Addition of Cationic Surfactants to Lipid-based Formulations of Poorly Water Soluble Acidic Drugs Alters the Phase Distribution and the Solid-State Form of the Precipitate upon *In Vitro* Lipolysis

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Abstract

It has been previously shown that the interaction of some weakly-basic drugs with oppositely-charged fatty acids during digestion can influence the solid-state form of the drug if it precipitates. The current study hypothesised the opposite effect for weakly-acidic drugs. Tolfenamic acid and an oppositely charged cationic surfactant, didodecyldimethylammonium bromide (DDAB), were combined in a model medium chain lipid formulation. The phase distribution upon *in vitro* lipolysis was determined using high performance liquid chromatography and the solid-state form of precipitated tolfenamic acid was determined using X-ray diffraction and crossed polarized light microscopy. Tolfenamic acid precipitated in a different polymorphic crystalline form to the starting reference material in the absence of DDAB, but precipitated in an amorphous form when DDAB was included in the same formulation. The solubility of tolfenamic acid upon dispersion and digestion of the formulation was considerably higher in the presence of DDAB. The findings point to ionic interactions between tolfenamic acid and DDAB as the reason for the improved drug solubility throughout digestion, and precipitation of drug in an amorphous-salt form, analogous to what has been observed in the past for some poorly-water soluble weakly basic drugs with anionic co-formers.

Introduction

Lipid based formulations (LBFs) can enable the oral delivery of poorly water soluble lipophilic drugs by circumventing the need for drug dissolution in the gastrointestinal tract (1). The

digestion of lipids by gastric and pancreatic lipase produces free fatty acids, which interact with endogenous amphiphilic molecules to form different self-assembled colloidal structures (2). Lipophilic drugs are transported to sites of absorption in the small intestine via these colloidal structures in a dissolved state.

An issue with LBFs, however, is the potential for reduction in solubilisation capacity of the formulation upon dilution in the gastrointestinal fluids (3). The solubilisation capacity can be further reduced upon lipid digestion, as the digestion products formed become increasingly hydrophilic. A decrease in the ability of the LBF to maintain drug in a dissolved state during digestion often leads to supersaturation of drug within the formulation and digestion products, and ultimately may result in precipitation of drug (4), which can decrease the total amount of absorbed drug and thereby limit bioavailability. Polymeric precipitation inhibitors have been used previously in an attempt to delay the onset of drug precipitation (5, 6). The polymers can stabilize the system in a supersaturated state, leading to a high absorptive flux of drug across the membrane (7-9). The inhibitory properties of polymers are clearly useful, however, precipitation of drug may not be deleterious to bioavailability if the drug does not precipitate in a poorly water-soluble crystalline form.

Recent studies have shown variation in the solid-state form of precipitated drug during the *in vitro* lipolysis of LBFs containing certain poorly water-soluble drugs (10-13). Specifically, it has been established that at appropriate pH, lipophilic weakly basic drugs, such as cinnarizine, interact with oppositely charged fatty acid molecules during *in vitro* digestion to form non-crystalline precipitates (14, 15). These amorphous-salt forms have a significantly higher dissolution rate in gastrointestinal media compared to low-energy crystalline forms (10-12). Using LBFs to form ionic interactions between weakly basic drugs and fatty acids provides a level of control over drug precipitation, and we hypothesize that the analogous approach would apply to weakly acidic drugs with LBFs containing cationic additives.

Weakly acidic drugs generally dissolve adequately in the gastrointestinal tract and are subsequently absorbed to a great extent, due to favourable pH conditions in the small intestine; however, there are some acidic drugs currently in the development pipeline where bioavailability is limited by solubility. The lipophilic weakly-acidic drug tolfenamic acid has been studied previously in LBFs with regard to the solid-state form of the precipitate (the chemical structure of tolfenamic acid is shown in Figure 1) (16). It was shown that during *in*

vitro digestion of different types of LBFs, tolfenamic acid precipitated either in its thermodynamically stable crystalline form, or as its 'yellow' polymorphic crystalline form (16). The high energy polymorph is expected to have different dissolution behaviour in the gastrointestinal tract compared to the thermodynamically stable crystalline form of tolfenamic acid. An approach to drive precipitation of the drug to an amorphous form may further improve dissolution characteristics.

The combination of a weakly acidic drug and oppositely charged surfactant with regard to the effect on the solid state form of precipitated drug upon *in vitro* digestion of LBFs has not been studied to our knowledge. The primary aim of the current study was to investigate whether the apparent ionic interactions that occur between fatty acids and weakly basic drugs to affect precipitation behaviour carries over to weakly acidic drugs and oppositely charged surfactants, and whether this interaction between the two species allows for a level of control over the precipitation of weakly acidic drugs. The cationic surfactant didodecyldimethylammonium bromide (DDAB) (chemical structure shown in Figure 1) was chosen as the oppositely charged species to include in the starting LBF to influence the precipitation of tolfenamic acid. This combination acts as a proof-of-concept to assess the ability of cationic components to influence the solid state characteristics of weakly acidic drugs upon lipid digestion, as outlined in Figure 1.

Materials

Captex 355® [medium chain triglyceride mixture composed of 59% caprylic acid (C_8), 40% capric acid (C_{10}), <1% lauric acid (C_{12}) as stated in the product information], and Capmul MCM® [mono/diglycerides composed of caprylic acid (C_8) in glycerol], were obtained from Abitec Corporation (Janesville, Wisconsin, USA). Cremophor EL® was purchased from BASF Corporation (Washington, New Jersey, USA). Didodecyldimethylammonium bromide (DDAB), Tris maleate (reagent grade), bile salt (sodium taurodeoxycholate (NaTDC), >95%), and 4-bromophenylboronic acid (4-BPBA, >95%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Tolfenamic acid was purchased from AK Scientific (Union City, CA, USA). Calcium chloride dihydrate (>99%) was obtained from Ajax Finechem (Seven Hills, New South Wales, Australia). Sodium chloride (>99%) was purchased from Chem Supply (Gillman, South

Australia, Australia). HPLC grade acetonitrile was purchased from Merck (Boston, MA, USA). Phospholipid (1,2-dioleoyl-sn-glycero-3-phosphocholine, DOPC) was obtained from Trapeze Associates Pty. Ltd. (Clayton, Victoria, Australia). USP grade pancreatin extract was purchased from Southern Biologicals (Nunawading, Victoria, Australia).

Methods

Preparation of LBF

A Type-3B MC self nano-emulsifying drug delivery system (SNEDDS) was chosen for this particular study (17). It has been shown previously that tolfenamic acid precipitates upon dispersion of this relatively hydrophilic LBF at drug loadings of 80 – 100% of its saturation solubility in these LBF, whereas upon dispersion and digestion of more lipophilic Type 1 - 3A formulations tolfenamic acid remains mostly in solution (16). The Type 3B MC-SNEDDS consisted of 0.125 g Captex 355, 0.125 g Capmul MCM, 0.5 g Cremophor EL and 0.25 g ethanol, as indicated in Figure 1, based on previous studies (18), and was prepared in 1 g batches. The above excipients were weighed into a glass scintillation vial and vortexed for 1 min to mix the different components. Tolfenamic acid was weighed (54 mg) into a separate vial, before 1 g of the formulation prepared above was added to give a final drug loading of 51 mg/g in the Type 3B MC-SNEDDS, and allowed to mix in an oven set to 37°C overnight before use. In instances where solid drug was visually observed after mixing overnight in the 37°C oven, the formulation was placed in a 60°C oven until all solid particles were dissolved. The second formulation additionally contained the cationic surfactant DDAB at a 1:1 drug/surfactant mol ratio. For these DDAB containing formulations, DDAB (95 mg) was weighed and added to the vial with the tolfenamic acid (providing a final drug loading of 47.0 mg/g), before the MC-SNEDDS was added and mixed in a 37°C oven as described above.

Preparation of Digestion Medium and Pancreatic Lipase

Digestion buffer was prepared and used to make up both the digestion medium and pancreatic lipase solution. The digestion buffer was comprised of 2 mM Tris-maleate, 1.4 mM $CaCl_2 \cdot 2H_2O$, 150 mM NaCl and adjusted to pH 6.5 using 1M NaOH and HCl solutions. Fasted state simulated digestion medium was then prepared by adding 5 mM NaTDC and 1.25 mM DOPC to the digestion buffer. The required amount of DOPC was weighed into a round-

bottom flask and dissolved in an appropriate amount of chloroform, before the chloroform was removed under vacuum to leave an evenly coated lipid film at the bottom of the flask. The required amount of NaTDC was then added to the flask and dissolved in digestion buffer under sonication. The digestion medium was kept refrigerated at 4 °C before use. To prepare the pancreatic lipase solution the raw pancreatin extract was weighed (2 g) into a small glass beaker and suspended in 5 mL of digestion buffer. This mixture was stirred for 5 min, transferred to a 12 mL plastic centrifuge tube and centrifuged at 2205 g at 4 °C for 15 min. The resultant supernatant was used as the pancreatic lipase solution (1000 TB units/mL of digest), which was collected and stored at 4 °C before use. A fresh batch of lipase solution was prepared as required, and each lipolysis experiment was initiated by adding 4 mL of this pancreatic lipase solution.

In vitro **Lipolysis**

The lipolysis experiments followed previously established protocols (19). Briefly, a Metrohm titrator with 5 mL dosing unit, autoburette and an iUnitrode pH probe (Metrohm AG, Herisau, Switzerland) was attached to a thermostatted glass vessel set to 37 °C. The MC-SNEDDS formulation containing tolfenamic acid was added to 36 mL of digestion medium in the glass vessel and allowed to disperse for 5 min. Adjustments to pH were made as necessary using 0.1M HCl and NaOH solutions during this 5 min dispersion period to reach the experimental pH value of 6.5. Tolfenamic acid has a pKa of 3.7 (20) and therefore is mostly ionised at the experimental pH of 6.5, thus the drug could potentially participate in ionic interactions with oppositely charged DDAB molecules. Immediately following the 5 min dispersion period, lipolysis was initiated by adding 4 mL of the previously prepared pancreatic lipase solution (to achieve an overall activity of 1000 TB units/mL of digest).

Upon addition of the lipase the MC-SNEDDS was digested and the enzymatic hydrolysis of lipids produced free fatty acids. This led to a decrease in the pH of the digestion medium, and 0.6 M NaOH was used as the titrant solution to restore the pH to 6.5. Upon digestion at pH 6.5, the liberated fatty acid molecules were partly ionised and partly unionised, only ionised fatty acid molecules were titrated.

To account for the unionised fatty acids at the end of lipolysis the pH of the digestion medium was rapidly shifted to pH 9.0, which fully ionised all fatty acids (21). The amount of titrant

required to shift to pH 9.0 at the end of the 60 min digestion period indicated the amount of unionised fatty acid present during lipolysis. This shift to pH 9.0 was performed only for experiments concerned with calculating the extent of lipolysis of the formulations, and was not performed for experiments where the solid-state of precipitated drug was analysed. The extent of digestion was calculated according to Equation 1.

Equation 1:

$$Extent\ of\ LBF\ digested\ (\%) = \frac{Ionised\ fatty\ acid\ (mol) + unionise\ \ fatty\ acid\ (mol)}{Theoretica\ \ fatty\ acid\ in\ LBF\ (mol)} \times 100$$

Quantification of Phase Distribution of Tolfenamic Acid

The phase distribution of tolfenamic acid was assessed upon *in vitro* digestion of the Type 3B MC-SNEDDS formulation in the presence and absence of DDAB. The HPLC system included a Shimadzu CBM-20A system controller, LC-20AD solvent delivery module, SIL-20A auto sampler and a CTO-20A column oven set at 40°C, coupled to an SPD-20A UV-detector (Shimadzu Corporation, Kyoto, Japan). A reverse-phase C_{18} column was used (4.6 × 75 mm, 3.5 µm; Waters Symmetry®, Boston, MA, USA).

During the *in vitro* digestion experiments, aliquots (200 µL) of the digesting formulation were taken at specific time points (t = 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min). These aliquots were transferred to 1.75 mL Eppendorf tubes prepared with 20 µL of lipase inhibitor (0.05 M 4-BPBA), which were then centrifuged at 7708 x g for 1 hr. The resultant aqueous colloidal phase (supernatant) was separated from the precipitated drug (pellet phase) for analysis. Mobile phase was used to dilute the aqueous colloidal phase until the concentration of drug fell within the concentration range of the standard curve. For analysis of the pellet phase the samples were first dissolved in 200 µL of acetonitrile, before diluting with mobile phase as described above. Lipolysis experiments and HPLC quantification of drug within the digested phases were performed in triplicate for the MC-SNEDDS with and without DDAB.

The mobile phase was prepared with acetonitrile and water at a v/v ratio of 80:20, with 0.1% formic acid added to the aqueous phase, using isocratic elution. The flow rate was 1 mL/min and the injection volume was 50 µL. The concentration of drug in the digested samples was determined by comparison to a standard curve.

The equilibrium solubility of tolfenamic acid in the starting formulations was also assessed. Excess tolfenamic acid was transferred to 1 g of Type-3B MC-SNEDDS in a 1.75 mL Eppendorf tube. The sample was vortex mixed and placed on a roller to mix overnight in an oven set to 37 °C. The solubility of tolfenamic acid was measured at different time points (24, 48 and 72 hr) until the dissolved concentration remained unchanged (± 5% w/w). The experiment was also conducted in parallel on the Type-3B MC-SNEDDS containing DDAB. Samples were prepared and analysed using the above HPLC method in triplicate (n=3).

Determination of Morphology and Birefringence of Precipitated Drug using Polarised Light Microscopy

Crossed polarised light microscopy (CPLM) was used to visualise the precipitated drug from the lipolysis experiments. Tolfenamic acid in its crystalline forms is birefringent under crossed polarised light, whereas amorphous materials do not exhibit birefringence, due to the lack of long range order in their molecular arrangement (22). Therefore, CPLM was used to indicate the crystallinity and morphology of precipitated drug. A Nikon ECLIPSE Ni−U upright microscope fitted with crossed polarising filters and a DS-U3 digital camera control unit (Nikon, Tokyo, Japan) were used. Following the lipolysis experiments the pellet phases were collected and placed onto glass microscope slides to air dry before analysis.

Determination of the Solid State Form of Precipitated Tolfenamic Acid using X-ray Diffraction

The solid state form of the precipitated drug was determined using X-ray Diffraction (XRD). Pellet phases from the lipolysis experiments were isolated and a Bruker D8 Advance X-ray diffractometer with Ni-filtered Cu Kα radiation (1.54 Å) at 40 kV and 40 mA was used to collect XRD data. The samples were analysed in the range of 5–50° 2θ, with a step size of 0.02° and a scan rate of 0.5 seconds per step. The diffraction peaks from the pellet samples were compared to the reference crystalline tolfenamic acid starting material. The presence of diffraction peaks in the samples confirmed the precipitation of tolfenamic acid in a crystalline form, whilst a halo and a lack of diffraction peaks indicated the presence of amorphous material.

Results

The Type-3B MC-SNEDDS formulation immediately dispersed upon addition to the digestion medium. Visually, an immediate increase in opacity of the medium during dispersion was observed, changing from a transparent micellar solution to a cloudy oil-in-water emulsion upon contact of the formulation with the medium. The dispersion period was set to 5 min to allow for complete mixing and to determine any initial propensity for precipitation, and necessary pH adjustments were made during this time, before pancreatic lipase solution was added to initiate digestion.

Lipid formulations containing MC lipids have been shown previously to digest completely under the lipolysis conditions used in this study (11, 16). Moreover, the Type-3B MC-SNEDDS used in the current study contains only 30% w/w lipids with the remainder consisting of surfactant and co-solvent. Rapid and complete digestion of the glyceride components was therefore expected, however, it was unknown how the inclusion of the charged non-digestible surfactant DDAB might influence the rate and extent of lipolysis for the DDAB containing formulations. Consequently, the *in vitro* lipolysis experiments were performed for the Type-3B MC-SNEDDS containing tolfenamic acid with and without addition of DDAB. The resultant lipolysis titration profiles for the two formulations tested are presented in Figure 2.

The rate and extent of lipolysis for the Type 3B MC-SNEDDS were similar for both formulations tested, as can be seen from the lipolysis profiles from Figure 2. The MC-SNEDDS underwent rapid digestion upon addition of the pancreatic lipase and digested to completion within the 60 min period of lipolysis. The addition of DDAB in the formulation did not affect the overall extent of formulation digested during the experiment, and appeared to only slightly affect the rate of lipolysis. The similar digestion profiles obtained for the two different formulations indicate that the rate and extent of lipid digestion would not be an important factor in any differences in precipitation behaviour.

Phase Distribution of Tolfenamic Acid During Lipolysis

At the beginning of the lipolysis experiments approximately 1 g of formulation was added to the digestion medium. The viscous nature of the formulation precluded accurate transfer of the 1 g of formulation that had been weighed out, to the digestion vessel, so the amount of drug recovered from the samples taken at various time points throughout lipolysis was calculated relative to the initial amount of drug in the dispersion prior to the addition of lipase.

For the MC-SNEDDS without DDAB it was evident upon dispersion that a fraction of the tolfenamic acid had precipitated, as a pellet was visually observed after centrifuging the sample. The pellet from t = 0 min was analysed and it was found that 30.1 ± 6.43% w/w of the drug had precipitated during this dispersion period. The precipitation of drug upon dispersion of the formulation (but not necessarily digestion) is generally believed to be indicative of a poorly-performing formulation. Relatively hydrophilic lipid formulations, such as the Type 3B MC-SNEDDS used in this study, are less able to maintain lipophilic drugs in solution when the formulation is dispersed, in comparison to Type 1 – 3A formulations.

However upon initiation of lipolysis of the DDAB-free formulation, tolfenamic acid continued to precipitate as the lipid components were hydrolysed (Figure 3a). A gradual increase in the amount of tolfenamic acid recovered from the pellet phase was observed throughout lipolysis, with 51% w/w of the initial tolfenamic acid in the pellet phase after 60 min.

In contrast, the MC-SNEDDS with DDAB presented a very different phase distribution profile for tolfenamic acid upon *in vitro* dispersion and digestion. Notably, there was no drug precipitation during the dispersion phase when DDAB was included in the formulation. This could be a result of ionic interactions between tolfenamic acid and DDAB in the starting formulation, which may have in turn improved the solubility of tolfenamic acid in the dispersed lipid droplets, as outlined in Figure 1. The above scenario is highly likely, as the mol amount of DDAB added to the formulation was equal to the mol amount of tolfenamic acid.

In addition, during lipolysis a significant increase in the amount of drug was retained in the aqueous colloidal phase throughout digestion in comparison to the formulation without DDAB, as shown in Figure 3b. The precipitation of tolfenamic acid from the formulation

containing DDAB was gradual and began only after 15 min of lipolysis. This means that close to 80% of the formulation needed to be digested before drug precipitation ensued. This difference in behaviour between the two formulations tested is significant given that the formulation without DDAB was unable to maintain drug in solution during dispersion, yet with the addition of DDAB at a 1:1 mol ratio of drug to surfactant the tolfenamic acid remained solubilised throughout dispersion, and remained solubilised up to a point where 80% of the formulation was digested.

After 60 min of lipolysis the formulation with the DDAB had digested completely, and only 28% w/w of the initial tolfenamic acid was in the pellet phase. Therefore, the final amount of precipitated tolfenamic acid was much less for the formulation with DDAB. It was clear from the phase distribution study that DDAB was having a significant effect on the precipitation behaviour of tolfenamic acid during *in vitro* digestion, as the addition of DDAB was the only variable between the two formulations examined. It remained unknown at this stage, however, whether DDAB was also affecting the solid-state form of the precipitated tolfenamic acid during the experiment.

The equilibrium solubility of tolfenamic acid in the undigested Type-3B MC-SNEDDS, with and without DDAB, was also assessed. The addition of DDAB slightly increased the solubility of tolfenamic acid in the formulation (64.6 ± 2.50 mg/g), as compared to the DDAB-free formulation (61.8 ± 3.51 mg/g), however, a t-test on the data revealed no statistically significant difference ($p > 0.05$) in solubility between the two data sets for the respective formulations. Therefore, the addition of DDAB did not allow for superior drug loading in the starting formulation, but it did enable the drug to remain in solution throughout dispersion and digestion, whereas the DDAB-free formulation gave rise to drug precipitation much earlier during the experiment.

Solid-State Analysis of the Pellet Phase Arising from In Vitro Digestion

After performing *in vitro* digestion on both formulations (with and without DDAB) the pellet phase was isolated and viewed under a crossed polarized light microscope. The pellet obtained from the formulation without DDAB was bright yellow in colour. This gave a strong indication that tolfenamic acid had precipitated during the experiment in its 'yellow'

polymorphic form, which has previously been reported (16, 23). The pellet obtained from the digestion of the formulation with DDAB, however, did not display this distinct yellow colour, but was brown, much like what is normally seen from pellets obtained from similar drug-free digestion experiments. From Figure 4 it can be seen that the formulation without DDAB gave rise to crystalline tolfenamic acid upon precipitation. This was evident in the form of sharp needle-like structures observed under the crossed polarized light. The pellet obtained from the *in vitro* digestion experiment performed on the formulation with DDAB did not display the same birefringence under crossed polarized light. There were no thin needle-like structures observed for the formulation with DDAB, indicating the absence of crystalline drug in the precipitated components.

The pellet phases from the lipolysis of both formulations were also analysed using XRD to identify the solid-state form of the precipitated tolfenamic acid. It has been shown previously that tolfenamic acid precipitates in two different crystalline forms depending on the type of lipid formulation. The 'yellow' polymorphic form, observed in the current study, was also observed previously when XRD was performed on the pellet phase from the *in vitro* digestion of a Type 4 lipid formulation (16). The thermodynamically stable crystalline form, however, was identified upon precipitation during the lipolysis of a similar Type 3B lipid formulation to the one used in this study. The XRD data from the current study, presented in Figure 5, reveals that the 'yellow' polymorphic form of tolfenamic acid was formed during digestion of the model Type 3B MC-SNEDDS, indicated by different peak positions to the reference crystalline form, but the same diffraction pattern as previously reported for this polymorph by Williams *et al*. (16). The components that made up the Type 3B MC-SNEDDS varied slightly between the current study and the study conducted by Williams *et al.*, although the drug loading was similar (51 mg/g). This minor adjustment in the formulation composition may have led to different drug supersaturation behaviour during dispersion, which caused the tolfenamic acid to precipitate as the 'yellow' polymorphic form in the current study.

For the formulation containing DDAB there was a clear lack of diffraction peaks in the XRD data. This reflected the absence of precipitated drug in the crystalline form, and the presence of a halo region in the diffractogram suggested that the tolfenamic acid had precipitated in an amorphous form (24). It is worth noting that the amount of tolfenamic acid that

precipitated after lipolysis of the formulation with DDAB was around 20% w/w less than from the formulation without DDAB.

This absence of crystalline drug with DDAB present was not a result of a lack of sensitivity with the XRD instrument. The amount of material required for XRD measurements is several mg, which is sufficient for the current study given that 30% w/w of the initial tolfenamic acid precipitated during lipolysis of the formulation with DDAB. Therefore, if tolfenamic acid had precipitated in a crystalline form it would have been detected.

Discussion

The effects of LBFs on the precipitation behaviour of poorly water-soluble drugs have been examined in recent times, in particular with regard to the solid-state form of the precipitated drug (11-13, 16, 25-28). It is now established that some weakly basic drugs appear to precipitate in an amorphous form during *in vitro* digestion experiments, due to ionic interactions with oppositely charged fatty acids present in the digestion medium (14, 15). These fatty acids are liberated upon the enzymatic hydrolysis, or digestion, of the lipids in the formulation. At pH 6.5, which is the pH that lipolysis experiments are routinely conducted at and which is representative of the upper small intestinal contents, the majority of fatty acids present are ionised and available for ionic interactions with oppositely charged species, such as weakly basic drugs that are also ionised at this pH. In addition, fatty acid molecules are complexed with calcium ions to form precipitated soaps, and this allows for digestion to proceed in the closed *in vitro* model where absorption of digestion products is not an aspect (29, 30). By this mechanism the fatty acid molecules are removed from the surface of the digesting lipid droplet, allowing lipases continued access to the lipid substrate.

Analogous to the ionic interactions that occur between fatty acids and oppositely charged species during digestion, the weakly acidic drug tolfenamic acid was hypothesised to interact with the oppositely charged surfactant DDAB included in the Type 3B MC-SNEDDS examined in this study. It should be noted that tolfenamic acid was used as a model weakly acidic compound in this study, and real biopharmaceutical benefits for this particular drug were not expected by implementing such an approach. This study acts as a proof-of-concept, showing

that the precipitation behaviour of weakly acidic drugs can be controlled during digestion by using oppositely charged species in the formulation.

The DDAB was dissolved in the starting MC-SNEDDS and therefore likely formed ionic interactions with tolfenamic acid, forming a lipophilic ion pair, which enhanced the solubility of the drug in the formulation before digestion. Although the ion pair was not isolated to determine its solid state properties, transforming drugs to ionic liquid forms through similar ion pairing has been shown previously to improve the solubility of drug in lipid excipients, which allows for a greater initial drug loading in the formulation (31, 32). Acidic lipophilic counter ions were used to turn the weakly basic drug cinnarizine into an ionic liquid for this purpose, which resulted in a 7-fold increase in the solubility of cinnarizine in the starting lipid formulation (31, 34). This approach was also applied to weakly acidic drugs, where tolfenamic acid was used as one of the model compounds, which were combined with lipophilic counter ions to form ionic liquids (35). A similar approach was used to enhance the loading of the anticancer drug irinotecan into nanostructured lipid carrier particles (33).

It is also worth noting that DDAB inherently self-aggregates in water at low concentrations (critical micelle concentration = 0.05 – 0.15 mM) to form vesicles (36). The high hydrophobicity of DDAB, however, and the presence of lipid components from the formulation means it is unlikely for any free DDAB to be present during dispersion, as the DDAB had already interacted with tolfenamic acid within the dispersed oil droplets.

The inclusion of DDAB in the formulation did not negatively impact the rate and extent of digestion of the formulation, as can be seen from the *in vitro* lipolysis profiles in Figure 2. This is in contrast to what has previously been reported for the effect of a similar cationic surfactant, dodecyl trimethyl ammonium bromide (DTAB), on the digestion of corn oil (37). It was shown that at concentrations greater than 1.4% w/w a noticeable inhibitory effect was observed on lipid digestion. The inhibitory effect of DTAB on lipid digestion was attributed to its ability to displace lipase from the surface of the digesting lipid droplet, or due to DTAB induced denaturing of the lipase. These findings, however, did not appear to translate to the current study, where the effect of DDAB on the rate and extent of lipolysis was studied. The extent of drug precipitation and the solid state form of the precipitate was therefore exclusive of the effect of DDAB on the lipolysis of lipids in the formulation.

In addition to tolfenamic acid, which appears to interact with DDAB, there are other species present during the *in vitro* digestion of the MC-SNEDDS that could interact with DDAB, such as fatty acids and bile salt. Vesicles composed of fatty acids can be stabilised by interactions between the ionised head-group of the fatty acid molecules and DDAB (38). In a similar fashion it has been shown previously that DDAB self-assembly in excess water results in different types of vesicle structures, and this phase behaviour is affected by the addition of sodium taurodeoxycholate (39). Interactions between the ionised head-groups of the anionic (sodium taurodeoxycholate) and cationic (DDAB) surfactants and the complex geometric packing of the sodium taurodeoxycholate increased the size of the vesicles in the two-component system. Sodium taurodeoxycholate is able to incorporate within the DDAB vesicle aggregates up to an addition of 20 mol%, beyond which leads to a phase separation.

It is of course conceded that such use of cationic surfactants in lipid formulations administered to humans may not be suitable due to toxicity reasons, however, alternative, less toxic, compounds may be explored in the future that could be adopted for development of an actual product. One potential alternative to surfactants is lipopeptides, which combine the structural properties of lipids (aliphatic chains) and amino acids and have been shown in some cases to be non-toxic to human cell lines (40). They also offer high versatility in potential structures, where the amino acid sequence and the number and length of the aliphatic chains can be customised to achieve a desired structure. Ultimately peptidases are likely to break down these components in the gut, potentially after they have provided their formulation function. Another option is to explore the use of 'soft' cations, such as pyridinium and guanidinium. One study showed that the toxicity of ionic liquids based on guanidinium was largely determined by the nature of the anionic components, where the majority of combinations used exhibited a low toxicity level (41).

In addition to exploring the use of less toxic cationic excipients for LBFs, there is also a need to study the performance of these formulations and the precipitation behaviour of drugs *in vivo*. Recently, it was demonstrated that ionic liquids improve the absorption profile of poorly water-soluble drugs when administered in lipid formulations *in vivo* in rats, leading to a sustained exposure to the drug (35). The extent of drug precipitation *in vivo*, however, remains unclear. One recent study examined the precipitation behaviour and solid-state form *ex vivo* of two poorly water-soluble drugs, fenofibrate and danazol, during the *in vivo*

digestion of LBFs (42). A more suitable experimental model for the purpose of following the fate of drug and lipid during digestion might be to adapt the recently developed *in vitro* digestion – *in vivo* absorption model (43). This model combines the standard *in vitro* lipolysis experiment coupled to an intestinal perfusion experiment in an anaesthetised rat. Further combining this setup with *in situ* synchrotron small angle X-ray scattering (SAXS) could potentially inform directly on drug precipitation behaviour and the solid-state form of the precipitate.

Conclusion

The weakly-acidic drug tolfenamic acid was more soluble upon dispersion and digestion of a lipid-based MC-SNEDDS formulation containing DDAB likely due to ion pair formation between drug and surfactant, analogous to the situation with anionic lipids and weakly basic drugs. Dispersion of the formulation containing DDAB in aqueous media did not result in precipitation, whereas dispersion of the DDAB-free formulation induced precipitation of >20% crystalline drug. In addition, the solid state form of tolfenamic acid upon precipitation during lipolysis was amorphous according to XRD data and CPLM images when DDAB was included in the MC-SNEDDS, whereas the drug precipitated in a polymorphic crystalline form in the absence of DDAB. The results obtained suggest that ionic interactions occurred between tolfenamic acid and DDAB to form a lipophilic ion pair in the starting MC-SNEDDS, and this complex significantly improved the solubility of the drug during dispersion and in the aqueous colloidal phase during digestion.

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