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Silica-lipid hybrid microcapsules: Influence of lipid and emulsifier type on *in vitro* performance

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ABSTRACT

This study reports on the physicochemical characterisation and *in vitro* investigations of macro-porous silica-lipid hybrid (SLH) microcapsules when formulated using various lipids: long-chain triglycerides (LCT), medium-chain triglycerides (MCT), medium-chain mono-, diglycerides (MCMDG); and emulsifiers: anionic lecithin and cationic oleylamine. For the lipophilic compound coumarin 102 ($\log P = 4.09$), a complete and immediate *in vitro* release was attained for the SLH microcapsules under simulated intestinal sink conditions. The *in vitro* digestion study of various types of SLH microcapsules demonstrates: (i) reduced variability and enhanced lipid digestibility for the MCMDG-based microcapsules (*i.e.* 90–100% lipolysis) in comparison with an equivalent lipid solution and emulsion (50–90% lipolysis); and (ii) more controllable digestion kinetics for the LCT-based microcapsules which produce a lipolysis rate higher than that of a lipid solution but lower than that of a lipid emulsion. The drug phase partition results show approximately 5- to 17-fold increase in the drug solubilisation degree resulting from the digestion of MCT and MCMDG-based microcapsules (116 μg/mL), and LCT-based microcapsules (416 μg/mL) in comparison with the blank micellar medium (24 μg/mL). In conclusion, the SLH microcapsules could be tailored to manipulate the digestion patterns of both medium- and long-chain lipids in order to maximise the drug solubilisation capacity.

1. Introduction

According to recent estimates, approximately 40–70% of the newly discovered chemical entities are poorly soluble in water and the percentage is estimated to increase (Leuner and Dressman, 2000; Takagi et al., 2006; Hauss, 2007). Poorly water-soluble drugs generally have low and erratic bioavailability because of various challenges such as: (i) poor drug dissolution in the intestinal milieu despite the aid of mixed micelles as natural surfactants; (ii) precipitation when passing through the unstirred water layer; (iii) transporter-mediated efflux and oxidative metabolism in the intestinal wall which affects drug absorption; and (iv) first pass metabolism (Porter et al., 2007; Dahan and Hoffman, 2008; Trevaskis et al., 2008; Chakraborty et al., 2009).

Lipid-based delivery systems are attractive formulation strategies as they have been shown to enhance dissolution and simulate the positive food effect, *i.e.* increased drug bioavailability in the fed state (Fatouros et al., 2007; Porter et al., 2008). Lipid-based drug delivery systems potentially improve the bioavailability of poorly water-soluble drugs via several mechanisms: (i) prolongation of gastric residence time, (ii) enhanced drug solubilisation via the formation of various lipophilic colloidal systems in the gastrointestinal tract, and (iii) stimulation of the lymphatic absorption pathway in which drug molecules avoid the first pass metabolism (Humberstone and Charman, 1997; MacGregor et al., 1997; Porter et al., 2007, 2008; Chakraborty et al., 2009).

Lipid emulsions have proved to be effective in enhancing drug bioavailability mainly via an increased total surface area for enhanced drug diffusional release as well as lipid–enzymatic interactions (Humberstone and Charman, 1997). Due to the physical and microbiological instability of wet emulsions, dry emulsions are preferred to confer storage stability and dosage precision. The formation of dry emulsions can be achieved by using various drying techniques such as rotary evaporation (Myers and Shively, 1992), lyophilisation (Molina and Cadorniga, 1995) or spray drying (Takeuchi et al., 1992a,b; Pedersen et al., 1998). Dry emulsions are usually prepared in combination with a water-soluble carrier such as gelatin and sugars, or a water-insoluble carrier such as colloidal silica. Ideally, a dry emulsion formulation can be easily reconstituted in water prior to administration or readily redispersed in the gastrointestinal fluids after administration (Christensen et al., 2001). Dry emulsions are potentially useful in providing light (Takeuchi et al., 1992a,b) and oxidation protection (Heinzelmann

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and Franke, 1999), as well as improving dissolution and bioavailability of drugs (Jang et al., 2006).

Considering the various advantages associated with solid state lipid-based formulations, our previous work has led to the establishment of a dry, porous silica-lipid hybrid (SLH) microcapsule system, composed of medium-chain triglycerides (MCT) and lecithin, encapsulated by hydrophilic silica nanoparticles (Simovic et al., 2009, 2010; Tan et al., 2009, 2010). For two model BCS Class II drugs, *i.e.* celecoxib (Tan et al., 2009) and indomethacin (Simovic et al., 2009, 2010), the SLH formulations have been shown to offer several physicochemical and biopharmaceutical advantages in comparison with unmodified drug as well as conventional lipid-based solutions and emulsions. Orally dosed absorption studies in fasted rats demonstrated significantly improved bioavailability of celecoxib and indomethaicn resulting from the SLH microcapsules as compared to the aqueous drug suspension (Simovic et al., 2009; Tan et al., 2009). The major advantages of the SLH formulations are (i) physical stability of the oil-based formulation, (ii) preservation of drug molecules in the amorphous state in the microcapsule matrix, (iii) high porosity of the microcapsule structure resulting in an enhanced lipid digestibility and drug solubilisation by the lipolysis products, (iv) potential drug delivery into the lymphatic system by varying lipid composition and drug type, and (v) the absence of synthetic surfactants which eliminates the safety issues associated with chronic dosage form administration.

Recent mechanistic studies have demonstrated the role of hydrophilic fumed silica in controlling the digestion kinetics of lipid-based formulations (Mohanraj et al., 2010; Tan et al., 2010). Silica nanoparticles in the dispersed state produced an inhibitory effect on the digestion of submicron lipid emulsions and liposomes due to the formation of a protection layer at the oil–water interface. In contrast, the porous SLH microcapsules enhanced lipolysis; this emphasizes the significance of the internal porous silica-lipid matrix structure in enhancing lipid digestibility. In another study, indomethacin-loaded SLH microcapsules were engineered using a cationic emulsifier, oleylamine (Simovic et al., 2010). This resulted in the formation of (anionic) drug-(cationic) lipid electrostatic complexes that leads to an enhanced bioavailability of indomethacin, possibly via controlled drug release and hence minimisation of drug precipitation in the intestinal lumen.

In this study, various types of lipid and emulsifier were incorporated into the SLH formulation to investigate the resultant physicochemical as well as *in vitro* drug release and lipid digestion properties. Investigations were conducted for soybean oil, a long chain triglyceride and Capmul MCM, a mixed medium chain mono-/di-/triglycerides as the lipid phase; in combination with either cationic oleylamine or anionic lecithin as the emulsifier. The fluorescent compound coumarin 102 ($\log P = 4.09$) was used in this study to represent a highly lipophilic drug. Significantly, the current investigation provides a sound platform on which the SLH microcapsules can be tailored to optimise the release and solubilisation of poorly water-soluble drugs through a versatile selection of lipids and emulsifiers.

2. Materials and methods

2.1. Materials

Coumarin 102 (dye content 99%) and soybean oil (C_{18} triglyceride) were purchased from Sigma Aldrich (Australia). Miglyol® 812 (C_8/C_{10} triglyceride) was obtained from Hamilton Laborotaries (Australia). Capmul MCM (58% monoglyceride, 36% diglyceride, and 5% triglyceride) was a gift from Abitec Corporation. Soybean lecithin (containing 94% phosphatidylcholine and <2% triglycerides) and oleylamine (primary amine purity >98%) were obtained from BDH Merck (Australia) and Sigma Aldrich (Australia), respectively. Fumed hydrophilic silica nanoparticles (average primary particle diameter 7 nm) (Aerosil® 380) were supplied by Degussa (Germany). Phosphate buffered saline (PBS) tablets and sodium dodecyl sulphate (SDS) (for molecular biology, ≈99%) were obtained from Sigma Aldrich (Australia). Sodium taurodeoxycholate (NaTDC), trizma maleate, type X-E L-α-lecithin (approximately 60% pure phosphatidylcholine, from dried egg yolk), porcine pancreatin extract (activity equivalent to 8× USP specification), calcium chloride dehydrate, sodium hydroxide pellets and 4-bromophenylboronic acid (4-BPB) were purchased from Sigma Aldrich (Australia). Polyallomer centrifuge tubes (13.5 mL, 16 mm × 76 mm) were purchased from Beckman Instrument Inc. (USA). All chemicals were of analytical grade and used as received. High purity (Milli-Q) water was used throughout the study.

2.2. Preparation of SLH microcapsules

SLH microcapsules were produced in a two-step process: homogenisation followed by spray-drying. Oil-in-water (o/w) emulsions were prepared by dissolving 0.6% (w/w) emulsifier in 10% (w/w) oil, followed by the addition of 1% (w/w) coumarin 102 (weight relative to oil). Milli-Q water was then added into the mixture as the continuous phase. A range of precursor emulsions were produced using three types of oil: Miglyol 812 (MCT), soybean oil (LCT), and Capmul MCM (MCMDG); in combination with two types of emulsifier: anionic soybean lecithin and cationic oleylamine. The coarse o/w emulsions were homogenised (Avestin® EmulsiFlex-C5 Homogeniser) under a pressure of 1000 bar for 5 cycles. An aqueous dispersion of silica nanoparticles was added into the homogenised emulsions to produce a final silica concentration of 50% weight relative to the oil content and the mixture was tumbled for 12 h. The silica-stabilised emulsions were then spray-dried (Mini Spray Dryer B-290, BÜCHI Labortechnik AG) to produce powdery SLH microcapsules.

2.3. Physicochemical characterisation of SLH microcapsules

2.3.1. Lipid content analysis

The lipid content of SLH microcapsules was determined using thermogravimetric analysis (Hi-Res Modulated TGA 2950, TA Instruments) (Simovic et al., 2009; Tan et al., 2009). Samples of 15 mg were heated at a rate of 10 °C/min from 20 to 600 °C under nitrogen purging. The lipid content was evaporated in the range of 240–350 °C and the silica component remained thermally stable. The sample weight change was computed by using the associated TA Universal Analysis software and after correction for the water content of spray-dried silica (previously determined to be 1.5 ± 0.0%), the subtracted weight loss corresponds to the lipid content of the microcapsules.

2.3.2. Particle sizing

Particle sizing of blank SLH microcapsule powder was conducted using two techniques, *i.e.* laser diffraction using a Malvern MasterSizer instrument, and dynamic light scattering using a Malvern Zetasizer Nano instrument. For laser diffraction analysis, a small amount of powder was loaded into a sample dispersion unit containing water as the dispersant. The refractive index (RI) of 1.46 was used in the analysis for lipids. Dynamic light scattering analysis was performed to examine the redispersibility properties of SLH microcapsules in a simulated intestinal medium. Each formulation (5 mg/mL powder) was redispersed in phosphate buffer (0.01 M, pH 7.4) and tumbled for 2 h. Each sample was then diluted 100-fold with Milli-Q water prior to measurement at 25 °C.

2.3.3. Scanning electron microscopy (SEM)

The surface morphology of the SLH microcapsules was examined by high resolution analytical scanning electron microscopy, SEM (CamScan CS44FE). Each sample was mounted on double-faced adhesive tape and sputter-coated with gold/palladium (60%:40%) prior to imaging at an accelerating voltage of 10 kV.

2.4. Charaterisation of coumarin 102-loaded SLH microcapsules

2.4.1. Coumarin 102 assay

Two sets of calibration solutions were prepared by dissolving 10 mg coumarin 102 in 10 mL of ethanol, followed by dilution with Milli-Q water or 0.05 M PBS containing 0.5% SDS solution (pH 7.4) to produce series of calibration solutions with concentrations ranging from 15 to 500 μg/L. The calibration solutions were examined for fluorescence intensity using a Cary Eclipse Fluorescence Spectrophotometer (Varian Inc.) set at an excitation wavelength (λ_{ex}) of 395 nm and emission wavelength (λ_{em}) of 488 nm.

2.4.2. Determination of drug loading efficiency

A 10 mg sample of each drug-loaded microcapsules was dissolved in 1 mL of ethanol, vortex mixed and centrifuged at 10,000 rpm for 15 min. Each sample was then diluted 1:1000 in Milli-Q water prior to fluorescence spectroscopy analysis (described in Section 2.4.1).

2.4.3. Confocal microscopy

The drug-loaded SLH microcapsules were viewed using confocal microscopy (Leica SP5 spectral scanning microscope) at 40× magnification (HCX PL APO CS 40.0 × 1.25 OIL UV objective). The excitation wavelengths used was 405 nm for coumarin 102. The images were enhanced using the associated software (Leica Application Suite, Advanced Fluorescence, 1.5.0 build 767).

2.5. In vitro release studies

The *in vitro* release study of drug-loaded SLH microcapsules was performed in 500 mL of 0.05 M PBS (pH 7.4) containing 0.5% SDS, using U.S. Pharmacopoeia (USP) 23 type II apparatus (paddle method) operated at 50 ± 0.02 rpm. Each sample was added into the dissolution medium (37 °C) to achieve a drug concentration of 1.5 mg/L. Aliquots of 1 mL were drawn at fixed time points and replaced with an equal volume of dissolution medium maintained at 37 °C. The samples withdrawn were centrifuged at 13,000 rpm for 3 min to remove undissolved materials. The supernatant phase of the samples centrifuged was analysed by using the same fluorescence spectroscopy method (as described in Section 2.4.1). The microcapsules concentration during sampling remained constant and cumulative drug release profiles are presented.

2.6. In vitro lipolysis studies

2.6.1. Preparation of lipid digestion medium

The lipid digestion medium was prepared according to the method adapted from Sek et al. (2002). The fasted state mixed micelles, *i.e.* phospholipid/bile salt (1.25 mM PC/5 mM NaTDC), were prepared in the following sequence: egg lecithin was dissolved in chloroform (4 mL) followed by evaporation of chloroform under vacuum (Rotavapor RE, Buchi, Switzerland) to form a thin film of lecithin around the bottom of a 50 mL round-bottom flask; NaTDC and digestion buffer [50 mM Trizma maleate (pH 7.5), 150 mM NaCl, and 5 mM $CaCl_2{\cdot}2H_2O$] was added and the mixture was stirred for ~12 h to produce a transparent (light yellow) micellar solution. Pancreatin extracts (containing pancreatic lipase, colipase and other non-specific lipolytic enzymes such as phospholipase A_2) were freshly prepared each day by stirring 1 g of porcine pancreatin powder in 5 mL of digestion buffer for 15 min, followed by centrifugation (at ~5000 rpm, 5 °C) for 15 min. The supernatant phase was collected and stored on ice until use.

2.6.2. Lipid digestion kinetics studies

The progress of lipid digestion was monitored for 60 min by using a pH-stat titration unit (TIM854 Titration Manager, Radiometer, Copenhagen, Denmark) according to the lipolysis protocol as described by Sek et al. (2002). Briefly, a known quantity of sample formulation (equivalent to ~200 mg lipid) was dispersed in 18 mL of buffered micellar solution by stirring continuously for 10 min in a thermostatted glass reaction vessel (37 °C). The pH of the digestion medium was re-adjusted with 0.1 M NaOH or HCl to 7.50 ± 0.01. Lipolysis was initiated by addition of 2 mL of pancreatin extract (containing ~2000 TBU of pancreatic lipase activity) into the digestion medium. Free fatty acids (FFA) produced in the reaction vessel were immediately titrated with NaOH via an auto-burette to maintain a constant pH in the digestion medium at the pre-set value of 7.50 ± 0.01 throughout the experiment. A solution of 0.6 M NaOH was used as the titrant for medium-chain lipids, whereas 0.2 M NaOH was used for long-chain lipids as per the established experiment protocol (Sek et al., 2002).

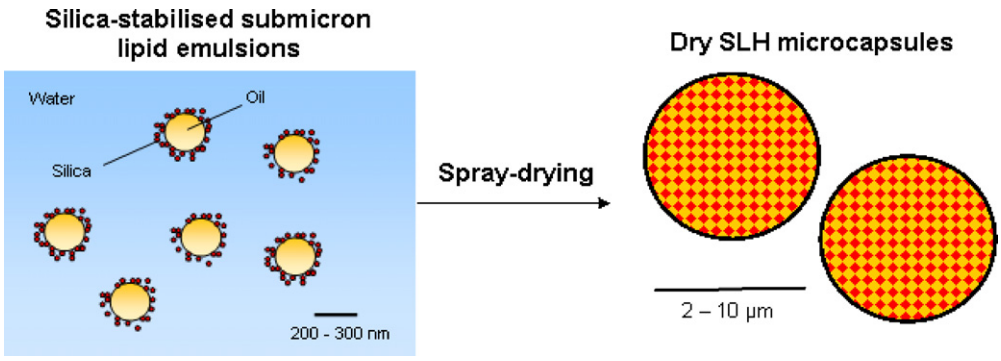
The enzyme-mediated lipolysis can be described as a pseudo first-order reaction because the reaction rate is strongly dependent on the concentration of the lipid substrates, while the concentration (*i.e.* activity) of the lipid-digesting enzymes is expected to remain constant throughout the reaction due to their presence in excess. The initial lipolysis processes were determined to be mono-, bi- or tri-phasic reactions depending on the types of lipid formulation (Tan et al., 2010). In this study, mono- or bi-exponential equations were fitted to the lipolysis data as appropriate using an iterative curve stripping technique (Mirfazaelian and Mahmoudian, 2006):

$$\%\,\text{Lipolysis} = 100 - (L_1 e^{-k_1 t} + L_2 e^{-k_2 t})$$

where L_1 and L_2 are the fractions of lipids undergoing lipolysis with a rate constant of k_1 and k_2.

2.6.3. Drug phase partition studies

The phase distribution of coumarin 102 was examined during the digestion process of the SLH microcapsules. At time 1, 5, 15, 30 and 60 min, aliquots of 1 mL of the lipolysis medium were collected into individual Beckman polyallomer tubes prefilled with 10 μL of 4-BPB (0.5 M in methanol equilibrated at 37 °C). 4-BPB was used as an enzyme inhibitor to stop the digestion process of the collected samples. The samples collected were ultracentrifuged (Beckman XL-80 Ultracentrifuge) at 40,000 rpm for 40 min at 37 °C to separate the different digestion phases. During ultracentrifugation, the lipid digests separated into an upper oily layer, an aqueous phase and a precipitated pellet. The aqueous phase was aspirated into a 1-mL syringe by penetrating the side of the tube with a 23-guage needle (Terumo 0.65 mm × 25 mm) and transferred into 1.5-mL Eppendorf tube. The aqueous phase was then analysed by fluorescence spectroscopy for drug molecules. The pellet fraction was dissolved in ethanol and acidified with 50 μL of 1 N HCl before being transferred into a 10 mL volumetric flask. The volume was made up with ethanol followed by vortex-mixing and sonication (Bransonic® Model 2510, Branson USA) for 15 min. The sonicated samples were centrifuged at 10,000 rpm for 10 min. The supernatant phase was taken and diluted with Milli-Q water to meet the calibration concentration range prior to analysis for the drug content using fluorescence spectroscopy.



Scheme 1. Formation of an internal matrix structure of SLH microcapsules.

Table 1
Average hydrodynamic diameters and zeta potentials of various submicron emulsions used to prepare SLH microcapsules (mean ± S.D., $n = 3$).

Emulsions	*z*-average (nm)	PDI	Zeta potential (mV)
MCT-lecithin	186.8 ± 4.0	0.081 ± 0.018	−55.6 ± 5.5
LCT-lecithin	360.3 ± 2.9	0.406 ± 0.014	−43.7 ± 0.3
MCMDG-lecithin	217.5 ± 3.0	0.047 ± 0.013	−59.5 ± 1.2
MCMDG-oleylamine	277.3 ± 0.9	0.318 ± 0.029	+76.8 ± 2.0

3. Results and discussion

3.1. Preparation and physicochemical characterisation of SLH microcapsules

The average hydrodynamic diameters and zeta-potentials of drug-free submicron emulsions prepared using various lipids and emulsifiers are shown in Table 1. The average particle size for LCT-based emulsions (≈360 nm) is relatively larger than that of the MCT- and MCMDG-based emulsions (≈187 nm and ≈250 nm, respectively). This is ascribable to the longer acyl chain length of LCT which results in a larger molecular spatial arrangement in comparison with MCT- and MCMDG-based emulsions. Each emulsion system acquired a positive/negative zeta potential according to the cationic/anionic properties of the emulsifiers. These emulsion systems were used as precursors for the formation of SLH microcapsules and as controls in the lipid digestion studies.

Scheme 1 illustrates the formation of the SLH microcapsules in which the dispersed silica nanoparticles are processed into a porous cross-linked network via the surface silanol groups during the process of spray-drying. It is proposed that during the water removal (spray-drying) process, lipids are adsorbed by the silica agglomerates as confirmed by observations of increased contact angle for hydrophilic nanoparticles at the oil–water interface in the presence of lecithin or oleylamine (Ghouchi Eskandar et al., 2007; Simovic et al., 2009). Fig. 1 shows the surface morphology of the microcapsules visualised by SEM (left panel) as well as the distribution of coumarin 102, which was dissolved in the lipid phase (right panel). The location of coumarin 102 indicates a homogenous distribution of lipids throughout the microcapsule matrix. Previous examination (visualized in cross section SEM imaging) showed pore sizes of 100–500 nm for the microcapsules prepared using MCT-lecithin (Simovic et al., 2009; Tan et al., 2009). It is noted that the porosity/internal surface area of the microcapsules is relatively independent of the lipid type, and controlled mainly by the silica types and its porosity/surface area. Given that the same type of silica has been used in all microcapsules, a minor variation in the microcapsule porosity is expected. The SLH microcapsules generated can thus be described as macro-porous (≥50 nm in pore sizes), originating from micro- or meso-porous fumed silica (7 nm in average primary diameters) according to the International Union of Pure and Applied Chemistry (IUPAC) definition. Irrespective of the type of lipid or emulsifier used, the general spherical structures of microcapsules were maintained (Fig. 1). The solid-state particle sizes were in the range of 1–10 μm. Table 2 shows the laser diffraction particle sizing of the microcapsules. In accordance with the trend of particle sizes of the precursor emulsions, LCT-based microcapsules were the largest (≈12.5 μm) compared to MCT-based (≈8 μm) and MCMDG-based microcapsules (9.0–11.5 μm).

SLH microcapsules prepared from all types of lipid/emulsifier combinations showed reproducible reconstitution properties in the simulated intestinal medium (0.05 M, pH 7.4). The *z*-average sizes of the microcapsules prepared from various combinations of lipid/emulsifier were in the range of 1–3 μm (Table 2). All microcapsules were negatively charged in the neutral medium (−67 to −17 mV). It is worth to note that the polydispersity indices for these microcapsules were rather high (PDI values > 0.4) when analysed using dynamic light scattering (DLS). Nevertheless, the laser diffraction analysis has confirmed the absence of particles greater than 15 μm for all microcapsule systems.

As shown in Table 2, the lipid content of the spray-dried SLH microcapsules prepared from MCT and LCT were in the range of 60–70% (w/w) as determined by using thermogravimetric analysis. This corresponds to a 100% lipid entrapment efficiency or recovery in the microcapsule systems. In contrast, a slightly lower lipid recovery (80–85%) was observed for the MCMDG-based microcapsules and this corresponds to a final lipid content of 50–60% (w/w) in the resultant microcapsule powder. This observation implies that the mass ratio of oil:silica at 1:0.5 is optimum for the formation of microcapsules using MCT and LCT, whilst the slightly reduced lipid entrapment efficiencies obtained for MCMDG-based microcapsules were possibly related to the surface activity of the lipid. Taken together, the lipid recovery levels are considered reasonably high in all formulations.

3.2. In vitro drug release

In the drug release study, SDS (0.5% (w/w)) was employed as a wetting and solubilising agent in the dissolution medium to simulate the gastrointestinal sink effect. It is noted that a concentration of up to 2% SDS has been employed in some of the standard USP/FDA recommended dissolution models (Baker, 2007). Drug release profiles of various SLH microcapsule systems are illustrated in Fig. 2. Among the four types of microcapsules examined, MCMDG-lecithin microcapsules exhibited the lowest drug release kinetics before reaching a 100% release at 60 min. In contrast, MCMDG-oleylamine, LCT-lecithin and MCT-lecithin microcapsules demonstrated a fast, burst initial drug release with near to completion at approximately 5–15 min. The initial extent of drug release (at 1 min) followed the trend of MCMDG-lecithin (≈28%) < MCT-lecithin (≈53%) < LCT-lecithin (≈67%) < MCMDG-oleylamine (≈85%). Importantly, all SLH

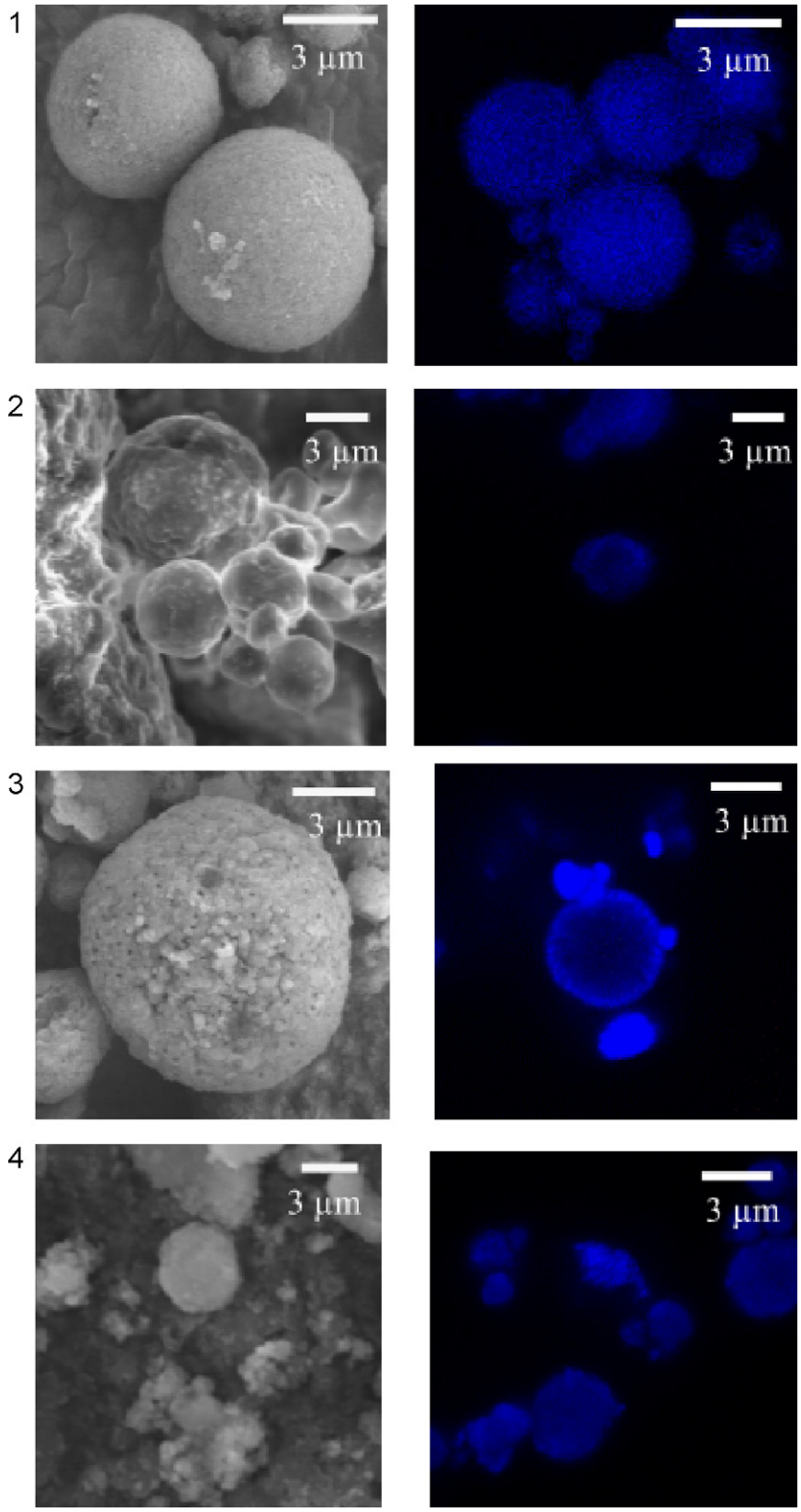


Fig. 1. Scanning electron micrographs (left panel) and confocal fluorescence micrographs (right panel) of SLH formulations: (1) MCT-lecithin, (2) LCT-lecithin, (3) MCMDG-lecithin and (4) MCMDG-oleylamine microcapsules.

microcapsules achieved more than 85% of drug release within 30 min under the simulated sink conditions; this complies with the FDA criteria for classification as an immediate-release dosage form (U.S. Food and Drug Administration, 2000).

The fast and complete release of coumarin 102 from the SLH microcapsules emphasised the significance of lipids and hydrophilic carriers (silica nanoparticles) in improving the drug release. Firstly, the presence of hydrophilic silica nanoparti-

Table 2
Physicochemical properties of SLH microcapsules: average hydrodynamic diameters and zeta potentials of SLH powder redispersed in PBS (0.01 M, pH 7.4), particle sizes determined by laser diffraction and oil content determined by thermogravimetric analysis (mean ± S.D., $n = 3$).

Microcapsules	Redispersibility (pH 7.4) – DLS			Laser diffraction, d (0.5) (μm)	Oil content (% w/w)
	z-average (μm)	PDI	Zeta potential (mV)		
MCT-lecithin	2.6 ± 0.4	0.704 ± 0.186	−66.1 ± 17.6	8.3 ± 0.2	64 ± 1
LCT-lecithin	2.5 ± 0.2	1.000 ± 0	−17.7 ± 4.3	12.5 ± 0.5	68 ± 1
MCMDG-lecithin	1.6 ± 0.4	0.939 ± 0.087	−65.9 ± 7.4	11.5 ± 0.3	58 ± 2
MCMDG-oleylamine	2.7 ± 0.6	0.782 ± 0.131	−5.21 ± 2.0	9.0 ± 0.2	52 ± 1

cles potentially enhanced dispersion of the drug-containing lipid droplets in the aqueous medium. Secondly, drug molecules are stabilised as a molecular solution in the SLH microcapsules, thus enhancing the release kinetics since the initial dissolution step of crystalline materials in aqueous medium was omitted. Previous X-ray diffraction (XRD) and differential scanning calorimetry (DSC) studies of drug-loaded SLH microcapsules have confirmed the non-crystalline form of drug in the microcapsules (Simovic et al., 2009; Tan et al., 2009). Hence, the crystallinity of the encapsulated coumarin 102 was not specifically examined in this study. Furthermore, the formation of micelles resulting from SDS also provided a lipophilic microenvironment in the aqueous medium which promoted drug diffusion from the oil cores into the surrounding medium. All these processes potentially contribute to the immediate release behaviour of the SLH microcapsule formulations.

3.3. *In vitro lipid digestion study*

3.3.1. *Digestion of medium-chain lipid formulations*

Previously, Tan et al. (2010) investigated the digestion kinetics of various MCT-based formulations. Their findings highlighted the importance of the highly porous matrix structure of the SLH microcapsules in facilitating a more predictable and enhanced lipid digestibility (*i.e.* ≈100% lipolysis with up to 20-fold higher initial digestion rate constants) as compared to equivalent lipid solutions and submicron emulsions (in the presence and absence of silica nanoparticles). The current work further evaluates a partially digested medium-chain lipid, *i.e.* MCMDG, as well as LCT to compare the lipolysis properties resulting from the microcapsule formulations.

Fig. 3 depicts the lipolysis profiles of various formulations based on MCMDG-lecithin, MCMDG-oleylamine and LCT-lecithin, along with semi-logarithmic plots showing the pseudo-first-order descriptions of the initial lipolysis process of each formulation. The lipolysis characteristics of each system are evaluated based on:

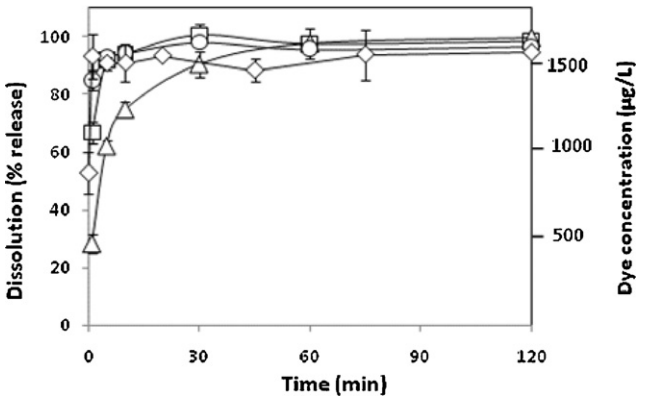


Fig. 2. Dissolution profiles for coumarin 102-loaded SLH microcapsules composed of various lipids and emulsifiers under sink conditions (0.01 M, pH 7.4 phosphate buffer, 0.5% SDS): MCT-lecithin (◊), LCT-lecithin (□), MCMDG-lecithin (△), MCMDG-oleylamine (○).

(i) the initial lipolysis rate constants (k's), which were calculated employing data points of every 5 s up to 10 min, and (ii) the percentage of lipid hydrolysis obtained at the end of each experiment, *i.e.* at 60 min (Table 3). The percentage of lipolysis obtained at 1 h for the MCMDG-lecithin systems follows the order of: MCMDG-lecithin SLH microcapsules (100%) > pure MCMDG (92%) = MCMDG-lecithin solution (92%) > MCMDG-lecithin emulsion (72%). The initial lipolysis process of all MCMDG-lecithin systems follows a biphasic first-order kinetics, in which a time-dependent reduction in the lipolysis rate was observed. Interestingly, the lipolysis rate constants (*i.e.* k_1 and k_2 values) are comparable across the investigated systems. This implies the minor role of droplet emulsification in governing the rate of lipolysis for MCMDG-based systems, presumably attributed to the self-emulsifying property of the MCMDG lipids. The lipolysis inhibitory effect of lecithin was prominent in the case of MCMDG-lecithin emulsion, but not for the MCMDG-lecithin solution. This is possibly due to the presence of lecithin at the oil–water interface which potentially formed a competitive binding site for the digesting enzymes in the case of emulsions (Pieroni and Verger, 1979). However, the competition-driven inhibition was not in effect in the case of crude oil-lecithin solution. A similar observation was reported by Nielsen et al. (2007) for a core–shell structured self nano-emulsifying drug delivery system (SNEDDS), where the shell consisting of ethylene oxide head groups of the CrRH40 surfactant encapsulates the nano-size emulsified droplets. According to their lipolysis data, both self micro-emulsifying drug delivery system (SMEDDS) (particle size, ≈5 μm) and SNEDDS (particle size, ≈45 nm) stabilised by surfactants exhibited a lower extent of lipolysis (59% and 58%, respectively) compared to a coarse oil solution (69%). This suggests that the lipolysis rate and extent are independent of the particle sizes, and highlights the inhibitory effect of specific surfactants on the digestion of emulsified lipid systems (Fatouros et al., 2008). The complete lipolysis of MCMDG-lecithin microcapsules is similar to the trend previously observed for MCT-lecithin microcapsules (Simovic et al., 2009; Tan et al., 2009). The presence of the internal porous matrix structure, which provides a larger surface area available for enhancing lipid–enzyme interactions, may explain the lipolysis enhancement of the MCMDG-lecithin microcapsules.

The digestion kinetics for MCMDG-oleylamine systems were found to resemble that of the MCMDG-lecithin systems, but at a relatively lower magnitude of percentage lipolysis: MCMDG-oleylamine SLH microcapsules (92%) = pure MCMDG (92%) > MCMDG-oleylamine solution (89%) > MCMDG-oleylamine emulsion (52%). MCMDG-oleylamine emulsions exhibited a lower extent of lipolysis as compared to MCMDG-lecithin emulsions. A possible reason for this observation is that silica nanoparticles are more closely packed at the interface of the oleylamine-based droplets and hence, producing a greater shielding effect than the lecithin-based droplets where the nanoparticles are sparsely dispersed around the droplets (Ghouchi Eskandar et al., 2007). MCMDG-oleylamine SLH microcapsules exhibited a lower extent of lipolysis than MCMDG-lecithin SLH microcapsules which is considered to be due to the electrostatic interaction between the positively charged oleylamine and the negatively charged

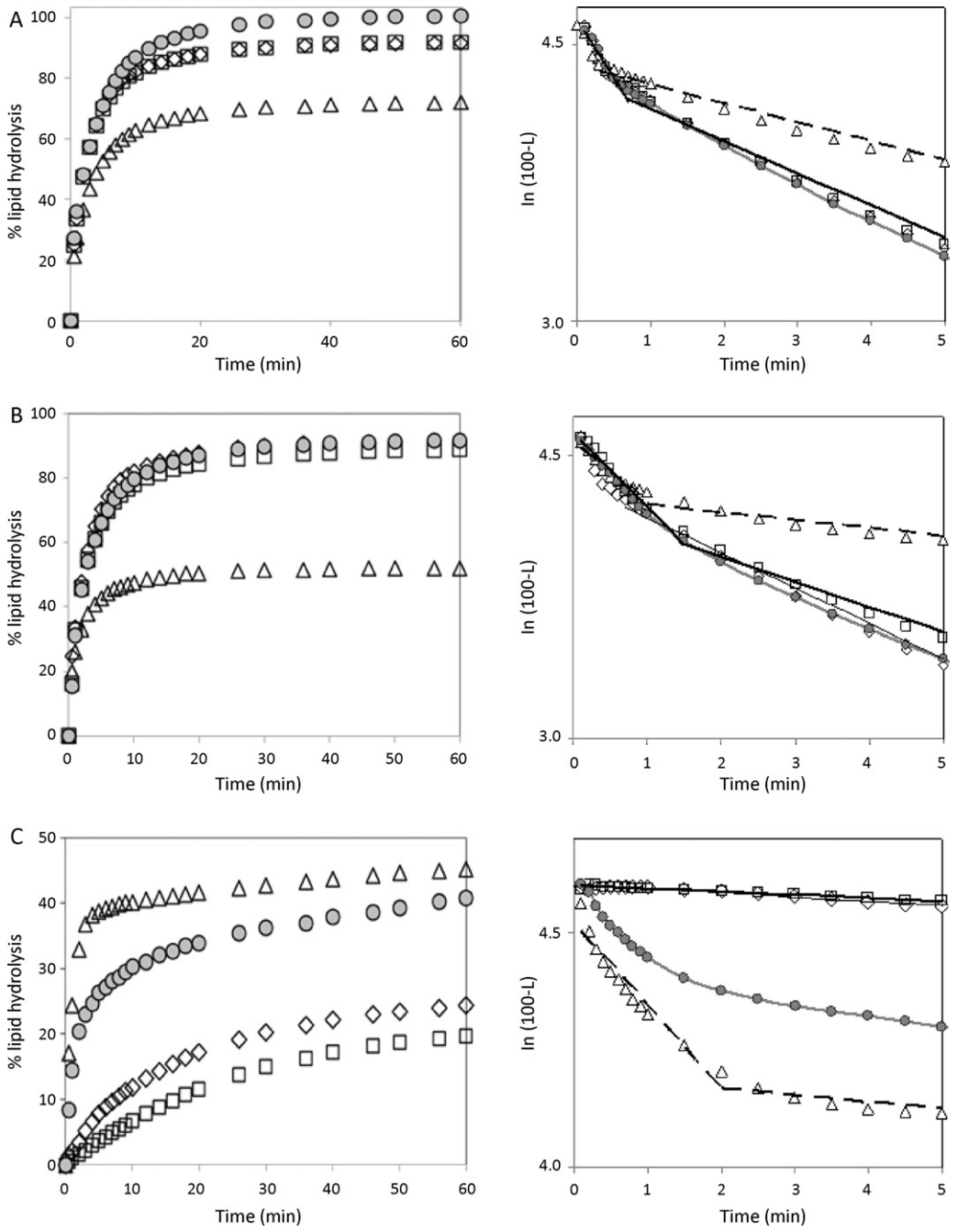


Fig. 3. Digestion profiles of (A) MCMDG-lecithin systems, (B) MCMDG-oleylamine systems and (C) LCT-lecithin systems under simulated fasted intestinal conditions: pure lipid (◊), lipid-emulsifier solution (□), lipid-emulsifier emulsion (△), SLH microcapsules (●) (Error bars with standard errors from triplicates are not shown as they are <3%).

hydrophilic silica nanoparticles; this resulted in slower release of the nano-sized droplets into the simulated intestinal medium for digestion (Simovic et al., 2010). An alternative explanation to this may be the electrostatic interactions between lipase and the lipid–water interface which retards the lipase activity (Peters et al., 2000). It is known that fatty acids generated during lipolysis are removed from the droplet interface via an electrostatic interaction with the calcium ions present in the lipolysis medium (Duinhoven et al., 1995; Zangenberg et al., 2001). This process further enhances enzyme adsorption and thereby allowing the continuation of lipolysis. However, the presence of oleylamine may decrease the efficiency of such a product removal process due to electrostatic attractions between oleylamine and fatty acid molecules. This subsequently reduces the efficiency of enzyme adsorption to the oil substrate interface and causes a reduction in lipolysis.

3.3.2. Digestion of long-chain lipid formulations

The lipolysis patterns of various LCT formulations are illustrated in Fig. 3(C). The pseudo first-order kinetics showed a monophasic lipolysis pattern for pure LCT and the LCT-lecithin solution at a rate constant of 0.01 min^{-1}, demonstrating a slow and constant rate of lipolysis throughout the process (up to 60 min). In contrast, both lipid emulsion and SLH microcapsules showed biphasic lipolysis kinetics in which the reaction was initiated at a relatively higher rate constant (k_1 = 0.46 min^{-1} and 0.12 min^{-1}, respectively) and followed by a reaction rate similar to that of a LCT solution (k_2 = 0.01 min^{-1} and 0.02 min^{-1}, respectively). The

Table 3
Lipolysis characteristics of various lipid-based formulations: initial lipolysis rate constants and the percentage of lipolysis at 60 min (mean ± S.D., $n = 3$).

Lipid formulations	Lipolysis rate constant, k ($min^{-1} \times 10^{-2}$)		Lipolysis at 60 min (%)
	k_1	k_2	
MCMDG	64 ($R^2 = 0.96$)	15 ($R^2 = 0.97$)	91.5 ± 2.3
MCMDG-lecithin solution	64 ($R^2 = 0.99$)	15 ($R^2 = 0.97$)	91.9 ± 3.0
MCMDG-lecithin emulsion	89 ($R^2 = 0.97$)	8 ($R^2 = 0.94$)	72.1 ± 1.3
MCMDG-lecithin SLH microcapsules	59 ($R^2 = 0.97$)	17 ($R^2 = 0.99$)	100.0 ± 1.2
MCMDG-oleylamine solution	22 ($R^2 = 0.93$)	11 ($R^2 = 0.97$)	88.9 ± 0.9
MCMDG-oleylamine emulsion	14 ($R^2 = 0.90$)	2 ($R^2 = 0.90$)	52.0 ± 8.3
MCMDG-oleylamine SLH microcapsules	20 ($R^2 = 0.95$)	12 ($R^2 = 0.98$)	91.6 ± 6.5
LCT	1 ($R^2 = 0.97$)	–	23.7 ± 5.1
LCT-lecithin solution	1 ($R^2 = 0.99$)	–	19.6 ± 0.9
LCT-lecithin emulsion	46 ($R^2 = 0.96$)	1 ($R^2 = 0.91$)	45.2 ± 1.6
LCT-lecithin microcapsules	12 ($R^2 = 0.95$)	2 ($R^2 = 0.95$)	40.3 ± 4.2

lipid emulsion showed the highest rate and extent of digestion (45%), this is followed by LCT microcapsules (40%), pure LCT (24%) and LCT-lecithin solution (20%) at 60 min. Emulsification markedly increased the rate and extent of the LCT hydrolysis mainly due to the increase in total surface area available for digestion. LCT-based SLH microcapsules demonstrated a lipolysis extent lower than that of the equivalent emulsion, but higher than that of the pure oil and lipid–lecithin solution. This contrasts the previous reports of enhanced lipid digestibility for the SLH microcapsule system in comparison with lipid solution and emulsion (Tan et al., 2010). This may be simply because LCT has longer acyl chain lengths and therefore, the LCT droplets are released at a lower diffusional rate from the microcapsule pores in comparison with the MCT-based microcapsules. In addition, the removal of the LCT lipolytic products from the microcapsule substrate surface may not be as efficient as in the case of bare LCT emulsions and shorter-chain MCT-based systems. This renders a slower enzymatic activity for the LCT-based microcapsule system. The more controllable rate of digestion obtained for the LCT-based microcapsules may be beneficial and lead to the avoidance of drug precipitation during digestion, hence the highly lipophilic drugs could remain associated with the undigested lipid phase and gradually transferred into the micellar species formed in the aqueous environment (Humberstone and Charman, 1997; Porter et al., 2004, 2008).

3.4. Phase partition of drug during in vitro lipolysis

The distribution behaviour of coumarin 102 between the aqueous and the pellet phases was elucidated for various SLH microcapsules during digestion. Fig. 4(A) illustrates the drug phase partition pattern resulting from the digestion of MCT-lecithin microcapsules. There is a clear trend of increasing percentage of drug solubilised in the aqueous phase (*i.e.* from ≈30 to ≈70%) with increasing percentage of lipid digestion (≈40 to ≈90%) throughout the 60-min study period. This signifies the important role of MCT digestibility on the release and solubilisation of drugs in the

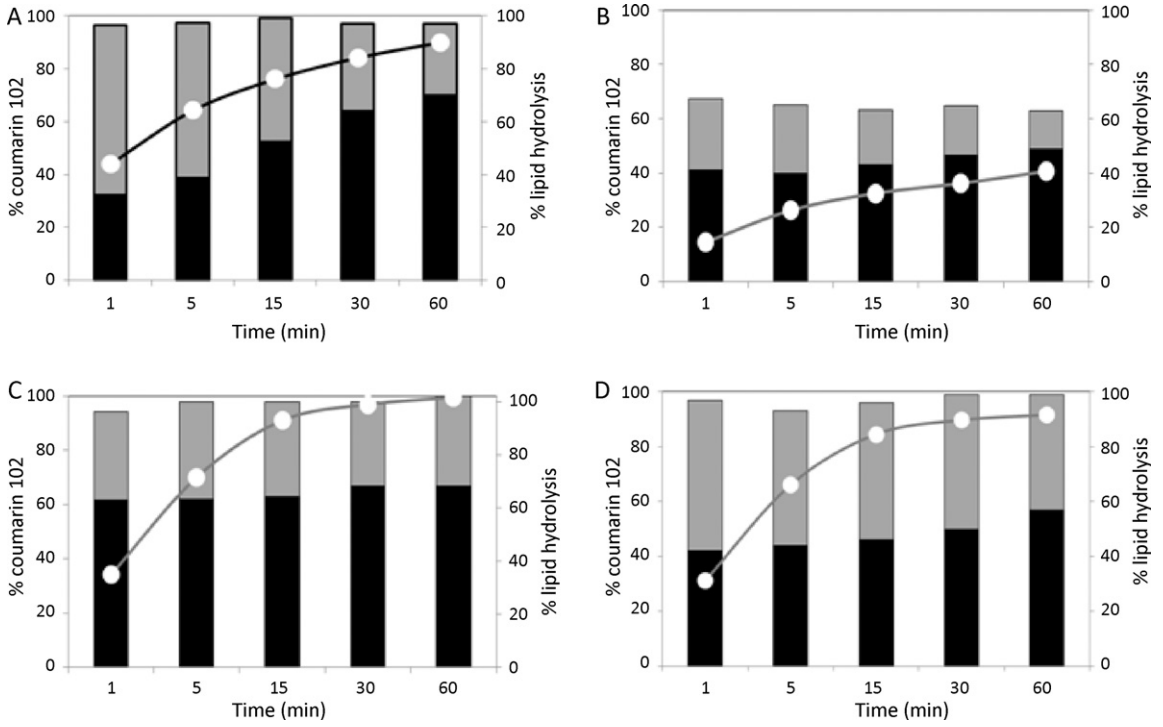


Fig. 4. Partition of coumarin 102 into the aqueous phase (black column) and pellet phase (grey column) during the digestion of coumarin-loaded (A) MCT-lecithin SLH microcapsules, (B) LCT-lecithin SLH microcapsules, (C) MCMDG-lecithin SLH microcapsules and (D) MCMDG-oleylamine SLH microcapsules. The line graphs represent the % lipid hydrolysis of each type of SLH microcapsules.

aqueous medium. It is suggested that the digestion kinetics data can be used to infer the solubilisation state of the encapsulated lipophilic compounds for this particular system. Importantly, complete digestion of the MCT-lecithin microcapsules (at 60 min) has produced a significantly higher solubilisation level for coumarin 102 (*i.e.* 116 μg/mL) in comparison with its solubility in the blank, lipid-free digestion medium (*i.e.* 24 ± 0.1 μg/mL).

In the case of LCT-lecithin microcapsules, Fig. 4(B) depicts a gradually increasing but incomplete drug solubilisation in the aqueous phase as digestion progresses, *i.e.* from ≈40% to ≈50% over the time course of 60 min. Clearly, there is no direct correlation between the percentage of drug solubilised and the percentage of digestion for the LCT-based microcapsules. The total percentage of drug molecules solubilised and precipitated did not make up to 100%; this could be attributed to the incomplete digestion of LCT lipids. It is proposed that a portion of the drug was retained in the undigested oil phase which was not feasibly analysed in our experiments. Future work with extended period of lipolysis for the LCT-based microcapsules is necessary to further elucidate the post-digestive solubilising capacity provided by the LCT lipolytic products compared to that of MCT. The solubilisation level of coumarin 102 after 60 min of digestion of the LCT-lecithin microcapsules was 416 μg/mL, which is more than three-fold higher than the maximum drug solubilisation level observed for the MCT-lecithin microcapsules.

Fig. 4(C) shows the phase partitioning of coumarin 102 in the lipolysis medium when formulated as the MCMDG-lecithin SLH microcapsules. The solubilisation degree of drug molecules into the aqueous phase was found to remain almost constant (≈60% to ≈65%) during 1 h of lipolysis. Similar to that observed for LCT-based microcapsules, the release of coumarin 102 from both the MCMDG-lecithin and MCMDG-oleylamine microcapsules did not correlate well with the lipolysis kinetics [Fig. 4(C) and (D)]. It can thus be inferred that digestion of MCMDG is not critical in influencing the solubilisation state of the encapsulated coumarin 102 molecules. This can be explained by the compositions of MCMDG, which consists of glycerides in the partially digested forms (mono- and diglycerides) that possibly undergo interactions with the biliary compounds to form various solubilising colloidal species (e.g. multilamellar/unilamellar vesicles and bile salt-lipid mixed micelles) (Humberstone and Charman, 1997; Porter et al., 2008). It is also plausible that the pre-digested lipids (mono- and diglycerides) provide an equally solubilising environment for coumarin 102 as does the post-digestion products (fatty acids only) under the simulated lipolysis conditions. Comparison of the drug phase partition between the MCMDG-lecithin and MCMDG-oleylamine microcapsules [Fig. 4(C) and (D)] reveals that the drug molecules were released to a lower extent for the latter formulation. This could be attributed to the lower lipid digestibility of MCMDG-oleylamine microcapsules as compared to MCMDG-lecithin microcapsules (100% versus 90% at 60 min, respectively).

Overall, these studies elucidate the feasibility of manipulating lipid digestion and thus, post-digestive state drug solubilisation by incorporating a different type of lipid/emulsifier into the SLH system. This provides key information for the development of hybrid lipid carrier to be employed *in vivo* that could eventually lead to an enhanced and controllable rate of absorption of various poorly water-soluble drugs.

4. Conclusions

SLH microcapsules with an internal porous matrix structure can be effectively prepared using different types of lipid (*i.e.* MCT, LCT or MCMDG) and emulsifier (*i.e.* anionic lecithin or cationic oleylamine). Under sink conditions, the *in* vitro release study indicates an immediate and complete release of lipophilic drug (log *P* = 4.09) from the SLH microcapsules regardless of the types of lipid and emulsifier used. Under simulated fasted-state intestinal lipolysis conditions, the SLH microcapsules were shown to produce a more controllable and increased lipid digestibility in comparison with equivalent pure oil, oil-emulsifier solution or emulsion systems. The drug phase partition studies reveal high solubilisation profiles of drug in the aqueous phase (*i.e.* within the range of 30–70% of drug solubilised) resulting from lipolysis of SLH microcapsules. The current investigations provide an important foundation for future formulation design and optimisation of hybrid materials based on lipid excipients and silica nanoparticles.

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