This is the peer reviewed author accepted manuscript (post print) version of a published work that appeared in final form in:

Meola, Tahlia R, Joyce, Paul, Wignall, Anthony, Bremmell, Kristen E, Prestidge, Clive A 2021 'Harnessing the potential of nanostructured formulations to mimic the food effect of lurasidone', *International Journal of Pharmaceutics*, vol. 608, article no. 121098, pp. 1-10

This output may not exactly replicate the final published authoritative version for which the publisher owns copyright. It is not the copy of record. This output may be used for non-commercial purposes. This version is licensed under a Creative Commons CC-BY-NC-ND 4.0 license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The final definitive published version (version of record) is available at:
https://doi.org/10.1016/j.ijpharm.2021.121098

Persistent link to the Research Outputs Repository:
https://researchoutputs.unisa.edu.au/11541.2/26319

General Rights
Copyright and moral rights for the publications made accessible in the Research Outputs Repository are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the persistent link identifying the publication in the Research Outputs Repository

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

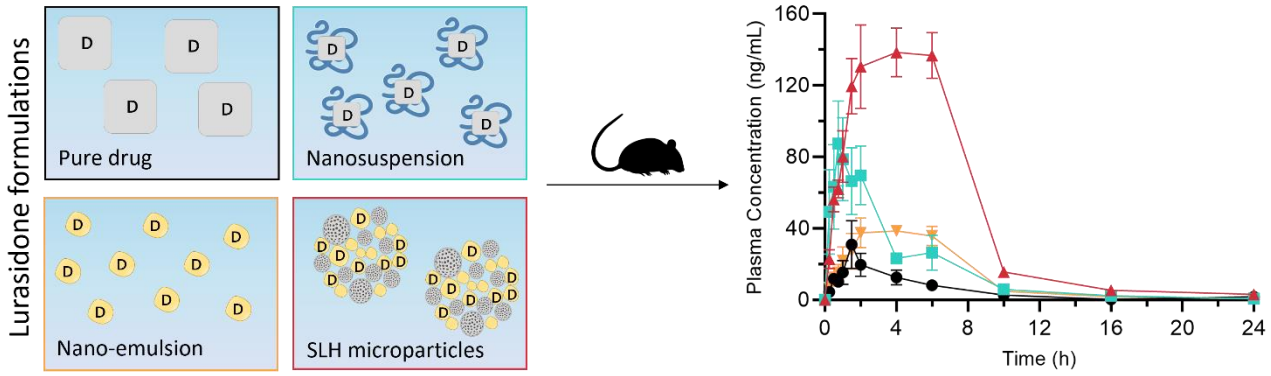
Harnessing the potential of nanostructured formulations to mimic the food effect of lurasidone

Tahlia R. Meola, Paul Joyce, Anthony Wignall, Kristen E. Bremmell and Clive A. Prestidge*
UniSA Clinical and Health Sciences, University of South Australia, Adelaide, South Australia 5000, Australia
ARC Centre for Excellence in Bio-Nano Science and Technology, Adelaide, South Australia 5000, Australia

*Corresponding Author - Clive.Prestidge@unisa.edu.au

Abstract:
Lurasidone is an important antipsychotic drug indicated for the treatment of schizophrenia and bipolar disorder, with an oral bioavailability of 9-19% owing to its poor aqueous solubility. Additionally, lurasidone exhibits a 2-fold positive food effect, such that patients must administer their medication with a meal, leading to significant non-compliance. The aim of this research was to evaluate the *in vitro* and *in vivo* performance of lurasidone when engineered as nanostructured systems. Specifically, a nanosuspension, nano-emulsion and silica-lipid hybrid (SLH) microparticles were formulated and the influence of composition and nanostructure on the mechanism of solubilisation was compared. Formulations were shown to enhance fasted state solubilisation levels *in vitro* by up to 5.9-fold, compared to pure drug. Fed- and fasted-state solubilisation profiles revealed that in contrast to the nanosuspension and nano-emulsion, lurasidone SLH mitigated the positive pharmaceutical effect of lurasidone. *In vivo p*harmacokinetic evaluations revealed that the nanosuspension, nano-emulsion and SLH enhanced the bioavailability of lurasidone by 3-fold, 2.4-fold and 8.8-fold, respectively, compared to pure drug after oral administration. For lurasidone, the combination of lipid-based nanostructure and porous silica nanostructure (SLH) led to optimal fasted state bioavailability which can ultimately result in enhanced treatment efficacy, easier dosing regimens and improved patient outcomes.

Graphical Abstract:



1. Introduction

The escalating burden of mental disorders is a growing concern worldwide, driving demand for more effective treatments, especially for severe disorders such as schizophrenia and bipolar, which affect approximately 65 million people globally [1]. Due to the chronic nature of schizophrenia and bipolar disorder, treatment adherence is essential to maintain symptom control and prevent relapse. However, statistics show that at least half of patients who are on long-term medication for these illnesses do not fully comply with their regimen leading to relapse, rehospitalisation, or suicide [2, 3].

Lurasidone (Figure 1) is an important second-generation oral antipsychotic indicated for the treatment of schizophrenia and bipolar disorder [4, 5]. Commercially marketed by Sunovion Pharmaceuticals Inc., under the brand name Latuda®, lurasidone was approved in 2010 by the Food and Drug Administration, and later in 2014 by the Therapeutic Goods Administration and European Medicines Agency [6-8]. Despite its clinical success, lurasidone has a limited oral bioavailability of 9-19% and exhibits a 2-fold positive food effect, owing to its poor aqueous solubility. Consequently, it is recommended that patients administer lurasidone with a meal containing at least 350 calories [6]. However, the dependence on food consumption to enhance drug exposure is erratic and heavily reliant on patient compliance, highlighting the need for an alternative formulation strategy to mitigate the food effect.

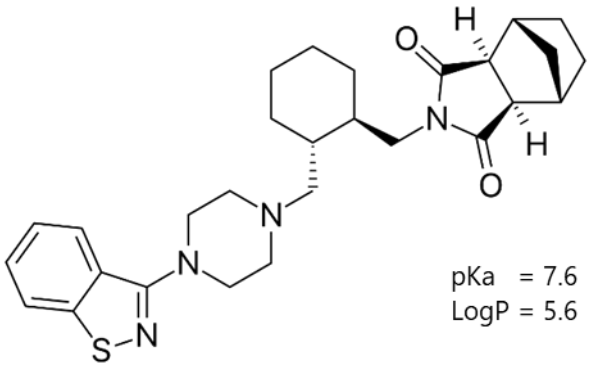


Figure 1. Chemical structure of lurasidone [7, 9]

Various formulation strategies have been developed to improve the oral delivery of poorly water-soluble drugs with similar physicochemical properties to lurasidone. Examples include amorphisation, solid dispersions, polymer-drug complexes, cyclodextrins, lipid-based formulations and particle size reduction [10, 11]. Importantly, the emergence of nanotechnology offers extensive advantages to conventional formulation strategies [12], since a wide range of nanostructured formulations can be fabricated through the application of specific excipients that promote drug dissolution and sustain drug solubilisation, including polymers, lipids and porous carriers. Specific attention within this study is attributed to comparing three commonly employed nanostructured formulations for mitigating the food effect of lurasidone, being a nanosuspension, nano-emulsion and silica-lipid hybrids (SLH).

Nanosizing or nanoisation refers to when the crystalline particles within the nanosuspension are less than 1000 nm, with an average size range of 200-600 nm [13, 14]. More recently, the European Commission have recommended that nanoparticles be defined as a particle with at least one external dimension between 1-100 nm [15]. Several commercial examples exist, including preparations for fenofibrate, aprepitant and megestrol acetate, which all remove the positive food effect associated with their original marketed formulations [16-18]. Elimination of the food effect is attributed to the small particle size and high surface area of the drug particles which facilitates dissolution regardless of the presence or absence of food. Notably, polymeric excipients, such as cellulose derivatives and poloxamers are generally incorporated within a nanosuspension for the dual-purpose of (a) stabilisation to prevent particle aggregation and (b) preventing precipitation and thus aiding in further drug solubilisation [19]. Mou *et al.* revealed that formulating itraconazole as a nanosuspension containing hydroxypropyl methylcellulose (HPMC) enhanced the rate of drug dissolution approximately 80% compared to the commercial formulation in the initial 5 min of dissolution [20]. Given that the food effect of lurasidone is reported to be independent of fat content [21], nanosizing may be a promising strategy to mitigate the food effect.

Alternatively, lipid-based formulations are renowned for their ability to reduce the influence of food on drug absorption [22, 23]. Specifically, nano-emulsions are colloidal systems comprising of lipid nano-droplets stabilised by a surfactant, dispersed in an aqueous phase [24]. The liquid lipid droplets can encapsulate drug in a molecularly dispersed state, removing the rate-limiting dissolution step associated with poorly water-soluble drugs and thus, promoting drug solubilisation [25, 26]. A megestrol acetate nano-emulsion formulated by Li *et al*. provided a 5-fold enhancement in oral bioavailability and eliminated the food effect, compared to a conventional suspension [27]. Though liquid-state lipid formulation approaches have proven effective for the delivery of numerous compounds, their solidification offers a multitude of additional benefits such as (i) improved drug solubilisation, (ii) enhanced physicochemical stability, (iii) controlled drug release and (iv) industrial and commercial advantages [28, 29].

As an extension of liquid lipid-based formulations, dry nanostructured particles can be produced by hosting liquid lipid nano-droplets within a solid carrier, such as silica, polymers or clays. One key example is that of SLH microparticles, which have been investigated for enhancing the bioavailability of various poorly water-soluble drugs [30-33]. SLH are fabricated by stabilising an oil-in-water nano-emulsion with silica nanoparticles, prior to spray-drying to form spherical microparticles composed of a nanostructured porous matrix [34]. The mechanisms of drug release from SLH have been thoroughly explored; briefly, the highly porous matrix facilitates lipase interaction which promotes lipid digestion and the formation of colloidal solubilising species, and subsequently, drug release and absorption [35, 36].

A wealth of research has investigated the impact of various parameters (e.g. particle size, surface chemistry) on oral drug solubilisation and absorption from individual nanostructured formulations [37-40]. For example, Shah *et al.* demonstrated that an increase in lurasidone solubilisation correlated with a decrease in nano-crystal particle size for crystals ranging from 200 – 1000 nm [41]. However, very few studies have compared the oral drug performance across varying nanostructured formulation types. That is, how does formulating lurasidone in a nanosuspension compare to a nano-emulsion or SLH? As such, the current study aimed to investigate the ability to increase lurasidone oral absorption through formulation with three nanostructured formulations with varying compositions and structures, being a nanosuspension, nano-emulsion and SLH, all of which have shown the ability to mitigate the food effect of poorly water-soluble drugs, similar to lurasidone [42-44]. Despite these three systems presenting contrasting physicochemical properties, which can impact solubilisation and absorption (e.g. particle size), it is essential that studies exist that directly compare commonly used formulation strategies to best direct future research and clinical translation of such technologies. Therefore, this study assesses the impact of nanostructured systems of varying composition (lipids versus nanocrystals) and structure (porous versus non-porous) on lurasidone performance, with the aim to fabricate a (i) nanosuspension where drug nanocrystals are combined with a polymeric excipient (HPMC), (ii) nano-emulsion where liquid lipid nano-droplets encapsulate drug in a non-crystalline form, and (iii) SLH microparticles where liquid lipid nano-droplets containing non-crystalline drug are hosted in a porous silica matrix. Physicochemical properties, *in vitro* dissolution and solubilisation under non-digesting and digesting (fed versus fasted) conditions and *in vivo* pharmacokinetics were critically evaluated. Furthermore, the unique solubilisation mechanism provided by each system was explored. It was hypothesised that the nanostructured formulations of lurasidone will improve solubilisation and oral absorption, with the extent of enhancement dependent on the composition and structure of the formulation. Based on the impact of food on oral absorption, it is predicted that formulating lurasidone with lipid nano-droplets, both in the liquid and solid dosage form, will be the most effective solubilisation approach leading to improved bioavailability, compared to the nanosuspension.

2. Materials and Methods

2.1. Materials

Lurasidone and ziprasidone powders (crystalline, purity > 99.0%) were purchased from Hangzhou Dayangchem Co. Ltd. (Hangzhou City, China). Capmul MCM (medium chain mono-, di-, and triglycerides, medium chain mixed glycerides) was kindly supplied by Abitec (Ohio, USA). Maisine 35-1 and Miglyol was obtained from Gattefossé (France) and Hamilton Laboratories (Adelaide, Australia), respectively. Soybean lecithin (containing >94% phosphatidylcholine and <2% triglycerides) was obtained from Merck (Bayswater, Australia). Fumed hydrophilic silica nanoparticles with the specific

surface area 300 m^2g^{-1} (Aerosil® 300 Pharma) was supplied by Evonik Degussa (Essen, Germany). FaSSIF/FeSSIF/FaSSGF powder was purchased from Biorelevant.com Ltd (London, United Kingdom). Porcine pancreatin extract (activity equivalent to 8 × USP specification) was supplied by MP Biomedicals (Seven Hills, Australia). 4-bromophenylboronic acid and carboxymethyl cellulose was purchased from Sigma Aldrich (Castle Hill, Australia) and Methocel E50 premium LV hydroxypropyl methylcellulose (HPMC E50) was purchased from Dow (Michigan, USA). Polysorbate 80 (Tween 80), sodium hydroxide (NaOH) and potassium dihydrogenphosphate (KH_2PO_4) were purchased from Chem-Supply (Adelaide, Australia). Heparin sodium and saline 0.9% was purchased from Lyppard Australia (Beverley, Australia). All chemicals and solvents were of analytical grade and used as received. High purity Milli-Q water was used throughout the study.

2.2. Liquid chromatography–mass spectrometry

A Shimadzu LCMS 8030 (Kyoto, Japan) equipped with a Kinetex C18 100A column (50 x 3 mm x 1.7 um) was utilised to determine unknown concentrations of lurasidone, using ziprasidone as an internal standard. A gradient elution at a flow rate of 0.5 mL/min was employed with mobile phase A and B consisting of 5 mM ammonium acetate: acetonitrile: formic acid at 95: 5: 0.1 and 5: 95: 0.1, respectively. The time program for mobile phase B was 10% (0.5 min), 100% (1.25-3.0 min) and 10% (3.5-4.0 min). Ion detection was performed in multiple reaction monitoring mode using positive electrospray ionisation. Mass to charge rations (m/z) for lurasidone was 413.2/194 and 493.2/166.1 for ziprasidone. The column oven was maintained at 40 °C and the injection volume was 10 µL. Linear calibration curves were obtained by plotting the internal standard peak area ratio against analyte concentrations. The limit of detection and limit of quantification for lurasidone was 0.3 ng/mL and 0.5 ng/mL, respectively.

2.3. Solubility studies

The equilibrium solubility of lurasidone in soybean oil, Maisine 35-1, Miglyol 812 and Capmul MCM was determined at room temperature (25 °C). Excess lurasidone was added to a centrifuge tube containing approximately 1 g of lipid. The drug-lipid suspension was sonicated (Soniclean, Adelaide, Australia) for 30 min prior to rotating for 24 h, protected from light. Samples were centrifuged (Hermle, Germany) at 35170 g for 20 min at 24 °C to sediment any undissolved drug. Dissolved drug was extracted from the supernatant using methanol, prior to dilution with mobile phase and quantification of lurasidone using LCMS.

The solubility of lurasidone was also determined in pH 7.4 phosphate buffer and FaSSIF/FeSSIF media. Excess lurasidone was added to a centrifuge tube containing 1 mL of media. The tubes were rotated 37 °C for 24 h, protected from light. Equilibrated samples were centrifuged at 35170 g for 20 min at 37 °C. The supernatants were diluted with mobile phase prior to LCMS analysis.

2.4. Preparation of lurasidone formulations

2.4.1. Nanosuspension

A 0.5% w/v lurasidone nanosuspension containing HPMC E50 was prepared using a Vibra-Cell ultrasonic processor (Sonics&Materials, Connecticut, USA). Lurasidone powder was added to an aqueous dispersion of HPMC E50 (0.25% w/v) and sonicated for 30 min to produce a uniform dispersion. Ultrasonication was then applied to the drug suspension at 90% amplification for a duration of 8 min using a 10 s on and 10 s off pulse cycle, whilst on ice. The nanosuspension was stored at 4 °C until use.

2.4.2. Nano-emulsion

Soybean lecithin (6% w/w) was dissolved in Capmul MCM (5 g) with the aid of sonication. Lurasidone (1.4% w/w) was then dissolved in the lipid solution prior to the addition of water. The resultant coarse oil-in-water emulsion was sonicated for 1 h to produce a nano-emulsion.

2.4.3. SLH microparticles

Lurasidone SLH microparticles were prepared following a previously described method [34]. An oil-in-water nano-emulsion was prepared as described above. Subsequently, an aqueous dispersion of silica nanoparticles (5% w/v) was added to the nano-emulsion to achieve a final lipid: silica ratio of 2:1. The silica-stabilised nano-emulsion was stirred

overnight at room temperature and spray dried (Büchi Mini Spray Dryer B-290 apparatus, Flawil, Switzerland) to form solid SLH microparticles. The following spray drying conditions were used: inlet temperature 160 °C, aspirator setting 100%, pump flow rate 5 mL/min and air flow rate 0.6 m^3/min.

2.5. Physicochemical characterisation

2.5.1. Determination of drug load

Solvent extraction was performed to analyse the content of lurasidone in each formulation by adding 10 mL methanol to the nanosuspension (10 µL), nano-emulsion (10 mg), or SLH microparticles (10 mg). The resulting dispersions were sonicated for 45 min to facilitate drug extraction, prior to centrifugation at 29066 g for 20 min at 24 °C. Supernatants were diluted appropriately with mobile phase for LCMS analysis.

2.5.2. Surface morphology

The surface morphology of pure lurasidone powder, nanosuspension and SLH microparticles was studied by high-resolution analytical scanning electron microscopy (SEM) (Zeiss Merlin, Oberkochen, Germany). Samples were mounted on double-faced carbon tape and sputter-coated with 10-20 nm platinum, prior to imaging at an accelerating voltage of 1-2 kV.

2.5.3. X-ray diffraction

The degree of crystallinity of encapsulated lurasidone in the nanosuspension and SLH was determined used X-ray diffraction (XRD). XRD patterns were obtained using a Malvern Panalytical Empyrean XRD (Worcestershire, United Kingdom) with θ – θ geometry. Samples were scanned between 5° and 50° (2θ) at a rate of 5 s/point and step size of 0.02°.

2.5.4. Particle size analysis

The particle size distributions of lurasidone nanosuspensions and nano-emulsions were analysed by dynamic light scattering using a Malvern Zetasizer Nano ZS (Worcestershire, United Kingdom). The formulations were redispersed in Milli-Q water (5 mg/mL) via hand-shaking prior to measurement at 25 °C. The particle size of lurasidone SLH was measured by laser diffraction using a Malvern Mastersizer (Worcestershire, United Kingdom). SLH powder was added into the sample dispersion unit until a laser obscuration of 10-20% was reached.

2.5.5. Thermogravimetric analysis

The lipid content within the SLH particles was determined using thermogravimetric analysis (Discovery TGA, TA Instruments, New Castle, DE, United States). Each sample was heated at a scanning rate of 10 °C/min from 20 to 600 °C under a nitrogen gas. As the lipid content decomposes in the temperature range of 150-450 °C whilst silica remains stable, the weight loss corresponded to the lipid content within the formulation.

2.6. *In vitro* dissolution study

In vitro dissolution studies for pure lurasidone powder, nanosuspension, nano-emulsion and SLH microparticles were performed using a USP type II apparatus (paddle method) operating at 50 rpm. Formulations equivalent to 5 mg lurasidone were added to 700 mL of pH 7.4 phosphate buffer BP with Tween 80 (1% w/v) as a surfactant, maintained at 37 °C. Aliquots were taken at pre-determined time points over a 120 min period, and replaced with an equal volume of fresh media. Samples were immediately centrifuged at 29066 g for 10 min at 24 °C. The resulting supernatant was diluted appropriately with mobile phase prior to LCMS analysis.

2.7. Drug-phase partition studies during *in vitro* lipolysis

2.7.1. Preparation of simulated intestinal lipolysis buffer

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared with biorelevant concentrations of bile salts (3 mM and 15 mM, respectively) and phospholipids (0.75mM and 3.75 mM, respectively) [45]. FaSSIF was prepared by dissolving FaSSIF/FeSSIF/FaSSGF powder (2.24 g/L) in simulated fasted-state intestinal buffer containing sodium hydroxide (0.420 g/L), sodium phosphate monobasic (3.44 g/L) and sodium chloride

(6.19 g/L) in Milli-Q water. FeSSIF was prepared by dissolving FaSSIF/FeSSIF/FaSSGF powder (11.2 g/L) in simulated fed-state intestinal buffer containing sodium hydroxide (4.04 g/L), glacial acetic acid (8.65 g/L), and sodium chloride (11.9 g/L) in Milli-Q water. The mixtures were stirred for 2 hours to allow complete dissolution and stabilisation of excipients.

Pancreatin extract (containing pancreatic lipase, colipase and other nonspecific lipolytic enzymes and proteins) was prepared by stirring 2 g of pancreatin in 10 mL intestinal buffer for 10 min, prior to centrifugation at 8450 g for 20 min at 4 °C.

2.7.2. Drug-phase partitioning

Solubilisation of lurasidone in the aqueous phase was investigated during *in vitro* lipolysis studies using a Metrohm 902 Titrando pH-stat titration apparatus (Herisau, Switzerland). Briefly, 18 mL of FaSSIF or FeSSIF was equilibrated to 37 °C in a thermostated glass vessel. The pH was manually adjusted to pH 6.45 ± 0.01 using 0.5 M NaOH or HCl. Formulation equivalent to 2 mg lurasidone was added to the simulated intestinal fluid and stirred for 1 min at 600 rpm to facilitate dispersion. Digestion was initiated by the addition of 2 mL pancreatic extract (containing approximately 2000 TBU of pancreatic lipase activity). Liberated fatty acids were immediately titrated with 0.6 M NaOH to maintain a constant pH of 6.45. Aliquots were withdrawn at pre-determined time points up to 60 min and added to centrifuge tubes containing 0.5 M 4-bromophenylboronic acid to inhibit lipase activity. Samples were centrifuged at 35170 g for 10 min at 37 °C to separate the aqueous phase from the pellet phase. The aqueous phase was extracted with acetonitrile, sonicated for 10 min, followed by centrifugation at 21130 g and 24 °C for an additional 10 min. The resulting supernatant was diluted appropriately prior to LCMS analysis.

2.8. *In vivo* oral pharmacokinetic study

Animal experiments were approved by the South Australian Animal Ethics Committee under approval number U05-20. Pharmacokinetic studies investigating the oral administration of pure lurasidone, nanosuspension, nano-emulsion and SLH were performed on 6-7 week old male Sprague-Dawley rats, obtained from the Animal Resources Centre (Canning Vale, Australia).

The rats were surgically implanted with a jugular vein cannula. After surgery, the rats were housed singularly in the Raturn™, equipped with the BASi Culex NxT (Lafayette, IN, United States), an automatic blood sampling system. Rats were allowed to recover overnight with *ad libitum* access to food and water. Prior to dosing, the rats were fasted overnight with free access to water.

Formulations were suspended in water containing 0.5% w/v carboxymethyl cellulose via vortex mixing for 30 s and sonication for 3 min, immediately before administration to the rats at a lurasidone dose of 20 mg/kg via oral gavage. Blood samples (200 µL) were automatically withdrawn from the jugular vein and collected into pre-heparinised tubes, pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 10, 16 and 24 h post-dose. Food was returned to the rats at 2 h post treatment administration. Plasma was collected by centrifugation at 5000 g and 24 °C for 5 min and stored at -20 °C until analysis.

2.9. Analysis of plasma samples

Standard lurasidone concentrations were prepared over a concentration range of 0.5-500 ng/mL. Blank rat plasma (45 µL) was spiked with lurasidone standard (5 µL) by vortex mixing for 30 s. The spiked plasma was combined with acetonitrile (90 µL) and spiked with internal standard (10 µL) by vortex mixing for 30 s. Samples were centrifuged at 37560 g for 10 min at 24 °C to precipitate plasma proteins. The supernatant was analysed via LCMS.

Thawed unknown plasma samples (50 µL) were diluted with acetonitrile (90 µL) and spiked with internal standard (10 µL). Samples were vortexed for 30 s and centrifuged at 37560 g for 10 min at 24 °C. The resulting supernatant was analysed via LCMS.

2.10. Pharmacokinetic data analysis

Pharmacokinetic analyses were conducted using Phoenix® WinNonlin® Version 8.3 (Pharsight®, a Certara™ company). Parameters were determined using non-compartmental methods (Model 200-202, Extravascular Input) and a linear-

trapezoidal approach. The maximum plasma concentration (C_{max}) and the time required to reach the maximum plasma concentration (T_{max}) was obtained directly from the plasma concentration-time profiles. The area under the curve (AUC_{0-24}) was calculated using the trapezoid rule. The terminal rate constant (λ_z) was determined from the slope of the terminal phase of the log-linear concentration-time profile. Consequently, the area under the concentration-time profile extrapolated to infinite time (AUC_{inf}) was calculated as AUC_{0-24} + the last measurable concentration / λ_z. The elimination half-life ($T_{1/2}$) was calculated as Ln(2) / λ_z. Apparent clearance (CL/F) was calculated as Dose / AUC_{inf} and the apparent volume of distribution (V_d/F) was calculated as CL/F / λ_z.

2.11. Statistical analysis

Statistically significant differences were determined using an unpaired t-test and one-way analyses of variance (ANOVA) followed by Tukey's post-test for multiple comparisons. Data was considered statistically significance when $p < 0.05$.

3. Results and Discussion

3.1. Fabrication and physicochemical characterisation of nanostructured lurasidone formulations

The selection of excipients is crucial in determining the final physicochemical properties and performance of a formulation. Although comprising of a similar particle size; the structure, physical state and drug state of nanosuspensions and nano-emulsions vary significantly. Further, by hosting the nano-emulsion within a silica carrier to fabricate SLH microparticles as a solid formulation, additional advantages can be achieved. The composition and structure of lurasidone formulations are summarised in Table 1.

Table 1: Composition and structure of lurasidone formulations. Values represent the mean ± SD, *n*=3.

Formulation	Physical state	Drug state	Solubilising agent/s	Mean Particle size (nm)	Drug loading	Encapsulation efficiency (%)	Lipid loading (%)
Nanosuspension	Liquid	Crystalline	HMPC E50	434 ± 24	0.50 ± 0.0% w/v	100 ± 0.0	-
Nano-emulsion	Liquid	Non-crystalline	Capmul MCM & soybean lecithin	292 ± 2.3	1.39 ± 0.01% w/w	99.2 ± 0.7	98.6 ± 0.0
SLH microparticles	Solid	Non-crystalline	Capmul MCM, soybean lecithin & fumed hydrophilic silica	2986 ± 400	0.73 ± 0.4% w/w	82.4 ± 2.1	65.9 ± 0.2

A 0.5% w/v lurasidone nanosuspension containing HPMC E50 as a stabiliser and precipitation inhibitor was successfully fabricated utilising an ultra-sonic probe, with particles achieving a mean diameter of 434 ± 24 nm. The results were supported by SEM, where a significant reduction in lurasidone particle size was observed for the nanosuspension compared to pure drug (Figure 2). The surface morphology of the nanosuspension appeared irregular and rough, attributable to the adsorption of HPMC E50 onto the surface of the drug crystals. Notably, it is evident that the SEM preparation process induced particle aggregation for the nanosuspension. Literature describes that in some instances, the process of nanosizing can generate non-crystalline drug [46]. This phenomenon was observed during the current study, whereby the intensity of lurasidone peaks in the XRD diffractogram of the nanosuspension were significantly reduced, suggesting a reduction in drug crystallinity compared to pure drug (Figure 3A and 3C).

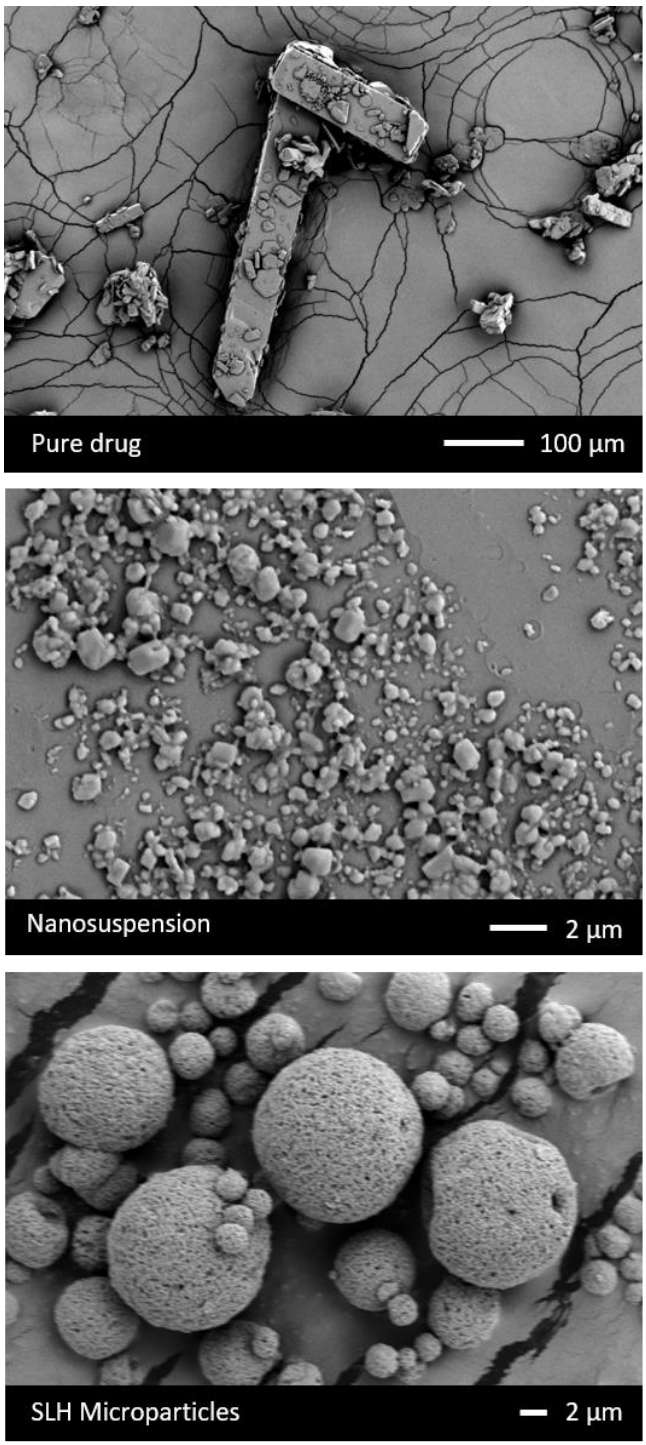


Figure 2. Scanning electron micrographs of nanostructured lurasidone formulations. Note: micrographs for the nano-emulsion are not included due to its liquid physical form.

Selection of a suitable lipid for the fabrication of the nano-emulsion and SLH formulation is critical to enhance the solubilisation of lurasidone and maximise formulation drug loading levels. Thus, the equilibrium solubility of lurasidone was investigated in various lipids of different compositions and chain lengths to determine which had the highest solubilising capacity. Among the lipids tested, soybean oil (long-chain triglyceride) provided the lowest solubility for lurasidone (8.0 ± 0.6 mg/g), followed by Maisine 35-1 (long-chain mono-, di-, and triglycerides) and Miglyol 812 (medium-chain triglyceride) with a lurasidone solubility of 12.0 ± 0.2 mg/g and 12.8 ± 0.3 mg/g, respectively. Capmul MCM, a mixture of medium chain mono- and di-glyceride medium chain fatty acids, exhibited the highest solubility for lurasidone at 14.0 ± 0.6 mg/g, and was thus chosen for further formulation development.

The nano-emulsion was fabricated by dissolving lurasidone in the lipid phase, Capmul MCM, at 100% of the equilibrium solubility to achieve a drug loading level of 1.39 ± 0.01% w/w. In order to fabricate a solid free-flowing lipid formulation, the nano-emulsion was spray dried with silica nanoparticles at a 2:1 lipid to silica ratio to reduce the impact of silica on the final drug loading and thus, ensure a satisfactory drug loading level could be achieved. In accordance with previous studies, a high lipid encapsulation efficiency of 82.4% w/w was attained within SLH, and the drug loading level reduced to 0.73 ± 0.4% w/w [43]. SLH particles appeared as porous and spherical structures with diameters of 1-4 µm (Figure 2). Tan *et al.* previously reported that the pore sizes range from 100-500 nm, attributed to the self-assembly of silica nanoparticles from the aqueous phase to the droplet surface during the dehydration process [47].

Lipid formulations are well-known for their ability to encapsulate and maintain poorly water-soluble drugs in a non-crystalline state and thus, XRD was used to characterise the state of lurasidone within SLH. A physical mixture of lurasidone in silica was examined to ensure XRD was capable of detecting lurasidone at a concentration of 0.5% w/w. No peaks were visible for SLH suggesting lurasidone was encapsulated in the non-crystalline state (Figure 3D). It was not possible to assess the nano-emulsion, however it can be hypothesised that lurasidone was also present in the non-crystalline state as it was loaded into the lipid at its equilibrium solubility.

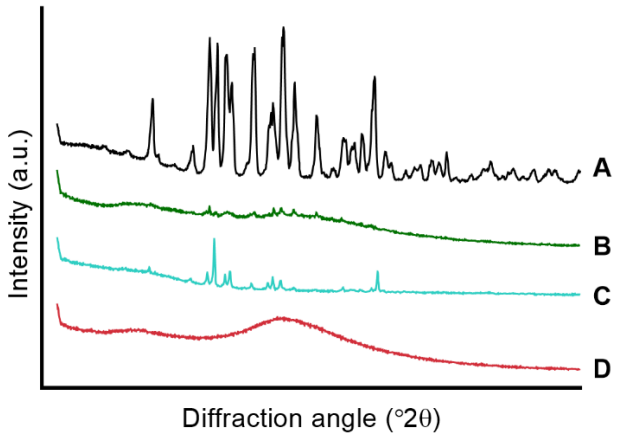


Figure 3. X-ray diffraction patterns of (A) pure lurasidone, (B) 0.5% w/w lurasidone in silica physical mixture (C) nanosuspension and (D) SLH microparticles.

3.2. *In vitro* dissolution studies

In vitro dissolution and release studies were conducted in simulated intestinal media (pH 7.4 phosphate buffer) under non-sink conditions. Due to the poor aqueous solubility of the drug in neutral pH media, even with the inclusion of a wetting agent (1% w/v Tween 80), sink conditions could not be achieved (equilibrium solubility = 1.8 ± 0.0 ug/mL).

Dissolution profiles for lurasidone are illustrated in Figure 4, with pure lurasidone powder exhibiting the lowest rate and extent of dissolution, reaching a maximum of 16.2 ± 0.7% after 120 min. Conversely, when formulated as a nanosuspension, dissolution was enhanced 4.9-fold compared to the pure drug over the experimental period (i.e. 80.4 ± 0.3% dissolution). This enhancement can be explained by the Noyes Whitney equation whereby reduced particle size and increased surface area of lurasidone nanoparticles enhances dissolution rate [48]. Furthermore, it is postulated that the inclusion of HPMC E50 in the nanosuspension reduced the prospect of drug recrystallisation and maintained a level of supersaturation by adsorbing to the surface of the particles to form a mechanical barrier for nucleation [49, 50]. It is also expected that the reduced crystallinity described in Section 3.1 may have contributed to the enhanced dissolution, compared to pure drug.

The rate of lurasidone release from the nanostructured lipid formulations was significantly faster than the pure drug and nanosuspension. Maximum drug was released from the nano-emulsion (56.5 ± 0.5%) after 10 min owing to encapsulation of the drug in a pre-dissolved state and thus, avoiding the rate-limiting dissolution observed with pure drug. In comparison, 100 ± 0.9% drug release was obtained from the SLH demonstrating the ability of silica to enhance

wettability and dispersion of the lipid droplets in the aqueous medium, thus further aiding drug release. Additionally, in comparison to the nano-emulsion, the porous structure of SLH presents a high surface-to-volume ratio which facilitates drug dissolution and solubilisation [47, 51].

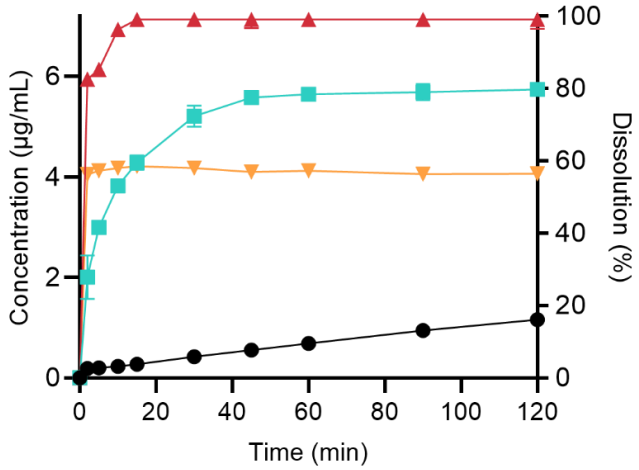


Figure 4. *In vitro* dissolution profiles of lurasidone dosed at 5 mg in 700 mL of phosphate buffer at 37 °C; pure drug (●), nanosuspension (■), nano-emulsion (▼) and SLH microparticles (▲) in simulated intestinal media (pH 7.4 phosphate buffer). Each value represents the mean ± SD, *n*=3.

It is well-established that particle size, irrespective of formulation type, is an essential factor which can alter the solubilisation and absorption of a drug; typically, the smaller the particle size, the greater absorption [48]. For example, Jinno *et al.* revealed that the bioavailability of cilostazol drug crystals was increased with reducing particle size, after oral administration to dogs [37]. Similarly, Salvia-Trujillo *et al.* demonstrated that a nano-emulsion was successful in enhancing the bioaccessability of β-Carotene, compared to a micro-emulsion [52]. However, key case studies have also shown that there is an optimal particle size for solubilisation, where further size reductions do not lead to correlate with enhanced oral absorption. This is evident in the study by Onodera *et al*. who investigated curcumin-loaded lipid nano-emulsions of various particle sizes (50, 100 and 200 nm) and found that the 100 nm particles were optimal for absorption and therapeutic efficacy [53]. In the current study, the size of SLH particles was significantly greater than the particle size of the nanosuspension and nano-emulsion. However, it is apparent that the large particle size of SLH did not have a negative impact on drug absorption and in fact, the agglomeration of silica and lipid drives release kinetics. Although dried SLH have a large particle size between 1 to 4 µm, previous studies reveal that SLH rapidly redisperse into their primary silica nanoparticles and lipid droplets upon dispersion in aqueous media [40, 43]. Therefore, favourable dissolution properties are retained even after agglomeration during spray-drying.

3.3. Solubilisation in simulated fasted and fed media under digesting conditions

As lurasidone possesses a clinically significant 2-fold positive food effect, *in vitro* solubilisation studies were conducted under simulated intestinal fasted and fed digesting conditions using biorelevant concentrations of bile salts, phospholipids and lipolytic enzymes. The selection of bile salts, phospholipid and pH can impact drug solubilisation. Here, it is hypothesised that using FaSSIF media would have negligible effect compared to media described by the Lipid Formulation Classification System (LFCS) Consortium [54]. The fasted- and fed-state solubilisation profiles for pure lurasidone, nanosuspension, nano-emulsion and SLH microparticles are depicted in Figure 5a and Figure 5b, respectively.

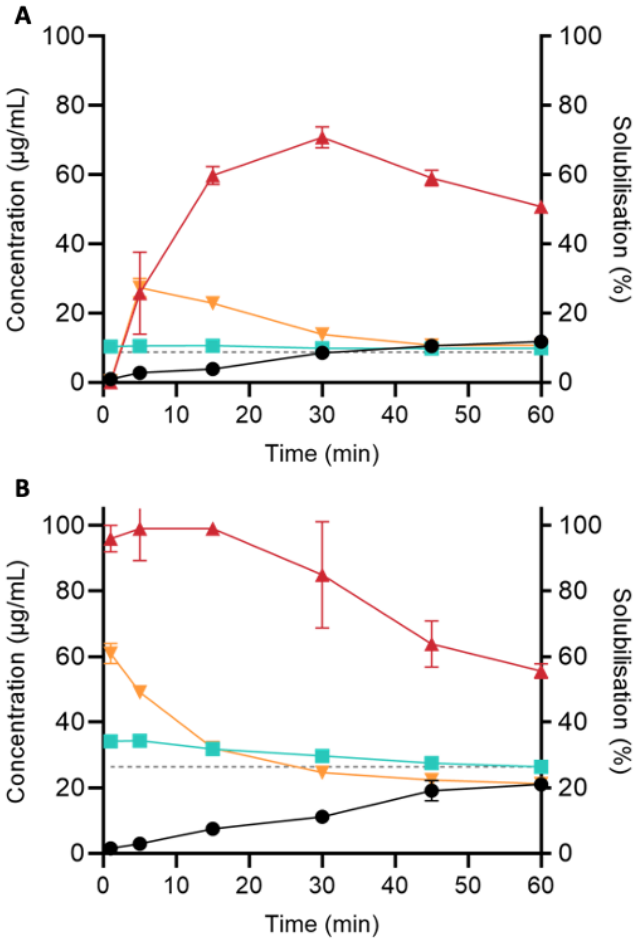


Figure 5. *In vitro* solubilisation profiles of lurasidone under digesting conditions; pure drug (●), nanosuspension (■), nano-emulsion (▼) and SLH microparticles (▲) under simulated (A) fasted and (B) fed conditions. Dotted line represents the equilibrium solubility of lurasidone in the media. Each value represents the mean ± SD, *n*=3.

It is evident that the formulation of lurasidone as a lipid-based formulation was most effective in enhancing drug solubilisation under simulated digesting conditions. In the fasted state, SLH reached a maximum lurasidone solubilisation of 70.8 ± 3.0% at 30 min, prior to undergoing gradual precipitation for the remaining experimental period (50.8 ± 1.3% at 60 min). In comparison, the nano-emulsion achieved 27.4 ± 2.6% solubilisation after 5 min decreasing to 10.7 ± 0.1% at 60 min. The enhanced performance of both lipid formulations can be attributed to (a) the encapsulation of drug in its molecularly dispersed state, thus avoiding the rate-limiting dissolution step and (b) the presence of the digestible Capmul MCM which leads to the formation of highly solubilising colloidal species, readily incorporating lurasidone and improving overall drug solubilisation, as illustrated in Figure 6. The superior drug solubilisation from SLH is in line with previous studies and is due to the porous silica matrix and high surface area facilitating lipase adsorption in its active conformation, which leads to enhanced lipid digestion kinetics [55]. Additionally, studies have demonstrated that fatty acid digestion products are repelled from the porous matrix into the aqueous phase via electrostatic repulsion due to the presence of negatively charged silica [56], and therefore, a greater concentration of solubilising digestion products is present within the simulated intestinal media.

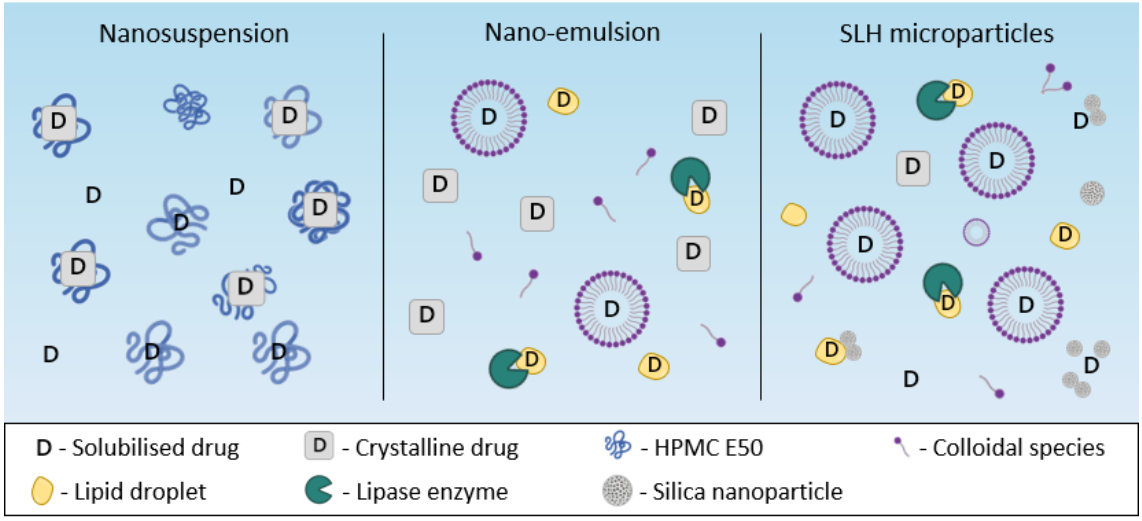


Figure 6. Schematic representation of the fasted-state drug solubilisation from nanostructured lurasidone formulations.

The fasted-state solubilisation of pure drug was slow over 60 min reaching 12.1 ± 1.0%, a significant reduction in solubilisation compared to SLH. The effect of nanosizing is highlighted whereby the nanosuspension exhibited rapid solubilisation reaching a maximum of 11.7 ± 0.9% after 1 min. The level of solubilisation was not significantly different to pure drug at 60 min ($p > 0.05$); however the nanosuspension was capable of maintaining levels of solubilisation up to 1.3-fold greater than the equilibrium solubility of lurasidone in fasted-state media throughout the 60 min period.

The lurasidone solubilisation ratio obtained in fed- and fasted-state media is depicted in Figure 7. Comparison of *in vitro* fasted- and fed-state solubilisation values showed pure lurasidone to exhibit a 1.9-fold food effect. This is comparable to the 2-fold food effect observed in humans [6], indicating that *in vitro* solubilisation experiments may be sufficient to predict the *in vivo* performance of lurasidone formulations. Although nanosizing has proven to reduce *in vitro* fasted and fed variation for numerous drugs including ziprasidone, cilostazol and MK-0869 [18, 37, 57], in the current *in vitro* solubilisation study the lurasidone nanosuspension displayed a 2.67-fold positive food effect.

A major advantage of lipid-based formulations is their ability to encapsulate drugs in a non-crystalline state which can in-turn promote supersaturated levels of drug in solution. Using the "spring and parachute" analogy which is widely described in polymer literature [58], the dispersion and rapid partition of drug from the lipid generates solubilised drug concentrations which are significantly greater than the equilibrium solubility of the drug alone, forming the 'spring'. However, as these formulations tend to contain digestible lipids, the solubilisation capacity of the formulation is reduced upon digestion, leading to drug precipitation [59, 60]. Therefore, suitable polymeric excipients should be utilised to reduce drug precipitation and form the 'parachute'. The nano-emulsion exhibited a 1.98-fold food effect. Additionally, gradual precipitation was observed over the 60 min period due to partition of drug from the lipid creating a supersaturated environment which the formulation was unable to stabilise. Therefore, recrystallisation of drug was favoured due to the thermodynamic driving force [61]. In contrast, SLH significantly reduced the food effect of lurasidone with only a 1.09-fold difference in fed/fasted solubilisation after 60 min demonstrating that silica and its nanoporous structure plays an important role in both driving solubilisation and maintaining a state of drug supersaturation in solution. Specifically, due to the presence of silica and through increasing lipid digestion products enhancing the concentration of colloidal solubilising species (i.e. lipid carriers and mixed micelles), the ability for precipitation was reduced. This highlights the benefit of a specifically engineered solid state lipid-based formulation for the delivery of poorly water-soluble drugs.

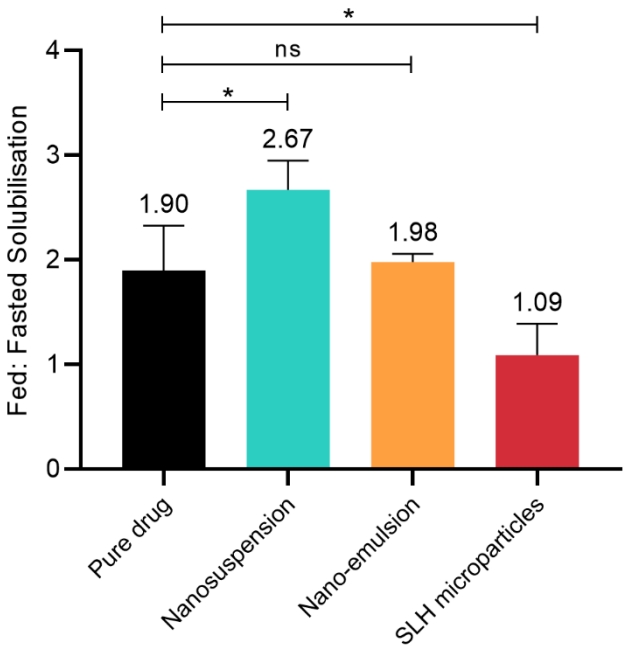


Figure 7. Ratio between lurasidone solubilisation in the fed- and fasted-state after 60 min. In comparison to pure drug, * indicates statistical significance ($p < 0.05$) and ns indicates no statistical significance.

3.4. *In vivo* pharmacokinetic study

The mean plasma concentration-time profiles for lurasidone following an oral dose of a pure drug suspension, nanosuspension, nano-emulsion and SLH to fasted Sprague-Dawley rats are illustrated in Figure 8. The corresponding pharmacokinetic parameters are summarised in Table 2.

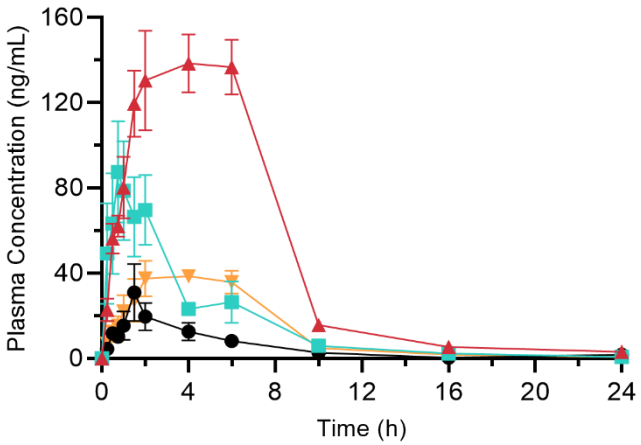


Figure 8. Plasma concentration-time profiles of lurasidone when orally administered as pure drug (●), nanosuspension (■), nano-emulsion (▼) and SLH microparticles (▲) to fasted Sprague-Dawley rats at a dose of 20 mg/kg. Values represent the mean ± SD, n=4.

Unformulated pure drug powder exhibited the lowest lurasidone exposure with an $AUC_{0\text{-}24}$ of 125 ± 36.4 ng.h/mL which can be attributed to the large crystalline particles limiting the rate and extent of dissolution and subsequent absorption. Dosing of the nanosuspension resulted in a 3-fold increase in lurasidone exposure ($AUC_{0\text{-}24}$) ($p < 0.05$) and a 1.4-fold reduction in T_{max} compared to the pure drug suspension, although statistically insignificant ($p = 0.91$). Rapid absorption from the nanosuspension emphasises the benefit of particle size reduction whereby rapid drug dissolution and solubilisation was observed. Similar observations were reported by Yu *et al*. where a 2.4-fold enhancement in bioavailability was observed when lurasidone was formulated as a nanosuspension compared to unformulated drug

[42]. It is noted that the lurasidone hydrochloride was used for this study and thus results are not directly comparable due to the difference in solubility parameters.

Table 2: Pharmacokinetic parameters for lurasidone after oral administration to Sprague-Dawley rats at a dose of 20 mg/kg. Values represent the mean ± SD, *n*=4.

Parameters	Pure drug	Nanosuspension	Nano-emulsion	SLH microparticles
T_{max} (h)	1.5 ± 0.629	0.875 ± 0.657	4.0 ± 2.0	4.0 ± 1.63
C_{max} (ng/mL)	35.4 ± 21.4	97.1 ± 37.3	46.3 ± 11	157 ± 25.4
AUC_{0-24} (ng.h/mL)	125 ± 36.4	375 ± 42.7	303 ± 41.8	1100 ± 176
AUC_{inf} (ng.h/mL)	130 ± 42.6	380 ± 40.4	308 ± 43.1	1190 ± 96.1
$T_{1/2}$ (h)	1.98 ± 0.525	4.44 ± 0.632	5.15 ± 0.545	5.42 ± 1.48
CL/F	167 ± 63.6	53 ± 5.11	65.8 ± 9.99	16.9 ± 1.36
Vd/F	456 ± 114	343 ± 76.1	485 ± 44.5	131 ± 25.4

Administration of lurasidone as a conventional liquid nano-emulsion achieved a 2.4-fold greater lurasidone exposure, compared to pure drug. The AUC_{0-24} of the nano-emulsion was not statistically different to the nanosuspension (p = 0.87) confirming that both approaches are a viable formulation strategy. Notably, formulation of lurasidone as a SLH improved exposure by 3.6- and 8.8-fold, in comparison to the nano-emulsion and pure drug, respectively ($p < 0.0001$). Lurasidone is pre-solubilised in Capmul MCM for the nano-emulsion and SLH, thus in alignment with *in vitro* findings of enhanced solubilisation, it is reasonable to suggest that the difference in performance is due to (a) the high surface area of SLH which facilitates lipase-lipid interaction supporting the formation of negatively charged and highly solubilising micelles and (b) the presence of negatively charged silica particles which repels the negatively charged micelles into the aqueous phase, promoting absorption into the intestine and enhancing bioavailability. It is noteworthy that the lurasidone bioavailability provided by SLH is significantly greater than reported in literature, whereby the exposure of lurasidone hydrochloride was enhanced 2.9-fold for a self-microemulsifying drug delivery system compared to a simple suspension [62] and 2.7-fold for a self-nanoemulsifying drug delivery system compared to the commercial formulation [9].

Furthermore, lipids and digestion products may stimulate the ileal brake, resulting in a delay in gastric emptying and prolonged small intestinal transit, thereby maximising the time for drug absorption [22, 63]. This supports the results in Table 2 whereby the $T_{1/2}$ of lurasidone increased from 1.98 h for pure drug to 5.15 h and 5.42 h for the nano-emulsion and SLH, respectively ($p < 0.005$). As $T_{1/2}$ is inversely proportional to CL, the increased $T_{1/2}$ correlates with reduced CL/F. Further studies are required for confirmation; however, it is predicted this would translate into extended therapeutic efficacy.

Rodents are the cornerstone of initial proof-of-concept formulation development [23]. Nevertheless, a sufficient rat model to mimic the gastrointestinal fed-state is yet to be developed. Furthermore, due to anatomical differences such as size, gastrointestinal length and transit time, extrapolation of rat pharmacokinetics to humans is challenging and potentially questionable [64]. More importantly, rats do not possess a gallbladder and thus secretion of bile is continuous, unlike humans, pigs and dogs where secretion is stimulated by the presence of food [65]. Hence, it is proposed that the constant secretion of bile may underestimate the solubilising potential of a lipid-formulation, compared to if administered to humans. As this study has proven that a nanosuspension and SLH are capable of mitigating the *in vitro* food effect and enhancing the fasted state oral bioavailability in rats, it would be extremely valuable to now evaluate these novel formulations in a larger animal model, such as a dog or pig, in both the fed and fasted state to elucidate if the influence of food can be entirely eliminated. Additionally, to enable clinical translation of such formulations, future direction should focus on strategies to enhance formulation drug loading levels. Our recent studies on supersaturated lipid formulations give an avenue for advancement [30, 66].

4. Conclusion

Lurasidone was successfully formulated and physicochemically characterised as a nanosuspension, nano-emulsion and SLH. All 3 nanostructured formulations were successful in enhancing the solubilisation and oral bioavailability of lurasidone, compared to unformulated drug. Specifically, SLH was capable of mitigating the *in vitro* pharmaceutical food

effect and significantly increased bioavailability of lurasidone by 8.8-fold when orally administered to Sprague-Dawley rats. Importantly, this serves as a framework for further development of SLH as a novel formulation approach for the oral delivery of lurasidone, without the need for co-administration with food to improve patient compliance.

Acknowledgments

The Australian Government is acknowledged for supporting this research by awarding Tahlia R. Meola with an Australian Government Research Training Program (RTP) Scholarship. Ian and Christine Gould are acknowledged for their generous contribution to this research. The Australian Research Council Centre of Excellence in Bio-Nano Science and Technology are recognised for their support. The authors gratefully acknowledge Ben Noll and the UniSA Core Animal Facility for their assistance during the *in vivo* study.

References

1. James, S.L., et al., *Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017.* The Lancet, 2018. **392**(10159): p. 1789-1858.
2. Vieta, E., *Improving treatment adherence in bipolar disorder through psychoeducation.* Journal Clinical Psychiatry, 2005. **66**(1): p. 24-29.
3. Perkins, D.O., *Predictors of noncompliance in patients with schizophrenia.* Journal of Clinical Psychiatry, 2002. **63**(12): p. 1121-1128.
4. Bawa, R. and J.R. Scarff, *Lurasidone: a new treatment option for bipolar depression-a review.* Innovations in Clinical Neuroscience, 2015. **12**(1-2): p. 21-23.
5. Meyer, J.M., A.D. Loebel, and E. Schweizer, *Lurasidone: a new drug in development for schizophrenia.* Expert Opinion on Investigational Drugs, 2009. **18**(11): p. 1715-1726.
6. Sunovion Pharmaceuticals Inc. *Latuda: Highlights of Prescribing Information*. 2018; Available from: https://www.latuda.com/LatudaPrescribingInformation.pdf.
7. Therapeutic Goods Administration, *Australian Public Assessment Report for lurasidone hydrochloride*, Department of Health, Editor. 2014: Australia.
8. European Medicines Agency, *Latuda (lurasidone)*. 2014: The Netherlands.
9. Miao, Y., et al., *Enhanced oral bioavailability of lurasidone by self-nanoemulsifying drug delivery system in fasted state.* Drug Development and Industrial Pharmacy, 2016. **42**(8): p. 1234-1240.
10. Van Hoogevest, P., X. Liu, and A. Fahr, *Drug delivery strategies for poorly water-soluble drugs: the industrial perspective.* Expert Opinion on Drug Delivery, 2011. **8**(11): p. 1481-1500.
11. Fahr, A. and X. Liu, *Drug delivery strategies for poorly water-soluble drugs.* Expert Opinion on Drug Delivery, 2007. **4**(4): p. 403-416.
12. Wais, U., et al., *Nanoformulation and encapsulation approaches for poorly water-soluble drug nanoparticles.* Nanoscale, 2016. **8**(4): p. 1746-1769.
13. Junghanns, J.-U.A.H. and R.H. Müller, *Nanocrystal technology, drug delivery and clinical applications.* International Journal of Nanomedicine, 2008. **3**(3): p. 295-309.
14. Keck, C.M. and R.H. Müller, *smartCrystals — Review of the Second Generation of Drug Nanocrystals*. 1st ed. Handbook of Materials for Nanomedicine. 2011: Jenny Stanford Publishing.
15. Bleeker, E.A.J., et al., *Considerations on the EU definition of a nanomaterial: Science to support policy making.* Regulatory Toxicology and Pharmacology, 2013. **65**(1): p. 119-125.
16. Guivarc'h, P.-H., M.G. Vachon, and D. Fordyce, *A new fenofibrate formulation: results of six single-dose, clinical studies of bioavailability under fed and fasting conditions.* Clinical Therapeutics, 2004. **26**(9): p. 1456-1469.
17. Jang, K., et al., *Novel nanocrystal formulation of megestrol acetate has improved bioavailability compared with the conventional micronized formulation in the fasting state.* Drug Design, Development and Therapy, 2014. **8**: p. 851-858.
18. Wu, Y., et al., *The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human.* International Journal of Pharmaceutics, 2004. **285**(1): p. 135-146.
19. Warren, D.B., et al., *Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility.* Journal of Drug Targeting, 2010. **18**(10): p. 704-731.
20. Mou, D., et al., *Potent dried drug nanosuspensions for oral bioavailability enhancement of poorly soluble drugs with pH-dependent solubility.* International Journal of Pharmaceutics, 2011. **413**(1): p. 237-244.
21. Preskorn, S., et al., *Effect of food on the pharmacokinetics of lurasidone: results of two randomized, open-label, crossover studies.* Human Psychopharmacology: Clinical and Experimental, 2013. **28**(5): p. 495-505.
22. Feeney, O.M., et al., *50 years of oral lipid-based formulations: Provenance, progress and future perspectives.* Advanced Drug Delivery Reviews, 2016. **101**: p. 167-194.
23. Hauss, D.J., *Oral lipid-based formulations.* Advanced Drug Delivery Reviews, 2007. **59**(7): p. 667-676.
24. Jaiswal, M., R. Dudhe, and P.K. Sharma, *Nanoemulsion: an advanced mode of drug delivery system.* 3 Biotech, 2015. **5**(2): p. 123-127.
25. Porter, C.J.H., N.L. Trevaskis, and W.N. Charman, *Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs.* Nature Reviews Drug Discovery, 2007. **6**(3): p. 231-248.
26. Porter, C.J.H., et al., *Enhancing intestinal drug solubilisation using lipid-based delivery systems.* Advanced Drug Delivery Reviews, 2008. **60**(6): p. 673-691.
27. Li, Y., et al., *Nanomemulsion of megestrol acetate for improved oral bioavailability and reduced food effect.* Archives of Pharmacal Research, 2015. **38**(10): p. 1850-1856.
28. Joyce, P., et al., *Solidification to improve the biopharmaceutical performance of SEDDS: Opportunities and challenges.* Advanced Drug Delivery Reviews, 2019. **142**: p. 102-117.

29. Tan, A., S. Rao, and C.A. Prestidge, *Transforming Lipid-Based Oral Drug Delivery Systems into Solid Dosage Forms: An Overview of Solid Carriers, Physicochemical Properties, and Biopharmaceutical Performance.* Pharmaceutical Research, 2013. **30**(12): p. 2993-3017.
30. Schultz, H.B., et al., *Enhancement of abiraterone acetate oral bioavailability by supersaturated-silica lipid hybrids.* International Journal of Pharmaceutics, 2020. **582**: p. 119264.
31. Tan, A., et al., *First in man bioavailability and tolerability studies of a silica–lipid hybrid (Lipoceramic) formulation: a Phase I study with ibuprofen.* Drug Delivery and Translational Research, 2014. **4**(3): p. 212-221.
32. Meola, T.R., et al., *A safety, tolerability, and pharmacokinetic study of a novel simvastatin silica-lipid hybrid formulation in healthy male participants.* Drug Delivery and Translational Research, 2021. **11**(3): p. 1261-1272.
33. Nguyen, T.-H., et al., *Silica–lipid hybrid (SLH) formulations enhance the oral bioavailability and efficacy of celecoxib: An in vivo evaluation.* Journal of Controlled Release, 2013. **167**(1): p. 85-91.
34. Tan, A., et al., *Silica-lipid hybrid (SLH) microcapsules: A novel oral delivery system for poorly soluble drugs.* Journal of Controlled Release, 2009. **134**(1): p. 62-70.
35. Joyce, P., H. Gustafsson, and C.A. Prestidge, *Engineering intelligent particle-lipid composites that control lipase-mediated digestion.* Advances in Colloid and Interface Science, 2018. **260**: p. 1-23.
36. Joyce, P., et al., *The Role of Porous Nanostructure in Controlling Lipase-Mediated Digestion of Lipid Loaded into Silica Particles.* Langmuir, 2014. **30**(10): p. 2779-2788.
37. Jinno, J., et al., *Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs.* Journal of Controlled Release, 2006. **111**(1): p. 56-64.
38. Lim, L.H., et al., *Silica-lipid hybrid microcapsules: Influence of lipid and emulsifier type on in vitro performance.* International Journal of Pharmaceutics, 2011. **409**(1): p. 297-306.
39. Tarr, B.D. and S.H. Yalkowsky, *Enhanced Intestinal Absorption of Cyclosporine in Rats Through the Reduction of Emulsion Droplet Size.* Pharmaceutical Research, 1989. **6**(1): p. 40-43.
40. Tan, A., et al., *Controlling the Enzymatic Digestion of Lipids Using Hybrid Nanostructured Materials.* ACS Applied Materials & Interfaces, 2014. **6**(17): p. 15363-15371.
41. Shah, S., et al., *Design, Optimization, and Evaluation of Lurasidone Hydrochloride Nanocrystals.* AAPS PharmSciTech, 2016. **17**(5): p. 1150-1158.
42. Yu, P., et al., *Enhanced oral bioavailability and diminished food effect of lurasidone hydrochloride nanosuspensions prepared by facile nanoprecipitation based on dilution.* Powder Technology, 2017. **312**: p. 11-20.
43. Dening, T.J., et al., *Silica encapsulated lipid-based drug delivery systems for reducing the fed/fasted variations of ziprasidone in vitro.* European Journal of Pharmaceutics and Biopharmaceutics, 2016. **101**: p. 33-42.
44. Charman, W.N., et al., *Effect of Food and a Monoglyceride Emulsion Formulation on Danazol Bioavailability.* The Journal of Clinical Pharmacology, 1993. **33**(4): p. 381-386.
45. Dressman, J., *Evolution of dissolution media over the last twenty years.* Dissolution Technol, 2014. **21**(3): p. 6-10.
46. Gao, L., D. Zhang, and M. Chen, *Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system.* Journal of Nanoparticle Research, 2008. **10**(5): p. 845-862.
47. Tan, A., et al., *Silica Nanoparticles To Control the Lipase-Mediated Digestion of Lipid-Based Oral Delivery Systems.* Molecular Pharmaceutics, 2010. **7**(2): p. 522-532.
48. Noyes, A.A. and W.R. Whitney, *The rate of solution of solid substances in their own solutions.* Journal of the American Chemical Society, 1897. **19**(12): p. 930-934.
49. Dash, R.N., et al., *Solid supersaturatable self-nanoemulsifying drug delivery systems for improved dissolution, absorption and pharmacodynamic effects of glipizide.* Journal of Drug Delivery Science and Technology, 2015. **28**: p. 28-36.
50. Gao, P., et al., *Characterization and optimization of AMG 517 supersaturatable self-emulsifying drug delivery system (S-SEDDS) for improved oral absorption.* Journal of Pharmaceutical Sciences, 2009. **98**(2): p. 516-528.
51. Tan, A., A.K. Davey, and C.A. Prestidge, *Silica-Lipid Hybrid (SLH) Versus Non-lipid Formulations for Optimising the Dose-Dependent Oral Absorption of Celecoxib.* Pharmaceutical Research, 2011. **28**(9): p. 2273-2287.
52. Salvia-Trujillo, L., et al., *Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions.* Food Chemistry, 2013. **141**(2): p. 1472-1480.
53. Onodera, T., et al., *Influence of particle size on the in vitro and in vivo anti-inflammatory and anti-allergic activities of a curcumin lipid nanoemulsion.* Int J Mol Med, 2015. **35**(6): p. 1720-1728.
54. Williams, H.D., et al., *Toward the Establishment of Standardized in Vitro Tests for Lipid-Based Formulations. 2. The Effect of Bile Salt Concentration and Drug Loading on the Performance of Type I, II, IIIA, IIIB, and IV Formulations during in Vitro Digestion.* Molecular Pharmaceutics, 2012. **9**(11): p. 3286-3300.
55. Simovic, S., et al., *An oral delivery system for indomethicin engineered from cationic lipid emulsions and silica nanoparticles.* Journal of Controlled Release, 2010. **143**(3): p. 367-373.

56. Joyce, P., et al., *Porous nanostructure controls kinetics, disposition and self-assembly structure of lipid digestion products.* RSC Advances, 2016. **6**(82): p. 78385-78395.
57. Thombre, A.G., S.M. Herbig, and J.A. Alderman, *Improved Ziprasidone Formulations with Enhanced Bioavailability in the Fasted State and a Reduced Food Effect.* Pharmaceutical Research, 2011. **28**(12): p. 3159-3170.
58. Guzmán, H.R., et al., *Combined Use of Crystalline Salt Forms and Precipitation Inhibitors to Improve Oral Absorption of Celecoxib from Solid Oral Formulations.* Journal of Pharmaceutical Sciences, 2007. **96**(10): p. 2686-2702.
59. Anby, M.U., et al., *Lipid Digestion as a Trigger for Supersaturation: Evaluation of the Impact of Supersaturation Stabilization on the in Vitro and in Vivo Performance of Self-Emulsifying Drug Delivery Systems.* Molecular Pharmaceutics, 2012. **9**(7): p. 2063-2079.
60. Brouwers, J., M.E. Brewster, and P. Augustijns, *Supersaturating Drug Delivery Systems: The Answer to Solubility-Limited Oral Bioavailability?* Journal of Pharmaceutical Sciences, 2009. **98**(8): p. 2549-2572.
61. Bhugra, C. and M.J. Pikal, *Role of thermodynamic, molecular, and kinetic factors in crystallization from the amorphous state.* Journal of Pharmaceutical Sciences, 2008. **97**(4): p. 1329-1349.
62. Patel, M.H. and K.K. Sawant, *Self microemulsifying drug delivery system of lurasidone hydrochloride for enhanced oral bioavailability by lymphatic targeting: In vitro, Caco-2 cell line and in vivo evaluation.* European Journal of Pharmaceutical Sciences, 2019. **138**: p. 105027.
63. Spiller, R.C., et al., *Further characterisation of the ileal brake; reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY.* Gut, 1988. **29**(8): p. 1042.
64. Henze, L.J., et al., *The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review.* Journal of Pharmacy and Pharmacology, 2019. **71**(4): p. 581-602.
65. Lentz, K.A., *Current Methods for Predicting Human Food Effect.* The AAPS Journal, 2008. **10**(2): p. 282-288.
66. Almasri, R., et al., *Porous Nanostructure, Lipid Composition, and Degree of Drug Supersaturation Modulate In Vitro Fenofibrate Solubilization in Silica-Lipid Hybrids.* Pharmaceutics, 2020. **12**(7).