly operating within reduced timelines with an emphasis on accelerating development for breakthrough therapies [17-19]. For PWSDs this is particularly challenging given that the optimal choice of bio-enabling formulation strategy is unclear at early stage preclinical development. In addition, for preclinical pharmaco- and toxico-kinetics evaluation there is a need to increase doses to up to 30 to 100 times greater (mg/kg) than might be envisaged with a clinically relevant dose and formulation in order to demonstrate dose limiting toxicity endpoints [12,17,19-21]. A simplified drug development process generally relies on obtaining dose proportionality in exposure, thus ensuring a predictable response [21]. Selection of formulations for early pharmaco- and toxico-kinetics studies is based on high throughput screening of drug solubility in different solvents, surfactants and lipid excipients and aims at finding suitable vehicles to maintain the drug in solution to allow oral dosing in preclinical animal models [18]. Generally, easily dose scalable formulations are preferred in preclinical studies and typically comprise of solutions and suspensions for both rodent and non-rodent species [18,20,21].

sLBDDS offer an advantage in terms of the ability to administer highly concentrated solutions of the drug. In contrast, using conventional LBDDS, particularly where drug solubility in the vehicle is low, dosing in toxicity studies may become limited by the large quantities of excipient often required to afford the higher exposure. sLBDDS can be manufactured without recourse to advanced processing approaches (e.g. salt or co-crystal formation or drug amorphization in solid dispersions) or to the use of co-solvents which may

promote drug precipitation upon dispersion in the aqueous GI media [18,20,21]. Furthermore, when a safe dose range is found in preclinical studies, first-in-human (FIH) clinical studies are commonly started typically using similar 'simple' formulations (i.e. solution or suspension) as used in preclinical studies [20,21]. Supersaturated lipid-based drug delivery systems are therefore highly suited to streamline the formulation process in early stage drug development, allowing ease of administration as simple lipid solutions in rodent and non-rodent models, which can be readily scaled to clinical formulation as lipid filled capsules.

To date, supersaturated drug delivery systems have most commonly been investigated for topical and transdermal administration with the advantage of increasing drug loading of drugs that otherwise may exhibit limited solubility in lipid vehicles [22,23]. More recently, sLBDDS, and specifically self-nano-emulsifying drug delivery systems (i.e. super-SNEDDS) have been shown to enhance oral absorption of PWSDs, for drugs such as halofantrine [14,15], simvastatin [13], fenofibrate [24] and R3040 [25] in dogs and mini-pigs pharmacokinetic studies. However, in these studies a relatively similar composition of SNEDDS in terms of lipid, surfactant and co-solvent was employed (e.g. lipid:surfactant:co-solvent = 55:35:10). The most commonly employed method to produce oral sLBDDS involves mixing the drug (at a dose exceeding drug equilibrium solubility in the lipid vehicle) with the lipid excipients, heating for a defined period of time (e.g. to 50-60°C) followed by cooling to ambient temperature [13-15].

While sLBDDS have gained biopharmaceutical focus due to their ability to provide good *in vivo* performance, there is a lack of studies exploring formulation design of sLBDDS. In particular, no study has, to the best of our knowledge, been reported on the influence of the lipid composition on the ability to supersaturate and capability to maintain supersaturation in sLBDDS during storage. The overall aim of this study was, therefore, to evaluate the influence of formulation complexity and lipid composition type on the ability to design sLBDDS for three PWSDs with different physico-chemical properties and their respective

ability to maintain supersaturation upon short-term storage. The three model drugs selected were a weak acid (celecoxib), a weak base (cinnarizine) and a neutral drug (JNJ-2A), with a clogP between 4.3 and 5.7 and different solid-state characteristics.

2. Materials and methods

2.1. Chemicals and materials

Celecoxib was purchased from Astatech Inc. (Bristol, PA, USA), cinnarizine and JNJ-2A were obtained from Janssen Pharmaceutica (Beerse, Belgium). Sesame oil (long chain triglycerides, LCT) was purchased from Croda (Snaith, United Kingdom), Capmul MCM C8 (medium chain mixed mono-/di-glycerides, MCM) was kindly donated by Abitec (Columbus, OH, USA). Maisine CC (long chain mono-/di-glycerides, LCM), Labrafac Lipophile WL1349 (medium chain triglycerides, MCT) and Labrasol ALF (hydrophilic surfactant, S) were a kind gift from Gattefossé (Lyon, France). All other chemicals and solvents were of analytical or HPLC grade and were purchased from WVR (Belgium).

2.2. Design of prototype lipid-based drug delivery systems

Composition of the different excipients used in this study is listed in (Supporting information Table S 1, [26]) and the components of the prototype lipid-based drug delivery systems are shown in Table 1. The mixtures contained either a single lipid component and surfactant (LCM+S, MCM+S), two lipid components (mono-di-glycerides blends and triglycerides) with same fatty acid length and surfactant (LCM+LCT+S, MCM+MCT+S) or were mixtures of two lipid components (mono-di-glycerides blends and tri-glycerides) with different fatty acid length and surfactant (LCM+MCT+S, MCM+LCT+S). Excipients for the LBDDS were mixed gently for 10 s at ambient temperature until a homogenous solution was obtained. These drug-free LBDDS were kept at ambient temperature, 37°C and 60°C for 24 h to mimic the drug loading conditions. Miscibility of these drug-free lipid systems was assessed visually

at ambient temperature and homogeneity or excipient separation was investigated for up to 28 days at 25°C/65% RH.

2.3. Crystallization tendency by Differential Scanning Calorimetry

The crystallization tendency of the three drugs from the undercooled melt state was evaluated using differential scanning calorimetry (DSC). Enthalpy of fusion (ΔH_{fus}), melting peak temperature (T_m), crystallization peak temperature during cooling ($T_{cryst,cool}$), onset and midpoint glass transition temperatures ($T_{g,onset}$, $T_{g,mid}$), and crystallization peak upon heating ($T_{cryst,heat}$) of the samples were determined in triplicate using a TA Q2000 DSC equipped with a refrigerated cooling accessory (TA Instruments, New Castle, DE). From the obtained data, entropy of fusion (ΔS_{fus}) and T_m/T_g ratio were determined (both using the Kelvin scale). The instrument was calibrated for temperature using adamantane, octadecane, lead, and indium. The enthalpic response was calibrated using indium. Nitrogen, 50 mL/min, served as the purge gas. For the crystallization screening experiments, samples were prepared in sealed pans, heated at 10°C/min to 178°C (celecoxib), 167°C (JNJ-2A) and 123°C (cinnarizine), held isothermally for 3 min, cooled to -75°C (10°C/min) and reheated at a rate of 10°C/min to the temperature mentioned above. The sample weights for each repeat were between 2-3 mg.

2.4. Equilibrium drug solubility in lipid-based drug delivery systems

The physico-chemical properties of the three model drugs used in this study are shown in Table 2. All three drugs belong to class II in the Biopharmaceutics Classification System (BCS) having good intestinal permeability, but poor water solubility.

Solubility of the drugs (i.e. celecoxib, cinnarizine and JNJ-2A) in the prototype LBDDS was determined by the shake-flask method. In short, an excess amount of drug was added to 1 mL of each blank LBDDS in vials containing a magnetic stirrer. Formed suspensions were continuously stirred at ambient temperature, 37°C and 60°C for 24 h. The

same experimental design was first pre-tested for the three drugs at 24, 48 and 72 h to determine if saturation solubility may be reached within 24 h. Aliquots of the mixtures were centrifuged at 17500 rpm for 30 min using an Eppendorf centrifuge 5430R (Eppendorf, Hamburg, Germany) at ambient temperature, 37°C and 40°C. The drug concentration in the supernatants was determined using an Acquity Ultra Performance Liquid Chromatography (UPLC™) H-class system (Waters, Milford, USA) consisting of a binary solvent manager, a sample manager and a photodiode array (PDA) detector. The output signal was monitored and processed using the Empower® software version 3.0. A reversed-phase Waters Acquity BEH C18, 50 mm × 2.1 mm; 1.7 µm column (Waters, Milford, USA) was used for the chromatographic analysis with a mobile phase containing a gradient mixture of solvents A (0.1% trifluoracetic acid in water) and B (100% acetonitrile – ACN) in the following A/B proportions: 60/40 for celecoxib and 70/30 for cinnarizine and JNJ-2A. The flow rate of the mobile phase was 0.60 mL/min for celecoxib and cinnarizine and 0.75 mL/min for JNJ-2A and the injection volume was 2 µL. The column temperature was maintained at 55°C and the wavelength was monitored at 251 nm (celecoxib), 253 nm (cinnarizine) and 280 nm (JNJ-2A). The calibration curves for the three drugs were confirmed linear between 5 – 100 µg/mL and samples were diluted accordingly. The solubility experiment was performed in triplicate.

2.5. Formulation of supersaturated LBDDS

The eight LBDDS were loaded with either celecoxib, cinnarizine or JNJ-2A at two elevated temperatures (37 and 60°C) with amounts equal to 85% of the equilibrium solubility determined at 37°C to prepare for an upcoming *in vivo* study and equal to 100% of equilibrium solubility at 60°C to induce more stress to the LBDDS. The required mass of drug was weighed into clean screw-top glass vials and blank LBDDS were added up to the target mass loading. Vials were sealed, mixed and incubated at 37°C and 60°C for 24 h and left to cool at ambient temperature for 2 h prior to analysis. The actual concentration in

sLBDDS was confirmed using the reversed phase UPLC methods described above for all three drugs.

2.5.1. Apparent degree of supersaturation

The apparent degree of supersaturation (aDS) was determined as the ratio of the concentration of the solubilized and molecularly dispersed drug in the supersaturated solution and the concentration in the saturated solution as previously reported by Blaabjerg et *al.* [27]. In this study, aDS was calculated according to equation (1) for the LBDDS loaded with drug at 37 and 60°C.

$$aDS = C_{supersaturation} / S_{equilibrium} \quad (1)$$

where $C_{supersaturation}$ is the concentration of the drug determined after heating the LBDDS (at 37 or 60°C for 24 h) followed by cooling to ambient temperature and $S_{equilibrium}$ is the equilibrium solubility at ambient temperature (as described in section 2.4).

2.6. Physical stability evaluation of supersaturated lipid systems

The physical stability of the LBDDS with different apparent degree of supersaturation was evaluated during storage at 25°C/65%RH for up to 28 days. Visual and analytical assessments at pre-defined timepoints were performed, immediately after drug loading (day 0) and after 1, 4, 7, 14 and 28 days. The samples were kept in plastic Eppendorf tubes for the duration of the stability evaluation.

2.6.1. Polarized Light Microscopy analysis

When formulations were noted to contain visible particles, they were microscopically analysed with a polarized light microscope (Nikon Eclipse CFI60, 4x) to detect changes in homogeneity and precipitate structure. Images were compared to corresponding images taken

of the starting drug material.

2.6.2. X-ray Powder Diffraction

Precipitate formed at the bottom of the test tubes was transferred onto zero background holders and analysed with X-ray powder diffraction (XRPD) from 3° to 50º 2θ. The analysis was carried out on a PANalytical (Philips, Amsterdam, The Netherlands) X'PertPRO MPD diffractometer, equipped with a Cu LFF X-ray tube. Diffractograms were compared to the ones corresponding to the starting drug material.

2.7. Data Analysis

GraphPad Prism Version 7.0 (GraphPad Software, San Diego, CA, USA) was used for all graphs.

3. Results

3.1. Solid-state characterization of model drugs

The crystalline characteristics of the three model drugs used in this study were confirmed by their respective XRD diffractograms (Supporting information, Figure S 1). In addition, pure drug was characterized microscopically under cross-polarized light to determine the shape of the crystalline material (Supporting information, Figure S 2). Differential scanning calorimetry was performed in the heat-cool-heat mode to investigate the effect of thermal treatment on the solid-state and the crystallization tendency of the three drugs. This analysis of undercooled drug melt provided information on glass forming ability (GFA) [28], which may be an early indicator of supersaturation propensity and hence the ability to generate sLBDDS .

The first heating cycle confirmed the melting point of the three drugs (T_m) (Table 3). In the second cycle, the drugs were cooled at a rate of 10°C/min with the purpose of

generating amorphous drug substance, i.e. to a macroscopic solid phase with no crystalline structure. Celecoxib crystallized during the cooling phase at a $T_{cryst,cool}$ of 138°C. No crystallization events were observed for the two other drugs. In the re-heating cycle, a glass transition ($T_{g,mid}$) of 8.5 °C was observed for cinnarizine and 91.2°C for JNJ-2A confirming formation of the amorphous material. On further heating, celecoxib and cinnarizine displayed endothermic peaks, while no endothermic or exothermic peaks were observed for JNJ-2A showing that the drug did not tend to recrystallize, and therefore indicative of the formation of an amorphous state. The entropy of fusion (ΔS_{fus}) and T_m/T_g ratio were used as indicators of crystallization tendency of the three drugs as illustrated previously by Baird and co-workers [28] and Fridgeirsdottir and co-workers [29]. JNJ-2A had a lower T_m/T_g ratio than the other model drugs suggesting lower crystallization tendency, which was also reflected in the lower value of the entropy of fusion. The experimentally determined values were for JNJ-2A were similar to internal J&J data and similarly data for cinnarizine and celecoxib (Supporting information, Table S 2) were in good agreement regarding enthalpy of fusion, entropy of fusion, and melting temperature [28].

3.2. Macroscopic and microscopic assessment of drug-free and drug-loaded sLBDDS

All selected lipid excipients displayed good miscibility at the selected ratio (i.e. lipid:surfactant = 4:1). In order to assess the suitability of the chosen LBDDS, a macroscopic and microscopic assessment was conducted in both drug-free and drug-loaded systems stored over 28 days at 25°C/65%RH. All drug-free LBDDS were found to be stable, with no phase separation or layering observed. Drug-free LBDDS did not display any traces of material resembling the three drugs when analysed microscopically under polarized light (data not shown) or after XRD analysis (Supporting information, Figure S 3).

Macroscopic evaluation of drug-loaded sLBDDS revealed clear and optically transparent solutions after 24h stirring at either 37 or 60°C with the exception of three LBDDS containing JNJ-2A (i.e. LCM+LCT+S, LCM+MCT+S and MCM+LCT+S) which displayed phase separation (Supporting information, Figure S 4). Due to this instability, it was methodologically challenging to determine accurate drug concentration for these three mixed excipient systems. Further characterisation studies on these three unstable systems with JNJ-2A were therefore not warranted and thus not continued.

3.3. Equilibrium drug solubility in LBDDS at ambient temperature

The equilibrium solubility of the three investigated drugs at ambient temperature in the tested LBDDS is shown in Figure 1.

Similar to previously reported studies, drug solubility of all three drugs was higher in MC compared to LC lipid vehicles [30]. Drug solubility was increased by inclusion of a surfactant in the LBDDS. For celecoxib, increases of between 4.5 – 6.3-fold were observed in the LCM lipid systems containing surfactant (LCM+S, LCM+LCT+S, LCM+MCT+S) compared to the surfactant-free LCM system, whereas in the MCM lipid systems (MCM+S, MCM+MCT+S, MCM+LCT+S) the increases were lower (1.6 – 2.0-fold) compared to the MCM system. In contrast, for cinnarizine, the drug solubility across the eight classes of LBDDS were broadly similar, suggesting a relatively low impact of formulation complexity on dose loading for this drug. A high increase in solubility (4.4-fold) was observed when a hydrophilic surfactant was added to the LCM system for JNJ-2A, whereas the drug solubility in MCM systems was similar across the stable LBDDS independent of surfactant inclusion. In general, a similar rank order of solubilisation capacity across the various LBDDS was similar for the three drugs. For celecoxib the following rank order of mean solubility was observed: MCM+S > MCM+MCT+S > LCM+MCT+S > MCM+LCT+S > LCM+S > LCM+LCT+S > MCM > LCM; for cinnarizine: MCM+MCT+S > MCM+S > MCM > LCM+MCT+S >

MCM+LCT+S > LCM+S = LCM > LCM+LCT+S and for JNJ-2A: MCM+S > MCM > MCM+MCT+S > LCM+S > LCM. All three rank orders confirm a higher solubilisation capacity of LBDDS based on MC mono-/di-glycerides and a poor solubilisation capacity of the single LC lipid component (i.e. LCM).

3.4. Influence of lipid composition type on drug solubility in LBDDS at elevated temperature

The solubility of celecoxib, cinnarizine and JNJ-2A in LBDDS on heating at 37 and 60°C, was determined in order to evaluate the influence of lipid composition type on drug solubility in LBDDS at elevated temperatures (Table 4).

For celecoxib, on heating to 37°C solubility in the LBDDS increased between 16.4 and 39.6% across the LBDDS, relative to solubility at ambient temperature. Similar to solubility trends at ambient temperature, the lowest solubility was observed in the LCM system (18.6 ± 0.3 mg/mL) and the highest solubility was obtained in MCM+S (138.5 ± 16.5 mg/mL). Solubility of cinnarizine at 37°C was similar across the eight tested LBDDS with increases of 27.5 to 88.9% relative to ambient temperature solubility and ranging from the lowest of 37.3 ± 1.2 mg/mL in LCM+LCT+S to the highest solubilized amount of 55.3 ± 2.5 mg/mL in the LCM system. In the case of JNJ-2A increases between 9.3 and 52.9% were observed compared to ambient temperature solubility, with the LCM system also showing the lowest solvent capacity for (55.3 ± 0.9 mg/mL) and the highest observed solubility observed for MCM+S (461.4 ± 11.9 mg/mL).

Solubility determinations at 60°C indicated that dose loading of celecoxib was increased by 71.8 to 172.8% in the tested LBDDS compared to ambient temperature solubility with the lowest solubilized dose in LCM (36.2 ± 2.8 mg/mL) and the highest in MCM+S (258.5 ± 51.2 mg/mL). The dose loading of cinnarizine increased between 55.0 and 196.0% in the tested LBDDS at 60°C compared to ambient temperature, with the lowest dose solubilized

in LCM+LCT+S (42.2 ± 0.3 mg/mL) and the highest in the corresponding MC systems, i.e. MCM+MCT+S (89.9 ± 8.7 mg/mL). For JNJ-2A, at 60°C, the solubilisation capacity of the tested LBDDS increased between 3.6 and 87.7% with the lowest solubilized dose observed for LCM (76.5 ± 2.9 mg/mL) and the highest for MCM+MCT+S (507.1 ± 28.4 mg/mL). Interestingly, in the case of MCM and MCM+S systems the solubility at 60°C was quantitatively lower than the solubility at 37°C, suggesting that no further gains in dose loading could be achieved at the higher temperature.

To get a better understanding of the solubility dependence on temperature in different LBDDS, the logarithmic drug solubility (expressed as logS) was plotted as a function of the tested temperature (Figure 2). A linear relationship was observed between celecoxib solubility with temperature increase for tested LBDDS and similarly in the case of cinnarizine, logS increased linearly with temperature in seven of the tested LBDDS, with the exception of LCM+LCT+S. For JNJ-2A, three systems, i.e. LCM, LCM+S, MCM+MCT+S, from the five tested, showed a linear dependence of LogS with increasing temperature, whereas for two systems (i.e. MCM, MCM+S), solubility decreased on increasing temperature from 37°C to 60°C.

3.5. Physical stability of supersaturated LBDDS

3.5.1. Celecoxib sLBDDS

Results for celecoxib are shown in Table 5 together with apparent degree of supersaturation. Over the 28-day period, at 25°C/65%RH, celecoxib sLBDDS formulated at 37°C were physically stable with no macroscopic changes in homogeneity, as concluded after microscopic and analytical evaluation (data not shown).

When formulating the sLBDDS by heating to 60°C and storage at 25°C/65%RH, higher overall degrees of supersaturation (aDS) were generated compared to sLBDDS

formulated at 37°C; however, the physical stability of these celecoxib LBDDS was adversely affected. Precipitation of celecoxib was observed within 14 days (Table 5), producing fine needle crystals, in seven of the eight tested systems as indicated by PLM and XRD analyses. The aDS appeared to have a substantial effect on the physical stability of sLBDDS. The LCM+S system was the only system where physical stability extended beyond 28 days, which most likely reflects that this system had the lowest aDS of 1.35, whereby for the sLBDDS with an aDS of ≥1.57, drug precipitation was observed. The mixed MC sLBDDS MCM+S, MCM+MCT+S, MCM+LCT+S were the least stable systems, relative to comparable LC sLBDDS. The precipitate from all sLBDDS was analysed by PLM and XRD and was confirmed to be crystalline and having similar shape and structure as the starting drug material (XRD diffractograms shown in Supporting information, Figure S 5.

3.5.2. Cinnarizine sLBDDS

For all cinnarizine sLBDDS precipitation was observed within 28 days (Table 6 and Supporting information, Figure S 6 and Figure S 7). While there was a trend towards poorer stability for sLBDDS prepared at 60°C, compared to sLBDDS prepared at 37°C, even at relatively low aDS (e.g. 1.1 for MCM+S) supersaturation was only maintained for up to 7 days on storage. The excipient type did not seem to affect the physical stability greatly, but rather an inherent poor stability of cinnarizine was observed in all sLBDDS independent of lipid composition.

3.5.3. JNJ-2A sLBDDS

For JNJ-2A, the aDS at both 37°C and 60°C were generally lower, compared to both celecoxib and cinnarizine, indicating that the propensity to generate supersaturation for JNJ-2A in LBDDS was lower. In fact, at 37°C, effectively no supersaturation was achieved (i.e. aDS ≤ 1.04) in the sLBDDS, which was in line with the relatively minor increases in

equilibrium solubility observed at 37°C (Table 4). At the higher processing temperature of 60°C, supersaturation was generated in four of the tested LBDDS (aDS 1.12-1.47), and supersaturation was maintained for all of these systems during the 28 days period, with no evidence of drug precipitation (Table 7).

4. Discussion

In the field of supersaturated LBDDS, a gap was identified in the pre-formulation of such drug delivery systems and thus there was a need to systematically assess the influence of excipient type, formulation complexity and drug physico-chemical properties on the ability to design sLBDDS. The current study has shown that lipid composition highly influences the dose loading ability and that the degree of supersaturation in sLBDDS was mainly drug-dependent. The apparent degree of supersaturation was a major factor impacting ability to maintain drug supersaturation upon storage.

A plot of logarithmic drug solubility versus temperature was useful for assessing the impact of temperature on drug solubility in lipids. The increases in solubility did not deviate greatly from linearity, indicating that the increased dose loadings in LBDDS at elevated temperature could be predicted based on extrapolation. While all three drugs displayed increased drug concentrations at higher temperature, the lines connecting the different data points for a lipid system were superimposable between the various LBDDS, indicative of a minor impact of composition type on propensity for supersaturation. In contrast, the lines of the thermal induced solubility increases were highly drug-dependent. Specifically, celecoxib and cinnarizine showed steep increases in solubility with temperature (i.e. up to 172.8% and 196.0% more drug solubilized relative to solubility at ambient temperature), which may be a consequence of the smaller size and more rigid/compact shape of the molecules. In contrast, JNJ-2A, which displayed the highest equilibrium solubility in lipids at ambient temperature possibly as a consequence of a more flexible structure, with a larger number of H-bond

donors, exhibited a relatively low overall thermal induced solubility increase in LBDDS (up to 87.7% more drug solubilized compared to ambient temperature drug solubility). To the best of our knowledge this was the first time that apparent supersaturation in LBDDS was studied more systematically for different temperatures and compositions. This approach was therefore a useful systematic approach in early development of supersaturated LBDDS to aid the identification of an optimised high dose load sLBDDS.

In this work, the apparent degree of supersaturation (aDS) was used as a measurement of drug supersaturation in different LBDDS and thus an indicator of the likelihood of designing sLBDDS -. Using celecoxib, cinnarazine and JNJ—2A as model drugs, supersaturated LBDDS were successfully prepared (aDS ≥ 1.1) after a mild heating at 37°C of seven LBDDS containing celecoxib (aDS = 0.92 – 1.36) and seven containing cinnarizine (aDS = 1.02 – 1.38). In the case of JNJ-2A it was not possible to generate sLBDDS at 37°C (aDS = 0.79 – 1.04). At a higher temperature of 60°C, sLBDDS were successfully prepared for all tested LBDDS containing celecoxib (aDS = 1.35 – 2.97) and cinnarizine (aDS = 1.33 – 3.33). Supersaturation of JNJ-2A using 60°C heating (aDS > 1.1) was only feasible in four LBDDS: LCM (aDS = 1.38), LCM+S (aDS = 1.12), MCM+S (aDS = 1.12) and MCM+MCT+S (aDS = 1.47). The relatively high solubility values obtained for JNJ-2A in LBDDS at ambient temperature and the lack of solubility increase with temperature elevation resulted in a lower degree of supersaturation obtainable using the suggested heating-cooling methodology. The effect may be explained by the solid-state characteristics of this drug (i.e. low crystallinity), which resulted in high solvation in lipid vehicles at ambient temperature, leaving little room for a solubilization gain from temperature elevation. A tabulated representation of the composition influence on the aDS after heating at 60°C and cooling at ambient temperature is illustrated in Table 8 as a summary for the keen reader.

The ability to maintain supersaturation upon storage was assessed in this study by monitoring the time to precipitate. Crystalline drug precipitate was observed with the 28-day

period in all cinnarizine sLBDDS (aDS of 1.02-– 3.33) and celecoxib sLBDDS with aDS >1.35, whereas no visible precipitate was observed for JNJ-2A in any of the sLBDDS (aDS = 0.88 – 1.47). The lipid composition type had a relatively minor impact on the risk of precipitation during storage; however, it appeared that the aDS value was a major determinant of the risk of drug precipitation from sLBDDS containing celecoxib and cinnarizine. A higher aDS, such as achieved upon heating drug and LBDDS mixtures to 60°C, tended to result in faster precipitation compared to LBDDS heated at 37°C for celecoxib and cinnarizine. Nonetheless, the risk of precipitation was also highly dependent on the drug, whereby in the case of celecoxib and JNJ-2A, sLBDDS with aDS up to 1.35 and 1.47 respectively, were stable over the 28 days study period. In contrast, for cinnarizine, precipitation was evident in all sLBDDS, even at relatively low aDS, which would indicate an intrinsically poor physical stability in sLBDDS. An inherently poor stability of cinnarizine in drug delivery systems containing lipids has been previously reported [16]. Poor physical stability of a liquid cinnarizine SNEDDS containing either MC or LC lipid excipients was reported by Shabba and co-workers [31]. Similarly, Siqueira and co-workers, reported poor physical stability and a tendency for precipitation of cinnarizine from supersaturated SNEDDS [16]. Considering all the above, it would appear that for certain PWSDs such as cinnarizine, caution is advised when such drugs have an inherently poor stability in lipid systems. Alternatively strategies to improve stability of the supersaturated formulations, such as solid-state formulations may be merited. For example Schultz and co-workers have reported an aproach to overcome instablity of sLBDDS of ibuprofen, using silica-lipid hybrids, where nanopores of porous silica microparticles inhibit the precipitation of the IBU and produce a solid-state LBDDS [32]. A focused representation of the LBDDS composition on the physical stability of sLBDDS formulated at 60°C is depicted in Table 9 and shows a general positive influence of additional lipid excipients or surfactant to the LCM excipient and a negative influence of such additions for the MCM-based LBDDS, especially for celecoxib and cinnarizine.

This study was also designed to evaluate the influence of lipid composition type on drug solubility in LBDDS at different temperature and thus on the ability to design supersaturated LBDDS using the heating-cooling approach. Drug solubility was higher in MC versus LC lipids at all tested temperatures. This was particularly the case for celecoxib and JNJ-2A, and to a lesser extent cinnarizine. Similarly, a greater effect on the solubility gain by incorporation of a hydrophilic surfactant was observed for celecoxib and JNJ-2A and had a limited influence on cinnarizine solubility. Lipid composition type influenced the equilibrium solubility, which in turn dictates drug loading in sLBDDS. In this study, it appeared that the supersaturation propensity for celecoxib and cinnarizine was higher in single-component systems (LCM, MCM) compared to the more complex LBDDS. Additionally, for both model drugs higher aDS were determined in the LCM system compared to MCM. Across the LBDDS with more than one component, slightly higher aDS were seen for celecoxib in MC sLBDDS and for cinnarizine in LC sLBDDS. This observation could be potentially explained by the logP values of the two drugs, where a lower value (i.e. 4.3) for celecoxib would indicate preference for more polar mixtures of lipid excipients (i.e. medium chain [8,33,34] and a higher value (i.e. 5.7) would suggest an affinity for more lipophilic mixtures of lipid excipients (i.e. long chain). No clear LC versus MC influence on supersaturation propensity was seen for JNJ-2A, however, results indicate that simple LBDDS (i.e. LCM, LCM+S, MCM+S), as in the case of the other two model drugs, had the ability to maintain drug supersaturation.

This work targeted a better understanding of the relevance of physico-chemical properties on the supersaturation propensity in LBDDS. The rank order of the supersaturation propensity of the three drugs was celecoxib ≥ cinnarizine > JNJ-2A based on the increases in drug solubility with elevation of temperature, i.e. 1.8 – 2.7-fold for celecoxib, 1.6 – 3.0-fold for cinnarizine and 1.0 – 1.9-fold for JNJ-2A. The more rigid structures with lower molecular weight and lower glass transition temperature of celecoxib and cinnarizine compared to JNJ-

2A may explain this ranking order and the clear influence of elevated temperature on drug solubility. Generally, the PWSDs investigated in super-SNEDDS were either weak bases (halofantrine [14,15], cinnarizine [16]) or neutral drugs (simvastatin [13], fenofibrate [24]). The present study was to our knowledge the first one to incorporate a weak acid (celecoxib) in comparison to a weak base (cinnarizine) and a neutral drug (JNJ-2A) in evalution of drug supersaturation in LBDDS. Interestingly, celecoxib showed the best potential for thermally-induced drug supersaturation in the tested LBDDS with suitable physical stability for preclinical trials. However, due to the rather limited number of drugs overall, it is difficult to extract general conclusions at this point based on the acid-base-neutral characteristics. The thermal characteristics, such as T_m/T_g ratio, correlated well with previously published observations that a high T_m/T_g ratio implies a higher crystallization tendency and a more unstable amorphous form, which resulted in the re-crystallization and precipitation of celecoxib and cinnarizine from sLBDDS. In a study by Baird and co-workers, a classification systems was suggested based on the crystallization tendency and GFA of 51 organic molecules using the heat-cool-heat mode of the DSC [28]. A similar protocol was used in this study to potentially explain the supersaturation propensity of the three model drugs in LBDDS. The present work was in line with the proposed crystallisation classification for cinnarizine as a drug which re-crystallizes above T_g upon re-heating (i.e. class II drug). Celecoxib displays crystallization upon cooling at a cooling rate of 10°C/min and thus can be considered a class I drug which is different from the classification made by Baird et *al*. Nevertheless, in the Baird et *al*. study re-crystallization of celecoxib upon cooling at a slow rate of 1°C/min was reported, which may indicate that the crystallization behaviour of celecoxib could be reflective of batch to batch variability for which class II drugs are more susceptible [28]. JNJ-2A does not crystallize upon cooling or reheating, and therefore is believed to remain in an amorphous form (i.e. a class III drug) with a lower tendency to re-crystallize from the undercooled melt, which was surprising regarding the comparatively low

entropy of fusion. The observable crystallization tendency of celecoxib and cinnarizine correlates well with the faster time to precipitate from sLBDDS upon storage. GFA of PWSDs was correlated previously with the supersaturation propensity of drugs upon aqueous dispersion in a study by Blaabjerg et *al*. [27]. In the present study, the poor glass forming drugs (i.e. celecoxib and cinnarizine) generated high degrees of supersaturation on heating in all tested LBDDS with the drawback of drug precipitation upon storage, while much lower aDS were determined for a drug with high glass forming ability (i.e. JNJ-2A), yet without any drug precipitation. Therefore, the crystallization behaviour of drugs may potentially explain the time to precipitate upon storage from sLBDDS, while properties such as high glass forming ability (i.e. class III drugs) and strong drug-lipid interactions and their influence on the drug solubility in lipids may be relevant factors for designing precipitation risk-free sLBDDS as seen for JNJ-2A.

5. Conclusions

This work provided a pre-formulation screening for assessment of composition influence and of drug physico-chemical properties on the design and short-term stability of sLBDDS. - The study demonstrated that drug loadings of between 130-150% could be successfully achieved in sLBDDS with associated stability in excess of 28 days. Even higher dose loadings in sLBDDS were achieved (i.e. up to 300% relative to LBDDS,) albeit the risk of precipitation on storage increased at higher aDS. The study therefore supports the utility of sLBDDS in bio-enabling strategy for PWSD candidates in preclinical toxicology studies where high doses are required and short-term formulation stability i.e. 1-28 days is considered sufficient. The solid-state properties of the drugs were useful in predicting the risk of precipitation on storage from sLBDDS, while properties such as high glass forming ability (i.e. class III drugs) and good lipid solubility were considered favourable for formulation as sLBDDS. Further studies are required involving a wider range of drugs to allow a more thorough understanding of the impact of drug properties on design and performance of sLBDDS. Additionally, *in vitro* dissolution and *in vivo* evaluations may be useful for identifying the performance of such delivery systems.

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Table 1. Composition of two groups of lipid-based drug delivery systems containing single lipid components (LCM, MCM) and mixtures of 2-3 lipid excipients (LCM+S, MCM+S, LCM+LCT+S, MCM+LCT+S, LCM+MCT+S, MCM+LCT+S)

Group	Abbreviation	Excipients	Composition (w/w, %)
LCM-based	LCM	Maisine CC	100
	LCM+S	Maisine CC + Labrasol	80 + 20
	LCM+LCT+S	Maisine CC + Sesame oil + Labrasol	40 + 40 + 20
	LCM+MCT+S	Maisine CC + Labrafac + Labrasol	40 + 40 + 20
MCM-based	MCM	Capmul MCM	100
	MCM+S	Capmul MCM + Labrasol	80 + 20
	MCM+MCT+S	Capmul MCM + Labrafac + Labrasol	40 + 40 + 20
	MCM+LCT+S	Capmul MCM + Sesame oil + Labrasol	40 + 40 + 20

Table 2. Physico-chemical properties of poorly water-soluble drugs

Drug	**Celecoxib**	**Cinnarizine**	**JNJ-2A[c]**
BCS class	II	II	II
Solubility in water (µg/ml)	3.3[a]	1.7[a]	< 0.2
MW (g/mol)	381.4	368.5	498.9
Melting point (°C)	163	121	142
clogP	4.3	5.7	5.4
pKa	-0.42[a] ;10.70[a]	1.95[b]; 7.40[b]	2.02 ;12.12

Molecular weight (MW), melting point and logP for celecoxib, cinnarizine from Baird et al.[28]
[a] https://www.drugbank.ca/drugs/DB00568
[b] pKa values for cinnarizine from Larsen et al.[35]
[c] Physico-chemical properties of JNJ-2A are results of in-house analysis

Table 3. Enthalpy of fusion (ΔH_{fus}), melting peak temperature (T_m), entropy of fusion (ΔS_{fus}), T_m/T_g ratio, crystallization peak temperature during cooling ($T_{cryst,cool}$), onset and midpoint glass transition temperatures ($T_{g,onset}$, $T_{g,mid}$), and crystallization peak upon heating ($T_{cryst,heat}$) for celecoxib, cinnarizine and JNJ-2A after heat-cool-heat cycle above melting point

Drug	**ΔH_{fus} (kJ/mol)**	**T_m (°C)**	**ΔS_{fus} x 10^{-2} (kJ/mol/K) ***	**T_m/T_g ^**	**Cooling**	**Glass transition**		**Re-heating**
					$T_{cryst,cool}$ (°C)	$T_{g,onset}$ (°C)	$T_{g,mid}$ (°C)	$T_{cryst,heat}$ (°C)
Celecoxib	34.1	162.4	7.8	1.32	138.0	N/A	N/A	160.5
Cinnarizine	37.5	121.4	9.5	1.41	N/A	7.2	8.5	82.5
JNJ-2A	22.9	140.2	5.5	1.14	N/A	89.3	91.2	N/A

*Entropy of fusion was calculated as the ratio of enthalpy of fusion (kJ/mol) and melting peak temperature (Kelvin), where T_m(K) is 435.55K (celecoxib), 394.55K (cinnarizine) and 413.35K (JNJ-2A)
^Mid T_g (Kelvin) used for calculation of T_m/T_g was 331.15 K (celecoxib) according to Baird et al. [28] and 280.35 K (cinnarizine) and 362.45 K (JNJ-2A) as determined in this study.

Table 4. Solubility values (mean ± SD) at ambient temperature (AT), 37°C and 60°C for celecoxib, cinnarizine and JNJ-2A in eight LBDDS

	Celecoxib			Cinnarizine			JNJ-2A		
Lipid system	AT	37°C	60°C	AT	37°C	60°C	AT	37°C	60°C
LCM	13.3±1.1	18.57±0.34	36.2±2.8	29.3±1.0	55.3±2.5	86.6±6.5	47.5±1.2	54.96±0.94	76.5±2.9
LCM + S	61.4±4.1	73.2±6.4	116±19	29.3±1.0	43.83±0.73	66.4±4.6	207.6±3.3	227±12	278.1±2.3
LCM + LCT + S	60.8±3.8	83.5±6.9	118±19	27.27±0.73	37.3±1.2	42.41±0.31	n.d.	n.d.	n.d.
LCM + MCT + S	83.4±6.8	97.1±3.0	143.3±8.2	34.24±0.83	46.7±1.6	67.2±4.0	n.d.	n.d.	n.d.
MCM	49.8±5.6	67.5±2.4	88.1±6.7	36.0±1.0	48.6±3.3	81.7±4.0	283±16	348±12	293±28
MCM + S	102±16	138±16	258±51	36.36±0.53	46.4±1.6	64.5±1.2	302±10	461±12	423.9±5.3
MCM + MCT + S	93.1±3.6	121±15	254±37	38.16±0.93	49.5±1.6	89.9±8.7	270.2±7.2	365±14	507±28
MCM + LCT + S	81.8±2.1	98.0±1.7	176±27	31.7±1.2	48.4±2.3	84.1±2.3	n.d.	n.d.	n.d.

n.d = not determined due to clear phase separation

Table 5. Apparent degree of supersaturation and physical stability evaluation for investigated supersaturated celecoxib lipid-based drug delivery systems at 25°C/65%RH for 28 days

	Celecoxib			
Lipid-based system	$aDS_{(37)}$	Stability (days)	$aDS_{(60)}$	Stability (days)
LCM	1.14	>28	2.97	<1
LCM+S	1.13	>28	1.35	>28
LCM+LCT+S	1.36	>28	1.73	7 - 14
LCM+MCT+S	1.13	>28	1.57	4 - 7
MCM	1.31	>28	2.04	1 - 4
MCM+S	0.92	>28	1.72	<1
MCM+MCT+S	1.23	>28	1.81	<1
MCM+LCT+S	1.33	>28	2.23	<1

Table 6. Apparent degree of supersaturation and physical stability evaluation for investigated supersaturated cinnarizine lipid-based drug delivery systems at 25°C/65%RH for 28 days

	Cinnarizine			
Lipid-based system	$aDS_{(37)}$	Stability (days)	$aDS_{(60)}$	Stability (days)
LCM	1.23	14 - 28	3.33	<1
LCM+S	1.27	14 - 28	2.55	1 - 4
LCM+LCT+S	1.19	14 - 28	1.33	1 - 4
LCM+MCT+S	1.02	7 - 14	2.63	7 - 14
MCM	1.24	1- 4	2.78	7 - 14
MCM+S	1.10	7 - 14	2.16	4 - 7
MCM+MCT+S	1.13	7 - 14	2.09	<1
MCM+LCT+S	1.38	7 - 14	2.42	4 - 7

Table 7. Apparent supersaturation degrees and physical stability evaluation for investigated supersaturated JNJ-2A lipid-based drug delivery systems at 25°C/65%RH for 28 days

	JNJ-2A			
Lipid-based system	$aDS_{(37)}$	Stability (days)	$aDS_{(60)}$	Stability (days)
LCM	0.94	>28	1.38	>28
LCM+S	0.79	>28	1.12	>28
LCM+LCT+S	n.d.	unstable	n.d.	unstable
LCM+MCT+S	n.d.	unstable	n.d.	unstable
MCM	0.92	>28	0.92	>28
MCM+S	0.87	>28	1.12	>28
MCM+MCT+S	1.04	>28	1.47	>28
MCM+LCT+S	n.d.	unstable	n.d.	unstable

Table 8. Schematic representation of findings on the influence of LBDDS composition on the apparent degree of supersaturation of celecoxib, cinnarizine and JNJ-2A

	Drug	**Single excipient**	**LCM-based**			**MCM-based**		
			+surfactant	+same FA length TGs	+different FA length TGs	+surfactant	+same FA length TGs	+different FA length TGs
$aDS_{(60)}$	Celecoxib	LCM>MCM	decrease	decrease	decrease	decrease	decrease	increase
	Cinnarizine	LCM>MCM	decrease	decrease	decrease	decrease	decrease	decrease
	JNJ-2A	LCM>MCM	decrease	n.a.	n.a.	increase	increase	n.a.

Table 9. Schematic representation of findings on the influence of LBDDS composition on the physical stability of supersaturated LBDDS containing celecoxib, cinnarizine and JNJ-2A

	Drug	**Single excipient**	**LCM-based**			**MCM-based**		
			+surfactant	+same FA length TGs	+different FA length TGs	+surfactant	+same FA length TGs	+different FA length TGs
Stability sLBDDS (heating at 60°C)	Celecoxib	MCM>LCM	increase	increase	increase	decrease	decrease	increase
	Cinnarizine	MCM>LCM	increase	increase	increase	decrease	decrease	decrease
	JNJ-2A	MCM=LCM	no effect	phase separation	phase separation	no effect	no effect	phase separation

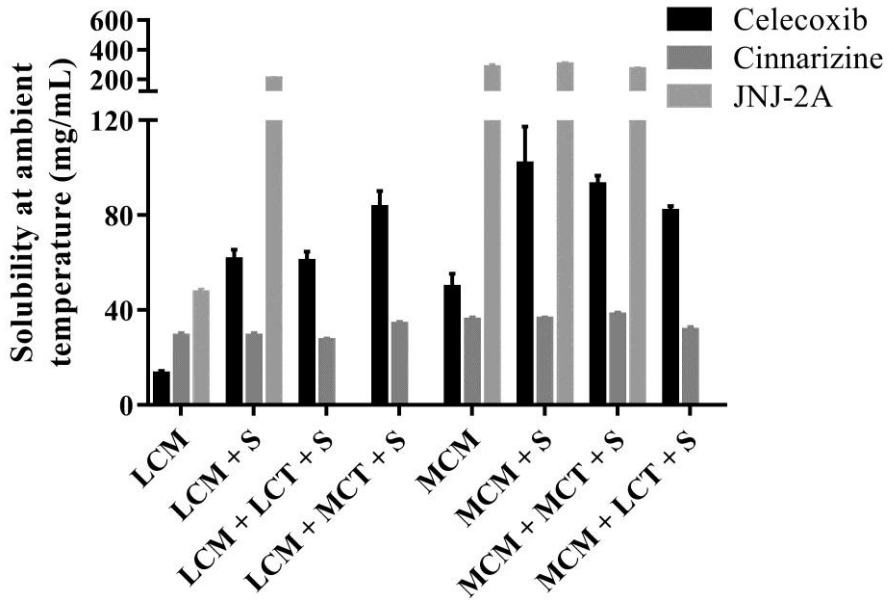

Figure 1. Solubility (mg/mL) of celecoxib (black bars), cinnarizine (dark grey bars) and JNJ-2A (light grey bars) in lipid -based drug delivery systems (mean ± SD) at ambient temperature (n=3).

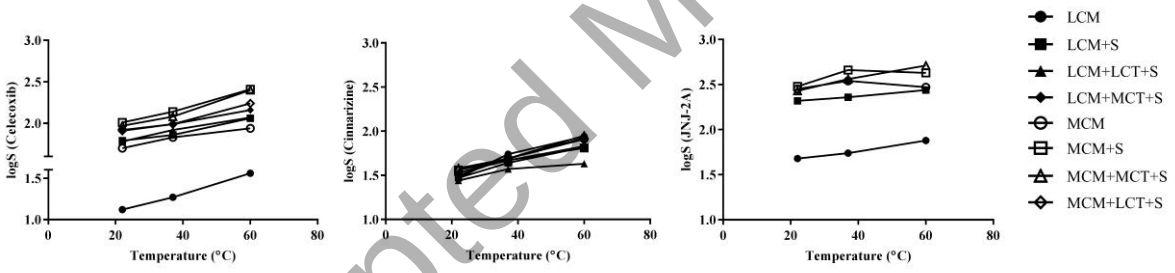

Figure 2. Plots of logS (log of drug solubility at ambient temperature, 37°C and 60°C) of drugs used in the study (celecoxib, cinnarizine and JNJ-2A) as a function of temperature. Lines are depicted as guides to the eye.