

Impact of Solidification on the Performance of Lipid-based Colloidal Carriers: Oil-based versus Self-emulsifying Systems

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Abstract: The study aims to develop and optimise lipid-based colloidal carriers (LBCC) for enhancing solubilisation and reducing fed/fasted variation for the poorly water-soluble danazol (DAN). Oil-based and self-microemulsifying delivery systems (SMEDDS) were developed, and the effect of solidification was investigated. Liquid SMEDDS (L-SMEDDS, Capmul MCM:Tween 80:Transcutol HP 1:2:1, w/w) and emulsion (Capmul MCM:soya lecithin 100:0.6, w/w) were developed. Solid-state formulations were prepared *via* (i) physical adsorption of L-SMEDDS (P-SMEDDS) or (ii) spray drying of emulsion (silica-lipid hybrid, SLH) and L-SMEDDS (spray-dried SMEDDS, S-SMEDDS) using Aerosil 380 silica nanoparticles as the solid carrier. *In vitro* lipid digestion and drug solubilisation under simulated intestinal conditions in both fasted and fed states were investigated. Solubilisation of unformulated DAN under both fasted and fed conditions was low, and a large fed/fasted variation was observed, *i.e.* 6.6-fold difference. All LBCC formulations provided enhanced drug solubilisation and significantly reduced the fed/fasted variation. For self-emulsifying LBCC, the fasted state drug solubilisation was ranked as L-SMEDDS > P-SMEDDS > S-SMEDDS, suggesting that solidification reduced the capability of SMEDDS in presenting DAN to the aqueous phase. However, in the case of oil-based LBCC, improved drug solubility was observed with the solid form SLH under both fasted and fed state in comparison to that of the equivalent liquid form. Overall, the SLH, which provided the highest drug solubilisation in the fasted state (*i.e.* 10-fold higher than the pure DAN) and the smallest fed/fasted variation, was considered an optimised solid LBCC to enhance the solubilisation of DAN and reduce the fed/fasted variation.

Keywords: Colloidal carriers, fasted state, fed state, food effect, *in vitro* lipolysis, lipid-based, self-microemulsifying, silica-lipid hybrid.

1. INTRODUCTION

Solubilisation of pharmaceutical compounds in gastrointestinal (GI) fluids is a prerequisite of oral drug absorption. Drug solubilisation in the GI tract is a complex process, and is determined by physicochemical properties of the compound (*e.g.* water solubility and particle size) as well as physiological factors (*e.g.* presence of food within the GI tract) [1]. For BCS class II drugs (*i.e.* low water solubility and high permeability), a positive food (or post-prandial) effect takes place when co-administered with a fat-rich meal and can significantly improve the oral bioavailability, *i.e.* by at least 25% [2]. Enabling lipid-based colloidal carriers (LBCC) have been developed to mimic the positive food effect and optimise the oral bioavailability of BCS class II compounds [3]. It was suggested that the ingested lipid excipients can enhance the drug solubility capacity in the GI tract by creating a lipophilic microenvironment and generate a concentration gradient favouring drug transport towards the intestinal absorptive sites [2c, 4].

A majority of the excipients used to construct LBCC are liquid or semi-solid at ambient temperature, and the marketed oral LBCC are typically filled in soft or hard capsules, such as Neoral[®] cyclosporine soft gelatin capsules and Solufen[®] ibuprofen hard gelatine capsules [5]. Whilst the capsule liquid-filling approach generally enables high drug loading potential, the risk of incompatibility between the fill component and the shell as well as the potential leakage or migration of liquid excipients into the capsule shell represent a major challenge. An alternative approach to convert liquid LBCC to the solid state involves the adsorption or encapsulation of liquid dosage forms onto/within inert porous solid carriers with high specific surface area, such as colloidal silicon dioxide (Aerosil[®]) [6], and magnesium aluminometasilicate (Neusilin[®]) [7]. Liquid-loaded powders are free flowing and can be easily compressed into tablets to allow convenient oral dosage [8]. However, literature data on the effect of such an adsorption or encapsulation process on the ability of the formulation to present the poorly water-soluble drug in a solubilised state has been sparse and contradictory. Speybroeck *et al.* reported the adsorption of liquid SMEDDS to Neusilin[®] US2 limited the release of danazol and provided a reduced oral bioavailability in comparison to the liquid SMEDDS in a fasted rat model [7a]. In contrast, Shanmugam *et al.* demonstrated comparable pharmacokinetic profiles for

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lutein-encapsulated liquid and solid SNEDDS following the oral administration to fasted rabbits [9]. In addition, solid-state silica-lipid hybrid (SLH) systems have shown improved oral bioavailability for a range of poorly water soluble compounds in comparison to the equivalent liquid oil-based or self-emulsifying formulations [6b, 10]. Furthermore, most of the literature data were focusing on the intestinal fasted state, little is known regarding the formulation factors that may affect the fed state solubilisation of poorly water soluble compounds and the fed/fasted state variation.

The current study therefore sought to assess the impact of solidification on the performance of LBCC under simulated intestinal digesting conditions in both fasted and fed states. A dynamic *in vitro* lipolysis model which simulates the physiological intestinal fed/fasted condition was used for the assessment. Danazol (DAN, water solubility = 0.42 µg/ml, logP = 4.53 [11]), for which food intake increases the oral bioavailability by more than three-fold over the fasted condition in healthy human subjects, was selected as the model BSC class II compound [12]. Two types of LBCC were compared, Lipid Formulation Classification System (LFCS) Type I oil-based formulation (*i.e.* only comprised of triglycerides or mixed glycerides surfactants) and Type IIIB SMEDDS formulation (*i.e.* comprising ≥ 80%, w/w of hydrophilic components) [13]. Together, the study provides important information on the role of solidification in enhancing or preserving the biopharmaceutical performance of various liquid-state LBCC, which will contribute to the future optimisation of solid-state lipid-based formulations for the oral delivery of poorly water-soluble drugs.

2. MATERIALS AND METHODS

Danazol powder and Tween 80 (Polysorbate 80) were supplied by Chem-supply (Gillman, SA, Australia). Capmul MCM (C8/C12 mono/diglyceride blend) and Transcutol HP were gifts from Abitech Corporation (Columbus, OH, USA) and Gattefossé (Lyon, France), respectively. Soybean lecithin (>94% phosphatidylcholine and <2% triglycerides) was purchased from BDH Merck (Kilsyth, VIC, Australia). Hydrophilic fumed silica (Aerosil® 380) with a primary average diameter of 7 nm and a specific surface area of 380±30 m²/g was purchased from Evonik Degussa (Essen, Germany). Sodium taurodeoxycholate (NaTDC) 99%, Trizma maleate, lecithin (60% pure phosphatidylcholine, from dried egg yolk), porcine pancreatin extract (activity equivalent to 8 × USP specification), 4-bromophenylboronic acid (4-BBB), calcium chloride dehydrate and sodium hydroxide pellets were purchased from Sigma-Aldrich (Sydney, NSW, Australia). All other chemicals were of analytical grade and used as received. High purity (Milli-Q) water was used throughout the study.

2.1. HPLC Analysis of DAN

DAN was assayed using a HPLC system (Shimadzu Corporation, Japan) consisting of a series of LC-20ADXR pumps, SIL-20ACXR auto sampler, CTO-20AC column oven, and SPD20A variable UV detector set at 286 nm, and a LiChrospher C18 analytical column (RP-18, 5 µm, 4.6 mm ID × 250 mm, Alltech, Grace Davison Discovery sciences, Columbia, MD, USA). The mobile phase was a mixture of acetonitrile and water (75:25 v/v), eluted at a flow rate of 1

ml/min. The limit of quantification (LOQ) of this analytical method was 50 ng/ml. The intra- and inter-day assay precision was assessed by coefficient of variance (≤ 10%) and the accuracy was assessed as percentage bias (≤ 10%). Linear calibration curve ($R^2 \geq 0.99$) was plotted for chromatographic peak areas against DAN concentrations (in mobile phase solution) over the range of 0.1–20 µg/ml, without the addition of an internal standard. All analytes were diluted suitably to meet the calibration concentration range.

2.2. Preparation of SMEDDS Formulation

Capmul MCM was selected as the lipid candidate, given its relatively higher solubility for DAN (21.6±1.2 mg/g at 37 °C) in comparison to other glycerides [14]. Tween 80 (Hydrophilic-Lipophilic Balance, HLB= 15) and Transcutol HP were selected as the surfactant and co-solvent, respectively. The lipid: surfactant: co-solvent ratio was optimised based on the solubility test and visual assessment of the propensity to self-emulsify as described below. The liquid SMEDDS (L-SMEDDS) were prepared by weighing the appropriate amount of DAN and excipient in glass vials, and mixed by magnetic stirring and sonication until DAN was completely dissolved.

To determine DAN equilibrium solubility, an excess amount of DAN was added into each L-SMEDDS pre-concentrate. The mixtures were covered with aluminium foil to avoid the exposure to light. The mixtures were sonicated (Bransonic® Model 2510, USA) for 90 minutes, and equilibrated by rotation at room temperature for 3 days. The mixture was then centrifuged (Hermle high speed table top centrifuge Z36HK, Germany) at 20,000 rpm (29,074 × g) for 20 minutes to precipitate the undissolved drug. The content of DAN in the supernatant, defined as the solubility, was quantified following drug extraction with methanol and sample analysis by HPLC as described above.

The self-emulsifying properties were assessed using a visual test, whereby 1 part of L-SMEDDS was added to 100 parts of water at room temperature (v/v), and the tendency to form bluish or transparent emulsion spontaneously was observed. Samples were gently agitated and the ease of emulsification was noted.

2.3. Preparation of Solid-SMEDDS

Two solidification approaches were investigated for the formation of solid SMEDDS, *i.e.* i) physical adsorption *via* blending the optimised L-SMEDDS with the solid carrier, and ii) bulk self-assembly and spray drying. Aerosil® 380 silica was selected as the solid carrier in both cases.

To prepare the spray-dried solid SMEDDS (S-SMEDDS), water was added to the prepared DAN-loaded L-SMEDDS (3%, w/w) as the continuous phase to form the o/w micro-emulsion (L-SMEDDS: water 1:10 w/w); after sonication for 10 min, the microemulsion was mixed with an equal volume of silica aqueous dispersion (5% w/v) *via* magnetic stirring for overnight; final S-SMEDDS was obtained following spray drying the mixture under the following conditions: inlet temperature 160 °C, outlet temperature 65 °C, flow rate 7 mL/min, aspiration setting 10 (BÜCHI Mini Spray Dryer B-290, Switzerland).

To prepare the physically adsorbed SMEDDS (P-SMEDDS), L-SMEDDS was physically mixed with silica using a spatula until the formation of a homogeneous and free-flowing powder. The ratio between L-SMEDDS and silica was kept at 2:1 (w/w). Prior to the physical mixture process, the silica particles were obtained following spray drying the silica dispersion (5%) under the same conditions as that described for the formation of S-SMEDDS to remove the excess of water adsorbed onto/within the porous silica particles.

2.4. Preparation of SLH Microparticles

SLH microparticles were prepared following a three-step preparation process as described previously [10c]. Capmul MCM and soybean lecithin were selected as the oil phase and emulsifier, respectively. Briefly, soybean lecithin (0.6 % w/v) was solubilised in Capmul MCM by sonication for 10 min. DAN (3%, w/w) was dispersed in the oil mixture by sonication for 10 min, after which Milli-Q water was added to the drug-loaded oil mixture. A coarse o/w emulsion was formed following sonication (1 hour), overnight stirring, and homogenisation (Avestin® EmulsiFlex-C5 Homogenizer) under a pressure of 1000 bar for 5-10 cycles. The coarse emulsion was then mixed with Aerosil® 380 silica dispersion (5% w/w) *via* magnetic stirring overnight. The final SLH formulation was obtained following water removal from the mixture by spray drying following the same conditions as that used for the preparation of S-SMEDDS.

2.5 Physicochemical Characterisation of LBCC

2.5.1. Particle Size

The particle sizes of solid formulations were characterised using laser diffraction (Malvern Mastersizer 2000, UK). Water (refractive index=1.33) was used as the dispersant, and the particle refractive index was pre-set as 1.45 for medium-chain lipids and silica particles.

In addition, the particle size and polydispersity index (PDI) of dispersions of liquid or solid formulations in aqueous medium were determined by dynamic light scattering technique (Malvern Nano-ZS Zetasizer, UK). Formulations were dispersed in water (25 °C) by hand-held shaking at a concentration equivalent to that obtained in the digestion medium. For solid formulations, the dispersions were centrifuged at low speed (450 g, 25 °C) for 5 min to avoid the interference of large silica particles prior to the size measurement of the redispersed lipid colloids [7a].

2.5.2. Lipid Content

Lipid content of solid formulations was determined using thermogravimetric analysis (Hi-Res Modulated TGA 2950, TA Instruments Australia). Approximately 10 mg of each formulation was accurately weighed. The sample was heated in an aluminum pan at a rate of 10 °C/min from 20 °C to 600 °C under a nitrogen gas purge. The lipid excipients were evaporated within the range of 200-500 °C, and the silica component remained thermally stable. After correction for the water content, the weight loss after heating was computed using the associated TA Universal Analysis software, and this corresponds to the lipid content of the microparticles.

2.5.3. Drug Loading

The amount of DAN loaded into solid-SMEDDS and SLH carriers was determined using a solvent extraction method. 10 mg of formulation was dissolved in 10 mL methanol and sonicated for one hour. The mixture was then centrifuged at 20,000 rpm for 10 min. The supernatant was collected and diluted with mobile phase prior to HPLC analysis for DAN content.

2.5.4. Solid State Characterisation

The degree of crystallinity of the encapsulated DAN was monitored using differential scanning calorimetry (DSC) technique (TA instruments Q100 DSC). Each sample (approximately 5 mg) was heated in an aluminum pan at a rate of 10 °C/min over a temperature range of 25-200 °C, under a flow of dry nitrogen gas (80 ml/min). The heat flow required to maintain an equal temperature between the sample and a plain reference was recorded using the associated Universal Analysis software. A DAN (2%, w/w)-silica physical mixture was prepared *via* mixing DAN with Aerosil® 380 silica particles at the mass ratio of 2:98 by overnight magnetic stirring. DSC analysis of the physical mixture was conducted to study the sensitivity of DSC for DAN in the presence of silica.

2.6. Lipolysis Study

2.6.1. Preparation of Digestion Medium

Fed/fasted state digestion media simulating human intestinal conditions were prepared according to protocols developed in previous research [15]. Standard buffer solution (pH 7.35-7.45) consisting of 50 mM Trizma maleate (pH 7.5), 150 mM NaCl, and 5 mM CaCl₂·2H₂O was prepared.

The fasted and fed state mixed micellar solutions consisting of bile salt (BS): phospholipids (PL) at the concentrations of 5 mM BS:1.25 mM PL (Fasted) and 20 mM BS: 5mM PL (Fed) were prepared, which resemble the levels of endogenous BS and PL in the fasted and post-prandial human intestine [2a]. Egg lecithin was weighed into a round-bottom flask and dissolved in 3-4 mL chloroform. The chloroform was then evaporated off under vacuum (BÜCHI Rotavapor-RE, Switzerland), forming a thin film of lecithin on the walls of the flask. NaTDC was weighed into the flask, and standard buffer was added. The resulting mixture was then covered with foil, and stirred overnight to form clear micelles. The fasted and fed state mixed micellar solutions were stored in the fridge (covered with foil) and used within two weeks of preparation.

2.6.2. In vitro Lipolysis Studies

The progress of lipid digestion was monitored for 60 min by using a TitraLab® 854 pH-stat titration apparatus (Radiometer Analytical, France) according to a previously established lipolysis model [16]. A known quantity of lipid formulation was dispersed in the digestion medium (37 °C), and the dosage of lipid was fixed at 200 mg/ 20ml digestion medium. The pH was re-adjusted to 7.50±0.01 with 0.1 M NaOH or HCl solution prior to the start of lipolysis. Lipolysis was initiated by adding 1 ml of pancreatin extract per 10 ml of lipolysis volume to produce a final lipase concentration of

~1000 TBU per ml of lipolysis volume. Free fatty acids (FFA) as a product of the lipolysis were titrated with 0.6 M NaOH *via* an auto-burette to maintain a constant pH at the pre-set value. The consumption of NaOH (after background correction with the blank micellar solutions) was used to calculate the number of moles of FFA liberated. It should be mentioned that oleic acid, which is the digestion product of Tween 80, is expected to be around 50% deprotonated at pH 7.5. Therefore, only 50% of the released oleic acid was detectable.

2.6.3. *In vitro* Solubilisation Studies

The solubilisation level of DAN in the aqueous phase under fed/fasted state digesting condition was examined for pure DAN and the lipid based formulations. At 1, 5, 15, 30 and 60 min, aliquots of 1 ml of lipolysis samples were collected into individual 1.5 ml Eppendorf® centrifuge tubes prefilled with 10 µl of 4-BBB (0.5 M in methanol) as an enzyme inhibitor to stop the lipolysis process in the collected samples. Each collected sample was separated into an upper aqueous phase and a pellet phase by centrifugation at 22,000 rpm for 45 min (37 °C). The aqueous phase was collected, extracted with acetonitrile, diluted with mobile phase, and analysed by HPLC for the DAN content.

3. RESULTS AND DISCUSSION

3.1. LBCC: Development and Physicochemical Characterisations

3.1.1. Development of L-SMEDDS

The solubilisation capacities of SMEDDS compositions for DAN and the propensity to self emulsify are presented in (Fig. 1). All SMEDDS formulations provided comparable solubility at room temperature, whilst the SMEDDS consisting of the highest proportion of Tween 80 provided the greatest solubility (34.54 mg/g in Formulation ②), and that consisting of the highest proportion of Transcutol HP was the least soluble (30.01 mg/g in Formulation ④). According to the visual assessment, it is apparent that the efficacy of emulsification increased as the % of Tween 80 increased in the composition. Upon 100-times dilution with water, the formulation composed of 25% Tween 80 (Formulation ④) provided a cloudy dispersion, and the formulation composed of 50% Tween 80 (Formulation ②) was clear in appearance. Therefore, SMEDDS formulation consisting of Capmul MCM: Tween 80: Transcutol HP at the mass ratio of 1:2:1

was selected as the optimised L-SMEDDS formulation for the following studies.

3.1.2. Physicochemical Characterisation of Solid LBCC

Two types of solid SMEDDS of essential similar theoretical compositions were fabricated *via* different drying methods. Each formulation was comprised of approximately 60% (w/w) lipids (including all the oil, surfactant and co-solvent fraction) encapsulated by or embedded in a porous silica-based matrix at a L-SMEDDS:silica mass ratio of approximately 1:0.5 (Table 1). The lipid and drug loading efficiency were above 88% and 100%, respectively, indicating the efficient loading of SMEDDS and drug encapsulation *via* either physical mixture or spray drying.

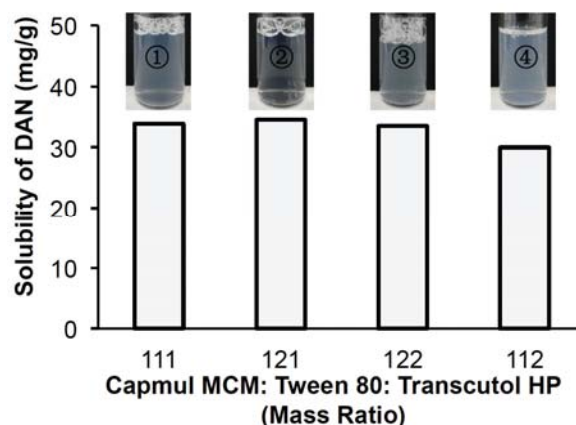


Fig. (1). Solubility of danazol in different SMEDDS pre-concentrates (bars). The top photos illustrate the dispersions of SMEDDS after 100 times dilution with water (the solubility data are expressed as the mean of three replicates, standard deviations are within 10% of the mean values; all studies were conducted at room temperature).

The SLH was characterised with 63.8% (w/w) lipid loading, rendering a near complete lipid loading following the spray drying process. The experimental drug loading level of SLH was approximately two thirds of the initial loading level as the drug was added in excess compared to the maximum drug solubility in the lipid. In all cases, the final drug loading in the solid formulations was equivalent to the maximum solubility in the lipids (*i.e.* 21 mg/g of DAN in Capmul MCM [14, 17] and 34.54 mg/g in the L-SMEDDS), which

Table 1. The lipid and drug loading level and encapsulation efficiency of different solid lipid based colloidal carriers (results are expressed as the mean of three replicates, standard deviations are within 10% of the mean values).

Compositions Formulation	Lipids *		Drug	
	Content (% w/w)	Efficiency (%)	Content (% w/w)	Efficiency (%)
P-SMEDDS	63.4	97.0	2.0	100.0
S-SMEDDS	57.9	88.6	2.2	110.0
SLH	63.8	97.6	1.3	65.0

*The lipids include all fractions of oil, surfactant and co-solvent in the formulation.

indicates that the drug was encapsulated within the lipid phase after the solidification process.

Fig. (2) illustrates the DSC profiles of pure DAN, DAN (2%, w/w)-silica physical mixture, DAN-loaded S-SMEDDS and SLH. Pure DAN powder showed a sharp endothermic peak at 229 °C, which corresponds to its melting point and confirms its crystalline nature [18]. The sensitivity of DSC for DAN in the presence of silica was confirmed as the melting peak was retained at approximately 200-230 °C. DSC profiles of S-SMEDDS and SLH were found to exhibit no endothermic peak, indicating that DAN was non-crystalline and either solubilised in the lipid excipients or molecularly adsorbed on the surface of silica nanoparticles.

3.1.3. Solid-State Morphology and Redispersed Colloidal Properties

All solid formulations appeared to be white agglomerated and free-flowing powders. Microscopic observation by SEM showed that both S-SMEDDS and SLH consist of well-separated spherical structures with heterogeneous size distributions ranging from 1 to 19 µm in the solid state (Table 2). Particle sizing by laser diffraction indicates a comparable redispersed particle size for DAN-loaded S-SMEDDS and SLH.

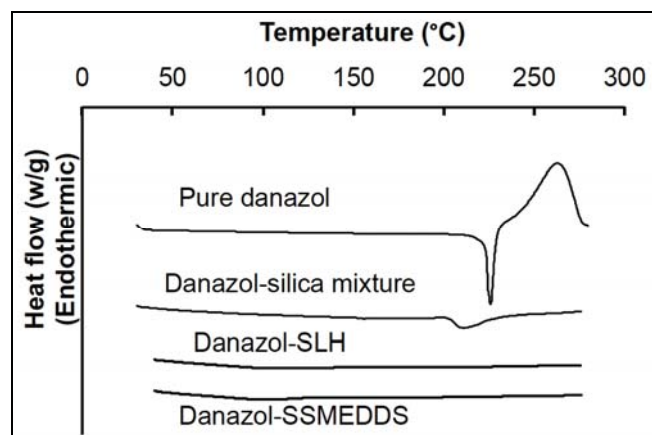


Fig. (2). Differential scanning calorimetric thermogram for pure danazol (DAN) powder, DAN (2% w/w)-silica physical mixture and DAN-loaded solid lipid-base colloidal carriers.

The average particle size and PDI of microemulsions generated by dispersing the solid or liquid-state LBCC in water were measured by dynamic light scattering. Ideally, the solid formulations should be able to readily disperse in the aqueous media and reform colloidal dispersions with comparable droplet size as the liquid formulations. As shown

Table 2. Physicochemical properties of DAN-loaded S-SMEDDS and SLH: redispersed particle size and solid state surface morphology (particle size are expressed as the mean of three replicates, standard deviations are within 10% of the mean values).

Colloidal Properties (in water)		Solid State SEM Images
S-SMEDDS	Diameter (µm)	
D (v, 0.1)	6.25	
D (v, 0.5)	10.93	
D (v, 0.9)	18.58	
SLH	Diameter (µm)	
D (v, 0.1)	6.21	
D (v, 0.5)	10.98	
D (v, 0.9)	18.93	

in (Fig. 3), L-SMEDDS self-emulsified rapidly in the aqueous media and formed fine micro-emulsions (188 nm). The droplet size of microemulsions formed by dispersion of the equivalent solid SMEDDS formulations (and subsequent removal of silica particles by low-speed centrifugation) were significantly larger (317.7 nm for S-SMEDDS and 329.7 nm for P-SMEDDS, respectively), indicating a reduced emulsification efficiency after solidification. This is in line with previous literature data which illustrate that solidification of SMEDDS formulations led to larger droplet size of the re-dispersed colloids [7a, 19]. A proposed explanation was the incomplete desorption of Tween 80 from the porous silica, as supported by the data showing a decreased capability of emulsification when the proportion of Tween 80 in the SMEDDS formulation was reduced (Fig. 1). Self-emulsifying is thought to occur *via* fracture of liquid crystals leading to formation of nanosized droplets, and the presence of hydrophilic surfactants enhances the penetration of the aqueous phase into liquid crystals and consequently facilitates self-emulsification [20].

By contrast, in the case oil-based systems, the micro-emulsions formed by the dispersion of SLH (and subsequent removal of silica particles by low-speed centrifugation) were significantly smaller than that of the equivalent Capmul emulsion (437.6 nm and 383.7 nm, respectively). It thus suggests a favourable increase in the emulsification capacity *via* the adsorption or entrapment of oil-based LBCC to the high surface area silica particles in SLH carriers.

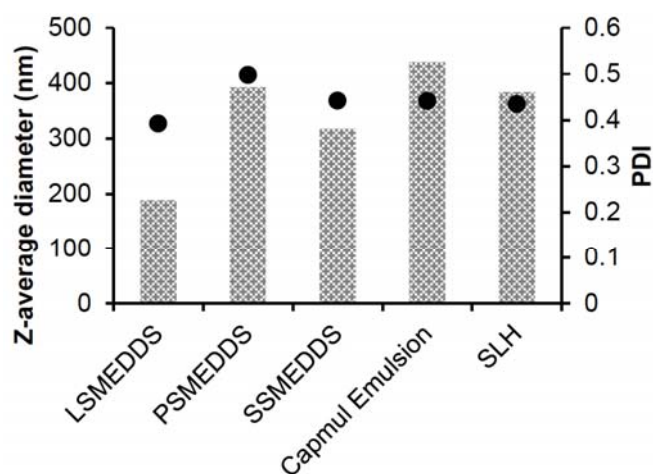


Fig. (3). Z-average diameter (bar, left axis) and polydispersity index (PDI, dot, right axis) of microemulsions generated by the dispersion of DAN formulations in water at 25 °C (results are expressed as the mean of three replicates, standard deviations are within 10% of the mean values).

3.2. *In vitro* Lipid Digestion Performance: Effect of Solidification

Fig. (4) shows the lipolysis-time profiles for DAN-loaded LBCC under simulated fasted and fed intestinal conditions. The time taken for approximately 66% fatty acid release from the lipid was calculated [21]. Considering the fasted-state lipolysis, for both oil-based and self-emulsifying LBCC, the solid formulations produced faster and more

complete lipolysis than the equivalent liquid formulations (Fig. 4). The values of $T_{66\%}$ can be ranked as S-SMEDDS (14 min) = SLH (16 min) > P-SMEDDS (24 min) > L-SMEDDS (36 min) = Emulsion (39 min). It thus suggests a possible role of silica nanostructured network in providing larger surface areas to facilitate the hydrolytic activity of interfacial active enzymes [3c]. Furthermore, it was also suggested that the lipolytic products that may interfere with the lipase action can be rapidly removed from the silica matrix due to the electrostatic charge repulsion between the negative charged silica surface and free fatty acids [22]. Differences in the lipolysis profiles, however, was observed between P-SMEDDS and S-SMEDDS, which is mostly likely due to the different solid-state structures and surface areas [6c]. Whist solid formulations produced *via* physical mixing are generally characterised with irregularly shaped mixtures [22], spray-drying which involves atomisation of lipid dispersions followed by instantaneous evaporation of the water phase often results in well separated, spherical particles (Table 2). Therefore, it indicates that structural and geometrical differences have a significant impact on the enzyme accessibility and lipolytic product removal, and consequently control the lipid digestion of solid-state LBCC. Furthermore, the faster and improved extent of lipolysis observed with solid SMEDDS may also be attributed to the limited release of Tween 80 from the silica matrix [7a], and thus resulting in a reduced level of free Tween 80, which can have an inhibitory effect on lipid digestion [23].

Introducing the LBCC's to the intestinal fed condition resulted in less significant differences in the lipolysis profiles between liquid and solidified formulations in comparison to that in the fasted condition. The $T_{66\%}$ for all SMEDDS formulations was approximately 30 min, and those for emulsion and SLH were 50 min and 40 min, respectively. Except for the L-SMEDDS, the fed state produced a decreased rate and extent of lipolysis than that in the fasted state. A proposed explanation was that the increased levels of bile salt, phospholipid, and pancreatic lipase may have an impact on the intermediate lipolytic products and the colloidal phases, and consequently reduces the rate and extent of lipolysis [24]. Furthermore, these surface active agents at increasing levels may also impart competitive adsorption of lipase at the lipid substrate surface, and thus interfere with the process of lipid digestion [25]. Further studies are required to fully understand the food effect on lipid digestion. The current study mainly focuses on the impact of these changes on the drug solubilisation capacities.

3.3. *In vitro* Solubilisation Enhancement of DAN by LBCC: Effect of Solidification

Fig. (5) illustrates the aqueous solubility of DAN under simulated fasted and fed intestinal conditions for pure DAN (a) and different types of LBCC (b, c). The solubilisation level of pure DAN during the 1-h lipolysis process was relatively constant, and the amount of drug solubilised was 11-14 µg/ml and 82-89 µg/ml in the fasted and fed condition, respectively. The solubilisation level was significantly improved when DAN was dosed in the form of LBCC. In all cases of LBCC, the drug was dosed at the same proportion of saturated solubility (*i.e.* 100%) in the lipid phase to ensure the same thermodynamic activity.

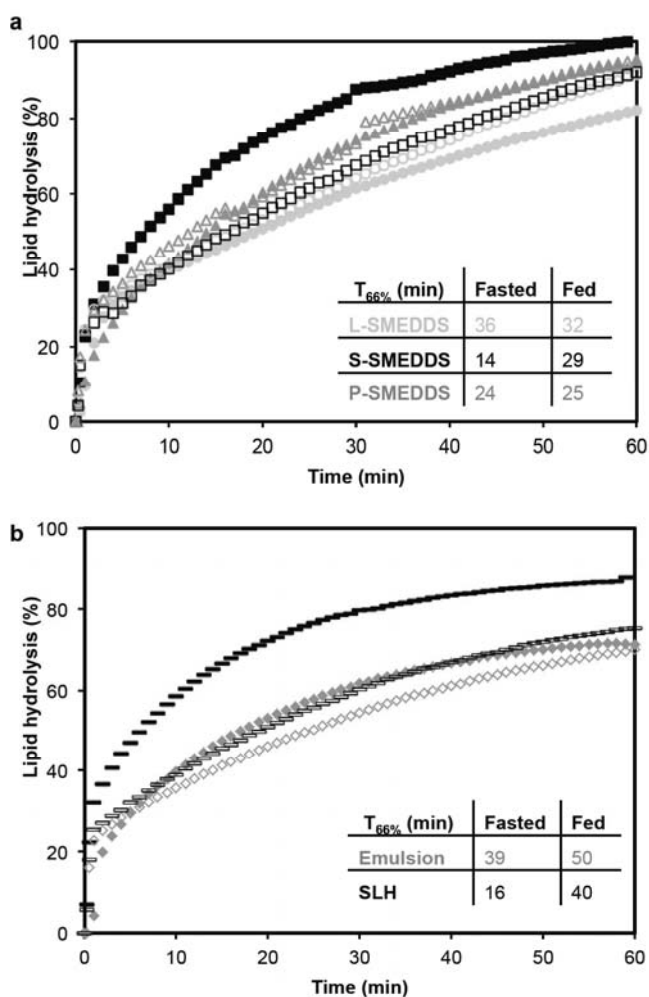


Fig. (4). Digestion profile of **a.** L-SMEDDS (○), S-SMEDDS (□) and P-SMEDDS (△), **b.** Capmul emulsion (◇) and SLH (—) under simulated fasted (*filled*) and fed (*unfilled*) intestinal conditions (dosage of lipid was fixed at 10 mg/ml in all cases; results are expressed as the mean of two replicates, error was within 10%).

3.3.1. Performance of Self-emulsifying LBCC

The SMEDDS formulations provided significantly improved drug solubilisation in comparison to that of the pure DAN (Fig. 5b). In the fasted state, approximately 100 µg/ml DAN was solubilised 1 min after the lipolysis of L-SMEDDS, and nearly 125 µg/ml was solubilised at 5 min (Fig. 5a); in the fed state, approximately 220 µg/ml DAN was solubilised within the first 5 min (Fig. 5b). In both cases, the drug solubility reached the peak level at 5 min and then levelled off for the rest 55 min. The solubilised level of DAN was, however, significantly lower than the initial dosage, 450 µg DAN/ml. Together, the data indicate that the solubilising power of SMEDDS has been diluted off by the digesting medium, and the lipolysis of SMEDDS may have negligible effect on the solubilisation of DAN. In accordance with the previous findings, it was suggested that the type IIIB SMEDDS formulation containing a large proportion of hydrophilic surfactant (HLB > 12, *i.e.* 50%) and co-solvent (*i.e.* 25%) has the risk of losing solubilisation capacity upon dispersion in aqueous medium [26] and that the lipid hy-

drolysis may not affect the solubilisation of DAN for such formulations [13].

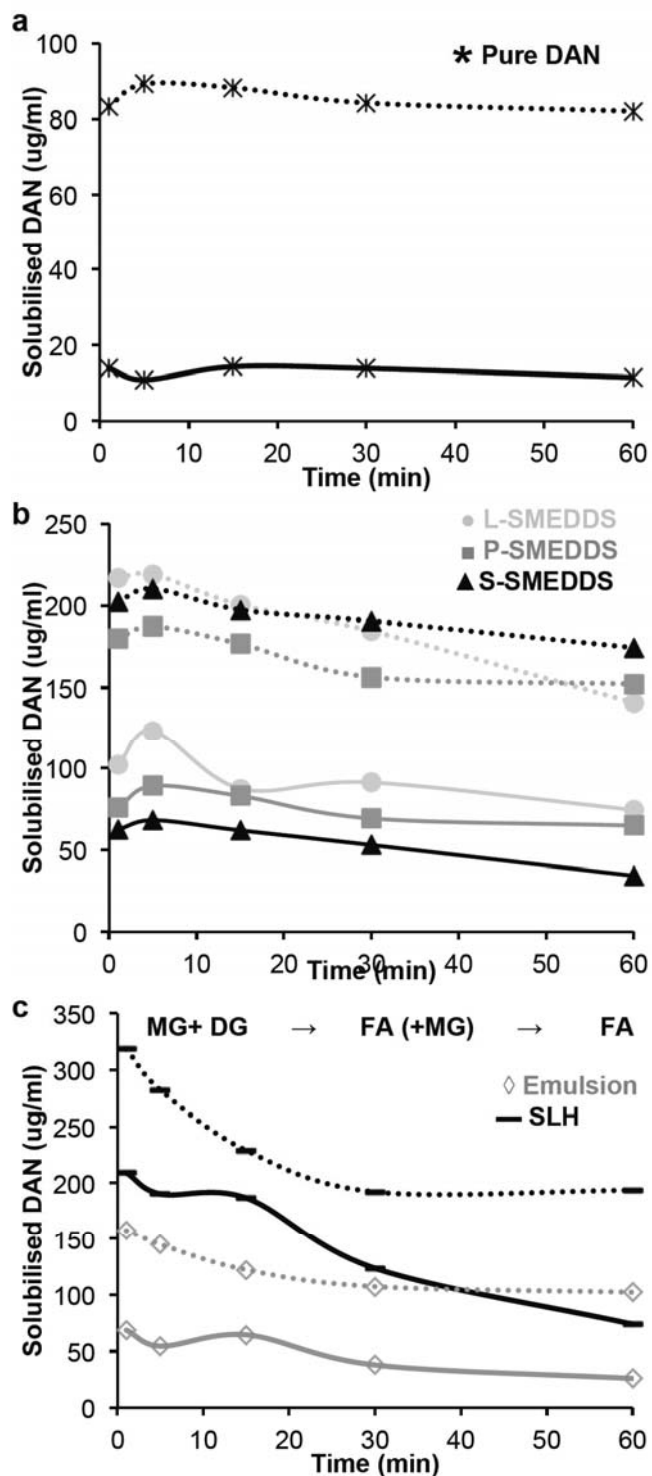


Fig. (5). The solubility of danazol (DAN) for unformulated drug (**a**), SMEDDS formulations (**b**), and oil-based LBCC lipid-based formulations (**c**) under simulated fasted (solid line) or fed (dashed line) intestinal conditions digestion (dosage of DAN was fixed at 450±50 µg/ml in all cases; results are expressed as the mean of two replicates, error was within 10%; MG= mono-glycerides, DG= di-glycerides, FA= fatty acids).

Solid SMEDDS formulations showed a comparable trend in the drug solubilisation profiles as L-SMEDDS under both fasted and fed states. However, the magnitude of drug solubilisation varied for the different SMEDDS formulations. The drug solubilisation level can be ranked as L-SMEDDS > P-SMEDDS > S-SMEDDS, thus indicating a reduced ability to present DAN in the solubilised state on digestion after solidification. This result is consistent with the previous observation that a Neusilin-loaded solid SMEDDS provided lower solubility of DAN in comparison to the equivalent liquid SMEDDS [7a], which suggested that solidification of self-emulsifying formulations *via* a high surface area carrier may limit the release of surfactant or co-solvent and compromise the drug solubilisation performance. Comparing physical adsorption and spray drying, it was found that physical adsorption may represent a more efficient solidification approach to retain the *in vitro* biopharmaceutical performance of liquid SMEDDS.

3.3.2. Performance of Oil-based LBCC

For the oil-based emulsion, approximately 69 $\mu\text{g/ml}$ DAN was solubilised within the first 1 min under the fasted digesting condition, and this was gradually decreased to 26 $\mu\text{g/ml}$ at 60 min. In the fed state, 158 $\mu\text{g/ml}$ DAN was solubilised at 1 min, and 102 $\mu\text{g/ml}$ at 60 min (Fig. 5c). The adverse effect of solidification on the *in vitro* solubilisation of DAN was absent when converting the oil-based emulsion to the solid SLH form. In fact, the SLH provided a higher drug solubilisation level than all other tested formulations in both fasted and fed states. Up to 209.5 $\mu\text{g/ml}$ DAN from the SLH was solubilised at 1 min post-digestion in the fasted state, which is more than 15-fold higher than that of the pure DAN and approximately 3 times than the emulsion. In the fed state, up to 318.7 $\mu\text{g/ml}$ DAN was solubilised, and this is nearly 4-times higher than that of the pure drug, and twice that of the emulsion. Correlating the enhanced solubility of SLH with the more efficient dispersion of SLH than the emulsion (Fig. 3), it suggests a significant role of silica in enhancing the dispersion of Capmul in the aqueous digesting medium to form microemulsions, which retain the drug in the solubilised state. Furthermore, the decreasing trend observed in the solubilisation profiles of the emulsion and SLH was likely due to that the dynamics of DAN solubilisation from the type I Capmul-based formulation is greatly influenced by the progress of lipolysis and thus the phase changes of the colloidal species (*e.g.* mixed micelles or vesicles) in the lipolysis media [14, 27]. Optimised drug solubilisation level can be achieved *via* optimising the digestion kinetics of the type I formulation.

3.4. Fed/Fasted Variation of the Drug Solubilisation Performance

It is well known that the intake of food can impact the pharmacokinetics and pharmacodynamics of concomitantly administered drugs. A three-fold increase in bioavailability of the commercial formulation of DAN has been reported after food intake [12]. Although improved absorption might generally be desirable, the situation is different for compounds with a narrow therapeutic window and requires careful monitoring of the patient to avoid under or over dosage.

Fig. (6) illustrates the area under the curve of the solubilisation profile (AUC_{0-60}) in the fasted or fed state of each formulation. For the unformulated DAN, the increased levels of bile salts and fatty acids in the fed state which simulates the physiological intestinal condition after food intake, led to a 6.6-fold increase in the solubility over that in the fasted state, indicating a significant food effect of DAN solubilisation. Comparing the AUC_{0-60} of LBCC in the fasted state with that of the pure DAN in the fed state, it demonstrates that the LBCC were capable of mimicking the positive food effect and enhancing drug solubilisation capacity, which is consistent with the previous clinical data that DAN-encapsulated mono-glycerides emulsion produced comparable bioavailability as that of the pure drug co-administered with food [12].

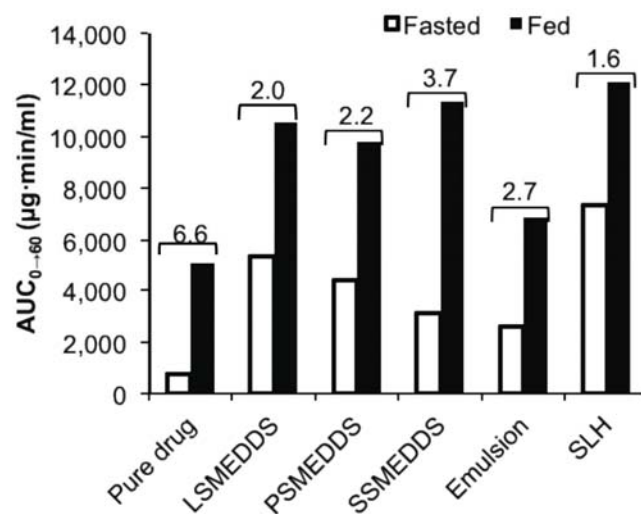


Fig. (6). The area under the curve of the *in vitro* solubilisation profile (AUC_{0-60}) in the fasted or fed state of each DAN formulation. The data labels represent the ratio of the AUC_{0-60} between the fed and fasted state for each formulation.

Furthermore, the ratio of the AUC_{0-60} in the fed and fasted state ($R_{fed/fast}$) was calculated for each formulation and presented in Fig. (6). Whilst a substantial fed/fast variation in the solubilisation of pure DAN was observed (*i.e.* $R_{fed/fast} = 6.6$), the $R_{fed/fast}$ is 3.7 in the case of S-SMEDDS, and approximately 2 for L-SMEDDS and P-SMEDDS. This thus indicates the potential of SMEDDS in reducing the effect of food intake on the absorption of DAN. A proposed mechanism to explain the reduced fed/fast response of SMEDDS is that diffusion of poorly water-soluble compounds to the absorptive regions occurs directly from the fine o/w microemulsions formed upon dispersion of SMEDDS in aqueous medium, and this process occurs independently of the concentration of the bile salt [28]. Solidification of SMEDDS *via* the physical adsorption to Aerosil showed no influence on the fed/fast variation. However, a larger fed/fast variation was observed with the S-SMEDDS prepared *via* spray-drying, consequently indicating that caution is required in converting self-emulsifying LBCC to the solid state to preserve the capability to reduce the food effect.

In comparison to the SMEDDS, the solid form of the oil-based LBCC, SLH microparticles, outperformed the equivalent liquid form, Capmul emulsion, in providing a higher solubilisation capacity for DAN in both fasted and fed states and a lower $R_{fed/fasted}$ value, *i.e.* 1.6, confirming a small fed/fasted variation in the solubilisation of DAN. The SLH, thus, can be considered as an optimal solid-state delivery approach to improve the oral bioavailability and reduce the food effect of DAN.

4. CONCLUSION

The physicochemical properties of DAN-encapsulated LBCC and the *in vitro* solubilisation performance under simulated fasted and fed intestinal digesting conditions were studied. Whilst all solid-state LBCC were free flowing and may allow convenient oral administration, the impact of solidification on drug solubilisation capacity was different in the case of type IIIB self-emulsifying and type I oil-based LBCC. Solidification of self-emulsifying LBCC was found to limit the release of lipid excipient and drug and consequently reduce the *in vitro* solubilisation performance. Physical adsorption was found to be an easy and more efficient method of SMEDDS solidification in comparison to spray drying. On the contrary, converting oil-based type I LBCC to the SLH form *via* spray drying enhanced the solubilisation capacity for DAN and reduced the food effect. In particular, the SLH provided 10-fold and 2.4-fold improvement in the AUC_{0-60} in the fasted and fed state, respectively, in comparison to that of the pure DAN. The fed/fasted solubilisation variation ratio was reduced from 6.6 to 1.6 when DAN was formulated as SLH, and this is significantly better than solid-SMEDDS (*i.e.* 2.2-3.7). This thus renders the SLH as a promising solid-state LBCC for the oral delivery of DAN. To this end, subsequent studies employing drug models of varying lipophilicity and acid-base properties will be conducted to further enhance our understanding on the development and optimisation of solid-state lipid-based formulations for poorly water-soluble compounds.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The Australian Research Council, the Australian National Health and Medical Research Council, ITEK Pty. Ltd., Bioinnovation South Australia and the Australian Biotech Ceridia Pty. Ltd. are greatly acknowledged for research funding and support. Dr Ben Boyd is acknowledged for useful discussion.

PATIENT CONSENT

Declared none.

REFERENCES

- Charman, W.N.; Porter, C.J.H.; Mithani, S.; Dressman, J.B. Physicochemical and physiological mechanisms for the effects of food on drug absorption: The role of lipids and pH. *J. Pharm. Sci.*, **1997**, *86*(3), 269-282.
- (a) Porter, C.J.; Charman, W.N. *In vitro* assessment of oral lipid based formulations. *Adv. Drug Deliv. Rev.*, **2001**, *50* Suppl 1, S127-47;(b) Shukla, D.; Chakraborty, S.; Singh, S.; Mishra, B., Lipid-based oral multiparticulate formulations - advantages, technological advances and industrial applications. *Expert Opin. Drug Del.*, **2011**, *8*(2), 207-224;(c) Porter, C. J. H.; Trevaskis, N. L.; Charman, W. N., Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.*, **2007**, *6*(3), 231-248.
- (a) Christiansen, M. L.; Holm, R.; Kristensen, J.; Kreilgaard, M.; Jacobsen, J.; Abrahamsson, B.; Müllertz, A. Cinnarizine food-effects in beagle dogs can be avoided by administration in a Self Nano Emulsifying Drug Delivery System(SNEDDS). *Eur. J. Pharm. Sci.*, **2014**, *57*, 164-172 (b) Christophersen, P. C.; Christiansen, M. L.; Holm, R.; Kristensen, J.; Jacobsen, J.; Abrahamsson, B.; Müllertz, A. Fed and fasted state gastrointestinal *in vitro* lipolysis: *In vitro in vivo* relations of a conventional tablet, a SNEDDS and a solidified SNEDDS. *Eur. J. Pharm. Sci.*, **2014**, *57*, 232-239(c) Tan, A.; Martin, A.; Nguyen, T.-H.; Boyd, B. J.; Prestidge, C. A. Hybrid nanomaterials that mimic the food effect: Controlling enzymatic digestion for enhanced oral drug absorption. *Angew. Chem. Int. Edit.* **2012**, *124*(22), 5571-5575(d) Rao, S.; Tan, A.; Thomas, N.; Prestidge, C.A., Perspective and potential of oral lipid-based delivery to optimize pharmacological therapies against cardiovascular diseases. *J. Control. Release*, **2014**, *193*, 174-87. DOI: 10.1016/j.jconrel.2014.05.013.
- (a) Humberstone, A. J.; Charman, W. N. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Deliv. Rev.*, **1997**, *25*, 103-128(b) Kossena, G. A.; Boyd, B. J.; Porter, C. J. H.; Charman, W. N. Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. *J. Pharm. Sci.*, **2003**, *92*(3), 634-648.
- Strickley, R. Solubilizing excipients in oral and injectable formulations. *Pharm. Res.*, **2004**, *21*(2), 201-230.
- (a) Balakrishnan, P.; Lee, B.-J.; Oh, D. H.; Kim, J. O.; Hong, M. J.; Jee, J.-P.; Kim, J. A.; Yoo, B. K.; Woo, J. S.; Yong, C. S.; Choi, H.-G. Enhanced oral bioavailability of dexibuprofen by a novel solid self-emulsifying drug delivery system(SEDSS). *Eur. J. Pharm. Biopharm.*, **2009**, *72*(3), 539-545(b) Rao, S.; Tan, A.; Boyd, B. J.; Prestidge, C. A. Synergistic role of self-emulsifying lipids and nanostructured porous silica particles in optimizing the oral delivery of lovastatin. *Nanomedicine-UK*, **2014**(in press);(c) Tan, A.; Rao, S.; Prestidge, C. Transforming lipid-based oral drug delivery systems into solid dosage forms: An overview of solid carriers, physicochemical properties, and biopharmaceutical performance. *Pharm. Res.*, **2013**, 1-25.
- (a) Speybroeck, M. V.; Williams, H. D.; Nguyen, T.-H.; Anby, M. U.; Porter, C. J. H.; Augustijns, P., Incomplete desorption of liquid excipients reduces the *in vitro* and *in vivo* performance of self-emulsifying drug delivery systems solidified by adsorption onto an inorganic mesoporous carrier. *Mol. Pharm.*, **2012**, *9*(9), 2750-2760;(b) Agarwal, V.; Siddiqui, A.; Ali, H.; Nazzal, S. Dissolution and powder flow characterization of solid self-emulsified drug delivery system(SEDSS). *Int. J. Pharm.*, **2009**, *366*(1-2), 44-52.
- Bremmell, K. E.; Tan, A.; Martin, A.; Prestidge, C. A. Tableting lipid-based formulations for oral drug delivery: A case study with silica nanoparticle-lipid-mannitol hybrid microparticles. *J. Pharm. Sci.*, **2013**, *102*(2), 684-693.
- Shanmugam, S.; Baskaran, R.; Balakrishnan, P.; Thapa, P.; Yong, C.S.; Yoo, B.K. Solid self-nanoemulsifying drug delivery system(S-SNEDDS) containing phosphatidylcholine for enhanced bioavailability of highly lipophilic bioactive carotenoid lutein. *Eur. J. Pharm. Biopharm.*, **2011**, *79*(2), 250-257.
- (a) Nguyen, T.-H.; Tan, A.; Santos, L.; Ngo, D.; Edwards, G. A.; Porter, C. J. H.; Prestidge, C. A.; Boyd, B. J. Silica-lipid hybrid(SLH) formulations enhance the oral bioavailability and efficacy of celecoxib: An *in vivo* evaluation. *J. Control. Release*, **2013**, *167*, 85-91;(b) Simovic, S.; Heard, P.; Hui, H.; Song, Y.; Peddie, F.; Davey, A. K.; Lewis, A.; Rades, T.; Prestidge, C. A. Dry hybrid lipid-silica microcapsules engineered from submicron lipid droplets and nanoparticles as a novel delivery system for poorly soluble drugs. *Mol. Pharm.*, **2009**, *6*(3), 861-872(c) Tan, A.; Simovic, S.; Davey, A. K.; Rades, T.; Prestidge, C. A. Silica-lipid hybrid(SLH) microcapsules: A novel oral delivery system for poorly soluble drugs. *J. Control. Release*, **2009**, *134*(1), 62-70.

- [11] Sunesen, V. H.; Vedelsdal, R.; Kristensen, H. G.; Christrup, L.; Müllertz, A. Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug. *Eur. J. Pharm. Sci.*, **2005**, *24*(4), 297-303.
- [12] Charman, W. N.; Rogge, M. C.; Boddy, A. W.; Berger, B. M. Effect of food and a monoglyceride emulsion formulation on danazol bioavailability. *J. Clin. Pharmacol.*, **1993**, *33*(4), 381-386.
- [13] Pouton, C. W., Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.*, **2000**, *11*, Supplement 2, S93-S98.
- [14] Kaukonen, A.; Boyd, B.; Porter, C. H.; Charman, W. Drug solubilization behavior during *in vitro* digestion of simple triglyceride lipid solution formulations. *Pharm. Res.*, **2004**, *21*(2), 245-253.
- [15] Sek, L.; Porter, C. J. H.; Kaukonen, A. M.; Charman, W. N. Evaluation of the *in-vitro* digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J. Pharm. Pharmacol.*, **2002**, *54*, 29-41.
- [16] Sek, L.; Porter, C. J.; Charman, W. N., Characterisation and quantification of medium chain and long chain triglycerides and their *in vitro* digestion products, by HPTLC coupled with *in situ* densitometric analysis. *J. Pharm. Biomed. Anal.*, **2001**, *25*(3-4), 651-661.
- [17] Prajapati, H. N.; Dalrymple, D. M.; Serajuddin, A. T. A comparative evaluation of mono-, di- and triglyceride of medium chain fatty acids by lipid/surfactant/water phase diagram, solubility determination and dispersion testing for application in pharmaceutical dosage form development. *Pharm. Res.*, **2012**, *29*(1), 285-305.
- [18] Williams, H. D.; Anby, M. U.; Sassene, P.; Kleberg, K.; Bakala-N'Goma, J.-C.; Calderone, M.; Jannin, V.; Igonin, A.; Partheil, A.; Marchaud, D.; Jule, E.; Vertommen, J.; Maio, M.; Blundell, R.; Benameur, H.; Carriere, F.; Mullertz, A.; Pouton, C. W.; Porter, C. J. H. Toward the establishment of standardized *in vitro* tests for lipid-based formulations. 2. The effect of bile salt concentration and drug loading on the performance of type I, II, IIIA, IIIB, and IV formulations during *in vitro* digestion. *Mol. Pharm.*, **2012**, *9*, 3286-3300.
- [19] (a) Li, L.; Yi, T.; Lam, C. Effects of spray-drying and choice of solid carriers on concentrations of Labrasol® and Transcutol® in solid self-microemulsifying drug delivery Systems(SMEDDS). *Molecules*, **2013**, *18*(1), 545-560;(b) Soottitawat, A.; Yoshii, H.; Furuta, T.; Ohkawara, M.; Linko, P. Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. *J. Food Sci.*, **2003**, *68*(7), 2256-2262.
- [20] Cuine, J.F.; Charman, W. N.; Pouton, C. W.; Edwards, G. A.; Porter, C.J.H. Increasing the proportional content of surfactant (Cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. *Pharm. Res.*, **2007**, *24*, 748-757.
- [21] Carrière, F.; Rogalska, E.; Cudrey, C.; Ferrato, F.; Laugier, R.; Verger, R., *In vivo* and *in vitro* studies on the stereoselective hydrolysis of tri- and diglycerides by gastric and pancreatic lipases. *Bioorgan. Med. Chem.* **1997**, *5*(2), 429-435.
- [22] Joyce, P.; Tan, A.; Whitby, C.P.; Prestidge, C.A. The role of porous nanostructure in controlling lipase-mediated digestion of lipid loaded into silica particles. *Langmuir*, **2014**, *30*(10), 2779-2788.
- [23] Li, Y.; McClements, D.J. Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions. *Eur. J. Pharm. Biopharm.*, **2011**, *79*(2), 423-431.
- [24] Fatourous, D. G.; Bergenstahl, B.; Mullertz, A., Morphological observations on a lipid-based drug delivery system during *in vitro* digestion. *Eur. J. Pharm. Sci.*, **2007**, *31*(2), 85-94.
- [25] (a) Delorme, V.; Dhoub, R.; Canaan, S.; Fotiadu, F.; Carrière, F.; Cavalier, J.-F. Effects of surfactants on lipase structure, activity, and inhibition. *Pharm. Res.*, **2011**, *28*(8), 1831-1842(b) Speranza, A.; Corradini, M. G.; Hartman, T. G.; Ribnick, D.; Oren, A.; Rogers, M. A. Influence of emulsifier structure on lipid bioaccessibility in oil-water nanoemulsions. *J. Agr. Food Chem.*, **2013**, *61*(26), 6505-6515.
- [26] Cuine, J. F.; McEvoy, C. L.; Charman, W. N.; Pouton, C. W.; Edwards, G. A.; Benameur, H.; Porter, C. J. H. Evaluation of the impact of surfactant digestion on the bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to dogs. *J. Pharm. Sci.*, **2008**, *97*, 995-1012.
- [27] Porter, C. J. H.; Kaukonen, A. M.; Boyd, B. J.; Edwards, G. A.; Charman, W. N. 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*, 1405-1412.
- [28] De Smidt, P. C.; Campanero, M. A.; Troconiz, I. F. Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification versus digestibility. *Int. J. Pharm.*, **2004**, *270*(1-2), 109-18.