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Controlling drug release by introducing lipase inhibitor within a lipid formulation

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ABSTRACT

Drug overdose connected to marketed pharmaceutical products, particularly opioids, occurs at an alarming rate. Novel strategies through innovative formulation approaches that reduce the likelihood of overdose while allowing safe therapeutic outcomes are urgently required. The current study provides a proof-of-concept for a new formulation approach by co-formulating drug with a lipase inhibitor within a solid lipid formulation in order to prevent or reduce the harmful effects of taking multiple doses of an oral solid dose form. Lipase inhibitor controlled-release (LICR) formulations were created using a simple hot melt method to co-formulate the inhibitor (orlistat) and ibuprofen, as the model drug, within the lipid matrix. The digestion and drug release kinetics were determined using an *in vitro* lipolysis model. Above a threshold level of orlistat there was decreased digestibility of multiple doses of the LICR formulations, leading to reduced drug release. Upon administration of the formulations in capsules to rats, the LICR formulation displayed the lowest exposure of ibuprofen during the pharmacokinetic studies. This novel formulation approach shows promise in preventing accidental drug overdose after oral administration of multiple doses of formulation.

1. Introduction

Drug overdose is commonly linked to illicit drugs such as heroin and cocaine, although recent trends show an alarming increase in deaths connected to marketed pharmaceutical products, particularly opioids (Martins et al., 2015). Accidental overdose, for example from an elderly patient misreading labels or a curious child accessing the family medicine cabinet and consuming paracetamol or ibuprofen can result in severe or even fatal health outcomes (Waring, 2012; Kanabar, 2017; Lodise et al., 2012). This highlights the immediate need for safer oral medication. A range of measures are taken to avoid overdose from occurring including legislative/prescription controls, education, packaging and formulation approaches, however the combination of these still have not made a significant impact on the problem (Martins et al., 2015; Paulozzi, 2012). Of these, formulation approaches such as encapsulation offer a means to prevent deliberate extraction of the drug (Litman et al., 2018; Alexander et al., 2014), but do not prevent unintentional overdose, or the impact of deliberately taking well over the prescribed or safe number of units for marketed medications. Some attempts have been made to form oral multi-dose abuse formulations (Nukala et al., 2019; Patki et al., 2020) although none have yet to become available on the market.

This study presents the exploration of a proof-of-concept for a new formulation approach in controlling oral drug release with the intention of being utilised to prevent accidental overdose. Poorly water-soluble drugs constitute approximately 80–90% of the new drug candidates (Wulff-Pérez et al., 2014), and it is well recognised that their bioavailability is often enhanced when co-administered with lipids in e.g. a fatty meal or in a formulation. It is recognised that the digestion of lipids in the gastrointestinal tract provides a colloidal lipidic environment into which a solid drug can dissolve and then subsequently be absorbed by the intestinal epithelium. The digestion process begins in the mouth under the action of lingual lipase, transitioning to the stomach where up to 30% of lipids are broken down by gastric lipase (Liao et al., 1984). The process of digestion produces diglycerides (and free fatty acids) which are then further digested in the small intestine primarily by pancreatic lipase. This lipase plays the most crucial role in efficiently digesting triglycerides into free fatty acids and *sn-2* monoglycerides through the hydrolysis of the ester linkage between the fatty acids and the glycerol backbone at the *sn-1* and *sn-3* positions (Widmaier, 2013). The presence of these lipids in the small intestine also acts as a signal to the gallbladder to contract and release biliary fluid, which contains

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phospholipids, cholesterol and bile salts, to further help with solubilisation and absorption (Salentinig et al., 2013; Kalantzi et al., 2006; Kalepu et al., 2013).

It is also known that the digestion of lipids can be reduced or prevented with the addition of compounds that inhibit pancreatic lipase (also known as pancreatic lipase inhibitors). These compounds inhibit the activity of lipase by occupying the binding pocket of the triglyceride which prevents lipolysis (de la Garza et al., 2011). In the absence of digestion, these lipids cannot be absorbed into the circulation and are therefore excreted through the faeces (Padwal and Majumdar, 2007). Consequently, lipase inhibitors have been typically marketed as anti-obesity drugs because the malabsorption of lipids limits calorie intake (de la Garza et al., 2011). Decreasing the level of absorption of dietary fats thereby can prevent multiple health risks such as diabetes, cardiovascular diseases and some forms of cancer, while keeping an individual in a healthier state (Mathus-Vliegen et al., 2004). Orlistat (tetrahydrolipstatin) is an inhibitor of gastric and pancreatic lipase and exerts its effect by binding with the active site of the lipase forming a stable complex and inducing a conformational change (Padwal and Majumdar, 2007; McNeely and Benfield, 1998). This leads to acylation of a hydroxyl group on serine within the active site, causing the lipase to become inactive. Orlistat has previously been loaded in gel lipid systems with the intention to control drug release showing its potential as a modified-release excipient (Dully et al., 2020).

By understanding the essential process of lipid digestion along with the nature of its corresponding inhibitory compounds, we considered the concept of using co-formulation of drug with an inhibitor within a solid lipid formulation in order to prevent or reduce lipase-induced drug release, thereby reducing the harmful effects of taking multiple inadvertent or deliberate doses of an oral solid dose form. While enabling release of a therapeutic dose of the drug, the coincidental release of the lipase inhibitor during digestion acts to block the further action of lipase in breaking down subsequently administered solid lipid dosage units, resulting in a self-regulating mechanism that can slow down or prevent release of drug after consumption above a number of units (Fig. 1). We have termed these formulations lipase inhibitor controlled-release (LICR) formulations. The need for a solid lipid arises due to the potential for immediate rapid release of drug by partitioning from an emulsified liquid lipid system even in the absence of digestion. It should be noted that although lipid formulations are typically used to increase the bioavailability of poorly water-soluble drugs, in this case the lipid matrix could conceptually host solid drug particles independent of their properties and thus could be a potential 'universal' formulation approach.

In this study we have sought to test the hypothesis behind these formulations. Ibuprofen sodium salt (as a model drug) was incorporated into a solid lipid matrix (Gelucire 43/01) along with orlistat (lipase inhibitor) to create lipase inhibitor controlled-release (LICR) formulations. The effects of differing concentrations of orlistat on the extent of lipid digestion were investigated and the link to the rate and extent of drug release was determined. Two LICR dosing methods were utilised in these experiments, sequential and concurrent. Sequential dosing refers to when the second formulation is introduced into the system at some nominal duration after the first dose (in this study 30 min) to allow for digestion and release of both drug and inhibitor prior to encountering the second dose. Concurrent dosing refers to the situation when multiple formulations are given together at the start of the digestion process. These dosing methods were selected as a simple model to understand the impact of the formulation on drug release after oral administration by simulating overdose scenarios. A subsequent *in vivo* study was also conducted to initially understand the impact of co-formulation of the lipase inhibitor with drug in a LICR formulation on the drug pharmacokinetics after oral administration of mini-capsules in rats. A schematic of the overall study is shown in Fig. 2.

2. Materials and methods

2.1. Materials and reagents

Ibuprofen sodium salt (>98%), 4-bromophenylboronic acid (4-BPBA, >95%), Trizma® maleate (reagent grade), sodium taurodeoxycholate hydrate (NaTDC, ≥95%) and triethylamine (TEA, ≥99 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gelucire® 43/01 (a mixture of C_8-C_{18} di- and tri-acylglycerols and free polyethylene glycol esters) was obtained from Gattefossé (France). Hydrochloric acid (HCl, 36%) was purchased from LabServ (Longford, Ireland). Sodium chloride (>99%) was purchased from Chem Supply (South Australia, Australia). Orlistat was purchased from both Sigma-Aldrich (St. Louis, MO, USA) (>98%) and Selleck Chemicals (Texas, USA) (>97.6%). DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine, >99%) was purchased from Cayman Chemical (Michigan, USA). Calcium chloride dihydrate (>99%) and sodium hydroxide pellets (>97%) were obtained from Ajax Finechem (Seven Hills, NSW, Australia). Sodium azide (NaN_3) (>99%), methanol, chloroform and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Orthophosphoric acid (OPA, >85%) was purchased from Univar (Illinois, USA). USP grade porcine pancreatin

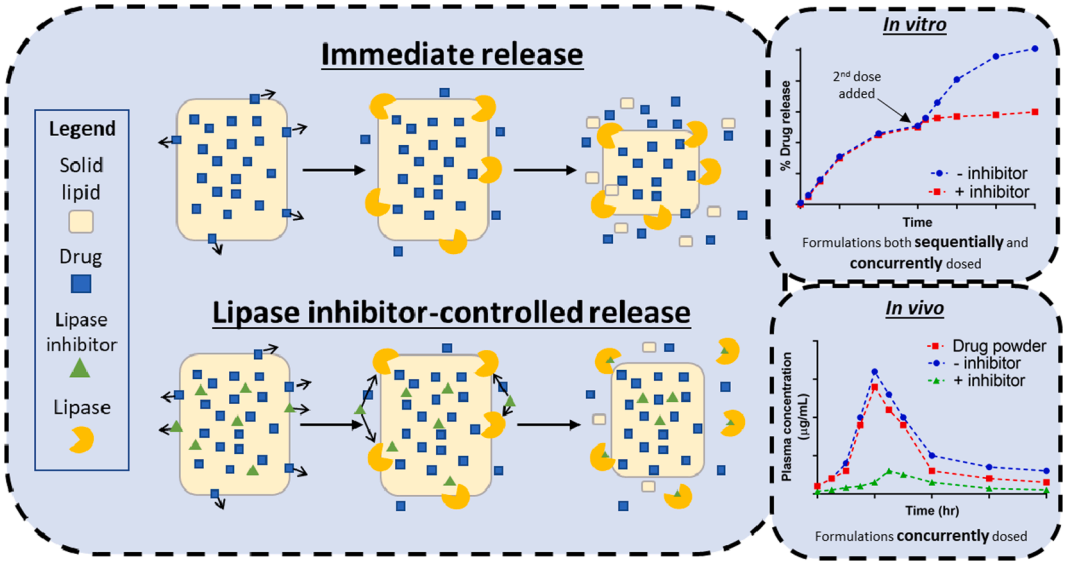

Fig. 1. Schematic of the lipase inhibitor-controlled release (LICR) concept along with hypothesised results from *in vitro* and *in vivo* experiments.

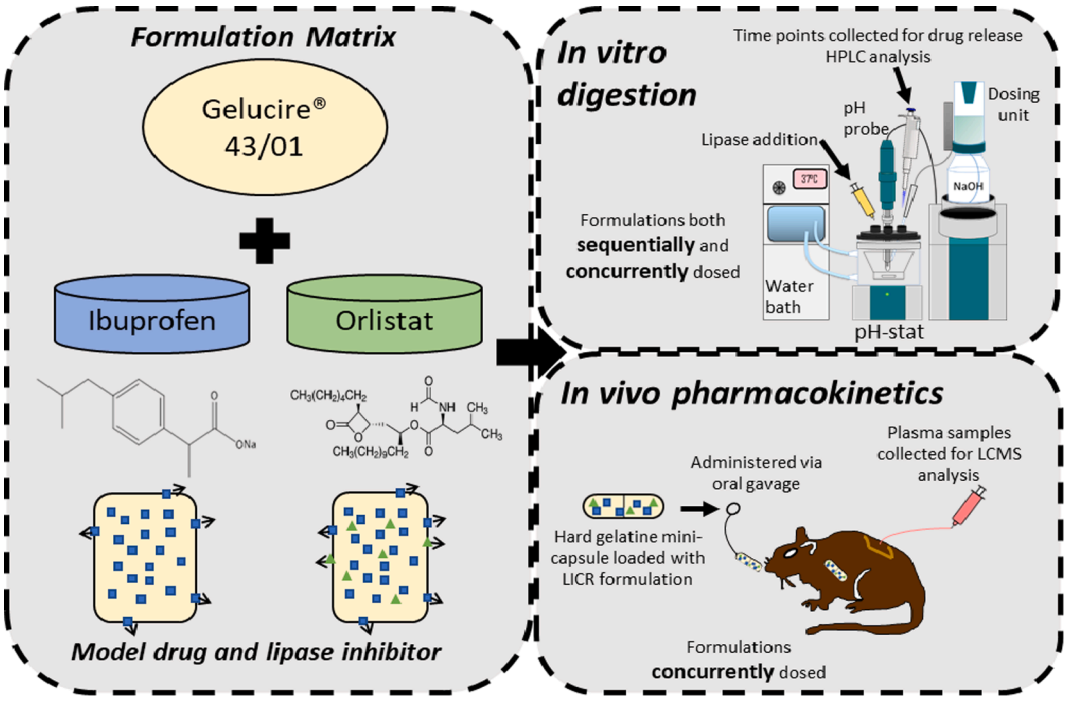

Fig. 2. Schematic of the proof-of-concept *in vitro* and *in vivo* study of novel lipase inhibitor controlled-release (LICR) formulations. The lipid formulations, with and without orlistat, were produced, subjected to *in vitro* lipolysis in a pH-stat model and drug release analysed with HPLC. LICR formulations were subsequently administered to rats in mini-capsules and the pharmacokinetics of the drug was compared to formulations without inhibitor and lipid.

extract was purchased from Southern Biological (Nunawading, Victoria, Australia). Water used in this study was from a Milli-Q purification system (Billerica, USA).

2.2. Preparation of LICR formulations

Lipid formulations comprised of Gelucire® 43/01 and differing combinations of ibuprofen sodium salt and/or orlistat (Table 1) were prepared using a hot melting method (Vithani et al., 2017). Ibuprofen, orlistat and Gelucire® 43/01 were weighed into 4 mL glass vials and melted over a hot plate at 40-45C. The molten formulation was drawn into a syringe (diameter = 15.8 mm) and placed into dry ice for 10 min to freeze the formulations in a cylindrical shape. The solid formulations were removed from the syringe and stored in clear glass scintillation vials (see Supporting Information section S1).

2.3. Thermal properties of formulations

Thermal properties of the non-dispersed lipid formulations and of each individual formulation component were assessed using differential scanning colorimetry (DSC). Experiments were performed using a PerkinElmer DSC 8500 system attached to a PerkinElmer Intercooler 2 cooling accessory. Approximately 3–5 mg of each component was weighed into 50 μL DSC aluminium pans and sealed with a pan lid. Samples were analysed under a dry nitrogen environment, from 0 to 200 °C, at a scanning rate of 5 °C/min against a blank reference.

Table 1
Composition of LICR formulations and the inhibitor-free control investigated in this study.

Substances	Control (0% ORL) (w/w)	0.5 % ORL (w/w)	0.1% ORL (w/w)	0.01% ORL (w/w)	0.005% ORL (w/w)
Gelucire® 43/01	95	94.5	94.9	95	95
Ibuprofen sodium salt	5	5	5	5	5
Orlistat	–	0.5	0.1	0.01	0.005

2.4. In vitro lipolysis of LICR formulations

A Metrohm Titrando 902 pH-stat apparatus was utilised for *in vitro* lipolysis of the LICR formulations, for both single and double dose (sequential and concurrent) experiments (Porter et al., 2007; Zangenberg et al., 2001; Phan et al., 2015). For a single dose experiment, LICR formulation (100 mg) was added to the digestion vessel containing 13.5 mL of fed-state simulated intestinal fluid (20 mM sodium taurodeoxycholate/5 mM DOPC in digestion buffer) at pH 7.5. Digestion buffer contained 50 mM Trizma® maleate, 150 mM NaCl, 6 mM NaN_3 and 5 mM $CaCl_2$. For double dose experiments simulating overdose scenarios, sequential (second singular dose was added 30 min after digestion of the first dose) and concurrent (two singular doses introduced at 0 min before digestion initiation) dosing of the LICR formulations was performed. Pancreatic lipase (1.5 mL with lipase activity about 700 tributyrin unit/mL) was added to the vessel after the initial 5 min dispersion period to initiate digestion of the lipid formulation. The pH was maintained at 7.5 during digestion using an auto-burette with 0.2 M NaOH as the titrant. All the digestion experiments were performed for 60 min at 37°C, and samples (200 μL) were aspirated at the specific time points into 1.5 mL microcentrifuge tubes pre-filled with 20 μL of lipase inhibitor (0.5 M 4-BPBA in methanol). A back-titration was performed at the end of the 60 min digestion period where the pH was increased to 9 using the NaOH titrant. This allowed for quantification of unionised fatty acid to determine to the final % digestion of the lipids.

2.5. HPLC assay for quantification of drug release

Collected timepoint samples were centrifuged at 16162 × g for 15 min, after which 100 μL of the supernatant was removed and diluted 1 in 10 v/v with buffer A and centrifuged again using the same parameters. The resulting supernatant (400 μL) was transferred directly into 2 mL HPLC vials with glass inserts. The amount of ibuprofen released during

the course of digestion was quantified using a reverse phase HPLC assay. A 4.6 × 75 mm Waters Symmetry® C18 (5 μm) analytical column (Waters corp., Milford, Massachusetts, USA) was utilised. The HPLC system consisted of a Shimadzu CBM-20A system controller, DGU-20A5 degasser, two LC-20AD solvent pump modules, SIL-20A auto sampler and a CTO-20A column oven, coupled to an SPD-20A UV/Vis detector (Shimadzu Corp., Kyoto, Japan). Ibuprofen was separated using a gradient method with ACN as the organic phase (buffer B) and an aqueous phase of ultrapure water with TEA and OPA at 0.1% and 0.04% v/v of the total solution (buffer A). The initial mobile phase composition was maintained at 5% B for 1 min, changed linearly to 65% B for 6 min (1–7 min), then changed to 95% B over 1 min (7–8 min) and finally returned to 5% B in 0.5 min with an extra 0.5 min hold (8–9 min). The injection volume was 20 μL and samples were eluted at a flow rate of 1 mL/min with the column temperature set to 40 °C. Ibuprofen was detected using a UV detector at the maximum wavelength of 221 nm. Ibuprofen standards were prepared directly from a 100 μg/mL stock solution and diluted to a range of 2–60 μg/mL with buffer A.

2.6. *In vivo studies with LICR formulations*

All animal procedures were approved and experiments were conducted in accordance with the guidelines of the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee (AE No. 19290).

2.6.1. *Preparation of LICR formulations for oral administration*

The LICR formulations for oral administration were prepared in the same manner as in the *in vitro* lipolysis experiments described in section 2.2. For *in vivo* experiments, the control (0% ORL w/w), ORL 0.5% w/w and neat ibuprofen powder were tested. Hard gelatin mini-capsules suitable for rats (Size 9, Torpac Inc, USA) were filled with 25 mg of the lipid formulations equivalent to 5 mg/kg of ibuprofen and, in the case of the ORL 0.5% w/w formulation, 0.42 mg/kg of orlistat per rat. Ibuprofen powder (1.25 mg) was directly added to the capsules for the drug-only treatment. The formulations were stored in clear glass vials until administration.

2.6.2. *Surgical and administration procedures*

Animal studies were conducted as described previously using male Sprague-Dawley rats weighing 250–300 g (Pham et al., 2017). Isoflurane (5% v/v for induction, 2.5% v/v for maintenance (Abbot Laboratories, NSW)) was used as the inhaled anesthetic for the duration of the surgical procedure. Cannulation of the right carotid artery was conducted using 0.8 × 0.5 mm (o.d. × i.d.) polyethylene tubing filled with heparinised saline (2 international units (IU) of heparin in 0.9% saline). A harness and swivel system were connected to the rats to allow non-invasive blood collection after the oral administration. Rats were fasted for at least 12 hr prior to and 8 hr after administration, with water provided *ad libitum*.

Capsules were administered via an oral gavage in a concurrent manner, *i.e.* two doses at the same time. Blood samples (200 μL) were collected from the cannula at t = 0, 30, 60, 90, 120, 150, 180 min, 4, 6, 8, and 24 hr after administration and dispensed into 1.5 mL microtubes pre-filled with 10 IU of heparin and centrifuged at 16162 × g for 3 min before removing and freezing 2 × 50 μL aliquots of plasma. Two capsules of each formulation were administered to n=4 rats per treatment.

2.6.3. *LCMS assay for quantification of ibuprofen in plasma*

Plasma samples were analysed using liquid chromatography coupled to mass spectrometry (LCMS) to determine ibuprofen concentrations in plasma. Ammonium formate buffer in water (10 mM pH 3.5, 150 μL) was added directly to the 50 μL aliquots of plasma and vortexed for 2 min. The internal standard (IS) solution of flurbiprofen (200 μL at 10 μg/mL in *tert*-butyl methyl ether (TBME)) was added and the mixture was vortexed for a further 2 min and centrifuged at 16162 × g for 20 min. The supernatant was collected and aspirated into new microcentrifuge tubes. The solvent was evaporated overnight under a fume hood and the drug reconstituted in 100 μL of ammonium formate in water (10 mM, pH 6.5). Samples were vortexed for 2 min and pipetted directly into HPLC vials for analysis. The samples were analysed on a triple quadrupole mass spectrometer with a Shimadzu Nexera Autosampler (Model SIL-30AC MP), a Shimadzu Nexera Liquid Chromatograph (Model LC-30AD), a Shimadzu Prominence Communications Bus Module (Model CBM-20A), a Shimadzu Prominence Degasser (Model DGU-20A5) and Shimadzu LCMS Liquid Chromatograph Mass Spectrometer. The system was controlled and data analyses were performed with the Shimadzu LabSolutions software. A Phenomenex Gemini, 50 × 2 mm C18 (3 μm) analytical column with a C18 cartridge security guard column (Phenomenex) was utilised. Ibuprofen was separated using a gradient method with ACN as the organic phase (buffer B) and an aqueous phase of ammonium formate in water (10 mM, pH 6.5) (buffer A). The initial mobile phase composition was maintained at 15% B for 3 min, changed linearly to 50% B for 2.5 min (3–5.5 min), then held at 50% B for 1.5 min (5.5–7 min) and finally returned to 15% B in 1 min with an extra 1 min hold (7–9 min). The injection volume was 4 μL and samples were eluted at a flow rate of 0.4 mL/min with the column temperature set to 40 °C. The ibuprofen peak eluted at approximately 3.5 min and flurbiprofen at 4.2 min. The MS was operated in the negative ion mode (ESI^-), detector voltage 10 kV, collision energy at −10, dwell time 100 msec, nebulising gas flow 3 L/min, drying gas flow 15 L/min and heat block and DL temperature both at 250 °C. The transitions from m/z 206 → 91 and 242 → 198 were chosen for ibuprofen and flurbiprofen respectively.

2.6.4. *Calculation of pharmacokinetic parameters*

The pharmacokinetic results were plotted as ibuprofen concentration (ng/mL) vs. time (hr) with errors calculated as standard error of the mean (SEM). Pharmacokinetic parameters C_{max} and T_{max} were determined from the normalised data for each formulation (concentrations normalised to a 5 mg/kg dose), and the truncated area under the curve ($AUC_{0\text{-}8}$) was calculated using the trapezoidal rule. GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA USA) was used to statistically analyse differences in the data using an independent-samples *t*-test for comparisons assuming equal variances and a one-way analysis of variance (ANOVA), with statistical significance assumed when $p < 0.05$.

3. Results

3.1. *Thermal behaviour of LICR formulations*

The thermal behaviour of the LICR formulations was compared to the individual bulk components using DSC (Fig. 3). The results for the bulk components in panel (a) show that Gelucire® 43/01 began its transition from a solid state to semi-solid state at approximately 36°C and continued until the lipid was completely melted at 54°C. This extended period of melting can be attributed to the different hydrocarbon chain lengths found within the lipid, which reflect the composition of Gelucire® 43/01 of C_{12} – C_{18} chain length fatty acids. The orlistat melting peak appeared at 46°C in the same region as Gelucire® 43/01, similar to that seen in Singh. A (Singh, 2011) although a sharper peak was observed, likely due to the slower scanning rate of 5°C/min compared to the reported 10°C/min. A peak at 102°C is observed for the ibuprofen sodium sample, which contradicts the literature melting point of 199°C (Zhang et al., 2003). To confirm this result, the ibuprofen sodium salt was placed under a hot stage microscope where melting was observed between 197 and 199°C as advised in the literature (see Supporting Information section S6). This suggests the peak seen in the DSC thermograph was a result of evaporating bound water or from impurities in the > 98% purity sample, rather than the melting of the crystalline drug (Ahmed, 2020). Fig. 3b highlights the similarities in phase transition for the formulations regardless of orlistat concentration. The LICR formulations are composed of approximately 95% lipid and therefore show

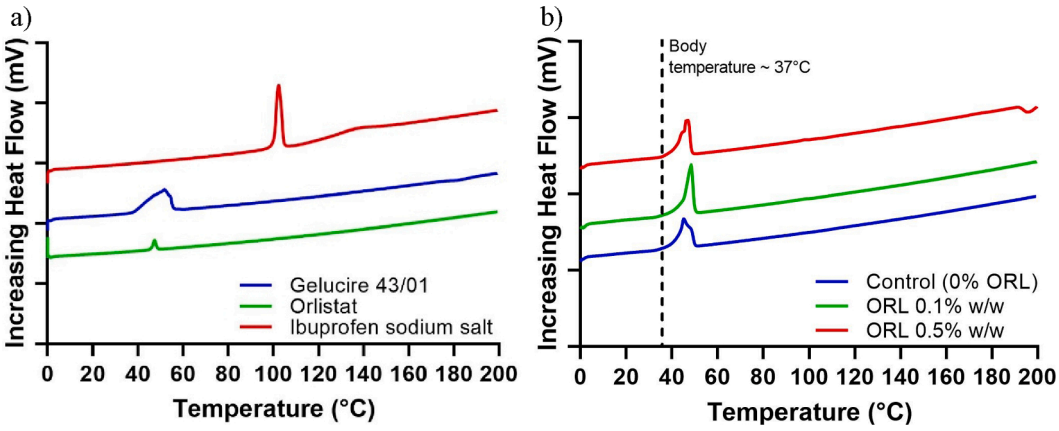

Fig. 3. Thermal analysis of (a) bulk components and (b) LICR formulations using differential scanning calorimetry (DSC). Peaks in the thermograms have been offset for ease of viewing.

thermal properties similar to the bulk lipid product.

As observed from Fig. 3b, the thermograms for the control and LICR formulation are very similar with the phase transition beginning at 40°C and complete melting occurring by 50°C, indicating that orlistat and ibuprofen do not alter the melting behaviour of the lipid. No peak was present in the 101–105°C range therefore it is assumed that the bound water or impurities have not affected the overall thermal behaviour of the formulations. It is also assumed that the ibuprofen sodium salt has molecularly dispersed throughout the lipid similar to formulations made by Grochowicz et al (Grochowicz and Kierys, 2015). All formulations began transitioning towards the melting at temperature slightly above 37°C. This suggests that in human body temperature conditions, the formulations would remain intact and no drug load dumping would occur from the matrix being compromised.

3.2. Digestibility of LICR formulations

Digestibility of the LICR formulations in both sequentially and concurrently dosed experiments are shown in Fig. 4. The digestion behaviour observed was similar between both dosing procedures of the LICR formulations; where an increase in orlistat concentration lead to a lower extent of lipid digestion. An additional concentration of orlistat at 0.01% w/w was included in these experiments in attempt to bridge the gap between the ORL 0.005% w/w and ORL 0.1% w/w formulations tested in the initial single dose experiments (see Supporting Information S4 for profiles for single dose digestions). The % extent of digestion is shown relative to the digestion of the control formulation as this allows for the easiest comparison between the formulations. All measurements have also been conducted after 5 min from the beginning of the digestion to allow for the pH to settle after the addition of fresh lipase.

Fig. 4 confirms that the extent of lipid digestion is not dependent on the dosing sequence as in both cases the control formulations digested to a greater extent than any LICR formulation. Panel (a) shows the digestion results from sequentially dosed LICR formulations. The digestion behaviour between formulations was indistinguishable until the 10 min mark. From 10 to 30 min the formulations acted similarly to the equivalent single dose experiment (Supporting Information section S4), with the ORL 0.1% w/w and ORL 0.5% w/w formulations plateauing and remaining at 13% digestion while the other formulations continued to digest to 37% (control 0% w/w), 25% (ORL 0.005% w/w) and 19% (ORL 0.01% w/w). After addition of the second dose, the control formulation continued to digest over the next 30 min while the final extent of lipid digestion for orlistat-containing LICR formulations varied between 18 and 41% depending on concentrations of the orlistat. Unlike the control formulation, addition of the second dose clearly inhibited the rate of digestion for the orlistat-containing formulations. The inhibition of the lipase is therefore noticeable suggesting that orlistat was

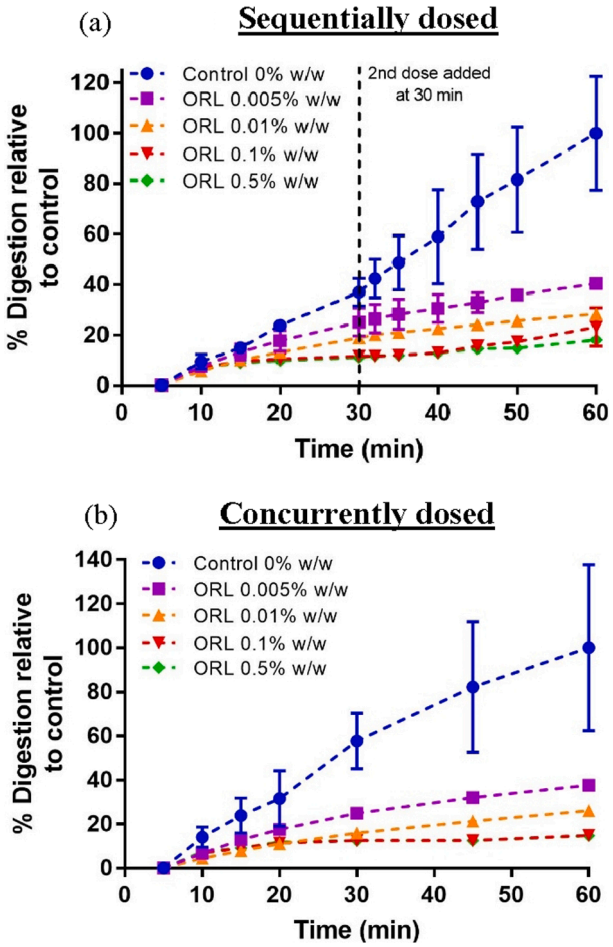

Fig. 4. Effect of orlistat concentration on the digestibility of (a) sequentially dosed and (b) concurrently dosed LICR formulations relative to the control (ORL 0% w/w). The presence of orlistat decreased the extent of digestion with a direct correlation between the amount of orlistat and digestion. Error bars represent standard deviation (n=3).

successfully released from the LICR formulations, and that the magnitude of inhibition correlates broadly with the orlistat content.

When the two doses were added concurrently, all of the LICR formulations digested considerably less than the control and with a similar trend to sequentially-dosed LICR formulations (Fig. 4 panel (b)). All orlistat-containing formulations digested less than the control at 10 min

from where the differences between the digestion behaviour of the formulations became evident. The formulations containing orlistat at the higher levels (≥ ORL 0.1% w/w) behaved identically for the duration of the experiment, digesting to 15%, while the other orlistat-containing formulations gave a final % extent of digestion of 26% (ORL 0.01% w/w) and 38% (ORL 0.005% w/w) respectively. More rapid inhibition of the lipase was noticeable when compared to sequentially-dosed experiments, presumably as a consequence of twice the amount of orlistat being available right from the beginning of the experiment compared to the sequential dosing scenario. At the 20 min mark, the orlistat-containing formulations only digested to approximately half the extent of the control, whereas for the sequential dosing experiments all formulation digestions were closer with respect to extent of digestion.

3.3. Drug release from digesting LICR formulations

Samples were retrieved during *in vitro* digestion of the LICR formulations and drug release was determined over time (Fig. 5). In both dosing sequences a burst release phenomenon was evident from all formulations with this initial release being attributed to drug present on the surface of the formulations dissolving initially, while the rest of the drug remained within the lipid matrix requiring digestion to be released over time. The burst release for concurrently dosed experiments was twice the amount seen from the sequentially dosed formulations as two doses were present from the start. Burst release is also seen between 30 and 32 min during the sequential dosing, as any drug present on the surface of the second dose is released into the digestion medium. The burst release was present in all formulations regardless of the presence of orlistat.

With respect to drug release from the sequentially dosed LICR formulations (Fig. 5 panel (a)), two distinctive groups of formulation behaviour were apparent. The two formulations containing a high level of orlistat (≥ ORL 0.1% w/w) showed clear suppression of drug release, achieving a total of 12–14% drug release prior to the addition of the second dose. After 60 min digestion, about 37% of the ibuprofen was released from the ORL 0.1% w/w formulation and 34% of the ibuprofen was released from the ORL 0.5% w/w formulation. The drug release profiles from the less concentrated formulations (≤ ORL 0.01% w/w) behaved similarly to the control. Only after 40 min was there any clear difference between the formulations as the drug release from both orlistat-containing formulations began to plateau finishing at 65% (ORL 0.1 w/w) and 80% (ORL 0.005% w/w) respectively.

Panel (b) of Fig. 5 shows the drug release profiles for concurrently dosed LICR formulations. Similar to the sequential dosed experiments, two distinctive groupings for the formulations can be seen. The high orlistat content formulations released about 25–30% of ibuprofen after 60 min, with the rate of release remaining consistent throughout the digestion process. Meanwhile, drug release from the less concentrated formulations (≤ ORL 0.01% w/w) was similar to the control formulation until approximately 20 min, after which the orlistat-containing formulations showed a decreased rate of drug release. After 60 min digestion, the ORL 0.01% w/w formulation released 73% and the ORL 0.005% w/w formulation released 84%. These values are slightly higher than their sequentially dosed counterparts, most likely due to presence of two doses in the digestion medium from the beginning.

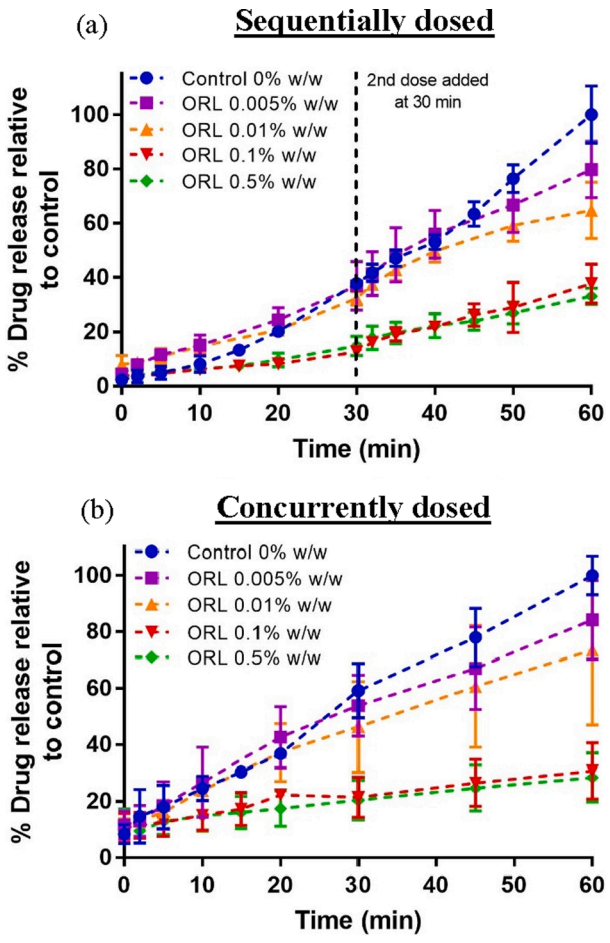

Fig. 5. Effect of orlistat concentration on the drug release of (a) sequential dosed and (b) concurrently dosed LICR formulations relative to the control (ORL 0% w/w). The presence of orlistat decreased the drug release with a direct correlation between the amount of orlistat and drug release. Error bars represent standard deviation (n=3).

3.4. Pharmacokinetics of ibuprofen when administered in LICR formulations

Plasma profiles for ibuprofen released from concurrently dosed formulations are given in Fig. 6 with the lipid formulations showing an increased T_{max} (time at which maximum drug concentration in plasma is achieved) compared to the ibuprofen powder. The highest orlistat concentration used in the *in vitro* experiments (ORL 0.5% w/w) was selected for these *in vivo* experiments in anticipation of that formulation showing the greatest influence on pharmacokinetics between the LICR formulation compared to the control (ORL 0% w/w). The formulations were all loaded into size 9 hard gelatine capsules due to their quick disintegration times and ease of performing an oral gavage. Both lipid containing formulations showed a delayed T_{max} as is often observed for lipid

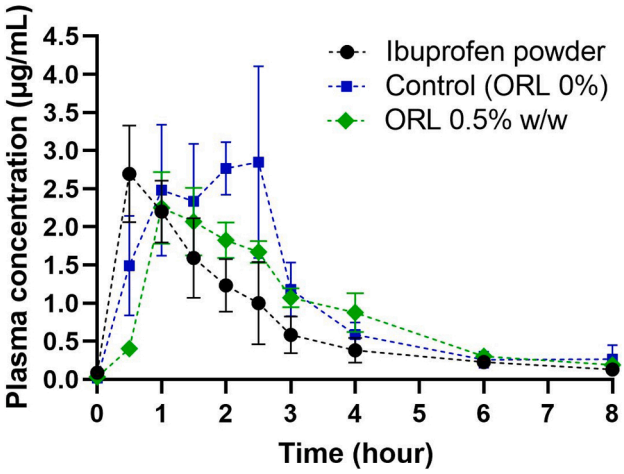

Fig. 6. Effect of orlistat concentration on ibuprofen concentrations in plasma after concurrent dosing of LICR formulation and ibuprofen powder given to Sprague Dawley rats. Data are mean ± SEM (n = 4).

formulations due to delays in gastric emptying and the requisite digestion to facilitate drug absorption. However, the LICR formulation had a shorter T_{max} and a reduced C_{max}. The orlistat-containing LICR formulation did not maintain elevated plasma levels around the T_{max} for as long as the control formulation, with the elimination phase occurring immediately after the peak was reached. It is interesting to note that the averaged plasma concentration of the LICR formulation at 30 min was three-fold lower than the control and nearly six-fold lower than the ibuprofen powder. This suggests that the presence of orlistat decreased the plasma uptake of ibuprofen in the initial period after administration, which could be an important mechanistic aspect of the LICR formulations.

Table 2 describes the pharmacokinetic statistical parameters for ibuprofen and reflect the plasma profiles. The control formulation had the highest exposure followed by the LICR formulation and the ibuprofen powder according to their respective $AUC_{0\text{-}8\ hr}$.

4. Discussion

The capacity for oral medications to cause unintentional harm is currently at an extreme high (Martins et al., 2015; Miller et al., 2020; Lessenger and Feinberg, 2008). The opioid epidemic has made it clear that changes need to be made for the safety of the consumer. Abuse-deterrent formulation (ADF) technology has been implemented with some success although further advancements are required to achieve long-lasting effects (Moorman-Li et al., 2012). Historically, lipid-based drug delivery systems (LBDDS) have been used to increase the solubility of poorly water-soluble drugs with digestion playing a crucial part. The digestion process in which triglycerides are broken down into fatty acids and a monoglyceride is well-established, with many formulations relying on this for boosting drug bioavailability. However, the concept of deliberately preventing this process to limit drug release has to our knowledge not been previously studied. In this research, a novel lipid formulation was created using Gelucire® 43/01 as the matrix, ibuprofen sodium salt as the model drug and orlistat as the lipase inhibitor. These new lipase inhibitor controlled-release (LICR) systems were made with the intention of determining whether the body's endogenous lipid digestion mechanism could be manipulated to limit the release of drug when an overdose quantity is ingested. Orlistat has previously been incorporated within a gel lipid formulation (Dully et al., 2020), although the purpose of these systems differ considerably.

Less than 10% of the drug was released upon dispersion of the formulations implying that digestion of the formulations by lipase was required for quantitative drug release. This is an intended characteristic as the majority of the payload should remain within the formulation for the controlled release mechanism to be successful. It is also important for the formulations to remain solid or sufficiently semisolid to retain the drug at the temperature of the gastrointestinal tract (37°C), as melting might result in unintended drug release. As most of the formulation is comprised of lipid, it is vital that the selected lipid exhibit these traits. Gelucire® is a family of lipids with differing properties. They are derived from mono-, di- and triglycerides and are produced with varying hydrophilic-lipophilic balances (HLB) (Chauhan et al., 2005). Currently, Gelucire® lipids are used in many applications, including sustained release formulations (Chauhan et al., 2005). Gelucire® 43/01 is a highly non-polar lipid made up of only triglycerides, with a melting temperature of 43°C and an HLB of 1. The low HLB is attributed to the absence of PEG esters, which are usually synonymous with the Gelucire family (Chauhan et al., 2005). It should be noted that while Gelucire® 43/01 was used in these experiments, the use of alternative lipids or combinations could better control the initial burst and modify digestibility if required.

Ibuprofen was chosen as a model drug but also represents the non-steroidal anti-inflammatory (NSAID) class of over-the-counter medications where overdose can be harmful (Higton and Rainsford, 2015). The salt form was selected over its acid due to its superior water solubility (100 mg/mL) meaning that it would be a tougher challenge than the lipophilic acid form which would be more readily retained in the lipid formulation (Legg et al., 2014). It was also chosen over any drugs of abuse for this initial proof-of-concept study due it not being confined to schedule 8 restrictions of use. Orlistat (tetrahydrolipstatin) has been approved for use as a lipase inhibitor by the FDA and EMA (de la Garza et al., 2011; Lunagariya et al., 2014). Boronic acids were considered as an alternative lipase inhibitor as they are readily used to prevent digestion during *in vitro* experiments, but they are not safe for use in humans (Khan et al., 2016). Orlistat is an irreversible inhibitor of gastric and pancreatic lipase and has been used to effectively manage obesity in adults (Padwal and Majumdar, 2007). Orlistat has also been shown to inhibit other digestive lipases such as pancreatic lipase-related proteins 2 (PLRP2) and carboxyl ester hydrolase (CEH) (Point et al., 2012). Currently, orlistat is sold under the trade name Xenical® with a recommended daily dosage of 120 mg three times a day. Compared to Xenical®, a very low amount of 5–500 μg had sufficient effect for the LICR formulations, therefore the chance of orlistat-related side effects would be extremely low. The optimal loading concentration of orlistat in the formulations was based on its IC_{50} of 0.72 μg/mL of lipase (800–1600 tributyrin unit/mL) (Habtemariam, 2013). Using this value, the amount of orlistat required to inhibit 50% of the lipase utilised in each experiment was 1.08 μg. Analogous to the drug release, it is expected that not all the orlistat would have been able to dissolve or have access to the lipid/aqueous phase interface, therefore the lowest amount of orlistat used in the formulations was set at 5 μg. Orlistat is also known to degrade when treated to temperatures above 25 °C for extended periods of time (Assessment Report For Alli (Orlistat). Agency, E. M., Ed. European Medicines Agency: London, UK, 2009). As our formulations were produced at 40–45°C there may have been some slight decrease in orlistat activity, although the orlistat was only exposed to these elevated temperatures for < 5 mins. Therefore, any significant change in orlistat activity is unlikely with the digestion results showing successful inhibition of lipid lipolysis. Other orlistat-containing formulations have also been processed at these temperatures (Park et al., 2020).

LICR formulations were made to exploit the human body's own digestive system as a means to control drug release. Traditionally LBDDS utilise digestion to create a favourable solubilising environment for poorly soluble drugs (Hauss, 2007; Rahman et al., 2011). In this case, instead of improving solubility, the insoluble Gelucire® 43/01 in an LICR formulation acts as a barrier from the digestive juices, allowing the majority of the drug (~90%) to remain within the formulation, but for digestion to drive drug release and the converse.

Fig. 7 illustrates the relationship between digestion and drug release more clearly than the time-dependent data presented earlier, showing the dependence of the extent of drug release vs extent of digestion at the 60 min time point. As hypothesised, the addition of orlistat within the formulation lead to lower digestion which in turn correlated to lower drug release. In all experiments, the ORL 0.1% w/w and ORL 0.5% w/w formulations acted similarly suggesting that the lipase within the digestion vessel was fully inhibited at these higher levels of orlistat. With this being the case, the least amount of drug release possible from a single LICR dose is 20% due to the delay in the onset of action from the orlistat. Importantly, from the results in Fig. 7 it is apparent that the drug release kinetics can be selected by changing the orlistat loading.

Table 2
Pharmacokinetic statistical parameters for orally administered ibuprofen and ibuprofen-loaded LICR formulations. Data is normalised to a dose of 5 mg/kg by accounting for the actual dose and the body weight of the animal. Data are mean ± SEM (n = 4).

Formulation	C_{max} (ug/mL)	T_{max} (hr)	$AUC_{0\text{-}8h}$ (hr. ng/mL)
Ibuprofen powder	2.97 ± 0.72	0.5 ± 0	6653 ± 1767
Control (0% ORL w/w)	4.55 ± 0.78	1.88 ± 0.38	8514 ± 1330
ORL 0.5% w/w	2.77 ± 0.33	1.38 ± 0.24	7620 ± 1014

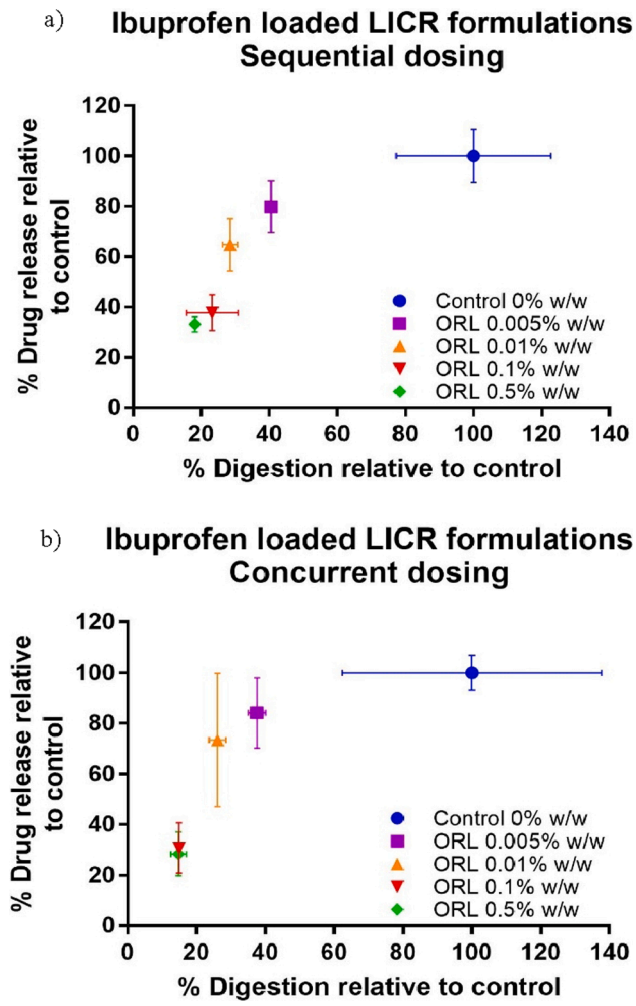

Fig. 7. Dependence of drug release on the extent of digestion for ibuprofen-loaded LICR formulations based on a) sequential dosing and b) concurrent dosing. The points represent the final digestion and drug release values after 60 min. The error bars represent the standard deviation (n=3).

Our study tested four different orlistat concentrations but depending on the active pharmaceutical ingredient (API), the LICR formulations can be modified to suit any drug strength and risk profile. A critical aspect of these formulations is that they are still required to release a therapeutic dose, while retaining the qualities that help reduce or prevent the release of dangerous quantities of drug. Two clear modes for drug release are shown in the *in vitro* experiments, the less concentrated LICR formulations release similar drug levels to that of the control differing only after 45 min, while the highly concentrated LICR formulations releases drug slowly, with their 60 min release values being comparable to drug release at 30 min from the control. This suggests that altering the orlistat concentration not only changes the final drug release, but can change the release behaviour from fast to slow release. These formulations could also be categorised as ADFs due to their composition primarily being lipid. ADFs are formulations which have properties that can deter the tampering of opioid medications, thereby limiting intentional dose dumping and potential drug toxicity issues (Moorman-Li et al., 2012).

The pharmacokinetic profiles provided initial evidence that the LICR formulations could have an impact *in vivo* in reducing undesirable drug release. It is known from the literature that orlistat inhibits lipid breakdown in rats with 5 mg/kg and 49.6 mg/kg concentrations inhibiting 20% and 70% of fat digestion respectively (Isler et al., 1995; Porsgaard et al., 2003). The ORL 0.5% w/w formulation contains an equivalent of 0.5 mg/kg of orlistat, considerably less than previous concentrations tested. This low quantity has no adverse effect on the experiments, as the aim was not to prevent overall fat digestion, rather to inhibit any lipase immediately surrounding the LICR formulations. Even slowing down digestion and flattening the plasma concentration vs time profile would help in reducing the incidence of toxicity events. Concurrently dosed formulations were administered *in vivo*, with the exposure level of ibuprofen being the lowest when administered in the LICR formulation, thus confirming the hypothesis that the orlistat impacted the digestion and thereby less drug was released compared to the average control formulation. The overall $AUC_{0\text{-}8hr}$ was not significantly differently, nevertheless this decrease could be the difference between drug concentration levels reaching toxicity or remaining in the safe range. Interestingly the LICR formulation also appeared to result in a plasma concentration at 0.5 hr that was considerably lower than the control lipid formulation. This slow uptake of drug may be a result of an initial release of orlistat, potentially from burst release, inhibiting the surrounding lipase sufficiently to reduce or prevent drug release. Of course it was anticipated that this first *in vivo* study would show a large difference between the orlistat and control groups, however without extensive further *in vivo* studies establishing the full correlation with *in vitro* studies, especially the optimal lipase concentration, the fact that the shape of the profiles are clearly and statistically different (albeit with similar AUCs) is consistent with the hypothesis and the slower absorption from the orlistat group is consistent with mechanism. This proof-of-concept study was conducted in rats as they are commonly used as models for digestion of lipid-based formulations (Steingoetter et al., 2019), with the next logical step being a phase 1 human study.

The results in this study act as a proof-of-concept to ascertain whether the addition of a lipase inhibitor within a lipid formulation could impact drug release. The promising results act as the first step towards creating safer medication, with a wide scope of possible future research including different lipid systems, different drugs and further *in vivo* proof-of-concept studies in larger species to optimise release of a therapeutic dose while minimising subsequent release. New formulation techniques are being explored with goals to create a wider range of inhibitor controlled-release (ICR) formulations with multiple different matrix compositions.

5. Conclusion

Lipase inhibitor controlled-release (LICR) formulations were developed in this proof-of-concept study with the aim of limiting drug release during overdose scenarios. LICR formulations were produced using Gelucire® 43/01 as the lipid matrix, ibuprofen sodium salt as the model drug and orlistat as the lipase inhibitor. *In vitro* lipolysis experiments confirmed that exposure of the LICR formulations to lipase resulted in a self-inhibiting effect on digestibility, with an inverse relationship being apparent between the orlistat concentration and extent of digestion. Due to the inhibited lipase, the breakdown of the LICR formulations was reduced, therefore leading to reduced drug release. This behaviour was observed for both single and double dose experiments. A lower exposure and modified pharmacokinetic profiles were observed during the *in vivo* rat studies after oral administration of the LICR formulation compared to an equivalent orlistat-free lipid formulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2022.121958.

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