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Towards simplified oral lipid-based drug delivery using mono-/di-glycerides as single component excipients

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Towards simplified oral lipid-based drug delivery using mono-/di-glycerides as single component excipients

Objective

This study aimed to systematically explore compositional effects for a series of lipid systems, on the *in vitro* drug solubilization and *in vivo* bioavailability of three poorly water-soluble drugs with different physico-chemical properties.

Significance

While many lipid-based drug products have successfully reached the market, there is still a level of uncertainty on the design guidelines for such drug products with limited understanding on the influence of composition on *in vitro* and *in vivo* performance.

Methods & Results

Lipid-based drug delivery systems were prepared using either single excipient systems based on partially digested triglycerides (i.e. mono- and/or di-glycerides) or increasingly complex systems by incorporating surfactants and/or triglycerides. These lipid systems were evaluated for both *in vitro* and *in vivo* behaviour. Results indicated that simple single component long chain lipid systems are more beneficial for the absorption of the weak acid celecoxib and the weak base cinnarizine compared to equivalent single component medium chain lipid systems. Similarly, a two-component system produced by incorporating small amount of hydrophilic surfactant yields similar overall pharmacokinetic effects. The lipid drug delivery systems based on medium chain lipid excipients improved the *in vivo* exposure of the neutral drug JNJ-2A. The higher *in vivo* bioavailability of long chain lipid systems compared to medium chain lipid systems was in agreement with *in vitro* dilution and dispersion studies for celecoxib and cinnarizine.

Conclusions

The present study demonstrated the benefits of using mono-/di-glycerides as single component excipients in LBDDS to streamline formulation screening and improve oral bioavailability for the three tested poorly water-soluble drugs.

Keywords; Lipid-based drug delivery systems, Long versus medium chain lipid excipients, Biorelevant media, Dilution and dispersion testing, *In vivo* pharmacokinetics

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1. Introduction

Lipid-based drug delivery systems (LBDDS) have been widely explored to overcome pharmaceutical developability challenges for poorly water-soluble drugs (PWSD) [1-3]. Typical consequences of low solubility in the aqueous gastrointestinal environment upon oral administration are poor absorption and food dependent bioavailability, thus increased variability in therapeutic responses [4,5]. Formulation of a PWSD in a LBDDS confers numerous biopharmaceutical advantages. These include increased drug solubility, but co-administration of lipid excipients also stimulates a number of physiological events promoted by food, resulting in increases in endogenous biliary lipid concentrations in the intestine (i.e. bile salts, phospholipids and cholesterol), leading to greater drug solubilization in the intestinal mixed micellar milieu [6,7]. The ability to (a) enhance drug solubility on dispersion in intestinal fluid and (b) maintain drug solubilisation within the gastrointestinal tract (GIT) are considered key bio-enabling characteristics for LBDDS [2,3].

Despite numerous commercially available lipid-based drug products and significant pre-clinical research in the field [8], there is still a reticence in many industry sectors towards advancing LBDDS as a lead formulation for use in clinical trials and/or commercially licensed drug products. Possible reasons for this may include a lack of clear guidance on formulation design of LBDDS, limited comprehensive understanding regarding compositional influence on *in vivo* behaviour and a lack of predictive *in vitro*

models for assessment of drug absorption from lipid systems [4]. Typically, the rationale underpinning choice of LBDDS is highly influenced by the organisation's expertise, as well as availability of scientific knowledge and manufacturing platforms within the company. While recent approaches to publish improved industry guidance

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maps and decision trees are welcome, many questions on composition influence remain unclear [1,5]. The main objective of this study was to identify approaches that streamline formulation screening and allow the potential benefits of lipid excipients to be identified as early as possible in an industrial drug development setting.

While there has been considerable focus in the scientific literature on developing compositionally complex LBDDS, with multiple excipients including oils, surfactants, co-surfactants, and co-solvents (commonly referred to as LFCS type III and IV according to Lipid Formulation Classification System (LFCS) [9]), it is interesting to contrast this with the findings of two separate reviews of commercially available LBDDS which indicated the predominance of single component marketed LBDDS [6,8]. While the scientific rationale supporting the trend towards Type III/IV systems relates to advantages in terms of improving drug solubility and *in vitro* dispersibility with 'digestion-independent' systems, clearly, from an industrial development perspective, there is a preference for simple LBDDS compositions in terms of streamlining excipient screening and lowering the regulatory burden. In this study, the biopharmaceutical benefits of partially digested triglycerides (i.e. mono- and/or di-glycerides) were investigated as single excipient LBDDS or mixed in more complex LBDDS. This was, to the best of our knowledge, the first study to investigate such simple systems based on partially digested triglycerides from both *in vitro* and *in vivo* perspectives.

Lipid excipients containing partially digested glycerides are generally lipolytic products of triglycerides (i.e. mono- and di-glycerides) which have been reported to display higher solubilization capacities and improved dispersibility properties relative to triglycerides [10]. Due to their amphiphilic surfactant-like properties, monoglycerides are reported to form more stable emulsions [10]. Additionally, monoglycerides are

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endogenously produced upon digestion of triglycerides, and together with fatty acids, phospholipids, bile salts and cholesterol constitute the pre-absorptive colloidal solubilizing environment in the GIT. These highly dispersed colloids are efficient in the transport of lipophilic compounds across the unstirred water layer (UWL) to the absorptive surface of the intestine [3]. The use of blends of partially digested glycerides and further addition of hydrophilic surfactants is increasingly popular among LBDDS (classified as Type IIIA according to LFCS) as they exhibit less dependence on digestion in comparison to type I pure oil systems and upon dispersion and digestion these result in moderate degrees of drug supersaturation, with a lower precipitation risk.

The *in vitro* and *in vivo* behaviour of LBDDS vary considerably with the chain length of the lipid components. Previous studies have reported higher bioavailability when administered in LBDDS containing long chain (LC) instead of medium chain (MC) triglycerides for drugs including cyclosporine, probucol, vitamin D3, dicoumarol, danazol [11,12]. In contrast, the *in vivo* performance of vitamin E, SL-512, progesterone, penclomedine, acetylsulfisoxazole and griseofulvin was improved after administration in MC lipids when compared to LC lipids [11,12]. There are also reports where lipid chain length had no influence on performance of drugs such as dexamethasone, seocacitol, an investigational new drug candidate (CDA) and anethole trithione [11,13,14].

To date, numerous studies have attempted to develop *in vitro* screening approaches to predict *in vivo* relations (IVIVR) of LBDDS, ranging from simple dilution/dispersion tests to more complex lipolysis models and, more recently digestion-permeation models aimed to simulate *in vivo* scenarios [15,16]. While many of the more complex *in vitro* tools provide useful mechanistic insights to the IVIVR for LBDDS, there is a need in early phase industrial development for *in vitro* techniques that support

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high throughput screening (HTS) and provide developability guidance (or indeed risks thereof) in shorter timeframes (i.e. hours/days). Therefore, the aims of this study were: (1) to systematically investigate the influence of lipid components in LBDDS on *in vivo* behaviour of three PWSD with different physico-chemical properties; (2) to assess the differences in drug solubilization capacity, dispersibility under biorelevant conditions, and propensity for precipitation upon dilution of LBDDS with different composition complexities and (3) to explore the utility of IVIVR in early pharmaceutical development of bio-enabling formulations such as LBDDS.

2. Materials and Methods

2.1.Materials

Three poorly water-soluble drugs were used in this study. Celecoxib (weak acid, 381.4 g/mol, logP = 4.3) was purchased from Astatech Inc. (Bristol, PA, USA), cinnarizine (weak base, 368.5 g/mol, logP = 5.7) and JNJ-2A (neutral, 498.9 g/mol, logP = 5.4) were obtained from Janssen Pharmaceutica (Beerse, Belgium). Analysis of plasma samples containing celecoxib and cinnarizine was done using internal standards: ibuprofen (for celecoxib samples) and flunarizine (for cinnarizine samples) according to [17] and [18], respectively. Both ibuprofen and flunarizine were obtained from Janssen Pharmaceutica (Beerse, Belgium). Sesame oil (long chain triglycerides, LCT) was purchased from Croda (Chocques, France), Capmul MCM C8 (medium chain mixed 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 kind gifts from Gattefossé (Lyon, France). SIF powder was obtained from biorelevant.com (London, UK). All other chemicals and solvents were of analytical or HPLC grade and were

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purchased from WVR (Belgium).

2.2.Methods

2.2.1. Design of prototype lipid systems

The composition of the excipients used for the eight LBDDS designed in this study is shown in Supporting information Table S 1. These LBDDS were: 1) one-component systems containing LC or MC partially digested triglycerides (blends of mono- and di-glycerides; LCM, MCM), 2) two-component systems containing also hydrophilic surfactant (LCM+S, MCM+S), 3) three-component systems with the same fatty acid chain length (LCM+LCT+S, MCM+MCT+S), and 4) three-component systems with different fatty acid chain length (LCM+MCT+S, MCM+LCT+S). Excipients for each LBDDS were mixed gently for 10 s at ambient temperature until a homogenous solution was obtained.

2.2.2. Drug solubility in blank lipid systems

Solubility of drugs (i.e. celecoxib, cinnarizine and JNJ-2A) in the prototype lipid systems was determined by the shake-flask method at 37°C. Additionally, solubility in LC and MC pure triglycerides (i.e. sesame oil – LCT and Labrafac Lipophile – MCT) was determined, to evaluate differences between different classes of lipid excipients. In short, an excess amount of drug was added to 1 mL of each lipid excipient or mixture in vials containing a magnetic stirrer. Formed suspensions were continuously stirred at 37°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 equilibrium solubility (S_{eq}) was reached within 24 h. Aliquots of the mixtures were centrifuged at 17500 rpm for 30 min using an Eppendorf centrifuge 5430R (Eppendorf, Hamburg, Germany). The solubility experiment was

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performed in triplicate.

2.2.3. Preparation of drug-loaded lipid systems

Based on the solubility values at 37°C, the amount of drug to be weighted was calculated to correspond to an 85% saturation degree in each of the tested lipid systems. The required mass of drug (Table 1) was weighted into clean screw-top glass vials and drug-free lipid systems were added up to the target drug loading. Vials were sealed, mixed and incubated at 37°C for 24 h prior to testing.

2.2.4. In vitro evaluation: Drug solubility in biorelevant media

Drug solubility in six biorelevant media was determined in order to evaluate the influence of LC and MC lipid excipients on *in vitro* solubilization behaviour of the three drugs used in this study. The six media represent either: (1) level II biorelevant media (i.e. fasted state simulated intestinal fluid - FaSSIF and fed state simulated intestinal fluid – FeSSIF [19,20], (2) FaSSIF with dispersed LCM and MCM and (3) two post-digestive assembled media containing FaSSIF and digestion products of LCM (i.e. Maisine CC = mono- and di-glycerides of $C_{18:2}$, $C_{18:1}$, $C_{18:0}$, C_{16}) and MCM (Capmul MCM = mono- and di-glycerides of mainly C_8).

FaSSIF and FeSSIF were prepared according to general instructions suggested by biorelevant.com using SIF powder and phosphate buffer, whereby FaSSIF was only used after 2 h of room temperature storage and FeSSIF was used immediately after preparation. Dispersed media were prepared according to Gautschi and co-workers [21], with slight modifications, by dispersing the undigested lipid excipient (LCM, MCM) in FaSSIF and continuously stirring at 300 rpm in a climate chamber at 37°C in a dilution of 1:40 lipid to FaSSIF for 2 h. For the post-digestive biorelevant media containing digestion products of lipid excipients the same dilution of 1:40 was

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employed similar to Gautschi et al. [21]. In the present study, it was assumed that the monoglycerides in the lipid excipients were not further digested and that diglycerides (DG) and the traces of triglycerides (TG) digest to the respective monoglycerides and free fatty acids in molar ratio of 1:1 for DG and 1:2 for TG. For simplification purposes, only one fatty acid (i.e. linoleic acid, $C_{18:2}$), which was in the highest concentration according to the certificate of analysis of Maisine CC, was considered in the composition of LCM. Therefore, the digestion products of Maisine were assumed to be monolinolein and linoleic acid (1 g Maisine presumably digests to 0.71 g monolinolein and 0.29 g linoleic acid). Similarly, for Capmul MCM, only caprylic acid (C_8) was considered the predominant fatty acid, thus the proposed digestion products used were monocaprylin and caprylic acid (1 g Capmul MCM presumably digests to 0.83 g monocaprylin and 0.17 g caprylic acid). Assembled media containing digestion products of LCM and MCM were stirred continuously at 37°C for 2 h.

Equilibrium solubility of celecoxib, cinnarizine and JNJ-2A was assessed at 37°C in the six biorelevant media by the shake-flask method described above. Samples were collected at 2, 4, 6 and 24 hours, centrifuged using an Eppendorf centrifuge and clear supernatant was diluted with diluent containing 50% N-Methyl-2-pyrrolidone (NMP) in Milli-Q water (v/v). Apparent drug concentration was assessed using the reverse-phase methods described in section 2.2.6 and results are shown as mean + SD.

2.2.5. In vitro evaluation: Precipitation and drug solubilization upon dilution and dispersion

The dilution, dispersion and precipitation characteristics of the different lipid systems containing the three drugs at 85% saturation degree were evaluated in FaSSIF at a 1:250 dilution ratio in triplicate. The diluted samples were stirred with a magnetic stirrer to ensure complete dispersion at 37 °C at 200 rpm in sealed glass vials. At pre-determined

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intervals (0, 0.25, 1, 2, 4, 24 h), the samples were investigated both macroscopically for the presence of precipitate and analytically for determination of drug concentration in the aqueous dispersed phase using the methods described in section 2.2.6. To determine the apparent drug concentration in the aqueous phase of the dispersed lipid systems, samples were withdrawn using a 1 mL syringe and centrifuged for 10 min at 17500 rpm and 37 °C in a benchtop Eppendorf centrifuge. Clear supernatant was diluted with diluent containing 50% NMP in Milli-Q water and measured with an Acquity Ultra Performance Liquid Chromatography (UPLC™) H-class system (Waters, Milford, USA) as shown in section 2.2.6. Drug precipitate formed during dispersion of lipid systems in FaSSIF 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 crystalline drug material which was used for drug-loading of lipid systems.

2.2.6. Drug quantification in in vitro samples

The drug concentration in the supernatants obtained after centrifugation was determined using an Acquity (UPLC™) H-class system 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 (RP) Waters Acquity BEH C18, 50 mm × 2.1 mm column packed with 1.7 μm particles (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

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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 2.5 – 100 µg/mL and samples were diluted accordingly. The solubility experiment was performed in triplicate.

2.2.7. In vivo evaluation: Rat pharmacokinetic study

The protocol used for the *in vivo* pharmacokinetic studies 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. Male Sprague-Dawley rats weighting between 250-300 g, received 0.5 mL/kg lipid solution of celecoxib (n=4), cinnarizine (n=4) and JNJ-2A (n=6) by oral gavage. Lipid systems were stirred continuously the night before dosing and were clear upon oral administration. Blood was collected after oral administration at defined timepoints: 0.5, 1, 2, 4, 6, 8, 24 h for cinnarizine and celecoxib and at 0.5, 1, 2, 4, 8, 10, 24 for JNJ-2A. A small volume (30 µL) of blood was collected into EDTA-plasma tubes and 10 µL of the plasma were harvested after centrifugation in end-to-end pipettes and analysed with LC/MS-MS for six of the celecoxib administrations and the JNJ-2A LBDDS. For two celecoxib (CCX) LBDDS (i.e. LCM and MCM) and for the whole set of cinnarizine (CIN) lipid systems, 100 µL plasma was harvested and bioanalysis was performed on a Waters UPLC™ system with UV-detection.

2.2.8. Quantitative analysis of plasma samples

Quantification of celecoxib in plasma after oral dosing of six lipid systems of celecoxib

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and of JNJ-2A after oral dosing of all eight lipid systems was performed on a Sciex API-4000 triple quadrupole mass spectrometer (Sciex, Ontario, Canada) equipped with an Acquity UPLC™ (Waters, Milford, MA, USA).. Separation was done on an ACQUITY UPLC™ BEH C18 1.7 µm particles, 50 × 2.1 mm column, using a gradient of 0.1% formic acid and acetonitrile (for celecoxib) and 0.1% formic acid and 0.01M Ammonium carbonate (for JNJ-2A). The flow rate was kept at 0.80 mL/min and method linearity was between 1 – 10000 ng/mL (celecoxib) and 4 – 20000 ng/mL (JNJ-2A).

Ibuprofen (IBU) was added as internal standard for the quantification of celecoxib in plasma samples after oral dosing of single component lipid solutions (i.e. LCM and MCM) and flunarizine (FLU) as internal standard for cinnarizine measurements after dosing of all eight lipid systems [17,18]. Ibuprofen and flunarizine were solubilized in acetonitrile to obtain internal standard solutions, 140 µL of these solutions were added to 20 µL plasma sample containing either celecoxib or cinnarizine. Plasma protein precipitation was successful after centrifugation for 30 min at 17500 rpm using an Eppendorf centrifuge 5430R (Eppendorf, Hamburg, Germany). The extraction recovery of celecoxib was ≥ 93.3% and of cinnarizine ≥ 96.6%. All other analytical parameters were identical to the reverse-phase methods described above for quantification of *in vitro* samples. The concentrations of celecoxib and cinnarizine were determined by standard calibration curve analysis using linear fitting of a plot of CCX/IBU peak area ratios versus celecoxib concentrations and CIN/FLU peak area ratios versus cinnarizine concentrations, respectively. The standard calibration curves were linear in the range 62.5 – 10000 ng/mL for celecoxib and 62.5 – 5000 ng/mL for cinnarizine.

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2.2.9. 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 PKSolver add-in. Multiple sample comparison was tested by an analysis of variance (ANOVA) on ranks of dose-normalized data for the pharmacokinetic parameters C_{max}, t_{max} and AUC_{0-24h} using SigmaPlot 12.5 from Systat Software, Inc. (Chicago, IL, USA). A statistical p-value<0.05 was considered significant. For statistical contrast analysis a Tukey post-hoc test was used. Results are expressed as mean ± SD for C_{max} and AUC_{0-24h} and median [min, max] for t_{max}. Pearson correlation coefficients for *in vitro* and *in vivo* relations were computed using GraphPad Prism version 8.4.3 (San Diego, CA, USA) and are presented as coefficient of determination (R^2).

3. Results

3.1.Comparing drug solubility in partial glycerides versus triglycerides

Equilibrium drug solubility in four classes of lipid excipients was determined at 37 °C. Lipid excipients were chosen to represent long chain triglycerides (LCT – sesame oil), long chain blends of mono- and di-glycerides (LCM – Maisine CC), medium chain triglycerides (MCT – Labrafac Lipophile WL 1349) and medium chain mono- and di-glycerides (MCM – Capmul C8 MCM). Results are shown in Figure 1.

A clear difference in solvent capacity was observed between excipients composed of blends of mono-/di-glycerides and pure triglycerides for celecoxib and JNJ-2A for both long and medium chain lipid classes. For cinnarizine, the highest solubility was obtained in LCM and the lowest in LCT, while MCM only presented a

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slightly higher solvent capacity relative to MCT. Low drug solubility in triglyceride vehicles, leading to limited dose loading, is often a limitation for choosing a simple TG-based drug delivery system, particularly for low potency/high dose drug candidates [8,9]. Drug solubility was higher in blends of mono-/di-glycerides, in line with previous literature on other lipophilic drugs [22-24]. The enhanced solvent capacity of partially digested triglycerides may reduce the need for inclusion of hydrophilic surfactants and/or co-solvents.

3.2.Comparing drug solubility in FaSSIF/FeSSIF media versus biorelevant dispersions of partial glycerides

Drug solubility in six types of biorelevant media was determined to assess simulated *in vivo* drug solubility under fasting conditions (FaSSIF); fed state conditions (FeSSIF); conditions that simulate *in vivo* solubilisation on dispersion in fasted state intestinal fluids (FaSSIF + LCM) and FaSSIF + MCM (1:40)); conditions that simulate post-digestive conditions (FaSSIF and lipolytic end products of LCM and MCM digestion). Solubility values obtained after 6 hours of continuous stirring in the tested media are presented in Figure 2.

Solubility in FaSSIF was relatively low for the three tested drugs, ranging between 14 and 80 µg/mL, while an increase of approximately 3-fold (celecoxib), 15-fold (cinnarizine) and 7-fold (JNJ-2A) was observed in the fed state simulating media (FeSSIF) indicating enhanced drug solubility in the post prandial conditions for these three drugs and a potential food effect (Figure 2). For the biorelevant dispersions of partial glycerides, the solubility of each drug in the LCM dispersions was similar to FaSSIF solubility. This indicated that the addition of the LCM excipient had limited effect on drug solubilisation on initial dispersion relative to the fasted state. In contrast, the solubility determined in conditions that simulate post-digestive LC conditions

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closely matched the solubility in FeSSIF indicating that the enhanced solubilisation in the post prandial state could potentially be addressed by employing an LCM excipient in the formulation of LBDDS containing celecoxib, cinnarizine or JNJ-2A.

In case of MCM based biorelevant dispersion, all three drugs displayed lower drug solubility relative to FaSSIF. This may be a reflection of observed drugs tendency for forming complexes with the medium chain lipid excipient leading to reduce solubilisation of the drug in the assembled media. Under simulated post-digestive conditions, the solubility of JNJ-2A was low and unchanged compared to dispersed MCM in FaSSIF. For cinnarizine the solubility improved under simulated digestive conditions, but was in general in the same range as the solubility in FaSSIF. Collectively these results suggested there were no solubility advantages including MCM lipid excipient for either JNJ-2A nor cinnarizine. In contrast, celecoxib displayed a 17-fold higher solubility observed in the MCM simulated post-digestive conditions when compared to FaSSIF solubility. In summary, this relatively rapid solubility screening test, designed to simulate biorelevant dispersion and digestion conditions, may be a useful guide on the choice of lipid chain length in LBDDS. In the case of cinnarizine and JNJ-2A LCM-containing LBDDS were predicted to display higher *in vivo* solubility, whereas for celecoxib MCM-containing LBDDS were estimated to display higher *in vivo* solubility in the post-digestive environment.

3.3.In vitro dilution and dispersion in FaSSIF

The dispersion characteristics of the different LBDDS loaded with either celecoxib, cinnarizine or JNJ-2A were evaluated following dispersion in FaSSIF (1:250 v/v). All eight lipid systems formed turbid dispersions with no macroscopically visible drug precipitate. The single component (LCM and MCM) and two component (LCM+S and MCM+S) systems displayed the poorest dispersibility, as evidenced by the presence of

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small oil droplets at the surface of the dispersion. The three component systems displayed improved dispersibility (i.e. no visible oil droplets). At different timepoints, drug concentration was determined and is presented in Figure 3 as percentage of drug solubilized in the aqueous environment relative to initial (theoretical) drug concentration upon LBDDS dispersion.

For all three drugs, a trend towards higher percentage of drug solubilized in the aqueous environment during the 24 hours of testing was evident for the one component LC LBDDS. Percentages relative to theoretical drug solubilized in the whole dispersion medium of 20-40% for celecoxib, 10-25% for cinnarizine and 20–50% for JNJ-2A were determined. For the two-component LCM+S system high percentages were also calculated (10-30% for celecoxib, 15-20% for cinnarizine and 15-25% for JNJ-2A). One or two component MC LBDDS displayed the lowest percentage of drug solubilized in the aqueous phase (celecoxib – 2-20% [MCM], 1-2% [MCM+S]; cinnarizine – 1-2% [MCM], 4-9% [MCM+S] and JNJ-2A - 0.7-6% [MCM], 4-10% [MCM+S]). Despite displaying a better dispersibility in biorelevant media, for the three component systems percentages of drug solubilized were similar to LCM and LCM+S dispersions (15-27% for celecoxib and 10-15% for cinnarizine). In contrast, addition of triglycerides to MC systems improved both the dispersibility and the drug solubilization compared to the one and two component MC systems (13-20% for celecoxib, 10-18% for cinnarizine and 2-10% for JNJ-2A).

Celecoxib precipitation between 0-2 h was observed in all LBDDS, except for LCM which showed precipitate at 4 h (Supporting information, Table S 2). Cinnarizine only showed precipitate during the screening in MC LBDDS. Both celecoxib and cinnarizine precipitated as crystalline material as illustrated by XRPD diffractograms, which resembled the diffractograms of the material used for drug-loading of LBDDS

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(Supporting information, Figure S 1 and Figure S 2). Notably, there was no precipitation observed for JNJ-2A in all dispersions. However, phase separation or 'oiling out' occurred in all JNJ-2A dispersions following centrifugation of the sample, with distinct oil droplets forming either as an upper layer in the vial on top of the aqueous phase for LCM-containing systems or as a lower oil layer for MCM-containing systems.

3.4.Assessing compositional effect in vivo

The plasma concentration versus time profiles following oral administration to rats of three drugs in eight LBDDS is presented in Figure 4. In all cases the drug saturation degree in the drug delivery system was fixed at 85% and the dosing volume of 0.5 mL/kg was maintained constant across all tested lipid systems. As a result, the dose was different between groups depending on the drug solubility in each system (Table 2) and drug concentrations in plasma were hence dose-normalised to facilitate direct comparison of formulation effects. In the case of celecoxib and cinnarizine, reference to previous published studies in rats indicate dose proportionality at doses between 12.5-100 mg/kg (celecoxib) and 2-30 mg/kg (cinnarizine) [17,25]. In case of JNJ-2A, no previously published reports were available to confirm dose proportionality, however in-house internal pharmacokinetic studies demonstrated that dose proportionality was observed at <100 mg/kg whereas at doses between 100 – 200 mg/kg dose proportionality was evident for $AUC_{0\text{-}24h}$ but not for C_{max} (Janssen internal data). A comparison of dose-normalised $AUC_{0\text{-}24h}$ as a function of LBDDS composition is presented in Figure 5 and the corresponding pharmacokinetic parameters are illustrated in Table 3.

For celecoxib, C_{max} after dosing of LBDDS containing at least one LC lipid excipient was higher than the equivalent MC lipid systems, with C_{max} of the single

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component LCM system being statistically significant relative to all LBDDS except LCM+S. Similarly, celecoxib exposure, as expressed by $AUC_{0\text{-}24h,}$ was the highest after dosing of LCM and LCM+S. In case of cinnarizine, C_{max} after dosing of LCM+S was statistically higher compared to MCM, while the $AUC_{0\text{-}24h}$ after administration of LCM was statistically higher relative to MCM+S. The overall higher bioavailability of LC LBDDS was seen for both celecoxib and cinnarizine compared to the corresponding MC systems. For JNJ-2A, no statistically significant difference was observed for C_{max} in the tested LBDDS; however, a statistically significant difference was observed for the one component MC lipid system relative to its LC correspondent for $AUC_{0\text{-}24h}$. No statistically significant differences were observed for t_{max} amongst the tested LBDDS for the three model drugs, with a relatively fast absorption (≤ 4h) of celecoxib and cinnarizine and a longer absorption of JNJ-2A (3-9.5h). No statistically significant differences were found for triglyceride-containing LBDDS.

3.5.In vitro – in vivo relation

In an attempt to identify possible relations between *in vitro* observations and *in vivo* pharmacokinetics, plots of $AUC_{0\text{-}24h}$ of drug concentration determined in the aqueous phases after dispersion in FaSSIF (Table S 3) and of plasma concentration versus time after oral administration (Table 3) were constructed. Additionally, the Pearson correlation coefficients were calculated and are presented as R^2. While overall the *in vitro – in vivo* relations were poor (R^2 was between 0.006 and 0.046), the higher solubilization in biorelevant media after LCM+S dispersion correlated with high *in vivo* performance of the lipid system (full squares, Figure 6) for all three drugs. Individually, by visual observation (Figure 6), for celecoxib a good correlation between *in vitro* and *in vivo* results was seen for LCM, in case of cinnarizine for LCM, LCM+LCT+S and MCM+LCT+S and finally for MCM+S and MCM+MCT+S in the case of JNJ-2A. In

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addition, it seems that poor *in vivo* performance of MCM and MCM+S (empty circles and squares) containing celecoxib and cinnarizine (Figure 6, graph A and B respectively) could be linked to the low drug solubilization in FaSSIF upon dispersion.

4. Discussion

Pharmaceutical academic research has particular relevance when it focuses on the needs of the pharmaceutical industry, for example in advancing compositional understanding on LBDDS, thus increasing industrial uptake of this bio-enabling technology. A prevalence of single component LBDDS as commercial products (e.g. Rocaltrol®, Marinol®, Avodart®, Sustiva®, Prometrium®, Fortovase®, Depakene®) was identified, while considerable academic research is invested to study complex LBBDS with compositions in excess of three excipients, which can be highly resource intensive in an accelerated screening pharmaceutical development setting. A key focus of this study is therefore to identify the potential merits of one or two component LBDDS, which are well suited to HTS and accelerated pharmaceutical development paradigm, as well as to compare with more compositionally complex LBDDS.

This study demonstrated the benefits of simple, one and two component LBDDS, over more complex systems for an improved drug absorption. In particular, the use of blends of mono-/di-glycerides as single component LBDDS offered a balance in terms of streamlining drug-excipient screenings/formulation characterisation and showed enhanced *in vivo* exposure for celecoxib, cinnarizine and JNJ-2A in rats. Such simple blends therefore bear much potential either as components or final formulation of LBDDS [8,11]. In fact, several commercial products (e.g. saquinavir - Fortovase®, calcitriol - Rocaltrol®, dutasteride – Avodart®) are formulated using only blends of MC mono-/di-glycerides [8]. In contrast, the analysis of three reviews on bio-enabling formulations [3,8,26] revealed no commercially available LBDDS containing solely LC

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blends of mono-/di-glycerides, which the current work found to be suitable for enhancing the bio-performance of both celecoxib and cinnarizine. Several oral pharmacokinetic preclinical studies have suggested that inclusion of celecoxib in compositionally complex self-(micro)-emulsifying drug delivery systems (S(M)EDDS) improve the drug's bioavailability compared to aqueous suspension [27] and conventional capsule [28]. Celecoxib was also used for successful formulation design of a bio-enabling silica-lipid hybrid (SLH) microcapsule system with several physico-chemical and biopharmaceutical benefits over unformulated drug, lipid emulsion, dry emulsion and the commercial product Celebrex® [29,30]. Similarly, for cinnarizine it has been shown that administration as LBDDS in the form of lipid solutions [25], SEDDS, or self-nano-emulsifying drug delivery systems (SNEDDS) [18,31-36], sub-microemulsions [37], silica-stabilized lipid cubosomes, silica-solid lipid hybrid, and polymer − lipid hybrid particles [38] exhibited bioavailability advantages relative to conventional formulations.

There is clear evidence in the literature that highly lipophilic drugs administered in a LBDDS have improved absorption compared to conventional formulations [39]. However, while numerous studies focus their hypotheses on comparing LBDDS to alternative bio-enabling formulations e.g. amorphous solid dispersions, relatively few studies compared between LBDDS to elucidate the potential compositional effects on *in vivo* performance [11]. The present study identified that choice between LC or MC lipids was highly drug specific, with advantages of LC systems (i.e. LCM and LCM+S) for celecoxib and cinnarizine and of the single component MCM system for JNJ-2A. The *in vivo* exposure of cinnarizine has previously been reported to benefit from LBDDS containing LCT (as either a lipid solution or nanoemulsion) [25,37], whereas, to our knowledge, pharmacokinetics of single component LBDDS composed of

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digested LCT (i.e.blends of mono-/di-glycerides) was not reported before. While further studies on a broader set of drugs would be required for clear correlations to be established between drug properties and lipid chain length, the observations here indicate that preformulation screenings should be performed with both LC and MC based excipients.

In vitro dilution and dispersion testing in FaSSIF can be employed for early stage physiological characterization of LBDDS [5] and was thus performed in this study to assess the precipitation risk and kinetic drug concentration profile for the range of LBDDS. The three component LBDDS displayed improved dispersibility in FaSSIF, a relatively high drug concentration in the aqueous phase, but also evidence of drug precipitation (celecoxib and cinnarizine). Interestingly these systems displayed lower *in vivo* absorption which may reflect the higher precipitation potential for these systems. In contrast, despite limited dispersibility for the single or two component LBDDS (LCM, LCM+S, MCM, MCM+S) *in vivo* bioavailability tended to be higher and this was matched by a general trend towards longer time to precipitate (e.g. LCM, LCM+S, MCM for celecoxib and cinnarizine). These observations therefore provide stronger support to the claim that designing LBDDS to maximise *in vitro* dispersibility provides limited insights on to the likely *in vivo* effects. In contrast, factors such as precipitation and solubilisation capacity are more likely to provide predictive insights [15]. Furthermore, Larsen *et al*. have shown for danazol that higher bioavailability of Labrafil M2125CS formulations with low drug loadings were corelated to higher drug concentration in the aqueous environment of lipolysis media and limited drug precipitation [40,41].

By plotting the calculated AUC *in vitro* versus AUC *in vivo*, no strong correlations were identified, with coefficients of determination of 0.046 for cinnarizine

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to 0.017 for celecoxib and 0.006 for JNJ-2A. Nevertheless, the good performance of LCM+S for celecoxib, cinnarizine and JNJ-2A and the poorer performance of MCM and MCM+S for celecoxib and cinnarizine may have been anticipated from low drug solubilization in biorelevant media. The promotion of such simple *in vitro* screening tests for formulation ranking is encouraged in early development in lieu of more complex tests (e.g. dynamic lipolysis) for an easily accessible and HTS characterization tool [15]. The dilution and dispersion testing was beneficial to identify higher risk formulation strategies in early stages of development and was proposed as a first tier testing in the revised LFCS reports [42]. Yet, the power to predict *in vivo* scenarios is limited and this test needs adjustments in order to be considered as a platform for *in vitro* LBDDS screening in industrial development. For cinnarizine, a weak correlation (R^2 = 0.39) was previously reported between the *in vivo AUC* after administration of four LBDDS to dogs and *in vitro* AUC observed in the % cinnarizine in the aqueous phase of a pH-stat lipolysis test after 60 min digestion [3].

Another *in vitro* test investigated in this study was the drug solubilization in different biorelevant media containing dispersed lipid excipients or their respective digestion products, which may give early indication on the solubilization capacity of the system formed upon dispersion and digestion of LBDDS in the GIT. Gautschi *et al.* previously suggested that the assembled medium representative of post-digestive conditions for the MC lipid excipient used in this study (i.e. Capmul MCM) was indeed reflecting the solubilization capacity observed in medium containing enzymatically digested lipid excipient [21]. In the present study, the data set was enriched with the addition of LCM dispersed and digested media. It was observed that addition of digestion products of LCM could reach the same drug solubilization capacity as FeSSIF and hence be considered a solution that had the potential to overcome potential food

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effect presented by the three tested drugs. In almost all cases (exception being JNJ-2A in MC media) the solubilization capacity of assembled post-digestive was higher than the solubility in the dispersed media indicating that the digestion step was essential for the solubilization enhancement in the simulated GIT environment for the three investigated drugs. The results for celecoxib (weak acid) and JNJ-2A (neutral) were in contradiction to conclusions by Alskär *et al.* who presented drug solubility for 6 drugs (3 weak acids and 3 neutral) to be higher in dispersion media when compared to post-digestive media [43]. Nevertheless, the experimental design of the approach by Alskär and co-workers was different compared to the one used in this study as the LBDDS were type IIIA, IIIB and IV (i.e. containing 35% surfactant), the LBDDS were lipolysed and the pH adjusted to 6.5 during the solubility studies, which was not done in the present work.

In the current study, limited information can be extracted for cinnarizine and JNJ-2A in the MC media as a result of drug tendency to form agglomerates with lipolytic products; however, cinnarizine was shown to have a higher solubility in the aqueous phase of LCT digests relative to MCT in a study performed by Kaukonen and co-workers [44] which was in line with the present results. The test had limited power in predicting the *in vivo* differences observed for LCM and MCM systems, as it showed a higher solubility for MC media for celecoxib and for LC media in case of JNJ-2A while the *in vivo* performance was reversed. However, the test had indicative power for cinnarizine, and it may serve as a starting point to determine drug solubilization in physiologically relevant media in a simplified manner.

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5. Conclusions

This study adopted a simplified approach to LBDDS formulation design, and demonstrated the merits of using mono-/di-glycerides as single component excipients in LBDDS, offering a balance in terms of streamlining formulation screening and improving oral bioavailability. MCM and LCM displayed a higher solvent capacity for the three PWSD, relative to triglyceride equivalents. Despite relatively poor dispersibility *in vitro*, the observations from the *in vivo* pharmacokinetic dosing of a range of increasingly complex LBDDS to rats, confirmed the merits of one (LCM/MCM) or two component (LCM/MCM + surfactant) systems, relative to three component systems (LCM/MCM + surfactant + LCT/MCT). In terms of the choice of LC versus MC based systems, this study indicated that LC systems displayed higher drug solubilization in simulated biorelevant media and a lower propensity for drug precipitation relative to MC systems. However, in terms of *in vivo* observations, the choice of excipient type appears to be drug specific, with LC systems favorable for celecoxib and cinnarizine whereas MC based systems were preferred for JNJ-2A. While two *in vitro* methods were evaluated for assessing formulation dispersion, digestion and solubilization under biorelevant conditions, in general the *in vitro* results were poorly predictive of *in vivo* effects observed across the range of formulations explored. From an industrial drug development perspective, it would appear that further advances on high throughput and bio-predictive *in vitro* screening approaches are needed to guide LBDDS formulation design.

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Table 1. Quantity of drug (mg) loaded per mL of lipid-based drug delivery systems. This dose loading corresponds to 85% of the saturated solubility of the drug reported previously in Ilie *et al.* [45]. (LCM = long chain mono/di- glycerides blends (Maisine CC, Ma), MCM = medium chain mono-/di- glycerides blends (Capmul MCM, Ca), LCT = long chain triglycerides (Sesame oil, SO), MCT = medium chain triglycerides (Labrafac Lipophile, LL), S = surfactant (Labrasol ALF, L).

LBDDS	**Composition (w:w)**	**Quantity added (mg per ml of LBDDS)**		
		Celecoxib	Cinnarizine	JNJ-2A
LCM	Ma	15.8	47.0	46.8
MCM	Ca	57.4	41.3	296.1
LCM+S	Ma + L (4:1)	62.2	37.3	193.0
MCM+S	Ca + L (4:1)	80.9*	39.4	392.2
LCM+LCT+S	Ma + SO + L (2:2:1)	71.0	31.7	n.a.*
MCM+MCT+S	Ca + LL + L (2:2:1)	102.7	42.1	309.9
LCM+MCT+S	Ma + LL + L (2:2:1)	82.5	39.7	n.a.*
MCM+LCT+S	Ca + SO + L (2:2:1)	83.3	41.2	n.a.*

n.a.* = not prepared based on preformulation studies which showed lack of excipient compatibility after JNJ-2A loading [45]

* value based on the initial mean value out of 3 replicates, updated in Ilie *et al.* [45] with two additional measurements

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Table 2. Dose (mg/kg) administered orally to fasted Sprague-Dawley rats in a pharmacokinetic study.

LBDDS	**Composition (w:w)**	**Dose (mg/kg)**		
		Celecoxib	Cinnarizine	JNJ-2A
LCM	Ma	7.9	23.5	23.4
MCM	Ca	28.7	20.7	148.1
LCM+S	Ma + L (4:1)	31.1	18.7	96.5
MCM+S	Ca + L (4:1)	40.5	19.7	196.1
LCM+LCT+S	Ma + SO + L (2:2:1)	35.5	15.9	n.a.*
MCM+MCT+S	Ca + LL + L (2:2:1)	51.4	21.1	155.0
LCM+MCT+S	Ma + LL + L (2:2:1)	41.3	19.9	n.a.*
MCM+LCT+S	Ca + SO + L (2:2:1)	41.7	20.6	n.a.*

n.a.* = not administered based on preformulation studies which showed lack of excipient compatibility after JNJ-2A loading [45]

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Table 3. Pharmacokinetic parameters C_{max}, $AUC_{0\text{-}24h}$ (mean ± SD) and t_{max} (median, [min, max]) following single oral administration of lipid-based drug delivery systems containing celecoxib, cinnarizine or JNJ-2A at 85% saturation degree.

LBDDS	Celecoxib			Cinnarizine			JNJ-2A		
	C_{max} (ng/mL)/ (mg/kg)	AUC $_{0\text{-}24h}$ (ng/mL*h)/ (mg/kg)	t_{max} (h)	C_{max} (ng/mL)/ (mg/kg)	AUC $_{0\text{-}24h}$ (ng/mL*h) / (mg/kg)	t_{max} (h)	C_{max} (ng/mL)/ (mg/kg)	AUC $_{0\text{-}24h}$ (ng/mL*h) / (mg/kg)	t_{max} (h)
LCM	429 ± 78[a]	3564 ± 984	1.5 [1.0; 4.0]	34 ± 12	285 ± 76[d]	4.0 [2.0; 4.0]	37 ± 14	387 ± 119[e]	3.0 [2.0; 7.0]
MCM	237 ± 52[a]	1979 ± 244[b]	4.0 [2.0; 4.0]	13.7 ± 3.1[c]	176 ± 42	3.0 [1.0; 4.0]	44 ± 14	625 ± 202[e]	9.5 [7.0; 12]
LCM + S	326 ± 7.1	3127 ± 251[b]	3.0 [1.0; 4.0]	38 ± 13[c]	255 ± 59	2.0 [2.0; 4.0]	47.9 ± 3.1	574 ± 50	7.0 [4.0; 7.0]
MCM + S	270 ± 63[a]	2776 ± 354	3.0 [2.0; 4.0]	16.1 ± 4.4	133 ± 29[d]	2.0 [2.0; 2.0]	31.3 ± 8.7	431 ± 138	9.5 [7.0; 12]
LCM + LCT + S	246 ± 47[a]	2642 ± 748	2.0 [1.0; 4.0]	31 ± 18	193 ± 44	2.0 [1.0; 4.0]	Not available		
MCM + MCT + S	236 ± 32[a]	2576 ± 304	4.0 [4.0; 4.0]	15 ± 4.8	174 ± 62	2.0 [2.0; 4.0]	38.8 ± 8.0	564 ± 91	7.0 [7.0; 12]
LCM + MCT + S	305 ± 48[a]	2961 ± 324	4.0 [4.0; 4.0]	31.3 ± 4.1	196 ± 56	1.0 [1.0; 2.0]	Not available		
MCM + LCT + S	221 ± 21[a]	2658 ± 257	4.0 [4.0; 6.0]	26 ± 12	196 ± 63	1.0 [1.0, 2.0]	Not available		

[a]LCM statistically different from all other lipid systems, except LCM+S

[b] LCM+S statistically different from MCM

[c] LCM+S statistically different from MCM

[d] LCM statistically different from MCM+S

[e] MCM statistically different from LCM

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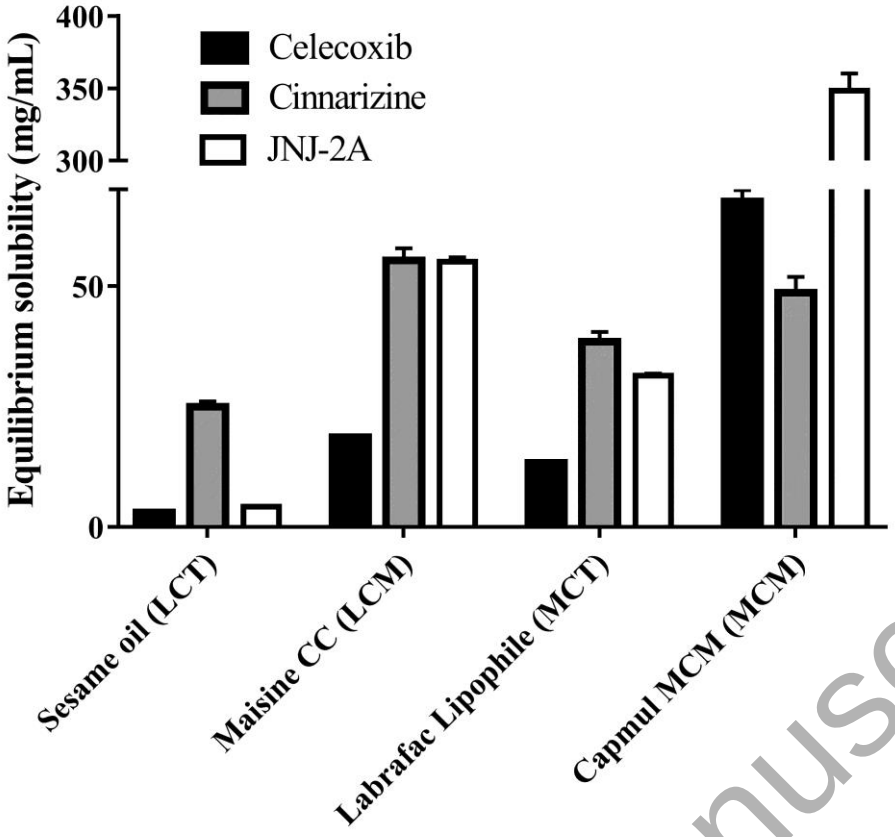


Figure 1. Solubility (mean + SD, mg/mL) in blends of mono- and di-glycerides (LCM-Maisine CC and MCM-Capmul MCM) and triglycerides (LCT-sesame oil and MCT-Labrafac Lipophile). Black bars depict data for celecoxib, dark grey bars for cinnarizine and white for JNJ-2A.

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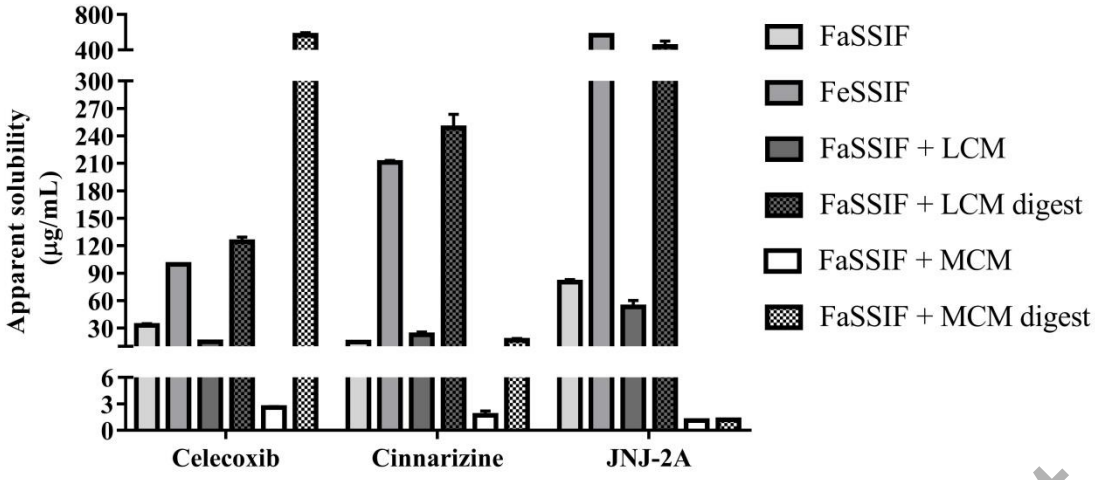


Figure 2. Apparent solubility (mean + SD, μg/ml) of celecoxib, cinnarizine and JNJ-2A in different types of biorelevant media.

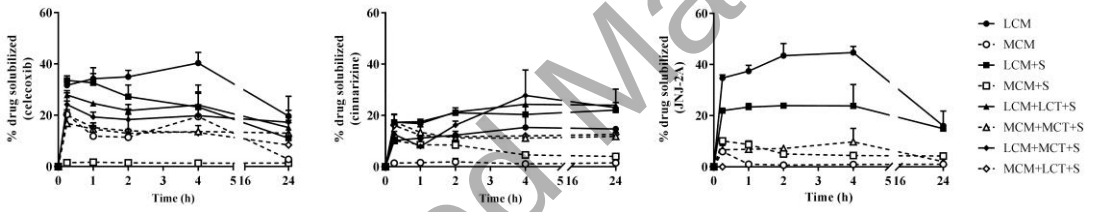


Figure 3. Percentage of drug solubilized in aqueous dispersed phase upon dilution and dispersion of lipid-based drug delivery systems containing celecoxib, cinnarizine and JNJ-2A at 85% saturation degree (mean + SD, n=3): continuous lines – LCM systems: LCM (full circles), LCM+S (full squares), LCM+LCT+S (full triangles), LCM+MCT+S (full diamonds); interrupted lines – MCM systems: MCM (empty circles), MCM+S (empty squares), MCM+MCT+S (empty triangles), MCM+MCT+S (empty diamonds).

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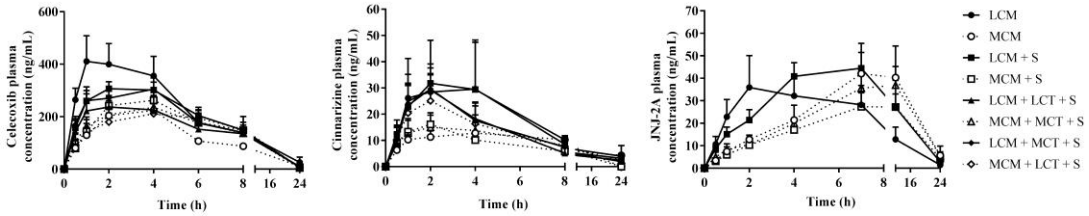


Figure 4. Plasma concentration-time profiles (mean + SD), n=4 for celecoxib and cinnarizine after dosing eight LBDDS and n=6 for JNJ-2A after oral dosing of five LBDDS. Continuous lines – LCM systems: LCM (full circles), LCM+S (full squares), LCM+LCT+S (full triangles), LCM+MCT+S (full diamonds); interrupted lines – MCM-based systems: MCM (empty circles), MCM+S (empty squares), MCM+LCT+S (empty triangles), MCM+MCT+S (empty diamonds).

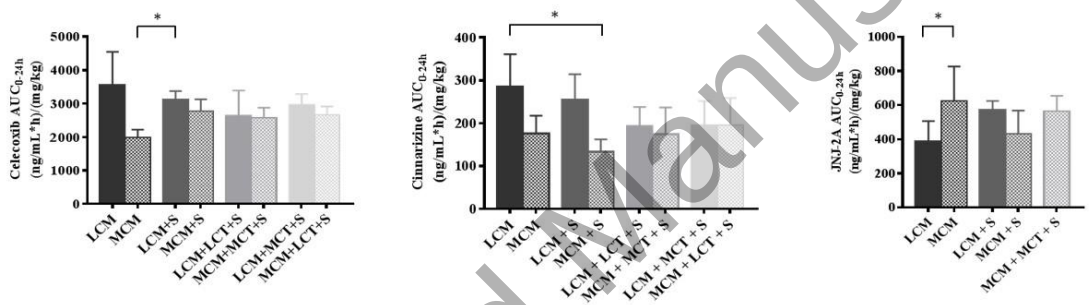


Figure 5. Area under the concentration-time plasma profiles obtained after oral administration of LBDDS containing celecoxib, cinnarizine and JNJ-2A. Full colour bars represent LCM lipid systems and dotted bars represent MCM lipid systems. Data shown as dose-normalized values (mean + SD).

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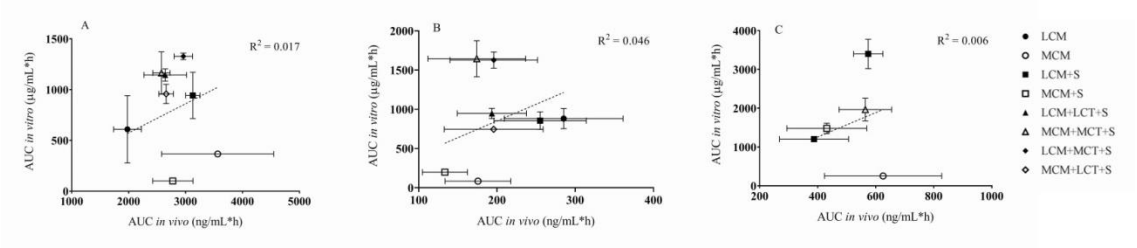


Figure 6. *In vitro - in vivo* relations of drug absorption after administration of LBDDS to male fasted rats ($AUC_{0\text{-}24h}$) and drug solubilization after dispersion of LBDDS as drug concentration in aqueous phase (total drug in free form in solution plus solubilized in dispersed phase); A) celecoxib, B) cinnarizine, C) JNJ-2A.

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