Pharm Res (2011) 28:1540–1551
DOI 10.1007/s11095-011-0387-8

RESEARCH PAPER

The Effect of Composition and Gastric Conditions on the Self-Emulsification Process of Ibuprofen-Loaded Self-Emulsifying Drug Delivery Systems: A Microscopic and Dynamic Gastric Model Study

Annalisa Mercuri • Antonio Passalacqua • Martin S. J. Wickham • Richard M. Faulks • Duncan Q. M. Craig • Susan A. Barker

Received: 16 October 2010 / Accepted: 31 January 2011 / Published online: 24 February 2011

ABSTRACT

Purpose To investigate the physical processes involved in the emulsification of self-emulsifying drug delivery systems (SEDDSs) and the use of the Dynamic Gastric Model (DGM) as a characterisation tool.

Methods SEDDSs based on soybean oil, Tween 80, Span 80 and ibuprofen were prepared and their equilibrium phase diagrams established. The emulsification behaviour in a range of media was studied using polarised light microscopy and particle sizing. The behaviour of the SEDDSs in the DGM and conventional testing equipment was assessed.

Results A range of liquid crystalline mesophases was observed, enhanced in the presence of the drug. Polarised light microscopy showed different emulsification processes in the presence and absence of the drug, which was also manifest in different droplet sizes. The droplet size distribution varied between the DGM and the USP II dissolution apparatus.

Conclusions The model SEDDS displays complex liquid crystalline behaviour which may be intimately involved in the emulsification process, which in turn may alter particle size on emulsification, although there remains a question as to the *in vivo* significance of this effect. Furthermore, we demonstrate that the DGM represents a very promising new method of assessing the biological fate of SEDDSs.

A. Mercuri • D. Q. M. Craig • S. A. Barker (✉)
School of Pharmacy, University of East Anglia
Norwich NR4 7TJ, UK
e-mail: Susan.Barker@uea.ac.uk

A. Passalacqua • M. S. J. Wickham • R. M. Faulks
Institute of Food Research
Norwich Research Park, Colney
Norwich NR4 7UA, UK

KEY WORDS droplet size · dynamic gastric model · mechanism of self-emulsification · phase diagram · self-emulsifying systems

ABBREVIATIONS

AGJ_{wp}	artificial gastric juice without pepsin
DGM	dynamic gastric model
HCl	hydrochloric acid
SEDDS	self-emulsifying drug delivery systems

INTRODUCTION

Lipid-based drug delivery systems have attracted considerable interest as a means of enhancing the bioavailability of drugs with poor water solubility (1,2). However, the physico-chemical processes associated with emulsification, the mechanism of absorption enhancement and the link between the two are not well understood. These dosage forms are classified into four categories: Types I, II, III (A and B) and IV (3), depending on the ease of emulsification and the product so formed. Of particular note are the self-emulsifying drug delivery systems (SEDDS); these are defined as an anhydrous mixture of drugs with oil, surfactant and (possibly) cosurfactant, which spontaneously form a fine emulsion or microemulsion upon contact with fluids present within the gastrointestinal tract (1). Several advantages of such systems have been described compared to basic oil formulations, not least being more consistent plasma profiles compared to the more erratic responses seen from conventional lipid formulations (4). In general, SEDDS are administered in hard or soft capsules,

as they are generally of liquid, gel or soft waxy consistency, although solid SEDDSs have also been investigated (5,6).

A wide range of SEDDS formulations have been studied in the literature, using a variety of naturally occurring oils, partially refined oils, triglycerides of defined composition, semi-synthetic mixtures of triglycerides, co-solvents such as ethanol, and surfactants of different chemical descriptions. The initial selection of the excipient mixture is usually based on the relative ability of the mixture to self-emulsify or self-micro-emulsify, the droplet size of the resulting emulsion or microemulsion, and the solubility of the drug within the vehicles. It should be noted that very few studies have investigated the effect of addition of the drug on the emulsification properties of these systems (7–9). Similarly, studies on the relationship between (micro-) emulsion droplet dimensions and enhancement of the drug bioavailability have produced ambiguous results. The work of some authors has indicated that a reduction in droplet size is followed by an increase in bioavailability of the drug, which was interpreted in terms of an increase in the surface area available for absorption (10–12). Cyclosporin A has been used extensively as a clinically important model drug in these studies. *In situ* perfusion studies in rats showed that a reduction in the droplet size of olive-oil-based emulsions resulted in an increased oral bioavailability (10). The human oral bioavailability of cyclosporin A was shown to be higher and more reproducible from a preformed microemulsion formulation (Sandimmune Neoral® containing polyoxyethylated castor oil and medium chain mono- and di-glycerides) than from the (at the time) marketed product, Sandimmune®, which produces a coarse emulsion on ingestion (11). Further work (12) examined a range of microemulsion formulations of cyclosporin A and again suggested that the improved human oral bioavailability compared to Sandimmune® was due to the reduced particle size of the microemulsion formulation. However, other authors found that droplets with similar size produced markedly different bioavailability results, indicating compositional dependence (13–15). For example, the oral bioavailability in dogs of halofantrine from a range of complex self-emulsifying and self-microemulsifying systems was found to be more dependent on their precise lipid composition than their droplet sizes (13), with formulations based on long-chain triglycerides appearing to give higher bioavailabilities than those based on medium-chain triglycerides. A subsequent study (14) examined this aspect in more depth, again using halofantrine as the model drug. For compositions of equivalent droplet size once emulsified, formulations containing triglycerides esterified at the 1- and 3- positions with a medium-chain fatty acid and at the 2-position with a long-chain fatty acid (MLM) showed a greater oral bioavailability than those containing triglycerides esterified at the 1- and 3- positions with a long-chain fatty acid and at the 2- position with a medium-chain fatty acid (LML). The authors ascribed this to a difference in the relative lymphatic and portal absorption of the two different formulations. The extent of lipid digestion after oral administration and the subsequent release of the drug, either into a precipitated pellet phase or solubilised into a colloidal structure, was suggested as an explanation for the marked differences in oral bioavailability of danazol seen in beagle dogs from self-microemulsifying systems composed of long- or medium-chain triglycerides (15), even though both formulations showed similar droplet sizes once emulsified and similar dispersion behaviour. In a further study, no difference in the oral bioavailability of probucol in mini-pigs was found when comparing self-emulsifiable formulations of the same composition based on sesame oil, Cremophor and Masine, but with a difference in droplet size of 100-fold (16).

There is, therefore, a limited understanding of the link between composition, emulsification behaviour and bioavailability, and a simple, direct relationship may not exist in all cases. Indeed, there is considerable evidence (e.g. 15) to suggest that the digestion of the SEDDSs plays a leading role in bioavailability enhancement, as is now well established for conventional lipid delivery systems (17,18). A recent study focused on the evaluation of the impact of gastric digestion on the bioavailability of the model drug cinnarizine from lipid-based formulations composed of medium- and long-chain lipids (19). The results showed that the bioavailability of the poorly water-soluble drug administered orally to rats was higher than that of the same formulation administered intraduodenally, suggesting a pivotal role played by gastric processing. Furthermore, the authors suggested that formulations containing medium-chain lipids are influenced by such processing more than formulations containing long-chain fatty acids, in turn indicating a composition dependence of bioavailability with respect to the digestibility of the oil phase.

In this investigation, we studied the link between the physico-chemical aspects of the emulsification process and the fate of those emulsions in the stomach using the Dynamic Gastric Model (described in more detail below). There is compelling evidence that the behaviour of SEDDSs is highly species-dependent, this being ascribed to the amount of fluid within the gastrointestinal tract having an effect on the extent of self-emulsification (19,20). In humans, gastric digestion of either coarse (>20 μm) or fine (1 μm) emulsions has been shown to result in droplets between 15 and 20 μm, irrespective of the initial size, as a product of the enzymatic digestion and shear forces in the stomach (21,22). It is therefore of considerable interest to develop our understanding of how SEDDSs behave in the human stomach, not least because it is necessary to establish whether there is indeed any evidence for the emulsification process *per se* having a significant influence on bioavailability.

The aim of this study is to provide a link between the formulation, the emulsification process and the fate of the emulsion in the stomach using the Dynamic Gastric Model (DGM). The DGM is an automated system which allows both the composition and mechanical movement of the stomach to be simulated, thereby providing a unique means of mimicking the effects of the human stomach on dosage forms without the need to resort to volunteer or patient studies or (unreliable) animal studies. More specifically, during the study, we examined the phase behaviour of a model emulsion system (Span 80, Tween 80, soybean oil) with and without inclusion of ibuprofen as a model drug. We then examined the mechanism and process of emulsification using microscopy and particle size analysis. Finally, we used the DGM to examine the effects of gastric conditions on the emulsification process, including a validation study to ascertain the reliability of the DGM as a means of mimicking *in vivo* behaviour. In this manner, we intended to derive a reliable impression of the emulsification process within the stomach in relation to composition and gastric conditions, thereby allowing insights into the likely emulsification behaviour of these systems on ingestion.

This study is part of a larger investigation into the behaviour of drug-loaded SEDDSs, including an examination of the location of the drug within the system during the emulsification process (to be reported in a subsequent paper); hence, a relatively simple model emulsifiable system was required to expedite the analytical procedures and to aid interpretation of the data obtained. Many self-emulsifying formulations reported in the literature contain a range of components additional to those in this study, such as co-solvents (e.g. ethanol) to improve the solubility of the drug in the formulation and partial glycerides to reflect the products formed after digestion. The simple formulation examined here is intended, therefore, to provide a baseline from which the behaviour of more complicated formulations can be extrapolated.

The three excipients used here (Span 80, Tween 80, soybean oil) are easily available in pharmaceutical grades and are commonly used in pharmaceutical formulations. Ibuprofen (log $P = 3.6$) was used as a model drug for three reasons: 1) it is a BCS Class II drug, i.e. low solubility and high permeability, and is therefore representative of a class of drug that should benefit from formulation in a SEDDS; 2) it is known to be weakly surfactant-like so may be expected to affect the emulsification behaviour of the system; 3) its clinical dose is relatively high so when used at these concentrations may represent something of a "worst case scenario" for formulations of SEDDS. Ibuprofen easily dissolves in the SEDDS formulations used here at 12%w/v (the highest concentration tested), but was used throughout the study at a concentration of 6%w/w to avoid issues of saturation and precipitation. Although it wasn't the purpose of this study to examine it in depth, initial experiments indicated that there was some solubility enhancement of ibuprofen in the final SEDDS formulation compared to the three individual components.

MATERIALS AND METHODS

Materials

Span 80 (Sigma, Poole, UK), Tween 80 (Sigma-Aldrich, Gillingham, UK), soybean oil (Sigma, Poole, UK), egg L-α-phosphatidylcholine (lecithin, grade 1, 99% purity) (Lipid Products, South Nutfield, UK), porcine gastric mucosa pepsin (activity 3,300 units/mg of protein calculated using haemoglobin as substrate) (Sigma, Poole, UK) and a gastric lipase analogue derived from Rhizopus orizae (Lipase F-AP15) (Amano Enzyme Inc., Nagoya, Japan) were used as received. Ibuprofen was a gift from BASF (Ludwigshafen, Germany). Licaps® capsules were a gift from Capsugel (division of Pfizer Inc., Bornem, Belgium). Other chemicals and solvents used in this work were of analytical grade. Three aqueous media were used as stated: water, 0.1 N HCl and artificial gastric juice without pepsin (AGJ_{wp}), which is composed of 2 g sodium chloride, 80 mL hydrochloric acid 1 N and water up to 1 L (23).

Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams of the system Tween 80:Span 80 (1:1)/soybean oil/water, in the absence and presence of model drug ibuprofen (6% w/w), were constructed by carefully weighing each anhydrous component mixture (total 1 g) into a glass vial. These mixtures were than titrated at room temperature ($25 \pm 1°C$) by drop-wise addition of MilliQ (18.2 mΩ) water. Equilibration of the system was promoted using a magnetic stirrer set at low speed. After equilibration (approximately 5 to 10 min), the appearance of the system was observed visually and using microscopy (Leica DM LS2 (Leica Microsystems GmbH, Wetzlar, Germany)), and the formed phases were classified as follows: L2, when addition of water produced a clear, transparent mixture; G, when a viscous gel-like phase was formed, which did not show birefringence under polarised light and no flow could be observed on tilting the vial at 90°; LC, when an opaque fluid system consisting of liquid crystalline mesophases were observed (detected via birefringence); and E when a free flowing o/w emulsion was formed. Based on these and visual observations of emulsification in larger volumes of water, the mixture chosen to represent self-emulsification for subsequent studies had the following composition: 65% soybean oil, 17.5% Span 80 and 17.5% Tween 80 (w/w).

Observation of Self-Emulsification Using Microscopy

The self-emulsification of the SEDDS was investigated using a modification of the method proposed by Lim and Miller (24). One end of a hollow borosilicate glass capillary (VitroCom, Mt. Lks., N.J.), with dimensions of 0.6×6.0×50 mm and wall thickness of 0.4 mm, was sealed with parafilm M (Alcan Packaging, Neenah, WI); the capillary was filled through the open side with an aqueous medium and mounted on a microscope glass slide. The self-emulsification mechanism was assessed at room temperature (25±1°C) by injecting between 0.2 and 0.5 μL of SEDDS (n=3) with a Hamilton syringe (Bonaduz, Switzerland) and observing the behaviour of the injected droplet using an optical microscope (as above) equipped with magnification lenses (x4 and x20) and a video camera (Panasonic WV CL310) interfaced to a computer through a capture device (o100vc.dll Osprey Capture Card 1). Polarised light was used in order to investigate the formation of liquid crystalline mesophases during self-emulsification. Images of the self-emulsification process in the various media were also taken using a bright field and polarising optics of an Olympus BX60 (Olympus, Japan) light microscope (x4 and x10). Images were recorded using a ProgRes Capture Pro 2.1 digital camera and software (Jenoptik, Germany).

Droplet Size Measurement

The droplet size was measured on a Coulter LS-230 laser light-scattering apparatus (Beckam, High Wycombe, UK), equipped with polarisation intensity differential scattering PIDS (0.04 to 2,000 μm) and a small volume modulus. Detector offset and beam alignment were performed every hour. The cell was rinsed twice and filled with degassed MilliQ water prior to running the background calibration. After calibration, an appropriate aliquot of the emulsion was injected into the cell with the aid of a glass pipette to obtain an obscuration of the PIDS between 45 and 55%. The refractive indices of soybean oil and water were used in order to calculate reliable droplet size values. The droplet size is given in terms of D_{50}, with the exception of the coarse meal emulsion, for which the average mean diameter was used in order to compare it with the *in vivo* data (21,25).

Preparation of the SEDDS Dispersions

Dispersions of SEDDS placebo (soybean oil 65%, Span 80 17.5% and Tween 80 17.5%) and drug-loaded (ibuprofen 6%) formulations were prepared for droplet size analysis by means of a) a volumetric flask method (n=3), b) USP dissolution apparatus II (n=4), and c) the Dynamic Gastric Model (n=4). Three aqueous media (water, 0.1 N HCl, AGJ_{wp}) were investigated with methods a and b.

Volumetric Flask Method

Fifty μL of SEDDS were dispersed in 10 mL aqueous medium at room temperature (c. 25°C). The self-emulsification was aided by gently inverting the flask 20 times prior to measuring the droplet size of the resulting emulsion.

USP Dissolution Apparatus II

Empty Licaps® capsules were filled with ~500 mg of SEDDS the day prior the experiment. A USP Dissolution apparatus II (Diss 8000, Copley Scientific, Nottingham, UK) equipped with a temperature controller unit (FH 16-D Copley Scientific, Nottingham, UK) was filled with 250 mL of aqueous medium in order to simulate the physiological amount of fluids in the stomach (between 250 and 300 mL (26)). The paddle speed was set at 60 rpm, and temperature was 37°C. Each capsule contained ten glass beads of 2 mm in diameter, to avoid floating of the capsule. Samples (approximately 30 mL) were collected after 25 min and stored under ice pending droplet size analysis.

Dynamic Gastric Model

The Dynamic Gastric Model (DGM) is the first dynamic *in vitro* model of the human stomach which is able to replicate its digestive functions of transforming the bolus into chyme. It has been developed at the Institute of Food Research (Norwich, UK) after intensive studies of the digestive process in healthy human volunteers using Echo-Planar Nuclear Magnetic Resonance Imaging and ileostomy patients (27–35). The DGM (Fig. 1) is composed of a main body and antrum. In the former, the dynamic addition of gastric juices occurs, while the latter is comprised of a piston and a barrel, the movements of which mimic the mechanical process of food breakdown and mixing. A valve allows the reflux of materials between the main body and antrum and also delivers the chyme into the "duodenum." In analogy to the human stomach, the "ingested" material is subjected to acid, enzymatic and mechanical processing. The gastric secretions present the same composition of the *in vivo* secretions, and the enzymatic digestion is accomplished by gastric lipase and pepsin. The addition of acid is regulated through a feed back mechanism, and the pH is monitored throughout by a pH meter; hence, a dynamic addition of acid with time can be achieved, which depends on the buffering capacity of the sample. The enzyme addition depends on the type and amount of material that need to be digested, as is the case *in vivo*. In addition, the

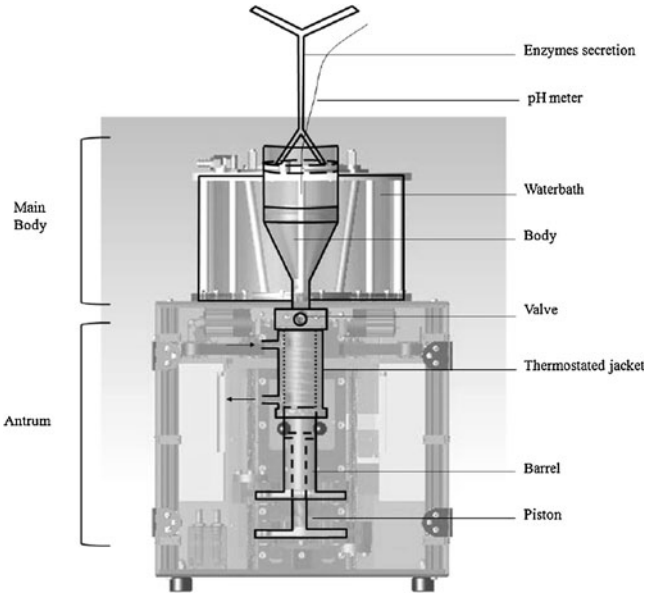

Fig. 1 Schematic diagram of the Dynamic Gastric Model showing the main components forming the main body and the antrum.

operator can set the rate of addition; thus, it follows the *in vivo* rate and is determined depending on the physico-chemical characteristics (e.g. calorific content, viscosity, density, etc.) of the meal. The delivery of the chyme follows a temporal release, in analogy to the release of chyme from the pylorus to the duodenum; hence, the samples collected with time will differ in composition and order of processing. The DGM not only replicates the physiological addition of gastric secretion as observed *in vivo*, but it is also able to reproduce, in the top section representing the main body of the stomach, gentle pulsatile contractions, and in the lower part, representing the antrum, the strong breaking forces (36) observed *in vivo* (34).

The DGM was primed with 20 mL of acid solution (0.01 N HCl solution containing salts) to simulate the acid residual in the fasted state. To compare more directly with the dissolution bath and volumetric flask methods, the DGM was run in the "fasted" state, i.e. 250 mL of water representing a standard glass of water taken with oral medication was added to the DGM with no "foodstuffs." A sample capsule (prepared as for the dissolution apparatus experiment but without the additions of the glass beads) was then added at the start of the experiment. Acid and enzymatic secretions were automatically added following a designed recipe through a feed back mechanism controlled by a computer. At predefined time intervals, samples were collected for droplet size analysis. A total of eight fractions were collected over even time periods over 25 min; the last two fractions (F8 and F9) were pooled together, as the latter comprised a very small volume. The enzymatic secretions were added according to the calorific content of the SEDDS, and the acid secretions were added through a feedback mechanism to reach a resting pH of 1.8 at the end of digestion. The DGM temperature was set to 37°C for the experiment.

Validation of the DGM. In order to ascertain that the droplet size obtained with the DGM can be reasonably considered to correspond to the *in vivo* situation, a test meal analogous to that previously used in human subjects (21,25) was used. The objective was to compare the particle size of the chyme produced by the DGM with the *in vivo* data. The meal consisted of 400 mL of a coarse emulsion containing 70 g commercial extra-virgin olive oil, one whole egg, one egg white, 70 g sucrose with an aqueous phase of 0.15 mM solution of NaCl. The total volume of the emulsion prepared was 400 mL and the final pH 7.5. Emulsification of the meal was accomplished using a food processor (Ultra-Turrax T25; Janke & Kunkel IKA® Labortechnik; Germany), and the mixture was warmed in a water bath (25°C) prior to the experiment. The DGM was fed with 20 mL acid solution, and then the meal was added in totality. The droplet size of the fractions collected from the DGM at 0, 20, 55, 90, 120, 160 and 190 min were measured in triplicate using the method previously described using the refraction index of olive oil to calculate the droplet size. The study was run in triplicate.

Statistical Analysis

SigmaPlot (version 11.0, build 11.0.0.75, Systat Software, Inc.) was used to analyse the results from the sizing studies. The data were compared for statistical significance by one-way analysis of variance (ANOVA) followed by pairwise comparisons adjusted for multiplicity by the Tukey method. A level of significance of $p < 0.05$ was used. All results are expressed as means ± their standard deviations (± s.d.).

RESULTS

Derivation of Pseudo-Ternary Phase Diagrams

Pseudo-ternary phase diagrams were constructed in order to examine the behaviour of the SEDDS components upon dilution with water. Several phases were formed during titration with water of the anhydrous placebo mixtures of Span 80, Tween 80 and soybean oil; the various phases were classified according to their macroscopic and, where appropriate, microscopic appearance (Fig. 2a). Addition of small amounts of water produced a visually clear system (L2), which may consist of reverse micelles or weakly aggregating systems or may otherwise be a w/o micro-

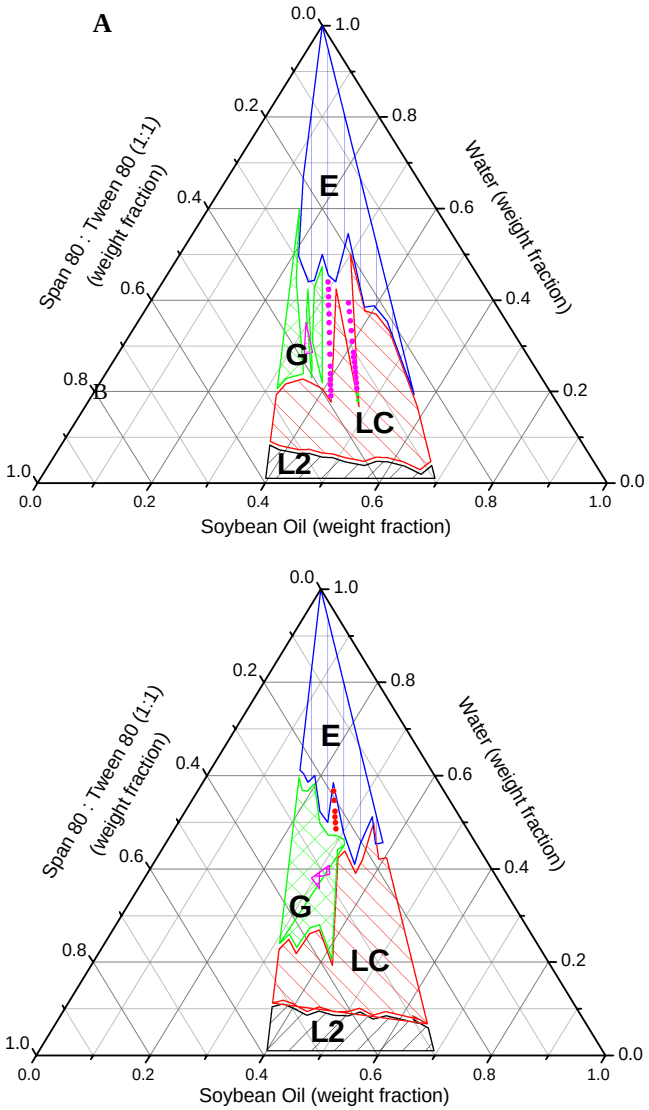

Fig. 2 (**A**) Phase diagram of the system Span 80: Tween 80 (1:1)/soybean oil/water. L2 indicates a w/o microemulsion, LC indicates an area in which liquid crystals can be observed, G is a gel-like viscous phase and E a o/w emulsion. The area in magenta represents an intermediate liquid-like phase. (**B**) Phase diagram of the system Span 80:Tween 80 (1:1)/soybean oil/water containing the model drug ibuprofen (6% w/w). The orange area represents a fluid-like gel phase. All units are weight fractions.

emulsion depending on the amount of water solubilised (37). The samples appeared isotropic when observed under polarised light.

Interestingly, the predominance of L2 was found to decrease with increasing concentration of the oil, as the solubilisation capacity of the water within the system is dependent on the amount of surfactant (38). As titration proceeded, the transparency of the system was lost, and an opaque and more viscous phase was observed. This area, defined as LC, was characterized by the presence of liquid crystalline mesophases dispersed within, as the samples showed birefringence when observed under polarised light. The LC predominance increased as the surfactant content was decreased. At increasing additions of water, a very viscous and gel-like phase (G) was formed; this phase appeared only when the oil concentration was lower than 37% and the surfactant concentration was between 25% and 75%. A milky o/w emulsion (E) was observed when higher amounts of water were added. The magenta circles in Fig. 2 represent those compositions for which a fluid system appeared to be formed within the gel area.

When 6% w/w ibuprofen was incorporated into the system, the corresponding phase diagram showed differences compared to the placebo (Fig. 2b). In particular, the most marked effect observed was the increase in area of the L2 phase, while the LC and G phases appeared to be slightly more prevalent than those observed in the absence of the drug; the E phase was significantly reduced in area. Also, a gel-liquid intermediate phase, indicated by the orange circles, was observed. The observation that the drug alters the phases formed during addition of water is an indication that the ibuprofen is actively participating and influencing the structuring of the system; in particular, the drug appears to be increasing the predominance of the L2 and LC phases. This may be a reflection of the surface-active nature of the drug, as suggested by previous studies (39,40). This implied influence on the formation of liquid crystalline mesophases may in turn reflect an influence on the ease of self-emulsification of the system, as studied in more depth below.

Microscopic Examination of the Self-Emulsifying Process

The mechanism of self-emulsification was studied via direct observation of the SEDDS/water interface, with a particular view to studying the effects of the model drug and the composition of the aqueous media used (water, 0.1 N HCl or AGJ_{wp}). After injection of the SEDDS placebo (65% soybean oil, 35% surfactant) into any of the aqueous media in the capillaries, fine droplets were observed to form from and around a main oil droplet, the appearance of the latter being characterised by transparency at the beginning of the experiment, although after all emulsification activity ceased, the droplet surface become opaque. Initially, the diameter of the droplet increased with time, but this process was counterbalanced by the ejection of material; hence, at the end of the process, the overall size of the droplet was reduced with respect to the initial size.

In the case of the placebo SEDDS injected into water, 0.1 N HCl or AGJ_{wp}, material was ejected from the surface of the droplet through a mechanism which we propose corresponds to the erosion process previously suggested by Wakerly and co-authors (41); this is illustrated in Fig. 3 for the AGJ_{wp} system. This proposed mechanism consists of

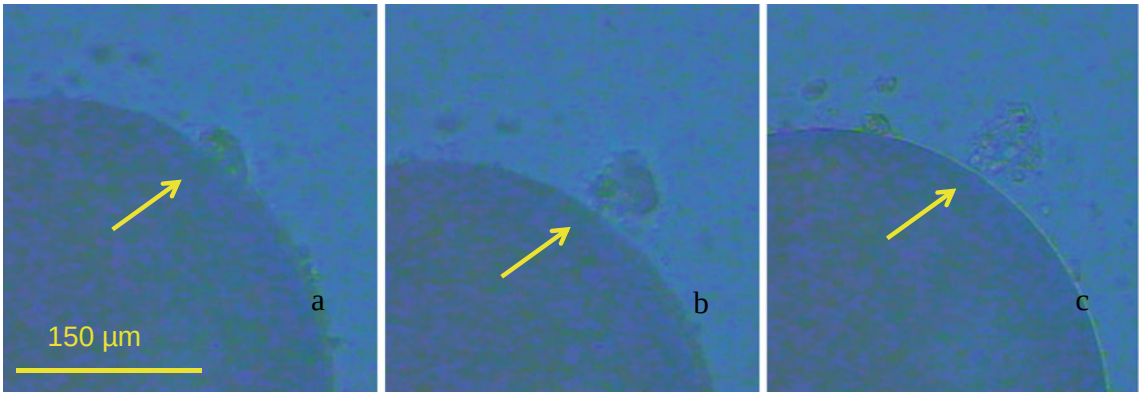

Fig. 3 Photomicrographs of the SEDDS placebo in contact with AGJ_{wp} observed using polarised light microscopy (Leica DM LS2; x4 magnification). Images show the interface during ejection, as indicated by the arrows (images a-c taken over 8 s). Bar = 150 μm.

penetration of water into the droplet surface, inducing the formation of a w/o microemulsion. As the water diffusing into the system increases, liquid crystalline mesophases are formed, while further penetration of water disrupts the surface and induces ejection of smaller droplets. Our data would suggest that this mechanism is applicable but that a mass of material, rather than individual droplets, is ejected. When the injected droplet was observed under polarised light, the formation of birefringent structures was clearly observed at the w/o interface at a location corresponding to the ejection point. The display of birefringence can be related to the formation of liquid crystalline mesophases which are intimately associated with the ejection of material from the droplet surface. This observation is shown in Fig. 4, in which the formation of birefringent structures precedes the ejection of material from the droplet. The texture of the liquid crystals formed during the self-emulsification process was identified as lamellar phase, as their appearance was characteristic of this type of mesophase (42) (Fig. 5). We therefore propose that the system forms lamellar liquid crystalline mesophases on contact with aqueous media and that a mass of such material is ejected during the emulsification process. It is logical to suggest that these masses form the subsequent individual droplets, although it was not possible to ascertain this for certain using the approach described here. Changing the aqueous media did not produce any modification of the behaviour observed in water, except that more material was ejected when the placebo SEDDS was injected into either 0.1 N HCl or AGJ_{wp} with a more vigorous ejection process.

Injection of the formulation containing 6% w/w of ibuprofen into water did not show any significant difference

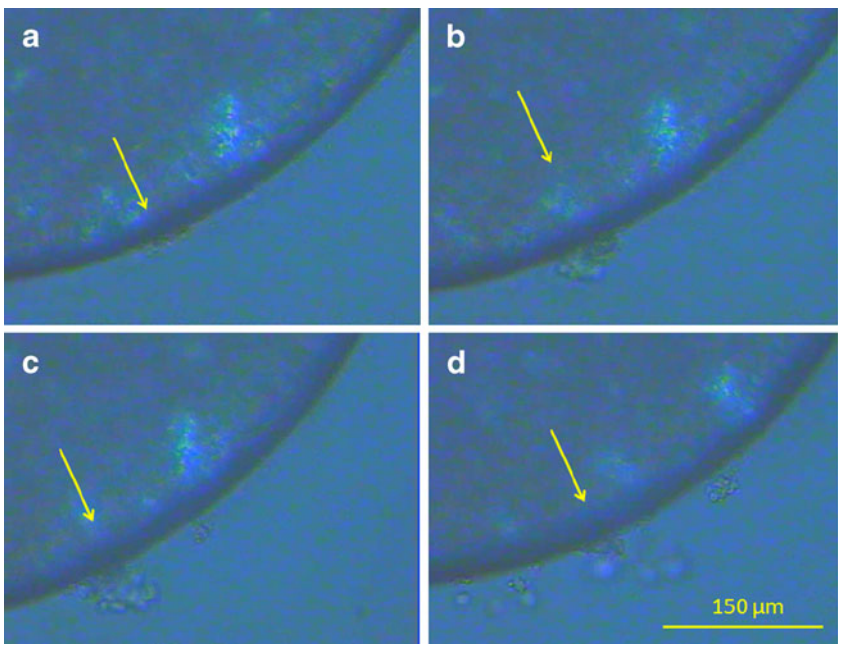

Fig. 4 Photomicrographs showing the erosion process for the SEDDS placebo formulation, involving ejection of material from the droplet surface on contact with water as a function of time (LeicaDM LS2; x20 magnification, polarized light). Images show the interface immediately prior to and during ejection, showing evidence for the presence of birefringent liquid crystalline mesophases at the site of subsequent ejection, as indicated by the arrows. Bar = 150 μm.

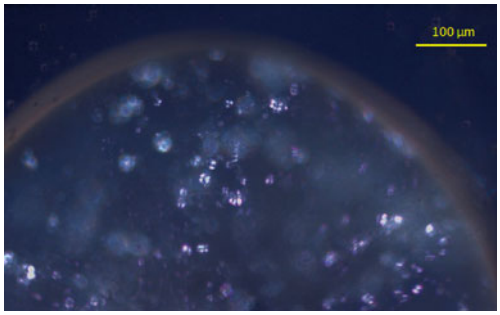

Fig. 5 Image of the texture formed by the SEDDS placebo formulation in water under polarised light (Olympus BX60). The textures formed are typical of the lamellar phase, in which Maltese crosses are easily identified. Bar = 100 μm.

compared to the placebo in terms of ejection of the material from the droplet surface and liquid crystals formation. However, a different mechanism was observed when the formulation was injected in 0.1 N HCl or AGJ_{wp} (illustrated for 0.1 N HCl in Fig. 6). Once the oil was injected, a dramatic change in shape in the "main" droplet was observed; the droplet assumed a cylindrical shape which formed a neck that eventually broke down to form smaller droplets (in the case of AGJ_{wp}, the cylinder did not always break down into smaller droplets). The formation of lamellar liquid crystalline mesophases was retained. Overall, therefore, the mechanism of droplet formation appears to be markedly different in the presence of the drug, at least for two of the dispersion media used. It is reasonable to suggest that this is in turn linked to the observed changes in the phase diagrams.

Validation of the DGM

In order to validate the novel use of the DGM as a means of modelling particle size effects following emulsification within the human stomach, we compared the outputs from the DGM with literature values for a model meal that had previously been studied *in vivo* (21,25). In the *in vivo* study, the meal was administered through a nasogastric tube, from which 100 to 200 mL were aspirated at 1 h interval for 3 h; 20 mL were retained for analysis and the remaining were readministered into the stomach. In the *in vitro* experiment conducted here, the samples were collected from the DGM ejection valve at regular time intervals.

Figure 7a shows the variation in mean droplet size of the meal *in vivo* (21,25) and *in vitro*. The droplet size obtained from the DGM appears to be of the same order of magnitude as that obtained *in vivo* but was numerically lower, though there was no statistical difference for the samples at times 0, 55 and 60, 180 and 190 min ($p > 0.05$), with the exception of the measurement at 120 min. We suggest that the differences such as were observed may be a function of the differences in sampling between the two methods.

It is likely that there is a degree of stratification of particle sizes in the stomach, with larger droplets being physically located at the top of the bolus and therefore more likely to be extracted during sampling. Due to the unavoidable shearing inherent in such a sampling process, the emulsion droplets may have undergone some particle size reduction during this process. The DGM has been designed to mimic the forces and fluid flow within the

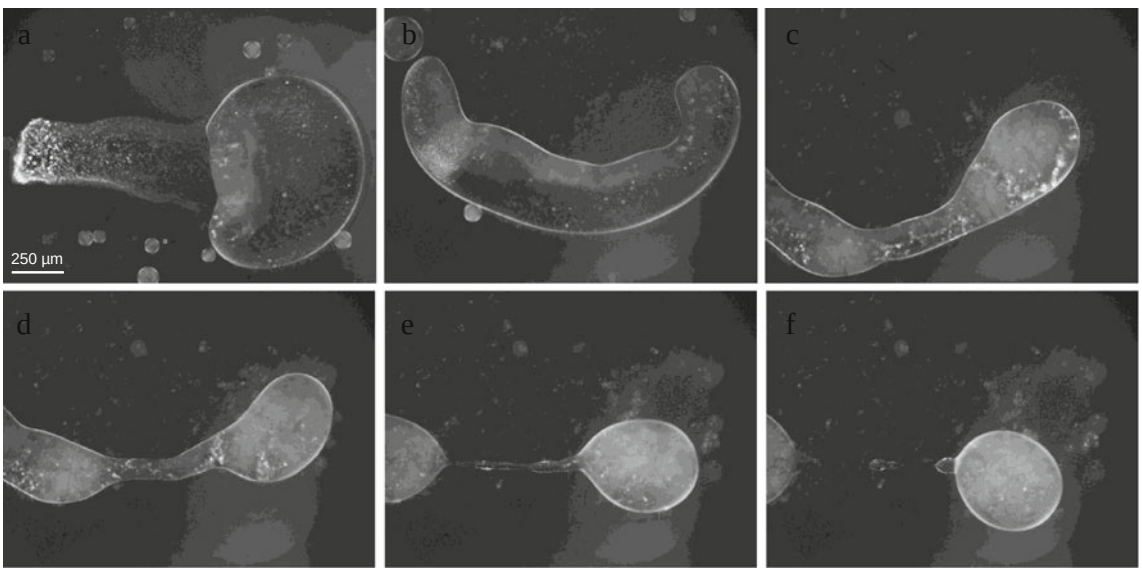

Fig. 6 Photomicrographs showing injection of a droplet of the ibuprofen-loaded SEDDS formulation into 0.1 N HCl under polarized light (Olympus BX60). The pictures show that self-emulsification occurs through a process whereby the droplet entirety becomes distorted and breaks down into individual droplets. Formation of lamellar liquid crystals is also observed. Images were taken over a period of 300 s. Bar = 250 μm.

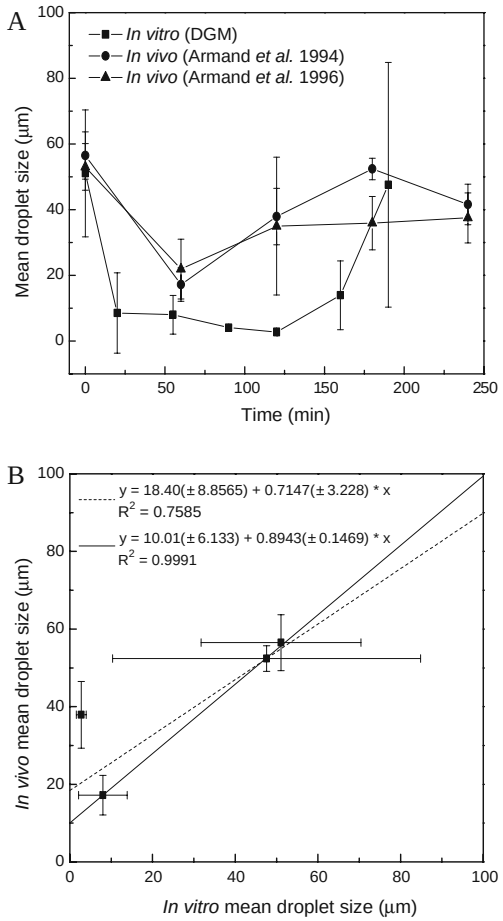

Fig. 7 (**A**) Variation of the droplet size (expressed as mean) of a test emulsion with time during gastric processing *in vivo* (21,25) and *in vitro* (DGM). (**B**) One-to-one correlations between the droplet size of a meal emulsion *in vitro* and *in vivo*.

stomach; therefore, the particle size stratification in the DGM should be equivalent to that in the stomach. Samples for analysis are taken with very low shear directly from the DGM exit valve, analogous to the pyloric sphincter, to mimic the *in vivo* transfer of material from the stomach to the duodenum, rather than from the bulk of the stomach contents, as was the case with the *in vivo* studies described here. Hence, particle size stratification will have resulted in smaller droplets being released through the DGM exit valve (pyloric sphincter) until the last sampling timepoint whereby the larger droplets floating on the surface of the stomach contents will have exited the DGM. Overall, therefore, a slightly smaller droplet size would be expected to be measured for the DGM, especially at the earlier timepoints, which indeed was the case, although as indicated above, this was not statistically significant.

The corrleation between the mean droplet size obtained from the *in vitro* DGM studies and *in vivo* studies is seen in 7b. The observation that the particle size following digestion in the DGM is similar to that of the human stomach indicates that this *in vitro* system provides a reasonable representation of gastric processing, which it is therefore reasonable to utilise to monitor the fate of SEDDS in the stomach.

A Comparison of the DGM and USP Methods

The droplet sizes of the placebo and drug-loaded SEDDS were found to be dependent on the method of preparation and also on the aqueous media used for the dispersion, as shown in Fig. 8a. When the droplets of the placebo SEDDS were prepared using the volumetric flask method, the size observed between the two acidic aqueous media was similar, while the droplet size in water was significantly higher ($p=0.031$ for HCl and $p=0.045$ for AGJ_{wp}). For the drug-loaded sample, the droplets showed again to have a smaller size in the acidic media ($p=0.004$ for HCl and $p=0.004$ for AGJ_{wp}). However, addition of ibuprofen resulted in droplets which were significantly lower in size than the corresponding placebo droplets, when dispersed in either water ($p<0.001$), HCl ($p=0.014$) and AGJ_{wp} ($p=0.038$).

In the case of the placebo droplets prepared using the USP dissolution apparatus II, similar sizes were obtained in all three media ($p=0.933$). When the drug-loaded formulation was analysed, a reduction in size was observed for the samples dispersed in both acidic media ($p=1.000$ for water, and $p<0.001$ for HCl and AGJ_{wp}) when compared to the placebo. Thus far, therefore, the most marked effect is that inclusion of the drug has resulted in a decrease of particle size for both methods. Perhaps surprisingly, examination of Fig. 8a also indicates that the absolute values of median particle size are greater for the USP dissolution apparatus method despite the more prolonged mechanical agitation induced by this approach compared to the volumetric method. This may indicate that it is the vigour of agitation rather than the time involved that is the critical factor in determining size.

The more extensive range of sampling times utilised for the DGM study allows the investigation of the droplet size of the SEDDS at different extents of gastric processing, thus allowing imitation of the range of conditions in which they would be delivered into the small intestine (Fig. 8b). It is interesting to note that the particle size did not appear to vary greatly until the last collection times (fractions F8 and F9), whereby a significant increase in size was observed ($p<0.001$). The drug-loaded formulation produced droplets of similar magnitude to the earlier samples, with the exception of samples F2 and F3, which were significantly smaller ($p=0.032$ and $p=0.036$, respectively). The increase in size for the F8 and F9 was again observed, albeit less marked than for the placebo.

There are therefore three key observations from this data set. First, the drug-loaded and placebo emulsions

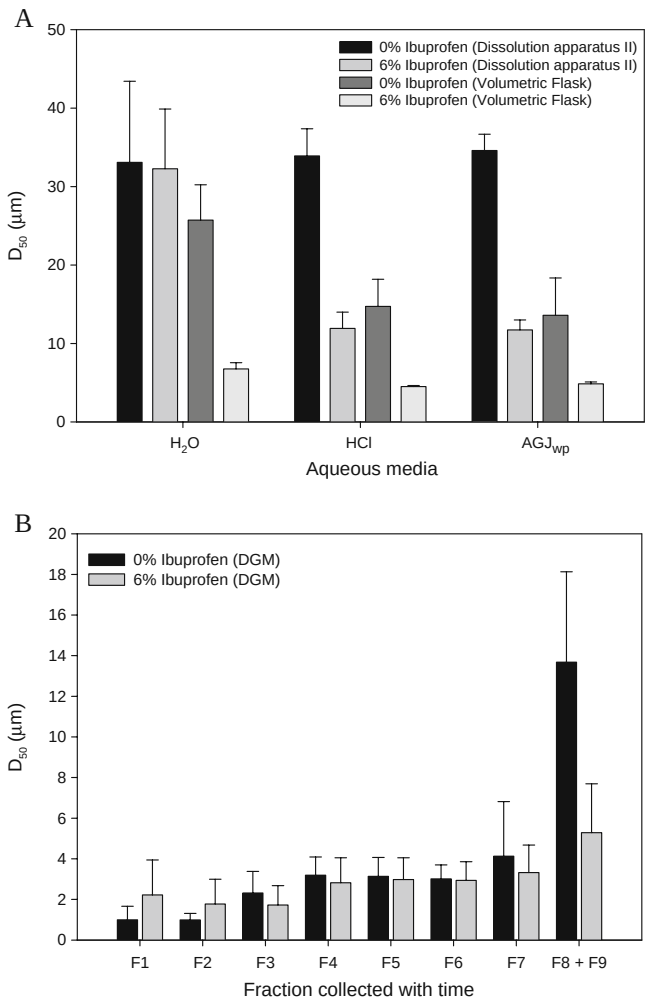

Fig. 8 (**A**) Droplet size (D_{50}) of SEDDS containing 0 and 6% of ibuprofen dispersed in water, HCl 0.1 N and AGJwp using the dissolution apparatus II and the volumetric flask methods. (**B**) Droplet size (D_{50}) of SEDDS containing 0 and 6% of ibuprofen for the eight fractions obtained during simulation of gastric digestion using the DGM. *Bars* represent the standard deviation.

showed broadly similar size distributions in the DGM, indicating that any changes in mechanism or emulsification tendency between the two systems have been superseded by other factors, the most logical suggestion being the mechanical agitation associated with the DGM. Second, surprisingly, the DGM results are closer to the volumetric flask results than they are to the USP dissolution apparatus approach, showing a much lower size for both types of emulsion. This further supports the suggestion that the vigour of mechanical agitation is highly important in determining size, despite these systems being theoretically self-emulsifying and hence requiring little or no agitation. The final observation is that the emulsion appears to be stable within the (model) stomach, with little change in size seen up until the final samples. It is feasible that this represents either poorly emulsified material with initially larger droplet size that floated on the stomach surface or instability and coalescence of the droplets. Nevertheless, the key observation is that the emulsion is predicted to be presented to the intestine in a remarkable uniform size for the majority of the digestion process.

DISCUSSION

The study has addressed three issues associated with the behaviour of SEEDSs: the mechanism of emulsification, the particle size of the resulting emulsion as assessed by a range of methods and the effect of the incorporation of a model drug on the emulsification process. In addition, we describe the use of a novel *in vitro* method to assess the behaviour of the emulsions in the stomach.

There appear to be two mechanisms by which emulsification takes place. The first involves the budding off or erosion of material from the surface of larger droplets, this involving the presence of lamellar liquid crystalline phases whose presence was confirmed by the establishment of pseudo-ternary phase diagrams. The second involves distortion and breakup of the larger particles. While some differences were observed depending on the aqueous medium used, the key factor influencing mechanism appeared to be the presence of the drug ibuprofen, which was also shown to influence the compositional dependence of the liquid crystalline phases for the phase diagram studies. It is suggested that the surface activity of the drug may well be of relevance to these observations, but overall, the study has clearly shown that the drug must be considered as a contributing factor to the emulsification properties of the SEDDS (although a corollary to this is outlined below).

Many different SEDDS formulations have been studied in the literature, although the exact phase behaviour of most of them has not been determined. It is reasonable to suspect that the phase behaviour of multi-component systems will be even more complicated than that of the relatively simple system studied here, with differing extents of the LC, L2 and G phases being observed. Particularly for systems which have a small amount of water added during the formulation stage (i.e. before oral ingestion), the phase behaviour is likely to be considerably different to that observed here for an anhydrous system. Given that the emulsification appears to be via a liquid crystalline intermediate stage, the relative extent of the LC phase would seem to be crucial. The observation here that the presence of the drug altered the phase behaviour of the system and resulted in a decreased droplet size on *in vitro* assessment is significant and suggests that the effect of incorporation of the drug into such a formulation should be investigated more routinely.

Particle size was assessed using a range of approaches ranging from microscopic, in which individual droplet sizes were assessed, to more conventional methods such as the volumetric flask and USP dissolution methods. In all cases, a trend of decreasing size with drug addition was noted, again suggesting the influence of the drug on the emulsification process. However, of particular significance is the introduction of the DGM as a novel means of modelling the action of the human stomach on the SEDDS emulsification. We present a validation study which supports the biorelevance of this model to the *in vivo* situation. For the SEDDS under study here, the DGM indicated that the particle size is lower than that predicted by the USP dissolution apparatus, while the effect of the drug was much smaller than the other *in vitro* methods would seem to suggest. We propose that the vigour of the mechanical movement of the stomach supersedes the formulation effects, leading to a small, uniform size distribution irrespective of the presence of the drug.

We have therefore attempted to provide a link between the physical chemistry of the emulsification process of SEDDSs and the emulsification behaviour *in vivo*. The study suggests that while the precise SEDDS formulation, including the presence or absence of drug, may significantly alter the emulsification process *in vitro*, the likely situation *in vivo* is that the mechanical agitation of the stomach may result in considerable and largely uniform particle size reduction irrespective of the formulation. This is in agreement with the previously observed effects of digestion and shearing in the stomach on pre-formed coarse emulsions, as discussed earlier (21,22). Clearly, caution is required in extrapolating this principle to other SEDDS formulations and drugs, but, nevertheless, the data clearly suggest that the interplay between the fundamental process of emulsification and the behaviour *in vivo* may be less than is sometimes assumed. Furthermore, the study opens up a means of assessment whereby issues such as the presence of food and changes in the degree of agitation as may be found, for example, in elderly patients may be modelled.

CONCLUSIONS

The investigation has explored the linkage between the formulation of a model SEDDS system with and without inclusion of the drug ibuprofen, the tendency to from lamellar liquid crystals, the emulsification mechanism and the droplet size profile, assessed using a range of methods including the Dynamic Gastric Model (DGM). The data indicate that the presence of the drug results in a lowering of the droplet size and an alteration of the emulsification mechanism, which may in turn be associated with changes to the tendency to form liquid crystals, as assessed using pseudo-ternary phase diagrams. However, the DGM studies indicated that the SEDDS generated fine droplets which remained stable within the gastric environment for up to two hours, irrespective of the presence of the drug. It is concluded that while the drug may alter the emulsification process and mechanism, in a biological environment such effects may be superseded by the mechanical movement of the stomach, at least for the system under study here. The investigation has also presented the DGM as a novel and potentially highly useful novel means of assessing emulsification in a biorelevant manner.

ACKNOWLEDGMENTS

The authors would like to thank the University of East Anglia for the financial support of A. M. and the University of Messina for the Erasmus placement of A. P. Access to the droplet sizer was kindly provided by the Institute of Food Research (Norwich). The authors are also grateful to Dr. Mary Parker from the Institute of Food Research for assistance with the microscopy images taken with the Olympus BX60.

REFERENCES

1. Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Deliv Rev. 1997;25:47–58.
2. Mullertz A, Ogbonna A, Ren S, Rades T. New perspectives on lipid and surfactant based drug delivery systems for oral delivery of poorly soluble drugs. J Pharm Pharmacol. 2010;62:1622–36.
3. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29:278–87.
4. MacGregor KJ, Embleton JK, Lacy JE, Perry EA, Solomon LJ, Seager H, *et al.* Influence of lipolysis on drug absorption from the gastro-intestinal tract. Adv Drug Deliv Rev. 1997;25:33–46.
5. Ito Y, Kusawake T, Prasad YVR, Sugioka N, Shibata N, Takada K. Preparation and evaluation of oral solid heparin using emulsifier and adsorbent for *in vitro* and *in vivo* studies. Int J Pharm. 2006;317:114–9.
6. Serratoni M, Newton M, Booth S, Clarke A. Controlled drug release from pellets containing water-insoluble drugs dissolved in a self-emulsifying system. Eur J Pharm Biopharm. 2007;65:94–8.
7. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res. 1992;9:87–93.
8. Craig DQM, Lievens HSR, Pitt KG, Storey DE. An investigation into the physico-chemical properties of self-emulsifying systems using low frequency dielectric spectroscopy, surface tension measurements and particle size analysis. Int J Pharm. 1993;96:147–55.
9. Thi TD, Van Speybroeck M, Barillaro V, Martens J, Annaert P, Augustijns P, *et al.* Formulate-ability of ten compounds with different physicochemical profiles in SMEDDS. Eur J Pharm Sci. 2009;38:479–88.
10. Tarr BD, Yalkowsky SH. Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. Pharm Res. 1989;6:40–3.

11. Kovarik JM, Mueller EA, Van Bree JB, Tetzloff W, Kutz K. Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. J Pharm Sci. 1994;83:444–6.
12. Gao Z-G, Choi H-G, Shin H-J, Park K-M, Lim S-J, Hwang K-J, *et al.* Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A. Int J Pharm. 1998;161:75–86.
13. Khoo S-M, Humberstone AJ, Porter CJH, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. Int J Pharm. 1998;167:155–64.
14. Holm R, Porter CJH, Edwards GA, Müllertz A, Kristensen HG, Charman WN. Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. Eur J Pharm Sci. 2003;20:91–7.
15. Porter CJH, Kaukonen AM, Boyd BJ, Edwards GA, Charman WN. Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation. Pharm Res. 2004;21:1405–12.
16. Nielsen FS, Petersen KB, Müllertz A. Bioavailability of probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. Eur J Pharm Biopharm. 2008;69:553–62.
17. Porter CJH, Charman WN. *In vitro* assessment of oral lipid based formulations. Adv Drug Deliv Rev. 2001;50:S127–47.
18. O'Driscoll CM, Griffin BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility–The potential impact of lipid-based formulations. Adv Drug Deliv Rev. 2008;60:617–24.
19. Lee KWY, Porter CJH, Boyd BJ. Gastric processing is a critical determinant of the ability of lipid-based formulations to enhance the oral bioavailability of a model poorly water-soluble drug. Transactions of the 36th Annual Meeting and Exposition of the Controlled Reslease Society, Copenhagen, Denmark; 2009, p. 681.
20. Grove M, Müllertz A, Pedersen GP, Nielsen JL. Bioavailability of seocalcitol: III. Administration of lipid-based formulations to minipigs in the fasted and fed state. Eur J Pharm Sci. 2007;31:8–15.
21. Armand M, Borel P, Pasquier B, Dubois C, Senft M, Andre M, *et al.* Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. Am J Phys. 1996;271:G172–83.
22. Armand M, Pasquier B, Andre M, Borel P, Senft M, Peyrot J, *et al.* Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. Am J Clin Nutr. 1999;70:1096–106.
23. The Department of Health. Appendix XII B. Dissolution guidance on dissolution testing. Artificial Gastric Juice. British Pharmacopoeia 2009 Volume I & II Monographs: Medicinal and Pharmaceutical Substances. London: The Stationery Office; 2008.
24. Lim JC, Miller CA. Dynamic behavior and detergency in systems containing nonionic surfactants and mixtures of polar and nonpolar oils. Langmuir. 1991;7:2021–7.
25. Armand M, Borel P, Dubois C, Senft M, Peyrot J, Salducci J, *et al.* Characterization of emulsions and lipolysis of dietary lipids in the human stomach. Am J Phys. 1994;266:G372–81.
26. Vertzoni M, Dressman J, Butler J, Hempenstall J, Reppas C. Simulation of fasting gastric conditions and its importance for the *in vivo* dissolution of lipophilic compounds. Eur J Pharm Biopharm. 2005;60:413–7.
27. Gowland PA, Marciani L, Fillery-Travis A, Spiller RC. MRI of gastric function. In: Webb GA, Belton PS, Gil AM, and Delgadillo I, editors. Magnetic resonance in food science, a view to the future. The Royal Society of Chemistry, Cambridge; 2001. p. 85–97.
28. Marciani L, Bush D, Wright P, Wickham MSJ, Pick B, Wright J, *et al.* Monitoring of gallbladder and gastric coordination by EPI. J Magn Reson Imag. 2005;21:82–5.
29. Marciani L, Faulks RM, Wickham MSJ, Bush D, Pick B, Wright J, *et al.* Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. Br J Nutr. 2009;101:919–28.
30. Marciani L, Ramanathan C, Tyler DJ, Young P, Manoj P, Wickham MSJ, *et al.* Fat emulsification measured using NMR transverse relaxation. J Mag Res. 2001;153:1–6.
31. Marciani L, Wickham MSJ, Bush D, Faulks RM, Wright J, Fillery-Travis A, *et al.* Magnetic resonance imaging of the behaviour of oil-in-water emulsions in the gastric lumen of man. Br J Nutr. 2006;95:331–9.
32. Marciani L, Wickham MSJ, Hills BP, Wright J, Bush D, Faulks RM, *et al.* Intragastric oil-in-water emulsion fat fraction measured using inversion recovery echo-planar magnetic resonance imaging. J Food Sci. 2004;69:E290–6.
33. Marciani L, Wickham MSJ, Singh G, Bush D, Pick B, Cox EF, *et al.* Enhancement of intragastric acid stability of a fat emulsion meal delays gastric emptying and increases cholecystokinin release and gall bladder contraction. Am J Phys. 2007;292:G1607–13.
34. Marciani L, Wright J, Bush D, Fillery-Travis A, Gowland PA, Spiller R. Assessment of antral grinding of a model solid meal with echo-planar imaging. Am J Phys - GI Liver Phys. 2001;280:G844–9.
35. Marciani L, Wickham M, Wright J, Bush D, Faulks R, Fillery-Travis A, *et al.* Magnetic resonance imaging (MRI) insights into how fat emulsion stability alters gastric emptying. Gastroenterology. 2003;124:A581–1.
36. Vardakou M. Personal communication, 2009.
37. Aboofazeli R, Patel N, Thomas M, Lawrence MJ. Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; The influence of oil. Int J Pharm. 1995;125:107–16.
38. Funari SS, Klose G. Phase behaviour of the ternary system POPC/C12E2/2H2O. Chem Phys Lipids. 1995;75:145–54.
39. Fini A, Fazio G, Feroci G. Solubility and solubilization properties of non-steroidal anti-inflammatory drugs. Int J Pharm. 1995;126:95–102.
40. Stephenson BC, Rangel-Yagui CO, Junior AP, Tavares LC, Beers K, Blankschtein D. Experimental and theoretical investigation of the micellar-assisted solubilization of ibuprofen in aqueous media. Langmuir. 2006;22:1514–25.
41. Wakerly MG, Pouton CW, Meakin BJ, Morton FS. Self-emulsification of vegetable oil-nonionic surfactant mixtures. Phenomena in Mixed Surfactant Systems, American Chemical Society, Washington, DC; 1986. pp. 242–255.
42. Rosevear FB. The microscopy of the liquid crystalline neat and middle phases of soaps and synthetic detergents. J Am Oil Chem Soc. 1954;31:628–39.