Colloidal aspects of dispersion and digestion of self-dispersing lipid-based formulations for poorly water-soluble drugs

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Abstract

Self-dispersing lipid-based formulations, particularly self-microemulsifying drug delivery systems (SMEDDS) have gained an increased interest in recent times as a means to enhance the oral bioavailability of poorly water-soluble lipophilic drugs. Upon dilution, SMEDDS self-emulsify in an aqueous fluid and usually form a kinetically stable oil-in-water emulsion or in some rare cases a true thermodynamically stable microemulsion. The digestion of the formulation leads to the production of amphiphilic digestion products that interact with endogenous amphiphilic components and form self-assembled colloidal phases in the aqueous environment of the intestine. The formed colloidal phases play a pivotal role in maintaining the lipophilic drug in the solubilised state during gastrointestinal transit prior to absorption. Thus, this review describes the structural characterisation techniques employed for SMEDDS and the recent literature studies that elucidated the colloidal aspects during dispersion and digestion of SMEDDS and solid SMEDDS. Possible future studies are proposed to gain better understanding on the colloidal aspects of SMEDDS and solid SMEDDS.

Keywords: Lipid-based formulations, self-dispersing formulations, self-microemulsifying drug delivery system, dispersion, digestion, colloidal phases, poorly water-soluble drugs

47 Table of Contents

72
73
74

1. Introduction

Many newly discovered active pharmaceutical candidates are poorly water-soluble and thereby pose problems of ineffective delivery and poor bioavailability after oral consumption. The oral route of drug administration is considered the most desirable due to the advantages of safety, non-invasiveness, cost-effectiveness, better patient convenience and thereby better compliance. The drugs need to be dissolved into the gastrointestinal (GI) fluids in order to be absorbed from the GI tract and to enhance oral bioavailability. The physicochemical properties such as poor aqueous solubility, low membrane permeability and instability of some drugs often result in poor or limited solubility in GI lumen and thereby low absorption and low oral bioavailability. In recent years, the high throughput screening and combinational chemistry in the drug discovery process raised the list of the new chemical entities that are poorly water-soluble [1-3]. More than 70% of these compounds are poorly water-soluble and lipophilic that are classified as Class II drugs in the Biopharmaceutical Classification System (BCS II) [4-6]. These compounds possess high membrane permeability for the intestinal membrane, but the low solubility in the GI lumen often results into poor or variable oral absorption [7]. These compounds often display slow dissolution in the GI tract that potentially results to incomplete dissolution during GI transit and thereby excretion of the undissolved material. The low solubility and/or low dissolution rate are considered as a rate-limiting step for the poor absorption of these active compounds. Many strategies have been explored in order to improve the bioavailability of such active compounds by modifying the drug solubility and/or dissolution characteristics or avoiding the need for dissolution by presenting the drug in the solution form [6, 8].

Among other approaches, lipid-based formulations (LBFs) have gained a significant popularity as a promising strategy for delivering poorly water-soluble lipophilic compounds [9-12]. The high lipophilicity of these compounds often results into high solubility in the natural or synthetic lipid-based excipients, thus the dosage forms are often prepared by dissolving lipophilic drugs into lipid-based excipients and presented into the highly solubilised state in GI lumen in a gelatin capsule. The interest in the lipid-based drug delivery systems has grown over the past few years and several lipid-based drug delivery systems have been developed with an aim to improve the absorption and oral bioavailability [9-11, 13-15]. The lipid-based drug delivery system is an umbrella term and the use of lipid-based drug delivery systems could range from simple triglyceride solutions to highly engineered complex LBFs [12].

Among the other developed LBFs, self-dispersing formulations such as self-emulsifying, self-micro/nano-emulsifying drug delivery system (SMEDDS/SNEDDS) are of special interest as a promising approach for the delivery of poorly water-soluble lipophilic drugs due to their self-dispersion behaviour and small droplet sizes upon dispersion that has been shown to improve drug absorption from the large interfacial area [16-18]. Typically, these self-dispersing formulations are complex mixture of oil, surfactant, co-surfactant and co-solvent that self-emulsify and form kinetically or (on rare occasions) thermodynamically stable oil-in-water (O/W) emulsion or microemulsion (depending on the ratio of oil-surfactants concentration and interfacial tension) in the GI tract under the mild agitation provided by GI motility [9, 19, 20]. Hence, in the current study, the term self-dispersing formulations is used interchangeably with SEDDS, SMEDDS or SNEDDS in order to reference all types of self-emulsifying lipid-based systems with self-emulsification capacity. The interest on SMEDDS has grown even further after the successful commercialisation of SMEDDS formulations of cyclosporine A, marketed as Sandimmune® and Neoral® [21]. Briefly, the SEDDS Sandimmune® formulation (Novartis Pharmaceutical UK Ltd), containing cyclosporine A, displayed intra- and inter-individual variability in pharmacokinetic profiles and bioavailability [22]. The absorption of the drug was greatly influenced by the presence of bile components, pancreatic enzymes, secretion and functionality of small bowel and the food [22]. Moreover, the absorption of the drug was reduced in the presence of cholestasis or gastroenteritis [23, 24]. The SMEDDS Neoral® formulation was developed by utilising hydrophobic and hydrophilic surfactants resulting in less dependence of the absorption on bile components [23]. The formulations further improved the drug exposure in adults and provided higher peak concentration in comparison to SEDDS Sandimmune® [25, 26]. Furthermore, in recent times, there has been a growing interest in transforming liquid SMEDDS into solid SMEDDS via solidification techniques in order to circumvent the drawbacks of liquid-based lipid systems and to improve the physicochemical stabilities and patient compliance [19, 27, 28].

Generally, the digestion of lipid is a prerequisite for LBFs to provide an optimal absorptive environment in order to maximise the bioavailability [9, 10, 12, 29-31]. During GI transit, the presence of endogenous and exogenous lipids, lipid digestion products and the formed colloidal phases upon interaction of the lipid digestion products with endogenous components often results into enhanced solubilisation of poorly water-soluble and highly membrane permeable active compounds [32, 33]. The overall performance of LBFs is highly dependent on the formed colloidal phases during dispersion and digestion and their morphologies [34, 35].

Briefly, after oral administration, LBFs are exposed to GI fluid where the self-dispersing formulation forms small-sized oil droplets and the presence of endogenous and exogenous dietary formulation components stimulates the secretion of endogenous amphiphilic components (i.e. bile salts, phospholipids and cholesterol) and digestive enzymes. The enzymatic digestion of the digestible components leads to the formation of digestion products (i.e. monoglycerides and free fatty acids) that interact with endogenous amphiphilic components and form a variety of self-assembled colloidal phases such as micelles, mixed micelles and vesicles at the interface of the droplets [35, 36]. The secretion of endogenous amphiphilic components and the generation of the colloidal phases further boost the solubilisation capacity of the GI fluid for poorly water-soluble lipophilic drugs and subsequently enhance the oral bioavailability of drugs [13, 30, 37]. These formed colloidal phases act as a transport medium for the absorption of lipidic species and lipophilic drugs [35, 38].

Despite the recognition of the importance of the self-assembled formed colloidal phases and its favouring impact on the drug solubilisation capacity in the GI tract, the earlier literature on SMEDDS is largely focused on the formulation development followed by its characterisation and drug release studies. Dispersion, digestion and structure formation are all important aspects of SMEDDS formulations, knowledge of which enables understanding of drug solubilisation and drug absorption. Several studies are reported where the dynamic *in vitro* digestion models have been utilised as a tool for a lipid-based system in order to study the formulation composition, digesting medium, the kinetics and extent of digestion, drug solubilisation and drug precipitation using analytical techniques [39-42]. However, the studies on the investigation of the self-assembled colloidal phases during dispersion and digestion of SMEDDS are limited due to the fact that SMEDDS are complex formulations where the excipients of the formulation can significantly impact on the processing. Additionally, the complex biological and physicochemical processing further increases the complexity of the process and making it difficult to monitor structural changes occurring during GI transit.

To date, several reviews have been devoted on SMEDDS with a major emphasis on formulation development, optimisation and characterisation with an ultimate aim to improve the bioavailability of poorly water-soluble drugs [19, 43, 44]. For instance, Dokania and Joshi reviewed SMEDDS formulations and reported the limitations and challenges associated with SMEDDS formulation development [19]. Gurram *et al*. highlighted the role of formulation substances in the development of SMEDDS formulations and Sudheer *et al*. summarised the

formulation techniques and dosage forms for the development of solid SMEDDS [45, 46]. However, there is no review paper focussing on the formation of colloidal phases during dispersion and digestion of SMEDDS. Thus, the present review aims to take the discussion one step further by summarising the recent advancement of the formed self-assembled colloidal structures during dispersion and digestion of SMEDDS and solid SMEDDS. Initially, SMEDDS and solid SMEDDS formulations and their properties are described very briefly. The process of self-emulsification, lipid digestion and the self-assembled colloidal phases for the lipid-based systems with several commonly used colloidal phase characterisation techniques for SMEDDS are illustrated in the following section. Then, the drug solubilisation and drug absorption behaviour are summarised in the subsequent section. Later, the most recently reported literature studies for SMEDDS focussing the formation of self-assembled colloidal structures during dispersion and digestion are reviewed and the formed colloidal phases during digestion of solid SMEDDS is highlighted with a particular emphasis on the potential impact of a solid-phase carrier on the formation of colloidal structures.

2. Lipid-based formulations

The use of lipids in order to improve the oral bioavailability of poorly water-soluble drugs could range from simple oil solutions to highly complex LBFs containing oil, surfactants, co-surfactant and sometimes co-solvent as a unit dosage form [9, 12]. LBFs has great potential to address the challenge of low oral bioavailability of poorly water-soluble lipophilic drugs by number of mechanisms including presenting the drugs into pre-dissolved form at the site of absorption, delaying gastric emptying process, promoting the lymphatic transport pathways thereby avoiding the first-pass metabolic effect and by driving the lymphatic update of some highly lipophilic drugs from the small intestine [13, 47, 48]. The absorption of drugs from LBFs is a dynamic process and the design of the formulation can directly influence the absorption process. The knowledge about the physiochemical properties of substances, thermodynamics and GI physiology is prerequisite to avoid drug precipitation upon dispersion and digestion of LBFs. Typically, LBF goes through several transition changes from solubilised state to an emulsified state upon dispersion in the GI tract and lastly, colloidal phases after digestion.

In the case of lipid-based drug formulations, Pouton classified the LBFs into four categories based on their formulation compositions, dispersibility into an aqueous environment and likely

behaviour during digestion (Table 1) [9, 12]. The main purpose of this classification system was to identify the type of formulation depending on the physicochemical properties and to predict the *in vivo* behaviour of the formulation [9]. The LBFs can potentially be composed of a range of formulations containing varying amount of oils, surfactants (hydrophilic or hydrophobic) and co-solvents. Briefly, type I formulations contain simple oil solutions (i.e. mono-, di- or triglycerides) that need digestion in order to achieve dispersion and to form colloidal phases. Type II formulations are a mixture of oils and water-insoluble surfactants (referred as a self-emulsifying drug delivery system (SEDDS)), type III are mixture of oils, water-soluble and/or water-insoluble surfactants and co-solvents (referred as self-microemulsifying (SMEDDS)/self-nanoemulsifying drug delivery systems (SNEDDS)) and type IV formulations are mixtures of water-soluble surfactants and co-solvents without oils [9, 12]. Type III has been further subdivided into type IIIA and type IIIB where the difference between formulations is distinguished by the concentration of hydrophilic components and co-solvents in the total formulation [9, 12]. The primary difference between SMEDDS and SNEDDS is the size and polydispersity of droplets upon dispersion into an aqueous phase where SMEDDS have a mean droplets diameter of < 250 nm with polydispersity of > 0.05 and SNEDDS have a mean droplets diameter of < 100 nm with polydispersity of > 0.1 [49]. However, the term SMEDDS and SNEDDS are used in parallel in the literature without further distinction of the dispersed solution. The technically incorrect 'microemulsifying' terminology is more common but tends to be used interchangeably with SNEDDS. Each system has some advantages and disadvantages such as type I being a poorly dispersible system but with less chance of drug precipitation upon dilution whereas type IV is a highly dispersible system but with higher chance of drug precipitation upon dilution.

Table 1. Lipid-based formulation classification system and associated characteristics, advantages and disadvantages of various types of LBFs. Reproduced with permission from reference [9, 12].

	Type I	Type II	Type IIIA	Type IIIB	Type IV
Materials	Oils (100%) without surfactants	Oils (40-80%) and water-insoluble surfactants (20-60%)	Oils (40-80%), water-soluble and/or water-insoluble surfactants (20-40%) and	Oils (< 20%), water-soluble and/or water-insoluble surfactants (20-50%) and	Water-soluble and (0-20%)/or water-insoluble surfactants (30-80%) and

			hydrophilic cosolvents (0-40%)	hydrophilic cosolvents (20-50%)	hydrophilic cosolvents (0-50%)
Characteristics	Non-dispersing, requires digestion	SEDDS without water-soluble substances	SEDDS/SMEDDS with water-soluble substances	SMEDDS with water-soluble substances and low oil content	Oil-free formulation
Advantageous	Simple solution, excellent capsule compatibility	Less likely to lose solvent capacity upon dispersion	Forms a clear or almost clear solution upon dispersion, drug absorption may occurs without digestion	Forms a clear and transparent solution upon dispersion, drug absorption may occurs without digestion	High solvent capacity for many drugs, forms micellar solution upon dispersion
Disadvantageous	Poor solvent capacity unless drug is highly lipophilic	Turbid O/W emulsion (particle size 0.25-2 µm)	Likely to lose solvent capacity upon dispersion, less easily digested	Likely to lose solvent capacity upon dispersion	Likely to lose solvent capacity upon dispersion, may not be digestible

2.1 Self-microemulsifying and solid self-microemulsifying drug delivery systems

Lipid solutions dose forms often pose drawbacks in dose uniformity, low stability and high dose volume [50]. Some poorly water-soluble drugs suffer from low solubility in triglycerides (limiting the drug-loading capacity) but have higher solubility in hydrophilic surfactants and co-solvent (enabling the high drug loading with maximum unit dose). Historically, to overcome these drawbacks, Groves *et al*. adapted the concept of self-emulsifying systems from the herbicide and pesticide industries and utilised for pharmaceutical applications [51, 52]. Subsequently, the field further expanded upon the successful commercialisation of cyclosporine A loaded Sandimmune® and Neoral® formulations [9, 53]. Self-microemulsifying drug delivery systems (SMEDDS), classified as type III formulations in the LBF classification system, are comprised of natural or synthetic lipids, surfactant, co-surfactants and co-solvents in addition to drugs and have received the most attention and emerged as a rational approach for an oral delivery of poorly water-soluble lipophilic drugs [9, 16, 19, 45].

Upon contact of the SMEDDS formulations with an aqueous fluid, the isotropic mixture rapidly and spontaneously self-emulsifies under mild agitation and arguably forms a kinetically stable oil-in-water (O/W) emulsion or microemulsion. In the GI tract, the mild agitation is typically

provided by the gastric and intestinal motility necessary for emulsification [9]. The emulsion provides submicron-sized droplets owing to low interfacial tension, large interfacial area and enhanced solubilisation capacity for poorly water-soluble lipophilic compounds (due to the presence of hydrophilic surfactants) that ultimately contributes towards the improved drug absorption and oral bioavailability [54, 55]. In addition, SMEDDS offer the advantages of enhanced oral bioavailability by reducing the dose, avoidance of gastric irritation caused by the prolonged contact between the drug and the GI wall, better stability compared to emulsions, less-production time and the protection of the drugs from the degradation by chemical and enzymatic means in the gut [19, 21]. SMEDDS also offers the advantages of improved stability, patient compliance and ease of manufacturing and scale-up over emulsion systems [19]. The lipid forms the core of the droplets and hydrophilic surfactants provide the emulsification efficiency upon dispersion in an aqueous fluid under mild agitation. In some cases, lipophilic surfactants and co-solvents are also utilised in order to improve the emulsification and dispersion efficiency [33, 56, 57].

The key challenges in formulating SMEDDS formulations are to identify and select the appropriate excipients that can solubilise the drugs in an acceptable volume. The emulsification of SMEDDS is known to be specific to the nature of lipid, surfactant and co-surfactant, the ratio of lipid to surfactant and/or co-surfactant and the self-emulsification temperature [58, 59]. It has been well reported that only specific combinations of the components can potentially result in efficient self-emulsification systems [9, 19, 60]. Additionally, the selection of excipients can significantly impact on the solubility of drugs in the formulation, the kinetics of dispersion, kinetics of digestion and thereby solubilisation of drugs during digestion, as well as the absorption and bioavailability of poorly water-soluble active compounds [18, 61-63]. Consequently, SMEDDS formulation development is often initiated by constructing the pseudo-ternary phase diagram to determine the best suitable combinations of the substances. However, the method is lacking the systemic approach in order to study the impact of excipients on the performance of the formulations. More recently, the design of experiment approach was implemented for SMEDDS in order to improve formulation screening and optimisation methods and the strategy proved a powerful tool for formulation optimisation [64, 65]. Design of experiment approach can be used to optimise SMEDDS formulations in order to simplify the optimisation process and to predict the optimised formulations [64, 65]. The compositions and selection of the suitable excipients for SMEDDS formulation development and

optimisation are well documented elsewhere and the interested reader is directed to references [9, 16].

Conventionally, SMEDDS are in liquid state at ambient temperature due to the liquid state of the most of the formulation components at the room temperature. Depending on the compatibility of the formulation components with capsule shell and the volume of the required dose, liquid SMEDDS are typically encapsulated into either soft or hard shell gelatin capsules for an ease of dosing. However, this can potentially lead to several drawbacks associated with capsule components. For example, the interaction of formulation components with capsule shells can change the taste of the formulation filling and can lead to hardness or softness of the capsule shell that can potentially cause bitterness and/or leakage of the filling [27, 66]. In regards to the capsule shell compatibility, the texture analysis technique has been a popular approach to evaluate the impact of filling on the mechanical properties of the capsule [67]. The presence of plasticizer in soft gelatin capsule shell may result in the migration of drug molecules from the formulation filling to the shell that can affect the drug release kinetics [68]. The sorption of the moisture from the infill material or from the environment can significantly affect the capsule properties and the presence of impurities can cause the cross-linking of the capsule shell affecting the kinetics of drug release [2, 69]. Additionally, the liquid state of the formulations often pose the problems of stability, handling and portability [19, 27, 28, 70].

Hence, the solidification of liquid-based SMEDDS into solid-based SMEDDS formulations has gained an increased interest in recent years in order to circumvent the drawbacks associated with liquid-based formulations and to have the additional advantages of SMEDDS in the wide range of dosage forms (i.e. powder, sachets, suspensions and tablets) [14, 28]. Additionally, the solid-based formulations further offer the benefits of improved stability, better control over the dose, better reproducibility, low production cost and better patient compliance [32, 43, 71-73]. Solidification is generally performed by transforming liquid formulations into solid-based formulations with the use of a solid-phase carrier or additives using solidification techniques or by using the high melting point lipids that are compatible to formulate solid-based formulations. Several solidification techniques including adsorption to solid carriers [74-76], spray cooling [12, 77, 78], spray drying [72], lyophilisation [79], melt granulation [80-83], supercritical fluid-based methods [84-88] and melt extrusion have been well utilised in order to convert liquid SMEDDS into solid SMEDDS. The final products are further processed into solid-based formulations such as powder sachets or tablets. The use of solidification techniques

for the preparation of solid SMEDDS formulations is well described in the literature [19, 27, 28].

The design of SMEDDS is primarily focussed on the optimisation of the solubility of the drug into the formulation components, self-emulsification efficacy and the size of droplets upon dilution into an aqueous fluid. Typically, the formulations are characterised for visual evaluation, turbidity measurement, droplet size measurement, dispersion time and digestion behaviours at physiologically relevant conditions using *in vitro* methods in order to predict the *in vivo* performance of the formulations [89]. More recently, the digestibility and propensity of solid SMEDDS for the formation of self-assembled colloidal phases of the digestion products with endogenous amphiphilic components have been examined in order to determine the fate of formulation and drugs [90, 91].

3. Self-emulsification process

The mechanism of self-emulsification is not entirely understood but there is a basic physical understanding [9, 21, 33]. It has been reported that the emulsification occurs when the entropy changes favouring the dispersion process is greater in comparison to the energy required to enhance the surface area of the dispersion [92]. For a conventional emulsion, the required energy to generate new surface between oil and water phases is a direct function to the free energy of the system. However, the formed oil and water phases tend to separate with time in order to reduce the interfacial area, resulting into the reduced free energy of the system. The surface-active components create a monolayer around the emulsion droplets upon dilution into an aqueous phase and assist in stabilising the droplets by reducing the interfacial energy and preventing coalescence. For SMEDDS, the emulsification occurs on very mild stirring (agitation) as the free energy required to form the emulsion is low [93]. The process of emulsification has been reported to be associated with the passage of water into the initial phases (i.e. liquid crystalline or gel phases) formed at the surface of the oil droplets [51, 58, 94]. Upon dilution, the emulsifying components of SMEDDS (i.e. surfactants and co-surfactants) reduces the interfacial tension between the oil and water phases and form an interface at the surface of the oil droplets. The aqueous penetration of water through the interface leads to the solubilisation of water into the core of oil phase until the maximum solubilisation capacity is achieved and further water penetration results in the formation of dispersed liquid crystalline phases. Hence, upon gentle agitation, the water will penetrate

346 rapidly into the core of oil droplets, resulting in disruption of the interface and droplet formation.
347 At the same time, the formation of the interface at the surface of the oil droplets stabilises the
348 formed droplets and makes SMEDDS stable to coalescence. Several studies have been
349 performed to quantify the role of liquid crystalline phases on the emulsification process and
350 the studies reported the complex relationship between those mechanisms [51, 58]. However,
351 the exact correlation between liquid crystalline phases and the emulsification process has not
352 been completely established and the phenomena is still largely unknown.

353 Primarily, the propensity of emulsification is assessed by visual assessment and the efficiency
354 of the emulsification is estimated by determining the dispersion rate and droplet size
355 measurement of the resultant emulsion [9, 21]. The dispersion rate of SMEDDS is generally
356 assessed under physiologically relevant conditions using the USP II apparatus attached with
357 rotating paddles or modified apparatus and the droplet size is typically determined by optical
358 microscopy or laser light diffraction methods [9, 19]. It has been assumed that the drugs are
359 dissolved in the anhydrous formulation thus, the focus of the dispersion has been to detect the
360 precipitation of the drug particles upon dilution into the GI fluids. However, to date, no
361 standard pharmacopeia method have been established in order to evaluate the dispersion
362 behaviour.

363 The formation of liquid crystalline phases upon dispersion and digestion of polar amphiphilic
364 lipids is an important aspect in order to understand the fate of formulations in the GI lumen. It
365 has been reported that the digestion of lipids plays a key role in the formation of colloidal
366 phases and subsequently drug solubilisation and drug absorption [13]. Thus, the formation of
367 colloidal phases upon dispersion of SMEDDS is less explored and the literature has been
368 largely focussed on the structure formation during digestion.

369

370 4. Lipid digestion

371 The digestion of lipids is a dynamic process *in vivo* and it is a complex combination of
372 biological and physiological processes. Digestion is an important feature to understand the
373 changes in solubilisation capacity of the GI lumen that might occur upon digestion of digestible
374 components. The impact of digestion and incorporated exogenous digestive components on the
375 performance of LBFs has led to the widespread use of *in vitro* lipid digestion models [34, 95-
376 98]. The enzymatic digestion of lipid occurs in the GI tract under the action of gastric lipases
377 and pancreatic lipase and co-lipase [99, 100].

A schematic illustration of lipid digestion and drug solubilisation process in the stomach and small intestine is depicted as Fig. 1. After oral administration of LBF, the hydrolysis of the lipid commences the action of the pre-duodenal lipases (lingual lipase secreted by the salivary glands in rats and mice, and the gastric lipase secreted by the gastric mucosa in humans and most mammals) leading to the partial hydrolysis of exogenous dietary triglycerides into diglycerides and partially unionised fatty acids [101]. These digestion products interact with dietary endogenous phospholipids and promote the formation of a crude emulsion (comprised of aqueous gastric fluid and lipid digestion products) [101]. The formed crude emulsion is propelled to the small intestine for the further hydrolytic processing. Quantitatively, the gastric lipolysis step is a minor contributor to the overall digestion process and it has been suggested to be approximately 10-25% responsible for the total digestion process [102, 103]. The major extent of hydrolysis occurs in the small intestine by pancreatic lipase and co-lipase.

Upon arrival of the crude emulsion in the small intestine, the pancreas secretes the mixture of fluid containing pancreatic lipase and co-lipase as well as other esterases (i.e. carboxyl ester hydrolases, phospholipases, pancreatic lipase related proteins) [104, 105]. These water-soluble enzymes facilitate the breakdown of triglyceride to diglyceride, monoglyceride and ultimately free fatty acids [106]. Principally, the hydrolysis of the ester bonds at sn-1 and sn-3 positions of triglyceride (linking the glycerol to fatty acids) by the pancreatic lipase leads to the breakdown of triglyceride to 2-monoglycerides and 2 moles of free fatty acids for each mole of triglyceride [104, 106, 107]. Additionally, the digestion of derived phospholipids also occurs in the small intestine where pancreatic phospholipase A_2 hydrolyses a single fatty acid molecule from the sn-2 position to produce lysophosphatidylcholine and free fatty acid [108, 109]. The presence of lipid digestion products stimulates the secretion of endogenous amphiphilic components such as bile salts, phospholipids and cholesterol from the gallbladder into the small intestine that act as emulsifying agents. The intercalation of the digestion products into the secreted endogenous amphiphilic components leads to the generation of lipidic reservoirs including colloidal phases, vesicles (i.e. multilamellar or unilamellar vesicles) and mixed micelles in the GI fluid [37, 110]. The mechanism of hydration, swelling and the self-assembly process of lipid digestion products results in the formation of liquid crystalline structures at the droplet surface and the dispersion of these phases into the intestinal fluid results in a range of colloidal phases such as lamellar, hexagonal and cubic where all mesophases possess differing solubilisation capacity to accumulate the digestion products or lipophilic drugs [37, 111]. These digestion products and formed colloidal phases provides a

solubilising medium for poorly water-soluble lipophilic drugs and further boost the solubilisation capacity of the GI lumen.

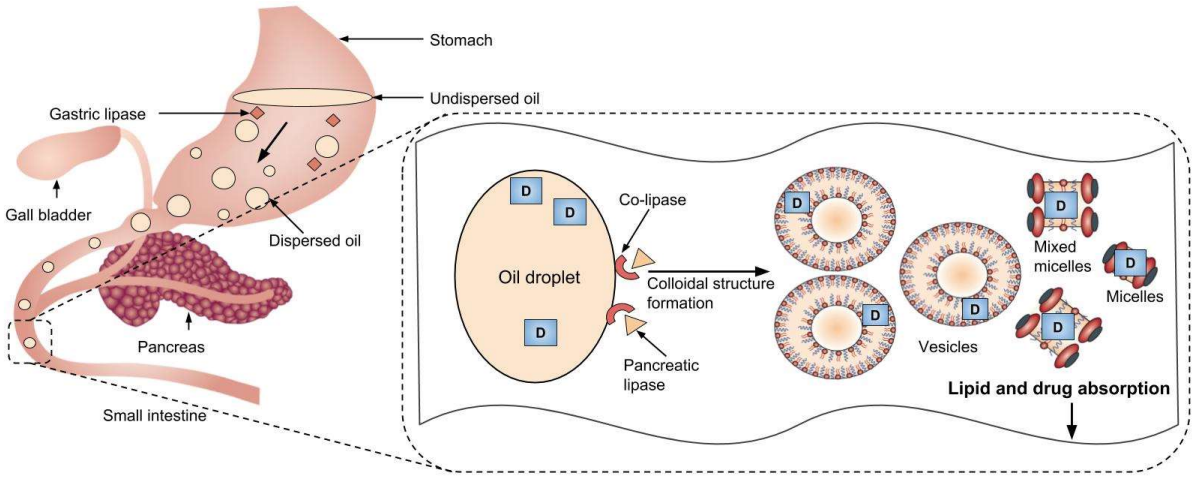


Fig. 1. Schematic overview of lipid digestion and drug solubilisation in the stomach and small intestine. Figure reproduced with permission from reference [13].

The pancreatic lipase/co-lipase complex is only active at the oil-water interface and the activity of the lipase is significantly affected by the surface of the substrate. The digestion products (i.e. monoglycerides and free fatty acids) possess high interfacial activity thus the formed colloidal phases act to remove the digestion products from the oil-water interface and assist in displacing the lipase from the interface [31, 112, 113]. This is specifically important as the amphiphilic digestion products accumulated at the surface of the substrate restricts the access of the lipase at the oil-water interface hindering the digestion [13, 114]. During the dynamic digestion process, the digestion products undergo a complex physiological sequence and various pH environments of the intestine that may have a pronounced effect on structure formation and transformation [36, 115, 116]. The formed mixed micelles are believed to constitute the final digestive phase prior to the absorption from the lumen in the gut and it is believed that the lipid is absorbed from the formed colloidal phases via partitioning from the intestinal mucus into the enterocyte [7, 35, 117, 118]. However, the phenomena of lipid absorption is poorly understand and the exact mechanism is still unknown [36, 119].

4.1 *In vitro* lipid digestion model

The *in vitro* digestion studies are performed with an aim to predict the *in vivo* performance of LBFs. In earlier studies, the digestion of the lipid-based systems was conducted by exposing lipid to lipase containing aqueous fluid on a microscopic slide and the formation of colloidal phases was observed under a microscope under static conditions [114, 120]. However, the digestion of lipid is a dynamic process *in vivo,* as the formulation passes through several complex physiological conditions where several variables such as osmolality, pH and viscosity of the fluids are varied at different stages [121]. Thus, more complex dynamic *in vitro* lipolysis models have been developed to better mimic the *in vivo* behaviour during digestion [119]. To date, several *in vitro* lipolysis models reflecting the *in vivo* digestion conditions have been employed in order to evaluate the lipolysis process of simple triglycerides to more complex self-emulsifying components and formulations [39, 40, 63, 90, 91, 122-127]. A schematic illustration of such model is depicted in Fig. 2.

Typically, *in vitro* lipolysis studies are conducted into the temperature-controlled digestion glass reaction vessel containing digestion medium reflecting the simulated intestinal fasted or fed state conditions. The LBFs are pre-dispersed into the digestion medium at 37°C into temperature-controlled digestion reaction vessel where the pH of the digestion medium is monitored by the pH-sensitive probe connected to pH-stat and autoburette. The pH of the digestion medium is adjusted 6.5 or 7.5 in order to reflect the physiological pH range of intestine (fasted and fed intestinal state pH range are 6.1-7.3 and 5-6.6, respectively) and to have optimal pH range for pancreatic lipase activity (pH range 6-10) [128, 129]. The lipolysis is commenced by adding pancreatic lipases after thermal equilibrium where the digestion of formulation triglycerides and digestible components leads to the production of free fatty acids, resulting in the drop of pH of the digestion medium. The drop in pH is recognised with the aid of the pH-stat controller and NaOH solution is titrated via autoburette to compensate the drop in pH in order to maintain the pH of the digestion medium at the pre-defined value. The consumed volume of NaOH to titrate the liberated fatty acids provides an indication of the extent of digestion. Upon completion of the initial lipolysis experiment at the pre-defined pH value, the pH of the digestion medium is increased to a higher value (i.e. to pH 9) in order to titrate all unionised fatty acids produced during *in vitro* lipolysis and the extent of digestion is calculated using the previously reported equation [90, 130, 131]. In most cases, the digestion of formulation-free blank digestion medium is carried out and subtracted (as background

subtraction) from the consumed NaOH volume from the formulation-titration profiles to have improved accuracy and better reproducibility [7, 17, 132].

The experimental models and parameters such as volume, pH, the concentration of medium components (i.e. ionic strength of buffer solution, bile salts and phospholipids) and degree of agitation slightly vary between the research group laboratories but the concept and operations are generally same [133]. Thomas *et al*. summarised several commonly used *in vitro* lipolysis models used by several laboratories [119].

During digestion, the liberated fatty acids can potentially accumulate at the digesting lipid surface and hinder the access of lipase to the digestible substrate. Thus, calcium is added either as a bolus at the start of the lipolysis process or continuously via peristaltic pump in order to prevent accumulation of liberated fatty acids at the surface and to precipitate the liberated fatty acids as calcium soaps [7, 39, 41, 42, 97]. All these variables between models can significantly impact on the kinetics of the process for the same formulation and can potentially affect the reproducibility. Consequently, the lipid-based formulation classification consortium proposed to develop a standardised model that can be used to evaluate the *in vitro* behaviour of lipid-based systems with higher accuracy and reproducibility [134]. However, the phenomena of colloidal phase formation during digestion was overlooked [36].

The formation of colloidal phases during digestion has been studied either by analysing digestion time point samples at static conditions or by monitoring the evolution of structures in real-time. For some studies, the sample time-points have been collected during lipolysis experiments and treated with lipase inhibitor to halt the lipase activity. Thereafter, the time-point aliquots have been used either directly or more often centrifuged/ultracentrifuged to separate the digest into a poorly dispersed oil phase (not common for SMEDDS and SNEDDS formulations), a highly dispersed aqueous phase and precipitate pellet phase, in order to quantify the formed colloidal phase during digestion, the lipid and lipid digestion products, [135], drug content in an aqueous phase or pellet phase [91, 96, 136] and solid-state form of precipitated drugs in pellet phase [136].

Alternatively, more recently, the in situ flow-through approach has been used by coupling *in vitro* lipolysis with small-angle X-ray scattering (SAXS) in order to examine the structural aspects during digestion in real-time (Fig. 2). The technique has been utilised to monitor the evolution of colloidal structures at shorter time scale and drug behaviours (drug solubilisation and drug precipitation) at a longer time scale during digestion in real-time [91, 130, 137]. This

approach avoids the need for sample time point collections, sample inhibition, storage and retrieval of the sample for analysis, further improving the efficiency and accuracy. As shown in Fig. 2, the capillary is mounted in the X-ray beam (source of X-ray can be a bench-top instrument or synchrotron SAXS) and the continuous flow of the digestion medium is enabled using the peristaltic pump.

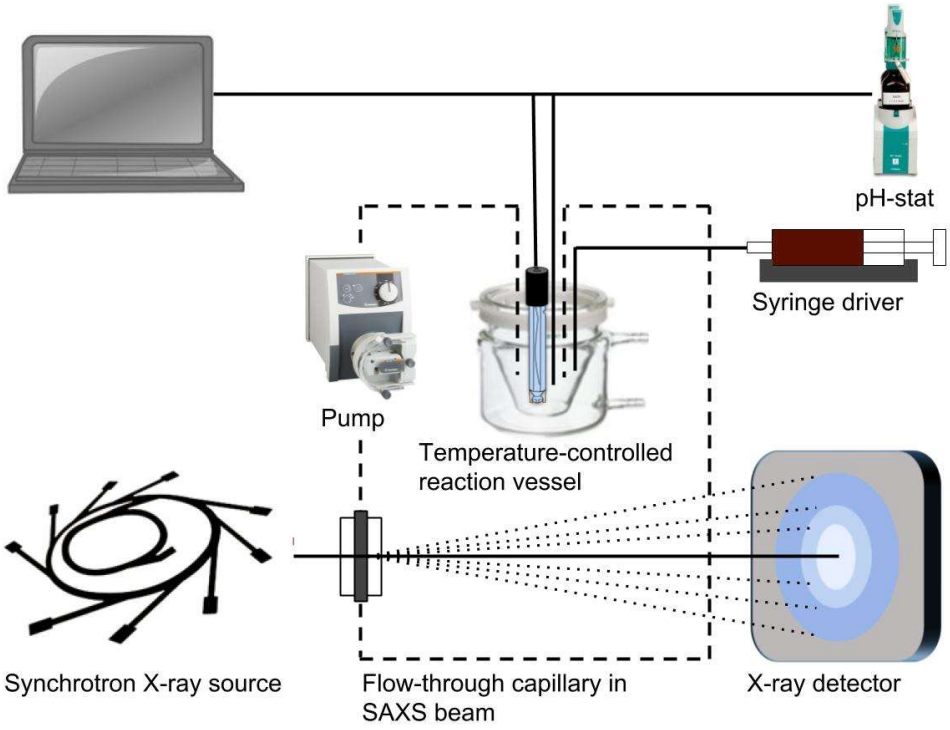


Fig. 2. Schematic representation of an *in vitro* digestion apparatus. The temperature-controlled reaction vessel is connected to the pH-stat comprising of pH meter and autoburette. A remotely controlled syringe driver is used to add lipase during synchrotron SAXS acquisition. A flow-through capillary can be mounted in the X-ray beam in order to monitor structure evolution during digestion in real-time. Figure reproduced with permission from references [10, 36].

5. Self-assembled colloidal phases for lipid-based systems

Lipids are biocompatible substances containing both hydrophobic and hydrophilic groups. Upon contact with excess water or an aqueous fluid, the self-assembly behaviour of these biocompatible amphiphilic molecules leads into the formation of a variety of ordered liquid crystalline phases with complex internal nanostructure [138]. The type of formed colloidal

phase depends on the packing of the self-assembly behaviour of amphiphilic lipids in order to prevent the direct contact between hydrophilic and hydrophobic regions. The concept of critical packing parameter (CPP) can be employed in order to understand the impact of surfactants and lipid on the geometry of the formed colloidal structures [139]. The CPP can be described by $CPP = V/al$, where, 'V,' is the volume of surfactant chain, 'a', is the effective cross-section area of the surfactant at the interface and, 'l' is the length of the surface chain [139]. These parameters are significantly affected by various factors such as the physicochemical properties of lipid, lipid compositions, lipid concentration, temperature, pressure and the presence of additives [140, 141].

A schematic illustration of commonly formed self-assembled liquid crystalline colloidal phases with associated CPP values are depicted in Fig. 3. The formed liquid crystalline phases can be separated into two categories: normal phases (Type 1) and inverse phases (Type 2). The normal phases are formed for the cone-shape amphiphilic molecules with a large polar head group where the interfacial curvature is pointed towards the lipid region. In contrast, the inverse phases are formed for the wedge-shape amphiphilic molecules with a small polar head group in comparison to large chain where the interfacial curvature is pointed toward the aqueous region [142, 143]. The inverse colloidal phases are most stable at physiological relevant temperatures and in excess water, and they can be formed by manipulating the temperature, salt concentration and hydration [144].

The most commonly observed liquid crystalline colloidal phases are the lamellar (L_α), inverse hexagonal (H_2) and the inverse bicontinuous cubic phases (V_2). The L_α phases, consists of a flat bilayer with no mean curvature, are formed at CPP = 1 and the normal and inverse phases are formed at CPP < 1 and CPP > 1, respectively. The normal phases such as normal micelles (L_1) and normal hexagonal (H_1) are formed for the hydrophilic emulsifiers and the inverse micelles (L_2) and inverse hexagonal (H_2) phases are formed for the lipophilic emulsifiers. The L_2 phases contains inverse micelles and the H_2 phases are cylindrical structures in a hexagonal pattern. They contain rod-shaped closed water channels in two-dimensional in a hexagonal pattern where the water channels are separated by the lipid bilayers [36]. The inverse bicontinuous cubic phases (V_2) are observed at CPP $\geq$ 1, between the H_2 and L_α phases. The V_2 phases are comprised of continuous non-intersecting curved water channels separated by a bicontinuous lipid bilayer which offers three-dimensional spatially organised liquid crystalline complex structures. The three different types of the observed V_2 phases are the gyroid (*Ia3d*),

the double diamond (*Pn3m*) and the primitive (*Im3m*) [145]. Close-packed spherical micelle aggregates form normal micellar cubic (I_1) and inverse micellar cubic (I_2) phases between the respective hexagonal and micellar phases.

These formed colloidal phases have the ability to solubilise a range of physiochemical compounds with the potential to control the release rate from the matrix, thus these structures are widely evaluated as a carrier for the drug delivery systems [140, 146].

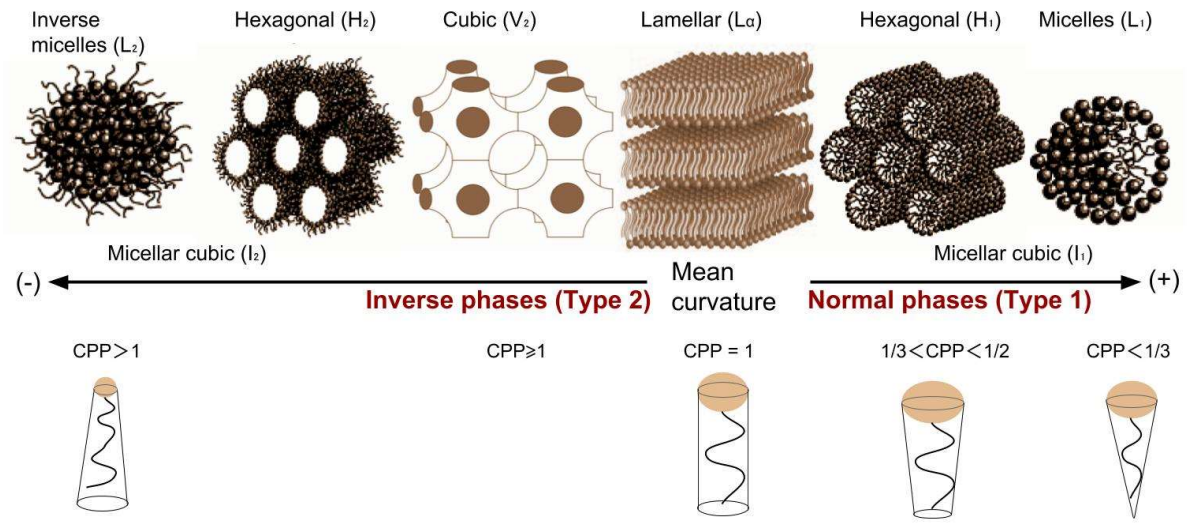


Fig. 3. Schematic illustration of common self-assembled liquid crystalline colloidal phases and their corresponding critical packing parameter. Figure reproduced with permission from reference [147] and cubic structure reproduced with permission from reference [148].

5.1 Conventional characterisation techniques for the study of formed colloidal phases during dispersion and digestion of SMEDDS

Lipids exhibits rich phase behaviour in the presence of water and form a variety of aforementioned colloidal structures at various stages of dispersion and digestion [114, 119]. During dispersion, the self-emulsification of SMEDDS in the GI fluid can potentially leads to the formation of a series of liquid crystalline colloidal phases at the interface of oil-water droplets prior to the conversation into stable oil droplets. Furthermore, the lipid species can potentially transition from simple unstructured oil droplets to complex geometrically organised liquid crystalline structures and eventually to simple micellar solutions during the dynamic digestion process. The formed colloidal phases, particularly upon digestion containing lipid digestion products and endogenous amphiphilic components, often possess complex structure

568 and dynamic compositions [37]. The fate of the formulation and the drug distribution in the GI
569 tract is dictated by the type of the formed colloidal phases during digestion and the performance
570 of LBFs is mediated by the interaction of poorly water-soluble lipophilic drugs with
571 endogenous and exogenous amphiphilic components, lipids and lipid digestion products that
572 can potentially enhance the solubilisation and thereby absorption [35, 126, 149]. Thus far,
573 several microscopic (light microscopy, cross-polarised light microscopy (CPLM), freeze-
574 fracture electron microscopy, cryogenic-transmission electron microscopy (cryo-TEM),
575 cryogenic field emission scanning electron microscopy (cryo-FESEM), atomic force
576 microscopy (AFM)), spectroscopic (nuclear magnetic resonance spectroscopy (NMR), Raman
577 spectroscopy and multiplex coherent anti-Stokes Raman scattering microspectroscopy (CARS),
578 Electron paramagnetic resonance (EPR)), scattering (dynamic light scattering (DLS),
579 small/wide-angle X-ray scattering (SAXS/WAXS), small-angle neutron scattering (SANS))
580 and more recently simulations (molecular dynamic simulation) techniques have been employed
581 in order to obtain the information about the formed structures and the transformation of the
582 self-assembled structures during dispersion and digestion of lipid-based systems [36]. Herein,
583 several commonly employed structural characterisation techniques for SMEDDS during
584 dispersion and digestion such as dynamic light scattering (DLS), transmission electron
585 microscopy (TEM), cryo-TEM, cryo-SEM, SAXS, fluorescence resonance energy transfer
586 (FRET) and Taylor dispersion analysis (TDA) are discussed.

587

588 ### 5.1.1 Dynamic light scattering

589 Dynamic light scattering (DLS) is a commonly used technique to obtain information about
590 particle size and size distribution of dispersed colloidal phases of diluted lipid-based systems
591 [150, 151]. The technique has been used to investigate the bulk structure of dispersed SMEDDS
592 formulations [64, 71]. In DLS, a laser beam is passed through the sample cell and the
593 autocorrelation function of the photocurrent is recorded at a fixed angle. DLS measures the
594 fluctuation in the scattering intensities at a single point as a function of time, caused by the
595 Brownian motion of the particles. The information about the particle size is obtained from the
596 mean diffusion coefficient calculated from the detected intensity correlation functions [152].
597 Although DLS does not allow a direct size measurement, it is a powerful and rapid screening
598 technique to measure the particle size distribution for SMEDDS formulations.

599

5.1.2 Transmission electron microscopy and cryogenic-transmission electron microscopy

Transmission electron microscopy (TEM) has been utilised to examine the morphology of SNEDDS formulations upon aqueous dispersion [153, 154]. In TEM, the sample is placed on the sample holder and a beam of electron is passed through a very thin layer of sample where the electrons interact with sample molecules and the transmitted electrons project an image of the sample on the detector with higher resolution. However, some biomaterials are susceptible to high vacuum condition and intense electron beams of TEM, thus, cryogenic-TEM (cryo-TEM) has been developed where the samples are transformed into frozen state and the analysis is carried out at low electron beam.

Cryo-TEM, with its superior resolution of 1-2 nm, allows the direct visualisation of the sample and enables to retrieve information about the morphology of particles and internal structure of the self-assembled systems [155]. Thus, it is a broadly used technique for the direct observation of the formed colloidal phases of the self-assembled lipid-based system [124, 156, 157]. However, cryo-TEM only provides the information about the small portion of the sample rather than global information about the sample. During *in vitro* digestion, the sample can be withdrawn at the desired time points and treated with lipase inhibitor and vitrified immediately. Briefly, the samples are placed on the carbon grid supported by the copper grid. Then, the samples are blotted dried using a filter paper in order to obtain a thin film of the sample on the grid and subsequently, the samples are vitrified by quenching in liquid ethane at -180°C and to liquid nitrogen at -196°C in order to preserve their structure in their native environment with minimal artefacts. Cryo-TEM offers the advantage of the avoidance of the artefact such as staining, fixation and adsorption process that can potentially occur during sample preparation. The technique has been utilised to elucidate the information of the formed colloidal structures during *in vitro* digestion of SNEDDS formulation (Fig. 4).

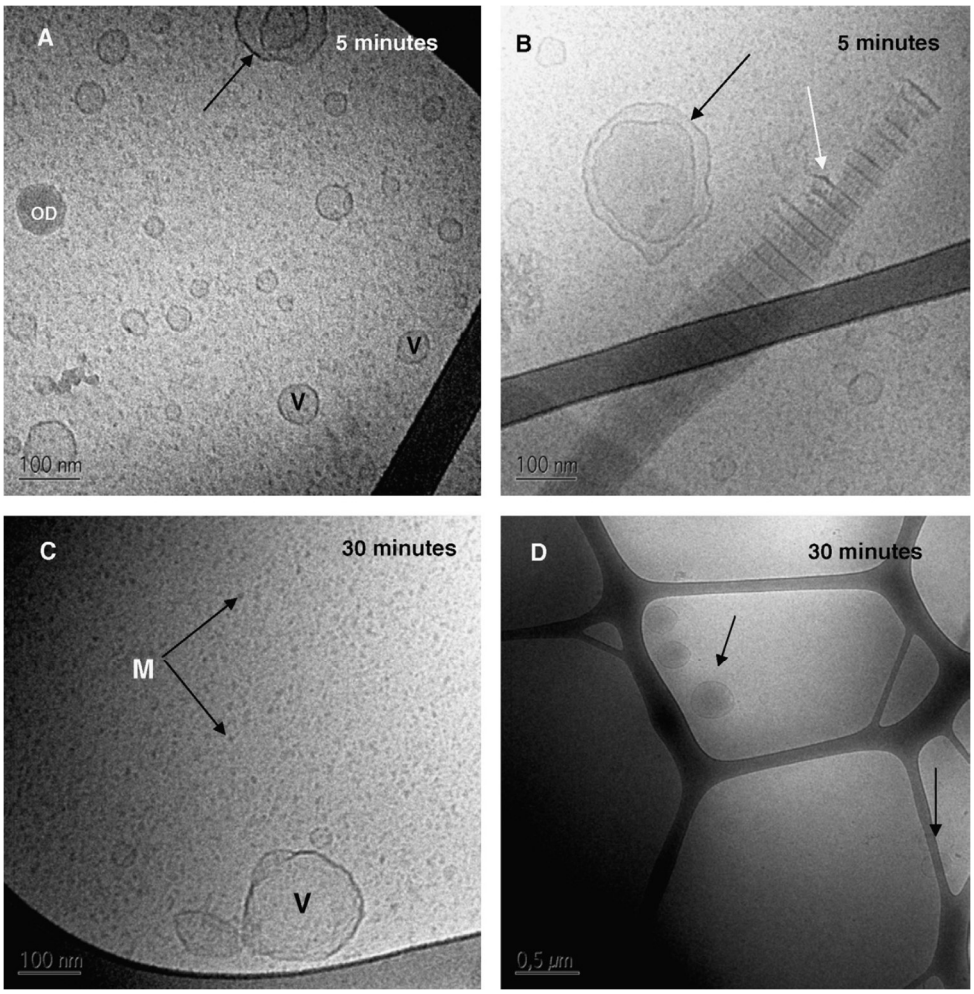


Fig. 4. Cryo-TEM images of lipolysis products of SNEDDS containing sesame oil, Maisine® 35-1, Cremophor® RH 40 and ethanol (30:30:30:10% w/w) (A and B) 5 min after lipase addition and (C and D) 30 min after lipase addition, at fasted state. (A) Oil droplet (OD), unilamellar vesicles, bilamellar vesicles (indicated by arrow) and vesicles were evident. (B) Bilamellar vesicles with rippled and angular surface (indicated by black arrow) and the ladder shape structure (indicated by white arrow) were apparent. (C and D) Irregularly spotted unilamellar vesicles (indicated by black arrow) and micelles (M) were observed. The scale bar of A, B and C represents 100 nm and D represent 500 nm. Figure adapted with permission from reference [124].

5.1.3 Cryogenic scanning electron microscopy

Cryogenic scanning electron microscopy (Cryo-SEM) enables a direct visualisation of the sample under the microscope and provides the morphological information of the formed colloidal phases upon aqueous dispersion of the polar amphiphilic lipids such as monoglycerides, phospholipids and formed mesophases [158, 159]. Briefly, the small amount

of sample is placed into the sample holder rivet at ambient temperature that is further treated with slushed liquid nitrogen at -196°C in order to achieve a frozen sample. Then, the sample is transferred to cryo-SEM sample preparation chamber under vacuum condition where the sample holder temperature is maintained at -150°C. Subsequently, the sample is fractured using a cold knife to achieve a clean surface of the frozen sample and the clean sample is sputter coated with gold or platinum and visualised under microscope. Cryo-SEM has been employed to identify the internal structure and three-dimensional structures of phytantriol cubic and hexagonal phases [160, 161]. However, the technique has not been utilised to date to identify the formed colloidal phases upon digestion of LBFs.

5.1.4 Small and wide-angle X-ray scattering

X-ray scattering techniques are broadly utilised to obtain structural information of solids or liquid crystals. Among other X-ray scattering techniques, small and wide angle X-ray scattering (SAXS/WAXS) technique has received the most attention for the identification of the liquid crystalline structures and solid state of drugs in solution [162]. As the name suggests, SAXS is typically used to detect the scattered X-rays at scattering angles lower than 10^0 (2θ). Briefly, the X-ray beam of the known wavelength (λ) is passed through a sample where the radiation are scattered at various angles (2θ) due to the interaction with the sample electrons. The inhomogeneity in the electron density within the sample causes the variation in scattering intensity depending on the scattering angle. The scattering intensities are measured as a function of scattering angle (θ) and two-dimensional scattering patterns are acquired [163, 164]. The angular scattering intensity provides the morphological information of the structures with a spatial dimension.

In the case of an aqueous dispersion, the technique can be used to retrieve the information on the size, shape and distribution of colloidal species in the liquid [163]. Additionally, the information about the formed colloidal structures can be retrieved by implementing 'structure pattern recognition' approach with the existence of the pronounced peaks, representing the presence of the highly ordered colloidal structure. The angular scattering intensities follow Bragg's law $n\lambda = 2d\sin\theta$, where 'n' is an integer, 'λ' the wavelength of X-rays, 'd' is the spacing between lattice planes and 'θ' is the scattering angle [165]. The positions of Bragg peaks for highly ordered systems are reciprocally related to the separation between molecules and/or lattice planes in the sample. The ratio of the peak positions are unique for each type of colloidal phases thus the specific type of colloidal structure can be determined from a relative peak

positions from a scattering pattern that corresponds to the *hkl* planes defined by the Miller Indices values [166]. Thus far, SAXS has been successfully implemented at static (ex situ) and dynamic condition (in situ) in order to monitor the structural evolution and colloidal phase transitions of digesting lipid-based systems for simple oil solutions and more recently for complex systems (Fig. 5) [137, 157].

In wide-angle X-ray scattering (WAXS) or more commonly X-ray powder diffraction (XRD), the scattering patterns are recorded at a wider angle (generally more than 10^0) in order to cover the size range from few nanometers to one angstrom [162]. WAXS is a useful tool to obtain the information of molecular packing and crystalline behaviour of drugs. WAXS approach has been employed to examine the drug/additive crystallisation, precipitation behaviour of drugs during digestion [130], and more recently the drug solubilisation behaviour during digestion of LBFs [91].

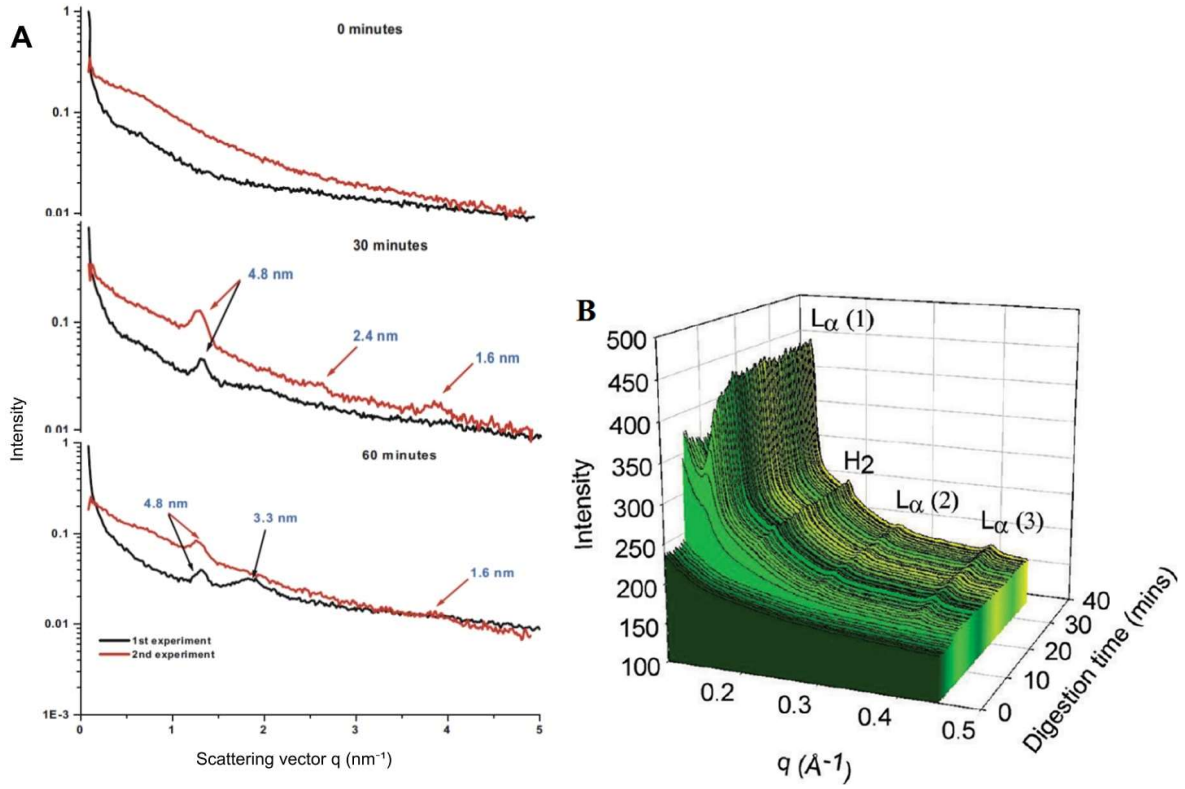


Fig. 5. SAXS scattering profiles of SNEDDS formulation containing sesame oil, Maisine® 35-1, Cremophor® RH 40 and ethanol (30:30:30:10% w/w) during digestion at fasted state acquired using (A) bench-top SAXS at ex situ condition and (B) synchrotron SAXS at in situ condition. (A) The scatter profiles illustrate no phase at 0 min, the formation of lamellar phases

(L_α) at 30 min and L_α phases and inverse hexagonal phases (H_2) at 60 min. The arrows denotes the spacing of Bragg peaks for L_α phases at q of 1.6, 2.4 and 4.8 nm^{-1} and for H_2 phase at q of 3.3 nm^{-1}. (B) The time-resolved scattering profiles exhibit the formation of L_α phases during initial stage of digestion and the evolution of L_α and H_2 phases at later stage of digestion. Figures adapted with permission from references [126, 137].

5.1.5 Fluorescence resonance energy transfer

Fluorescence resonance energy transfer (FRET) is a commonly used technique in the biological applications to investigate molecular interactions and to determine the stability of nanoparticles [167, 168]. FRET is a distance-dependent process in which the non-radiative energy transfer occurs between an excited molecular fluorophore (the donor) and another fluorophore (the acceptor). The energy transfer process from the donor to acceptor is mediated by the intermolecular long-range dipole-dipole coupling [169, 170]. The efficiency of the FRET is dependent on the distance between the donor and acceptor (the FRET pair) and the technique is highly efficient when the donor and the acceptor are aligned efficiently within the Förster radius. For instance, the efficiency is highest at the distance (typically 3-6 nm) where the half of the donor excitation energy can be transferred to the acceptor. The technique has been utilised to quantify the structural changes occurring at the interface during digestion of an emulsion and more recently, applied to SMEDDS systems as a complementary technique to monitor the structural changes occurring during digestion of SMEDDS at *in vitro* condition and at the mucus [171, 172]. Briefly, the FRET pair (the donor and the acceptor) are blended into the formulations prior to lipolysis experiment. The samples are collected at the desired time points during digestion and treated at a specific excitation wavelength and the emission scan range using a plate reader. The energy transfer between the FRET pair is quantified by examining the fluorescence intensities of the donor and the acceptor and consequently the appearance of the formed intermediate phases are monitored (Fig. 6).

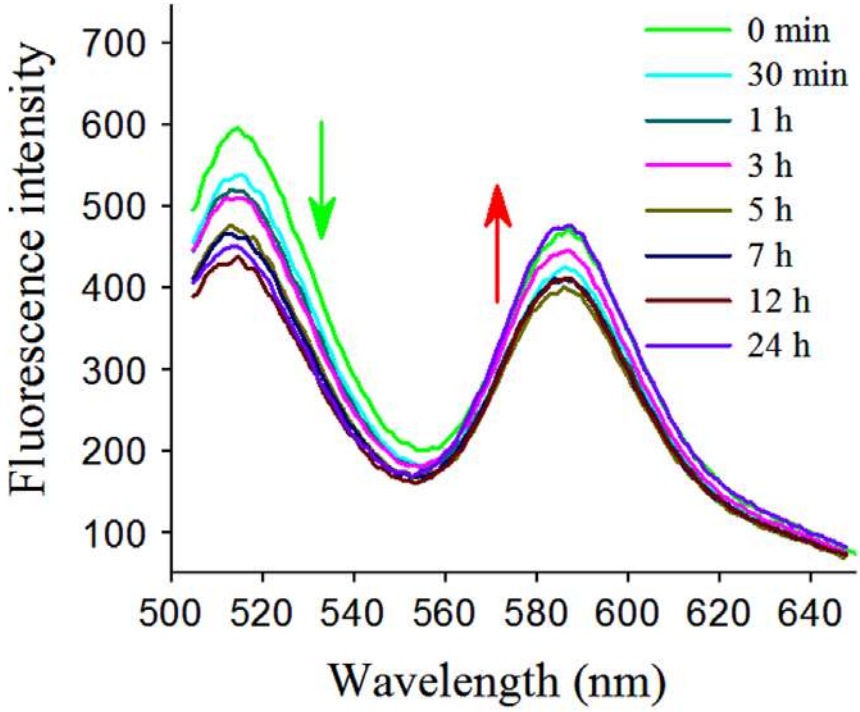


Fig. 6. The FRET fluorescence spectra of SMEDDS formulation containing ethyl oleate, Cremophor® RH 40 and 1,2-propanediol (40:40:20% w/w) at different digestion time points at fasted state in phosphate buffer saline solution with FITC-ODA (FITC-octadecylamine) as the donor and RhB-MP (rhodamine B-α-monopalmitin) as the acceptor. The decrease in the fluorescence intensity of the donor at 520 nm and variation in the fluorescence intensity of the acceptor at 580 nm indicated the generation of intermediate liquid crystalline phases during lipolysis of SMEDDS. Figure adapted with permission from reference [172].

5.1.6 Taylor dispersion analysis

Taylor dispersion analysis (TDA) is a microcapillary based flow technique that allows the characterisation of size and stability of small molecules in solution even for complex mixture systems. The application is not significantly affected by the presence of aggregates or by the viscosity of the solution, thus the samples can be run without dilution or filtration [173]. TDA works on the principle of quantifying the broadening of the peaks of a solute plug in a laminar Poiseuille flow in order to determine the molecular diffusion coefficient and subsequently, the hydrodynamic radius of the solute [174, 175]. Briefly, a small volume of sample is injected into a microcapillary with matched buffer solution under a constant driving force. Due to dispersion and diffusion at the axial and radial direction, the sample pulse gets broadens in the microcapillary as it flows and the detection is carried out (either by UV or fluorescence

detection) in order to analyse the absorbance at the cross-sections. Then, the Taylorgram is plotted as absorbance versus time and the hydrodynamic radius is calculated from the molecular diffusion coefficient [175].

TDA has been used as an alternative technique to characterise the micellar systems [173, 176]. For example, Chamieh *et al*. employed TDA in order to measure the molecular diffusion coefficient of micelles for micellar systems containing non-ionic and ionic surfactants and demonstrated the efficiency and suitability of technique for micellar systems [176]. More recently, the same group utilised TDA with fluorescence detection for the size characterisation of self-emulsifying pharmaceutical excipients (Labrasol® and Gelucire® 44/14) during digestion (Fig. 7) [173].

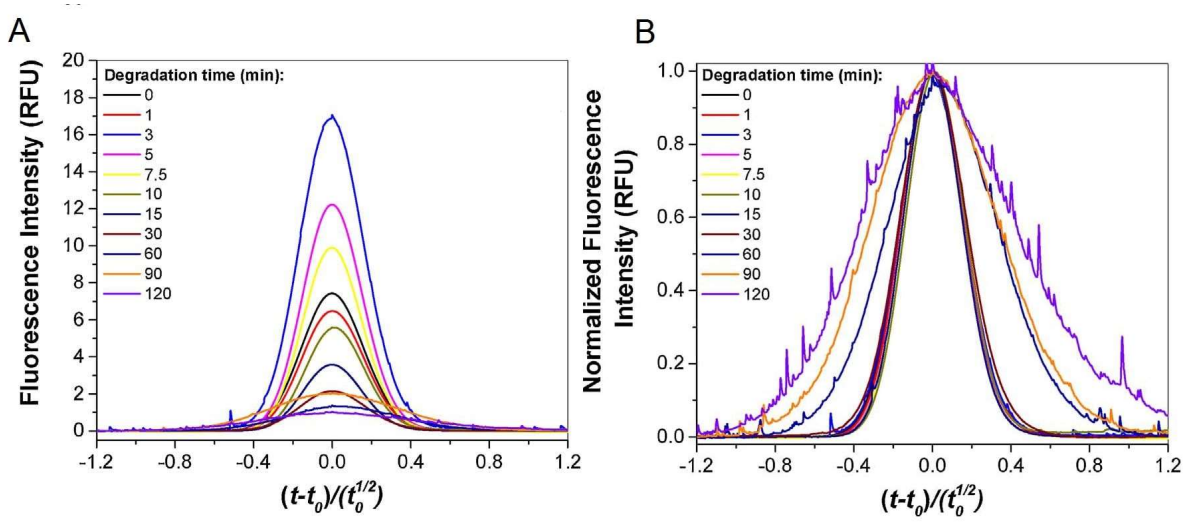


Fig. 7. Overlay profiles of obtained Taylorgrams of Gelucire® 44/14 at different time points during digestion at fasted state and at 37°C in (A) Relative Fluorescence Unit (RFU) and (B) normalised scale. (A) The image clearly shows the evolution of the Taylorgrams suggesting the presence of lipid solution under initial operating conditions and the formation of coacervates at advanced time points (t > 60 min). (B) The image shows broadening into Taylorgram profiles indicating the enlargement in size of the micelles. Figure adapted with permission from reference [173].

6. Drug solubilisation and drug absorption

After oral administration, drugs must be dissolved into the GI fluid in order to be able to cross the biological membrane and absorbed. Typically, poorly water-soluble lipophilic drugs exhibit a dissolution rate limited drug absorption in the GI tract [177]. The dissolution of drugs in the GI fluid is complex process and is determined by physiochemical properties of the compounds (i.e. aqueous solubility, molecular weight, solid-state properties (i.e. crystalline or amorphous form), partition coefficient, pKa, particle size, solvent property and pH variability) and physiological factors such as the presence of food within the GI tract [178].

The solubilisation capacity for incorporating drugs into LBFs during GI transit is attributed to the ability of the formulation component to maintain the drug in a solution state during dispersion and/or digestion [105]. The enhanced solubilisation and dissolution attributed to the presence of endogenous and exogenous amphiphilic components, lipid digestion products and the formed liquid crystalline colloidal phases may improve the drug absorption and oral bioavailability [32, 33]. Generally, the solubilisation capacity of the GI is correlated to the total bile salt concentrations in the absence of exogenous components that is low at fasted state and high at post-prandial state [11, 13]. However, the addition of exogenous digestible lipids leads to the generation of digestion products and colloidal phases where the nature and characteristics of the processing can significantly enhance the solubilisation capacity. Briefly, after oral administration of LBF, the exogenous formulation lipids lead to the change in the nature of the GI fluid and simulate the physiological process, resulting in the secretion of endogenous amphiphilic components. These endogenous amphiphilic components enhance the solubilisation capacity of the GI fluid for lipophilic drugs where the solubilisation capacity is generally higher at post-prandial state (fed state) [178]. Furthermore, LBF enhances the drug solubilisation by utilising the body's natural digestion process where the presence of lipid digestion products further induces the secretion of endogenous components [137, 179, 180]. The intercalation of digestion products with endogenous components results in the formation of an aforementioned variety of colloidal phases including micelles, vesicles and mixed micelles [36, 137]. These colloidal phases create a solubilising environment for lipophilic drugs where the colloidal phases act as a reservoir for lipophilic drugs by maintaining the drug into the aqueous phase of the solution [13, 91, 95]. It has been well-reported in the literature that micellar solubilisation can potentially enhance the luminal solubility by up to 1000-fold [180]. Furthermore, the swelling of the liquid crystalline phases due to the intercalation of digestion products into endogenous components may further boost the solubilisation capacity of the GI

fluid [95]. Hence, the nature of the formed colloidal phases by intercalation of formulation components and digestion products with endogenous components are considered as crucial factors affecting the fate of drug upon oral administration.

The use of lipids and surfactants generally leads to improved oral absorption and thereby bioavailability of poorly water-soluble lipophilic drugs. Lipids can enhance drug absorption by a number of mechanisms including accelerating the dissolution process, facilitating the formation of solubilised phases as a result of reduced particle size to the molecular level [32, 95], enhancing drug solubilisation in intestinal milieu, yielding a solid state solution with the carrier [181, 182], changing the drug uptake, efflux and disposition by altering enterocyte based transport and enhancing the drug transport to the systemic circulation via intestinal lymphatic system by avoiding first-pass metabolism [31, 183].

Several drug absorption models have been developed in order to understand the impact of lipid-based excipients on drug absorption and it has been reported that the digestion of LBF is prerequisite to promote the drug absorption [184]. The formed colloidal phases act as an effective tool to transfer the hydrophobic species (lipid and lipophilic drugs) across the viscous unstirred water layer to the absorptive site of the intestine [13]. However, the absorption mechanism is still unclear and it is believed that the drugs are absorbed from the free concentration that is in rapid equilibrium with the solubilised species into colloidal phases via the partitioning into the membrane of epithelial cells of the intestine [38, 185]. It is pivotal for the effective drug absorption that hydrophobic drug diffuses out of the colloidal phases into aqueous intestinal fluid either by diffusion from the droplets or by degradation of the vehicle into the media [7, 32]. More recently, Yeap *et al*. highlighted the role of lipid metabolite components on drug absorption and reported the improved drug absorption for weak base cinnarizine due to the local supersaturation near the intestinal epithelium and enhanced thermodynamic activity [186]. Therefore, it was concluded that LBF can enhance the bioavailability of poorly water-soluble drugs by two mechanisms (i) by maintaining the solubility of drugs in the formed colloidal phase upon digestion and (ii) by promoting the drug transport across the intestinal epithelium by creating local supersaturation due to the absorption of lipid metabolites from the formed colloidal phases [184].

In some cases, the absorption of lipid is rapid in comparison to lipophilic drugs due to a lower affinity for drug molecules to triglycerides [187]. This can potentially lead to reduced solvation capacity of digestion products resulting into drug saturation in the formulation that can lead to

either supersaturation (may lead to precipitation and potentially reduced bioavailability) or increased thermodynamic activity (may lead to enhanced drug absorption). Several efforts such as the addition of solid substrates to manipulate digestion kinetics and the addition of precipitation inhibitors have been reported in the literature [177]. The optimal balance between the drug solubilisation, supersaturation and precipitation is a prerequisite in order to achieve improved drug absorption. Thus, this interplay has been a hot topic of research in the field over the decade.

7. Formed colloidal phases during dispersion of SMEDDS

Upon dilution, self-dispersing formulations can potentially form a series of ordered liquid crystalline structures in presence of water prior to the conversion into spherical oil droplets. Despite the recognition of the formation of the liquid crystalline phases in excess water for some lipids, the formation of liquid crystalline phases upon dispersion of complex systems such as SMEDDS has not been fully characterised. The current reported literature studies are summarised in Table 2.

The morphology and structures of self-emulsifying LBFs after dispersion were visualised using transmission electron microscopy (TEM). The diluted formulations were placed on the film grid and the samples were visualised after drying. As shown in Fig. 8A, the TEM images displayed the oil droplets in spherical shapes [153, 154, 188-191]. Furthermore, the advanced methods such as cryo-SEM and cryo-TEM have been employed and the studies reported the presence of the spherical oil droplets with smooth surface (Fig. 8B).

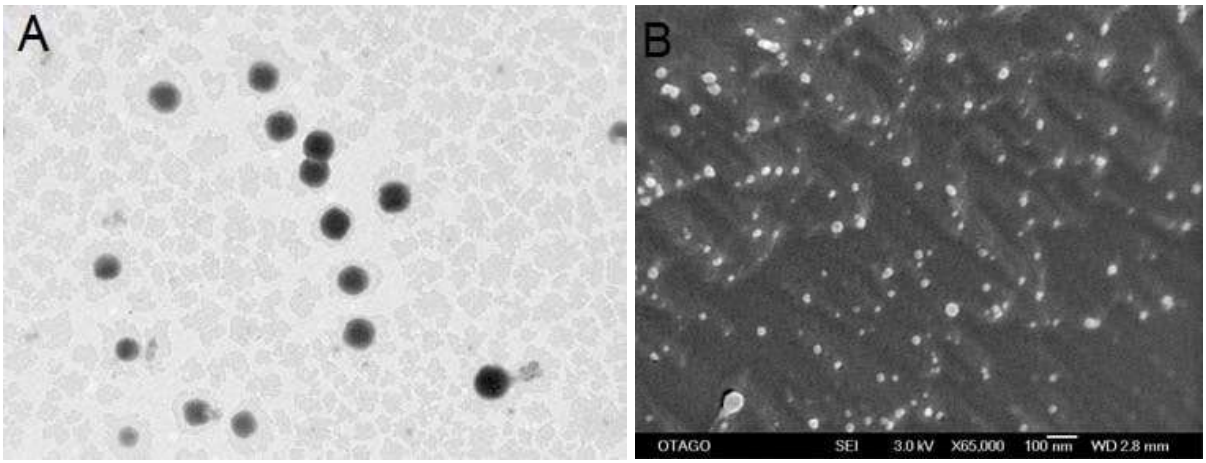
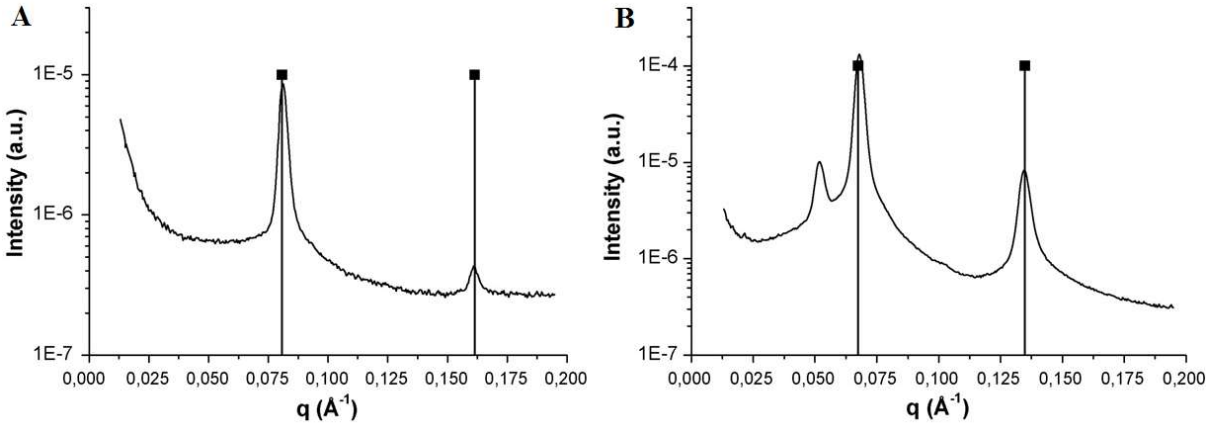


Fig. 8. (A) TEM image of SMEDDS containing Cremophor® ELP, Transcutol® HP and Capryol® 90 (43:7:50% w/w) with 25 mg exemestane and (B) Cryo-SEM image of SNEDDS containing a mixture of Captex® 300 and Campul® MCM (1:2 w/w), Cremophor® RH40 and 10% ethanol (55:35:10% w/w). Both images indicated the presence of spherical shape oil droplets. Figure adapted with permission from references [189, 192].

Although, these microscopic techniques are useful in detecting the presence of the oil droplets but they are not able to identify the type of liquid crystalline phases with confidence. Therefore, more recently, Goddeeris *et al*. implemented SAXS in order to characterise the formed liquid crystalline phases upon aqueous dilution of three SMEDDS containing propylene glycol mono- and dicaprylate and mono- and dicaprate (PGDCDC) and Vitamin E TPGS (22.7:77.3% w/w) or polysorbate 80 (25.3:74.7% w/w) or polyoxyl 40 hydrogenated castor oil (19.8:80.2% w/w) at different proportions of water. The samples were diluted with various proportions of water and the scattering patterns were recorded at 25 and 37°C [193].

The SAXS scattering patterns displayed the Bragg peaks indicating the presence of periodic or lamellar structures at 10% w/w water (Fig. 9A) that transformed into lamellar structures with increased layer-to-layer spacing distance upon adding more water (20% w/w water content) (Fig. 9B). Depending on the geometry of surfactants and critical packing parameters (CPP) values, further addition of water led to transformation of lamellar structures to hexagonal or lamellar structures and the structures were not affected by a temperature increment from 25 to 37°C [193]. The results from this study showed the pronounced effect of surfactant geometry and CPP on the self-assembly behaviour of lipid. The cone-shaped amphiphilic molecules (Vitamin E TPGS and polysorbate 80) influenced the formation of hexagonal structures in addition to lamellar structures as a result of low CPP values due to reduced volume of hydrophobic chains. In contrast, the rectangle shape of polyoxyl 40 hydrogenated castor oil led to the generation of lamellar structures as a result of high CPP value due to increased volume of hydrophobic chains. This results were in good correlation with literature reports of other lipid-based system where the structures were influenced by the surfactant geometries [194].



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878 Fig. 9. The SAXS scattering patterns of SMEDDS containing propylene glycol mono- and
879 dicaprylate and mono- and dicaprate (PGDCDC) and Vitamin E TPGS (22.7:77.3% w/w) at
880 (A) 10% w/w and (B) 20% w/w water content, recorded at 25°C. The solid line denotes the
881 positions of the observed Bragg peaks. (A) The formulation exhibited two characteristic peaks
882 at *q* of 0.08 and 0.16 $Å^{-1}$, suggesting the formation of liquid crystalline lamellar structures with
883 lattice distance of 78 Å. (B) At higher proportion of water, the produced lamellar peaks shifted
884 to lower *q*-range with increased lattice distance of 93 Å, indicating the incorporation of water
885 into lipid-rich layers. In addition, the second lamellar structures was observed at *q* of 0.05 $Å^{-1}$
886 and small peak at 0.10 $Å^{-1}$ which disappeared at 37°C. Figure adapted with permission from
887 reference [193].

888

889 Table 2. Summary of literature studies of the formed colloidal phases during dispersion of self-
890 dispersing LBFs.

Formulations (% w/w)	Characterisation technique	Observations	References
SNEDDS – 20% Sefsol 218, 18% T80, 18% Carbitol® and 44% standard pH 5 buffer solution with 5 mg ramipril (deionised water)	TEM	Dark spherical oil globules with bright surroundings	[153]
SNEDDS – 20% Sefsol 218, 18% CrEL, 18% Carbitol® and 44% standard pH 5 buffer solution with 5 mg ramipril (deionised water)	TEM	Dark spherical oil globules with bright surroundings	[154]
SMEDDS – 43-75.50% CrELP, 7-12.50% TrP and 12-50% Ca90 with 25 mg exemestane (deionised water)	TEM	Dark spherical oil droplets with bright surroundings	[189]

SMEDDS – 50% Ca90, 7% CrELP and 43% TrP with 25 mg exemestane (deionised water)	TEM	Dark spherical oil droplets with bright surroundings	[188]
SNEDDS – 34.20% a mixture of Labrafil®/CamMCM (2:1 w/w), 40.41% a mixture of CrRH40/T80 and (1:1 w/w) 25.39% TrP (deionised water)	TEM	Similar size spherical droplets for fresh and stored formulations	[190]
SNEDDS – 16.40% Ma35-1, 32.80% Ca90, 32.80% CrRH40 and 16.4% PG (deionised water)	TEM	Spherical shape oil droplets	[191]
SNEDDS – 55% a mixture of Cap300/CamMCM (1:2 w/w), 35% CrRH40 and 10% ethanol (Milli-Q water)	Cryo-SEM	Smooth surface spherical shape oil droplets	[192]
SNEDDS – 30% sesame oil, 30% Ma35-1, 30% CrRH40 and 10% ethanol (fasted state)	Cryo-TEM	Oil droplets and micelles	[124]
SMEDDS – (i) 22.7% PGDCDC with 77.3% TPGS (ii) 25.3% PGDCDC with 74.7% T80 (iii) 19.8% PGDCDC with 80.2% P40HC (at different proportions of water)	Synchrotron SAXS	At 10% water- Formulations (i) L_α phases (ii) & (iii) random periodic phases, At 20% water - L_α phases for all formulations with increased lattice distance, At 40% water – Formulations (i) and (ii) H_1 structures and (iii) L_α phases	[193]
SEDDS – 20 or 28.5% Cap300, 20 or 28.5% CamMCM, 60 or 43% Labrasol® and with or without MAPC (fasted state)	In situ SAXS and Cryo-TEM	Without MAPC - coarse emulsion droplets. With MAPC – nanoemulsion droplets	[127]

SNEDDS self-nanoemulsifying drug delivery system, *SMEDDS* self-microemulsifying drug delivery system, *SEDDS* self-emulsifying drug delivery system, *T80* Tween® 80, *CrEL* Cremophor® EL, *CrELP* Cremophor® ELP, *TrP* Transcutol® P, *Ca90* Capryol® 90, *CamMCM* Campul® MCM, *CrRH40* Cremophor® RH40, *Ma35-1* Maisine® 35-1, *PG* propylene glycol, *PGDCDC* propylene mono- and dicaprylate and mono- and dicaprate, *TPGS* Vitamin E TPGS, *P40HC* polyoxyl 40 hydrogenated castor oil, *Cap300* Captex® 300, *MAPC* monoacyl phosphatidylcholine, *TEM* transmission electron microscopy, *SAXS* small angle X-ray scattering, *Cryo-SEM/TEM* cryogenic scanning/transmission electron microscopy, L_α lamellar phase, H_1 hexagonal phase

The reported studies clearly indicate the phase changes of self-dispersing complex formulations from liquid to spherical oil droplets or liquid crystalline phases upon immersion in aqueous

fluids prior to digestion. This can potentially have a pronounced effect on overall stability and performance of the formulation. The current literature studies are limited to the visualisation of oil droplets at static conditions after complete dispersion of self-dispersing complex formulations. However, the phase behaviour of the formulations upon immersion into an aqueous fluids and prior to conversion into stable oil droplets has not been explored. Thus, it would be interesting to study the phase behaviour of self-dispersing complex formulations in real-time using in situ flow-through approaches to understand the fate of formulations prior to digestion.

8. Formed colloidal phases during digestion of SMEDDS

8.1 Liquid SMEDDS

Until recently, *in vitro* digestion models were utilised only to obtain the information of the changes in the chemical composition and distribution of compounds between separated phases during digestion. In recent times, the advancement of the methods to study the self-assembly behaviour of the lipid-based systems during digestion in real-time utilising more advanced techniques such as SAXS have evolved. The formation of self-assembled intermediate colloidal phases has been widely studied for simple lipid-based systems, however, much less study has been reported for self-dispersing complex lipid-based systems. Herein, the reported literature studies of structures for self-emulsifying formulations during digestion are summarised in Table 3.

922 Table 3 Summary of literature studies of the formed colloidal phases during digestion of self-dispersing LBFs.

Formulations (% w/w)	Characterisation technique	Observations	References
SNEDDS – 30% sesame oil, 30% Ma35-1, 30% CrRH40 and 10% ethanol (fasted and fed state)	Cryo-TEM	At fasted state – Presence of micelles throughout the digestion process. Oil droplets transitioned into spherical or elongated unilamellar vesicles in co-existence with the low number of bilamellar vesicles and open vesicles that further transitioned into micelles. At fed state – Higher fraction of bilayer fragments with multilamellar vesicles that transitioned into micelles	[124, 156]
SNEDDS – 30% sesame oil, 30% Ma35-1, 30% CrRH40 and 10% ethanol (fasted state)	Ex situ bench-top SAXS and cryo-TEM	L_α phases transitioned into H_2 phases	[126]
SNEDDS – 30% sesame oil, 30% Ma35-1, 30% CrRH 40 and 10% ethanol (at fasted state)	In situ synchrotron SAXS	L_α phases transitioned into H_2 phases at faster rate compared to ex situ study	[137]
SEDDS – (i) 20% Cap300, 20% CamMCM and 60% LB (ii) 20% Cap300, 20% CamMCM and 40% LB with 20% MAPC (iii) 20% Cap300, 20% CamMCM EP and 30% LB with 30% MAPC (iv) 28.5% Cap300, 28.5% CamMCM EP and 43% LB (fasted state)	In situ synchrotron SAXS, ex situ cryo-TEM and ex situ DLS	*SAXS:* Without MAPC – Oil droplets transitioned into vesicles that further transitioned into MLS structures where the transition rate was correlated with LB concentration With MAPC – formed vesicles that did not transitioned into MLS. *Cryo-TEM:* Presence of uni-, bi- and oligo-lamellar vesicles, opened or deformed vesicles for MAPC-free and MAPC-contained formulations after 60 min of lipolysis	[127]

SMEDDS – (i) 60% MCT, 27% CrRH40 and 13% 1,2-propanediol (ii) 40% MCT, 40% CrRH40 and 20% 1,2-propanediol (iii) 40% EO, 40% CrRH40 and 20% 1,2-propanediol (iv) 40% Castor oil, 40% CrRH40 and 20% 1,2-propanediol (fasted state)	Synchrotron SAXS and FRET	*SAXS:* Liquid crystalline phases for formulations (i), (ii) & (iii). *FRET*: Presence of micelles for formulation (iii) where the formation of micelles was influenced by the concentration of $CaCl_2$, bile salts and lecithin	[172]
LB and GEL (fasted state)	TDA	*LB:* Decrease in droplet size *GEL:* Increase in droplet size with sigmoidal shape.	[173]

SNEDDS self-nanoemulsifying drug delivery system, *Ma35-1* Maisine® 35-1, *CrRH40* Cremophor® RH40, *Cap300* Captex® 300, *LB* Labrasol®, *CamMCM* Campul® MCM, *MAPC* monoacyl phosphatidylcholine, *MCT* medium chain triglycerides, *EO* ethyl oleate, *GEL* Gelucire® 44/14, Cryo-TEM cryogenic transmission electron microscopy, SAXS small angle X-ray scattering, *FRET* fluorescence resonance energy transfer, DLS dynamic light scattering, *TDA* Taylor dispersion analysis, L_α lamellar phase, H_2 inverse hexagonal phase, *MLS* multilamellar structures

928 The formation of the intermediate colloidal phases during digestion of SNEDDS containing
929 sesame oil, Maisine® 35-1, Cremophor® RH 40 and ethanol (30:30:30:10% w/w) were studied
930 at simulated fasted and fed state using cryo-TEM [124, 156]. For these studies, the samples
931 were withdrawn at specific time points during *in vitro* lipolysis process and treated with lipase
932 inhibitor to halt the digestion process. The formulation exhibited the presence of oil droplets
933 and micelles prior to lipolysis process at both simulated intestinal conditions. The results of
934 fasted state showed the presence of micelles throughout digestion process, the transformation
935 of oil droplets to spherical or elongated unilamellar vesicles in co-existence with very low
936 number of bilamellar vesicles during early stage of lipolysis process and finally, high number
937 of micelles in combination with unilamellar vesicles and reduced number of oil droplets at
938 approximately 50% digestion (Fig. 4). In contrast to fasted state, the formulation exhibited the
939 transformation of oil droplets directly to multilamellar vesicles with bilayer fragments and
940 finally into mixed micelles (at approximately 42% digestion) at high level of bile salts and
941 phospholipids. However, it is challenging to determine the size and structural changes
942 occurring during digestion using cryo-TEM. Therefore, the same group employed bench-top
943 SAXS in combination with the *in vitro* lipolysis model as a complementary technique in order
944 to investigate the structural aspects of same composition SNEDDS formulation during
945 digestion at the simulated fasted state. Similarly, the sample time points were withdrawn at the
946 desired time point and treated with the lipase inhibitor. SAXS studies revealed the formation
947 of lamellar phases during the early stage of lipolysis process, the presence of hexagonal phases
948 in addition to lamellar phases and transformation of lamellar phases into inverse hexagonal
949 phases upon complete digestion due to the compositional changes induced by the increased
950 fraction of the lipolysis products [126].

951 The presence of these phases for the same composition SNEDDS was further confirmed by
952 Warren *et al*. using the advanced in situ flow-through approach where the *in vitro* lipolysis
953 model was attached to synchrotron SAXS and the evolution of structures during digestion was
954 monitored in real-time. As shown in Fig. 2, a continuous flow of the digestion medium through
955 a quartz capillary was achieved by using peristaltic pump and the time-resolved scattering
956 patterns were acquired in real-time during digestion at fasted state. SAXS measurements
957 complimented the previous observation and exhibited the evolution of lamellar phase and faster
958 evolution of inverse hexagonal phase [126, 137].

959 More recently, the structure formation of SEDDS containing Captex® 300, Campul® MCM EP,
960 Labrasol® (at varying concentrations) with or without a natural surfactant, monoacyl

phosphatidylcholine (MAPC), have been studied under simulated fasted state using in situ synchrotron SAXS in combination with ex situ cryo-TEM [127]. SAXS results revealed the presence of vesicles at initial stage of digestion that transformed into multilamellar structures after 10 min of lipolysis process in absence of MAPC (Fig. 10A) where the evolution and peak intensity of lamellar structures was rapid and increased at higher concentration of Labrasol®. Contrary to this observation, the poor digestion of MAPC by pancreatic extract prohibited the formation of multilamellar structures during digestion of MAPC-loaded formulations and exhibited the vesicles throughout the lipolysis process (Fig. 10B). The unilamellar, bilamellar and oligo-lamellar structures with deformed or opened vesicles were observed by cryo-TEM for the MAPC-free and MAPC-loaded formulations. Interestingly, the multilamellar structures of in situ SAXS were not evident most probably due to the artefacts induced by sample treatment such as the addition of lipase inhibitor, temperature variation experienced by sample before and during sample preparation. This indicates that in situ SAXS is more reliable technique for studying the evolution of structures during dynamic digestion process.

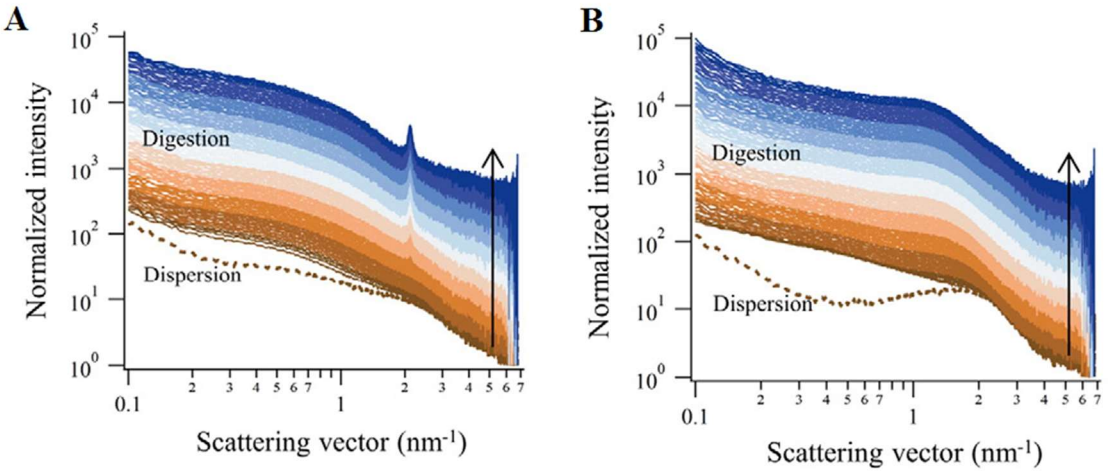

Fig. 10 The time-resolved SAXS scattering patterns of (A) MAPC-free SEDDS formulation containing Captex® 300, Campul® MCM and Labrasol® (20:20:60% w/w) and (B) MAPC-loaded SEDDS formulation containing Captex® 300, Campul® MCM, Labrasol® and MAPC (20:20:40:20% w/w), during digestion at simulated fasted state over 60 min. (A) The system formed vesicles after 1 min of digestion and a single peak evolved after 10 min of digestion at the scattering vector of 2.13 nm^{-1}, indicating the formation of multilamellar structures. (B) After 1 min of the lipolysis process, the system formed vesicles and the scattering vector value for the hump corresponding to the vesicle scattering patterns decreased over time, suggesting

985 the increment in the size of vesicles. SAXS scattering patterns are shifted vertically as a
986 function of time and the black arrow indicates the progression of lipolysis process. Figure
987 adapted with permission from reference [127].

988 Fluorescence resonance energy transfer (FRET) was utilised in addition to ex situ synchrotron
989 SAXS to monitor the structural changes occurring digestion at fasted state and at the mucus for
990 SMEDDS formulations containing different proportions of medium chain triglyceride, ethyl
991 oleate, Cremophor® RH 40 and 1,2-propanediol [172]. The ex situ SAXS confirmed the
992 formation of liquid crystalline phases and the ex situ FRET indicated the presence of mixed
993 micelles with small particle size during lipolysis process. Increasing the fraction of $CaCl_2$, bile
994 salts and lecithin induced the prevalence of mixed micelles. At the mucus, the liquid crystalline
995 phases interacted with mucin and transformed into other structures (i.e. smaller size micelles)
996 depending on the concentrations of the substances.

997 The Taylor dispersion analysis (TDA) in combination with fluorescence detection was
998 employed in order to evaluate the micellar size droplets of self-emulsifying excipients,
999 Labrasol® and Gelucire® 44/14, during digestion at fasted state [173]. In this study, the samples
1000 were withdrawn at the pre-defined time points and mixed with lipase inhibitor in order to halt
1001 the lipolysis process and subsequently mixed with fluorescence marker (9,10
1002 bis(phenylethynyl)anthracene) in order to detect the droplets via the fluorescence detector. The
1003 quantification of the droplets showed an exponential reduction of the micelle size during
1004 digestion of self-emulsifying Labrasol® (mainly due to the disappearance of the coacervate
1005 fraction). In contrast to Labrasol®, increment in the droplets size with sigmoidal shape after 30-
1006 40 min of lipolysis for Gelucire® 44/14 was observed. The number of droplets/micelles
1007 decreased with time as the surface area under the Taylorgrams decreased with time for both
1008 self-emulsifying components. The number of colloids available for the solubilisation of poorly
1009 water-soluble drugs is a critical performance attribute of SMEDDS that can be easily studied
1010 with TDA (Fig. 7) [195].

1011

1012 ## 8.2Solid SMEDDS

1013 In a more recent study, Vithani *et al*. utilised solid-based components and prepared solid
1014 SMEDDS containing Gelucire® 44/14, with non-digestible surfactant (Vitamin E TPGS and
1015 Lutrol® F 127) or digestible surfactants (Sucrose esters S-1670 and Span® 60) without a solid
1016 carrier using the in-house developed syringe-based melting method and studied the evolution

of the colloidal phases during digestion at fasted state in real-time by utilising the in situ synchrotron SAXS [90]. The results of this study showed the formation of lamellar phase (corresponding to Gelucire® 44/14) for non-digestible surfactants-loaded solid SMEDDS at 50:50% w/w lipid to surfactants ratio with a significant lag time (approximately 12 min) after lipase addition (Fig. 11A). However, the substitution of the non-digestible surfactants with digestible non-ionic sugar esters influenced the structure formation process and the formulation produced lamellar phase (corresponding to Gelucire® 44/14) without a lag time (Fig. 11B). The study reported the impact of surfactant digestibility on the formation of colloidal phases and demonstrated that the concentration of surfactants may have pronounced effect on the structure formation process [90]. Furthermore, the group observed the evolution of lamellar phases for fenofibrate or cinnarizine-loaded Gelucire® 44/14-based solid SMEDDS formulations at simulated fasted state, mixed micelles at the higher concentration of bile salt and phospholipids and mixed micelles for fenofibrate or cinnarizine-loaded glyceryl monooleate-based solid SMEDDS at both (fasted and fed) simulated intestinal conditions [91].

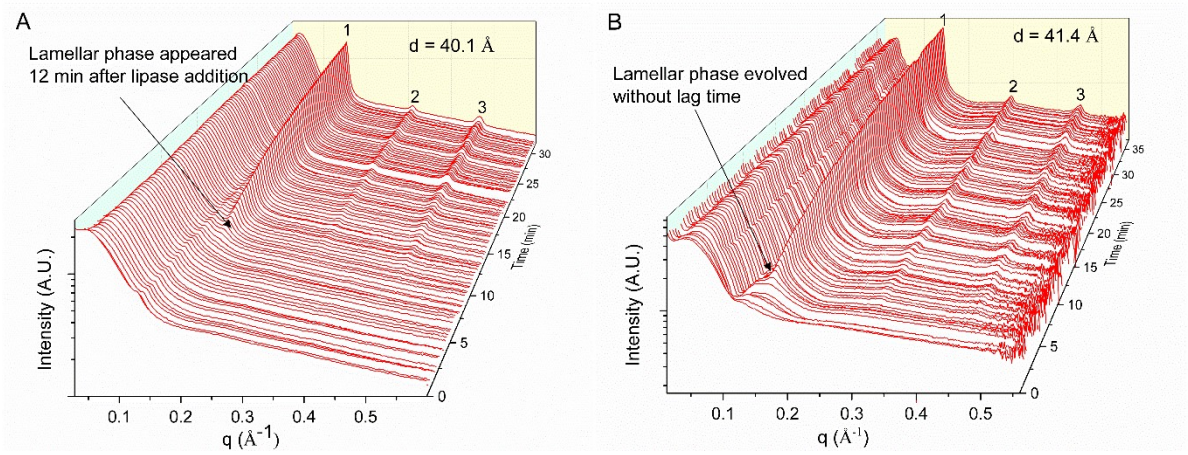

Fig. 11. The 3D waterfall SAXS scattering patterns of solid SMEDDS (A) containing non-digestible surfactants and (B) digestible surfactants, at 50:50% w/w lipid to surfactants ratio, during digestion at simulated fasted state. (A) The system formed lamellar phases (corresponding to main digestible components Gelucire® 44/14) after 12 min of lipase addition and (B) the system produced a lamellar phases (corresponding to main digestible components Gelucire® 44/14) without a lag time. Figure adapted with permission from reference [90].

Typically, solid SMEDDS are prepared by blending liquid formulations with an additional solid-phase carrier or additives and transformed into solid-based formulations via several solidification techniques. The solidification process typically involves the adsorption of liquid solution onto a solid-phase carrier via several solidification techniques such as physical adsorption, spray-drying, lyophilisation and melt-extrusion or melt-granulation [19, 27, 46]. The selection of solidification material is primarily based on the capacity of the carrier to enable high drug loading and the characteristics of the final product (i.e. good redispersibility, good flowability and tablet properties) [28]. It has been demonstrated that the solid formulations prepared by solidification can preserve the biopharmaceutical performance of the formulation and arguably provides further delivery advantages [196, 197].

Over the past few years, researchers have developed solid SMEDDS formulations using a variety of solid-phase carrier materials but very few have examined the impact of the additional component particularly on digestion and consequently structure formation. For instance, Speybroeck *et al*. prepared solid SEDDS via physical adsorption (using Neusilin® US2 as a solid carrier) and elucidated the impact of solidification on the *in vitro* and *in vivo* performance of solid SEDDS in comparison to the liquid counterpart formulation [198]. The *in vitro* dispersion and digestion studies showed reduced solubilisation (approximately 35%) of danazol for solid SEDDS compared to the liquid counterpart. Similarly, the *in vivo* results showed a reduced bioavailability of danazol (approximately 50%) from solid SEDDS compared to liquid SEDDS due to the incomplete desorption of the formulation components from solidified SEDDS [198]. More recently, Alinaghi *et al*. prepared solid SMEDDS via physical adsorption and spray-drying (Aerosil® 380 was used as a solid carrier in both cases) and studied the impact of solidification on the performance of the formulations compared to liquid SMEDDS [199]. Solid SMEDDS formulations displayed rapid and complete lipolysis under fasted and fed state attributed to the large surface area provided by the silica nanostructure network [200]. Interestingly, the results of this study showed the higher solubilisation of danazol into liquid SMEDDS compared to solid SMEDDS, indicating the inhibitory effect of solidification process on the solubilisation capacity of SMEDDS formulations [199].

These studied clearly indicated the pronounced impact of an additional solid-phase carrier or additive on the kinetics of digestion *in vitro* and *in vivo*. Hypothetically, this additional carrier may potentially affect the structure formation process during dispersion and digestion of self-dispersing formulations. However, to the best of our knowledge, the structural aspects of solid

self-dispersing formulations prepared by solidification technique has not been studied and the impact of solid phase carrier on the structure formation process during dispersion and digestion is unknown. Future work of correlating the generation of colloidal phases during dispersion and digestion of same composition SMEDDS and solid SMEDDS formulations are in great interest of the field. Overall, the literature studies of the colloidal aspects of SMEDDS and solid SMEDDS during dispersion and digestion are limited and the further work focussing on these aspects would greatly enhance the understanding of the fate of the formulation after oral administration.

9. Conclusions

SMEDDS have been a popular lipid-based formulation system for the delivery of poorly water-soluble lipophilic drugs due to their potential to improve the bioavailability of these active compounds. Liquid SMEDDS pose the problems of capsule compatibility, handling and portability thus producing solid SMEDDS could minimise those issues and further improve patient compliance. Drug may also be less chemically stable in the liquid formulation compared to a solid SMEDDS system. The characterisation of the colloidal phases formed during dispersion and digestion is an essential aspect in order to gain understanding on drug solubilisation and drug absorption during GI transit for an orally deliver poorly water-soluble drug. However, the process of structure formation can be complicated for a complex lipid-based system such as SMEDDS due to the presence of surfactant, co-surfactant, co-solvent and carrier that can significantly influence the processing. The major structure characterisation techniques for SMEDDS are DLS, cryo-TEM, cryo-SEM, SAXS, FRET and TDA. Particularly, the in situ flow-through approach using SAXS in combination with *in vitro* lipolysis model provides real-time information about the self-assembled structures formed during dispersion and digestion of complex SMEDDS system within a short time in the highly dynamic environment. The presence of a solid-phase carrier in solid SMEDDS can potentially impact on the formation of colloidal structures during dispersion and digestion and future work is required to enhance understanding on the impact of formulation excipients on the self-assembly behaviour of SMEDDS formulations. An alternative technique of producing solid SMEDDS without the need of a solid-phase carrier would be highly appreciated in order to avoid the potential impact of the additives on the overall performance of the formulation.

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Conflict of Interest

The authors report no conflict of interest.

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