ACS APPLIED MATERIALS & INTERFACES

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Controlling the Enzymatic Digestion of Lipids Using Hybrid Nanostructured Materials

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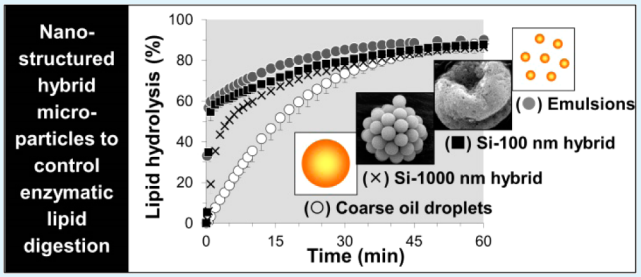
ABSTRACT: Solid nanoparticle–lipid hybrids have been engineered by using spray drying to assemble monodisperse hydrophilic silica nanoparticles and submicron lipid (triglyceride) emulsions together into composite microparticles, which have specific activity toward enzymes. The influence of silica particle size (100–1000 nm) and emulsifier type (anionic and cationic) on the three-dimensional structure of the composite particles was investigated. The nanostructure of the hybrid particles, which is controlled by the size of the voids between the closely packed silica particles, plays a critical role in lipase action and hence lipid digestion kinetics. Confining lipid droplets within the nanostructured silica aggregates led to 2- to 15-fold enhanced rate of lipolysis in comparison with dispersed coarse oil droplets. The composite particles were tailored to enhance, retain or sustain the lipolysis kinetics of submicron lipid emulsions. The presence of repulsive nanoparticle-droplet interactions favored aqueous redispersion and fast lipolysis of the hybrid composite materials, while attractive interactions hindered redispersion and delayed lipolysis of the confined lipid droplets. Such hybrid nanomaterials can be exploited to control the gastrointestinal enzymatic action and promisingly form the basis for the next generation of foods and medicines.



KEYWORDS: *silica particles, lipid emulsions, emulsifiers, spray drying, nanostructure, lipolysis*

1. INTRODUCTION

Understanding the mechanisms and factors controlling gastrointestinal digestion of lipids has important implications in health as well as the formulation and manufacture of food and pharmaceuticals. For example, minimizing fat absorption for the control of obesity-related diseases and optimizing the delivery of lipid-soluble nutrients and drugs. Gastrointestinal hydrolysis of lipids is mainly catalyzed by the water-soluble enzyme, pancreatic lipase–colipase, which is known to act on the surface of insoluble lipid substrates.[1] The cascade of lipase-catalyzed lipolysis essentially relies on the enzyme–substrate interfacial binding affinity and the removal efficiency of lipolysis products from the substrate surface.[1−3] To date, research in controlling pancreatic lipolysis has mainly focused on the inhibition of bulk enzyme activity (e.g., the antiobesity drug, Orlistat),[4] as well as modification of the substrate surface compositions.[5−10] The concept behind the latter approach is to create a physical barrier at the substrate–water interface for interfering with the accessibility of lipase (at its catalytic domain) to the substrate moieties. Emulsifiers and surfactants commonly used in pharmaceutical formulations have the tendency to affect partitioning of the digestive enzymes via competitive adsorption at the lipid–water interface.[11] Interfacial adlayers of various surface active agents have been shown to exert dose-dependent effects toward delaying or slowing down lipolysis of emulsions mainly via interfacial shielding and/or competitive binding. Examples include lecithin and bile salts,[5,12,13] Tween 20/80, sodium lauryl sulfate and cetyltrimethylammonium bromide,[3,5,9] and various types of biopolymers (e.g., chitosan, polylysine, poloxamers, or otherwise commercially known as Pluronics).[7,8,10]

In recent years, there has been a renewed interest to control enzymatic reactions with minimal substrate chemical modification by adsorbing either the enzymes or substrates onto a solid phase. Immobilizing enzymes on hydrophilic solid supports (such as mesoporous silica, alumina, and polystyrene nanoparticles) offers the potential advantages of enhanced reaction kinetics and increased product yield in comparison with free enzymes in solution.[14−18] The orientation and retention of the active site structure is crucial toward preserving the enzyme stability and activity when loaded into porous materials. Similarly, confining substrates within highly porous, nanostructured carriers also enabled more precise modulation of the rate of chemical conversion or breakdown of organic molecules.[19−25] In previous studies, we have explored the effect of nanostructure confinement on the digestion behavior of lipids by (i) spray-drying triglyceride emulsions stabilized by silica adlayers into three-dimensional hybrid microparticles,[21−24] and (ii) directly loading triglyceride substrates into porous silica particles via solvent immersion technique.[25] The physicochemical properties of the silica materials including pore dimensions, surface functional groups and particle sizes, play a major role in governing the flexibility of enzyme conformational changes and the ease of diffusion of substrate and lipolytic

Received: June 17, 2014
Accepted: August 13, 2014
Published: August 13, 2014

Table 1. Experimental Parameters for the Study of Silica Size Effect (approximately 100, 200, and 1000 nm) and Emulsifier Type (Negatively Charged Lecithin and Positively Charged Oleylamine) On the Three-Dimensional Structure and Lipid Encapsulation Capacity of Hybrid Materials Spray Dried from Submicron Lipid Emulsions Stabilised by Monodisperse Hydrophilic Silica Particles

			lipid-to-silica ratio				
type of silica particle	mean particle diameter (nm)	specific surface area (m^2/g)	mass ratio	theoretical particle number ratio	lipid	measured lipid load in solid state (w/w)[c] (%)	lipid encapsulation efficiency (%)
Si-100 nm (Levasil 30/50)	120	30.0[a]	1:1	1:2	lecithin	43	86
			1:1.5	1:3		34	
			1:2.5	1:5		25	
					oleylamine	19	38
						15	
						9	
Si-200 nm (microparticles R-L-2072)	190	15.8−17.5[b]	1:1.5	1:1	lecithin	40	100
			1:3	1:2		25	
			1:4.5	1:3		18	
			1:15	1:10		6	
Si-1000 nm (microparticles R-B1060)	1120	2.7−3.0[b]	1:3	1000:9	lecithin	22	88
			1:4	100:1		18	
			1:6	100:2		12	
			1:11.5	100:4		7	

[a]Reported by the supplier AkzoNobel/EKA Chemicals based on known particle density of 2.2 g/cm^3. [b]Estimated using the established equation SSA = $3/r\rho$ (where SSA = specific surface area, r = particle radius, and ρ = particle density) based on known particle density of 1.8−2.0 g/cm^3 (www.microparticles.de/properties.html). [c]All data are within the error limit of 2% of the mean.

molecules. While silica particles in the aqueous, dispersed state formed an interfacial shielding layer inhibiting the enzymatic digestion of triglyceride droplets and phospholipid liposomes,[23,26] confinement of glyceride lipids in hydrophilic silica pores increased lipid digestibility.[21−25] The latter is presumably due to reduced lipid droplet size in conjunction with enhanced lipolytic product removal and anchoring of enzymes via the silica surface silanol groups. On the other hand, hydrophobic porous silica reduced the rate and extent of lipid digestion, most likely attributed to suboptimal orientation of the lipid substrate molecules and/or digestive enzymes in the process of interfacial binding.[25] Importantly, these three-dimensional hybrid microparticles have been shown to increase the oral bioavailability of various poorly water-soluble, lipophilic drugs (e.g., celecoxib, indomethacin, and ibuprofen) in animal and human models.[22−24,27−29] Other potential biopharmaceutical advantages associated with such a silica−lipid hybrid drug delivery approach are enhanced drug-carrier encapsulation efficiency,[30] improved feasibility of dosage form transformation from liquid to solid,[31,32] and minimized diet-induced variations in drug absorption because of the food-mimicking effect of lipids.[33]

Given the enormous potentials and versatility that the hybrid materials could offer, the current study aims to take such investigations to the next level by examining the influence of emulsifier type and particle size on the structure of the hybrid materials, their redispersibility and the rate of lipid digestion. Our previous microparticle prototypes were engineered using silica nanoparticles with primary particle diameters of ≤20 nm (e.g., Aerosil fumed silica nanoaggregates and Ludox silica nanoparticles). In this work, monodisperse hydrophilic silica particles of larger and varying diameters (i.e., approximately 100, 200, and 1000 nm) were examined for the formation of different composite structures at varying lipid-to-silica mass ratio (Table 1). Two types of lipophilic emulsifiers, i.e., negatively charged lecithin or positively charged oleylamine, were incorporated to induce repulsive or attractive interactions between the emulsified lipid droplets and silica particles. Such variables are hypothesized to influence the pattern of particle packing and thus, lipid encapsulation capacity and lipolysis kinetics of the confined lipids in the presence of pancreatic enzymes. The current findings will be significant toward enabling quality formulation design and more predictable control over the gastrointestinal enzymatic action and thus, the biological fate of lipid-based pharmaceutical and nutraceutical delivery systems.

2. EXPERIMENTAL SECTION

2.1. Materials. Miglyol 812, a mixture of saturated caprylic/capric (C8/C10) triglycerides, was purchased from Hamilton Laboratories (Australia). Soybean lecithin (containing >94% phosphatidylcholine and <2% triglycerides) and oleylamine (primary amine purity >98%) were supplied by BDH Merck (Australia) and Sigma-Aldrich (Australia), respectively. Two types of monodisperse silica particles (as aqueous dispersions) were purchased from microParticles GmbH (Germany): SiO2-R-L2072 (average particle size 190 nm; for simplified discussion this is expressed as "Si-200 nm" hereafter) and SiO2-R-B1060 (average particle size 1.120 μm; expressed as "Si-1000 nm" hereafter). Another type of colloidal silica dispersion, Levasil 30/50 (average particle 120 nm; expressed as "Si-100 nm" hereafter), was obtained as a gift from Akzo Nobel/EKA Chemicals (Sweden). For lipolysis studies, Trizma maleate, calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), and type X-E L-α-lecithin (60% pure phosphatidylcholine, from dried egg yolk) were purchased from Sigma-Aldrich (Australia). Sodium taurodeoxycholate (NaTDC) 97% and porcine pancreatin (lipase activity ≥6 USP-U/mg, protease activity ≥25 USP-U/mg and amylase activity ≥25 USP-U/mg) were obtained from Chem-Supply (Australia). Sodium hydroxide (NaOH) pellets was supplied by Merck (Australia). All other chemicals were of analytical grade and used as received. High-purity (Milli-Q) water was used throughout the study.

2.2. Preparation of Spray-Dried Nanostructured Hybrid Particles. Nanostructured hybrid particles were prepared from precursor triglyceride emulsions stabilized by silica particles of varying colloidal sizes according to previously established method.[22,23] Briefly, the precursor oil-in-water emulsions were prepared by emulsifying 10% (w/w) of Miglyol 812 in water in the presence of either negatively charged lecithin (0.6% w/w) or positively charged

oleylamine (1.4% w/w). Submicron emulsions were produced via high-pressure homogenization (Avestin EmulsiFlex-C5 Homogenizer) under a pressure of 1000 bar for 5 volume cycles. This produced average droplet sizes of 200 and 230 nm (polydispersity index, PDI < 0.2) for the lecithin- and oleylamine-based emulsions, respectively. All silica aqueous dispersions were used at the same concentration (5% w/w) and ultrasonicated (Bransonic 2510 ultrasonic bath) for 1 h prior to incorporation to the homogenized emulsions. Varying mass ratio of lipid-to-silica was employed as specified in Table 1 (which corresponded to approximately 6% to 50% of theoretical lipid levels in the solid state). The silica-containing emulsion samples were mixed and equilibrated overnight (~18 h) to facilitate self-assembly of the silica particles and lipid droplets. The silica stabilized emulsions were then subjected to spray drying using a BUCHI Mini Spray Dryer B-290 operated under the following conditions: emulsion feeding rate of 6 mL/min, aspirator setting of 100%, air flow rate of 0.7 m^3/min, and inlet/outlet temperature of 190/75 °C.

2.3. Solid-State Microscopy Analysis. The surface structure of the spray dried hybrid particles was examined using a high-resolution scanning electron microscopy (Philips XL30 FEGSEM) at an accelerating voltage of 10 kV. Each sample was mounted on an aluminum stub using double-sided carbon adhesive tape, and sputtered with gold/palladium prior to imaging.

2.4. Redispersibility Study. Particle sizes of the spray dried hybrid particles redispersed in water (refractive index = 1.33) were characterized by laser diffraction using a Malvern Mastersizer 2000 instrument. Zeta potentials of the redispersed hybrid particles were measured by phase analysis light scattering (PALS) using a Malvern Zetasizer Nano ZS at 25 °C. The redispersion behavior of the spray dried particles was visualized using an Olympus transmitted light microscope. A small trace of each sample was dispersed in water and placed on a microscope slide with a coverslip prior to imaging.

2.5. Lipid Loading Capacity. The lipid content of the spray dried hybrid particles was determined by thermogravimetric analysis (TA Instruments, Hi-Res Modulated TGA 2950). Each hybrid particle sample (~10 mg) was heated at a scanning rate of 10 °C/min from 20−500 °C under a nitrogen gas purge. The lipid content (both oil and emulsifier) was evaporated in the range of 180−350 °C, whereas the silica component remained thermally stable. Changes in the sample weight represent the lipid content and these were computed using the associated TA Universal Analysis software.

2.6. In Vitro Lipid Digestion Performance Study. The lipid digestion profiles of various Miglyol-based systems were monitored under simulated human duodenal environment using a TitraLab 854 pH-stat titration apparatus (Radiometer Analytical). Based on a previously established protocol,[22,23] a mixed micellar medium containing bile salts (NaTDC) and egg phospholipids at a concentration of 5 mM:1.25 mM (in the reaction mixture) was used to simulate the human duodenal fluids in the fasted state. The mixed micelles were buffered at pH 7.5 (37 °C) in the presence of 50 mM Trizma maleate, 150 mM NaCl and 5 mM CaCl2.2H2O. Pancreatin extracts (containing pancreatic lipase, colipase, and other nonspecific lipolytic enzymes such as phospholipase A2) were freshly prepared each day and used as the lipid digesting enzymes at a lipase activity level of 1000 tributyrin units (TBU) per mL (in the reaction mixture). Each lipid system, including pure Miglyol oil (which dispersed to form coarse oil droplets in the medium) and the various hybrid particles, was digested at a dose of 200 mg oil per 20 mL of medium. As the digestion progressed, free fatty acids (FFAs) produced in the reaction vessel were titrated with 0.6 M NaOH via an autoburet; this maintained a constant pH (at 7.50 ± 0.02) in the lipolysis medium. The cumulative volume of NaOH dispensed from the autoburet was recorded by the associated TitraMaster 85 Software at a 5 s interval. Lipolysis of the blank micelles without the addition of any lipid samples was performed in the same way to account for the background FFA produced by components other than the formulated lipids. The consumption of NaOH (after background correction) was used to estimate the number of moles (n) of FFA released and thus, the magnitude of lipid digestion resulting from the various lipid systems

$$\text{lipolysis, } L(\%) = \frac{n(\text{FFA})}{3n(\text{triglycerides})}100 \quad (1)$$

The computation was based on the assumption that 1 mol of Miglyol oil (i.e., a triglyceride) is completely hydrolyzed to produce 3 mol of FFA in the presence of enzymes in excess (i.e., at least 2-times higher than the typical physiological concentrations of ~500 TBU/mL).[34] Given that complete intestinal absorption of triglyceride fat typically occurs at 66% of FFA release from a lipid substrate,[35,36] the lipolysis characteristics of each system were evaluated based on: (i) the time taken to reach approximately 60% of lipid digestion ($t_{60\%}$), and (ii) the maximum percentage of lipid hydrolysis achieved at 60 min ($L_{60\ \text{min}}$).

3. RESULTS AND DISCUSSION

3.1. Bottom-up Formation of Nanostructured Silica-Lipid Hybrid Microparticles. Spray-drying has continuously been investigated as an effective and scalable approach to construct various uniquely structured composites from colloidal particles. Using a benchtop spray drying unit, we systematically examined the influence of emulsifier type (cationic and anionic) and silica particle size (100, 200, and 1000 nm) on the formation of different three-dimensional composite structures (Figure 1). Submicron triglyceride droplets (approximately 200 nm), initially emulsified using either lecithin (anionic) or oleylamine (cationic), were encapsulated by monodisperse hydrophilic silica particles through self-assembly from the aqueous bulk. Spray-drying at elevated temperature rapidly removed the aqueous phase, resulting in aggregated silica-lipid composites decorated with unique nanostructures. The effects of composite void sizes and particle-droplet interactions on the lipid loading capacity, microparticle redispersibility, and in vitro enzymatic digestion of lipids are illuminated.

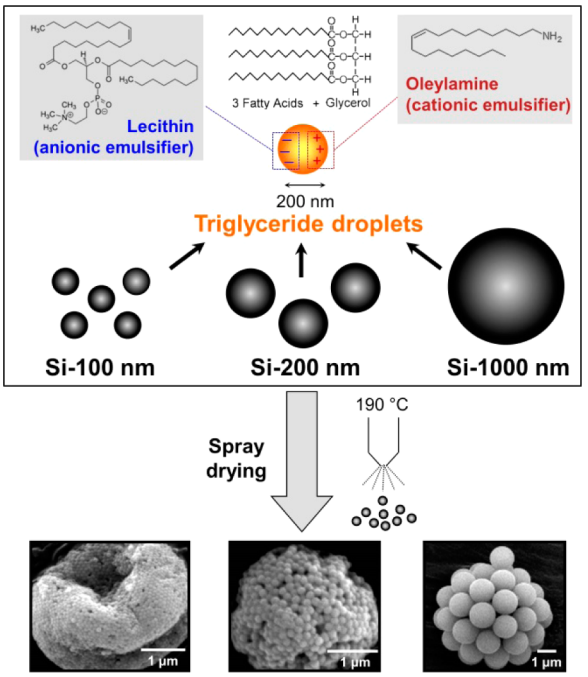


Figure 1. Schematic illustration of the formation of nanostructured silica-lipid hybrid microparticles via spray drying of submicron triglyceride emulsions stabilized by (i) different type of emulsifiers and (ii) monodisperse hydrophilic silica particles of varying sizes (100, 200, and 1000 nm).

dx.doi.org/10.1021/am5038577 | ACS Appl. Mater. Interfaces 2014, 6, 15363−15371

3.1.1. Influence of Emulsifier Type on Composite Structures and Lipid Load. In elucidating the influence of emulsifier charge on the three-dimensional compactness and lipid encapsulation efficiency of the hybrid entities, nonporous silica Si-100 nm was used as the solid carrier for triglyceride-based emulsions of an average droplet size of 200−230 nm.

In the precursor wet state, the Si 100 nm−negative emulsions displayed a monodisperse, sub-500 nm size distribution profile (Figure 2a, top figure). The adjacent

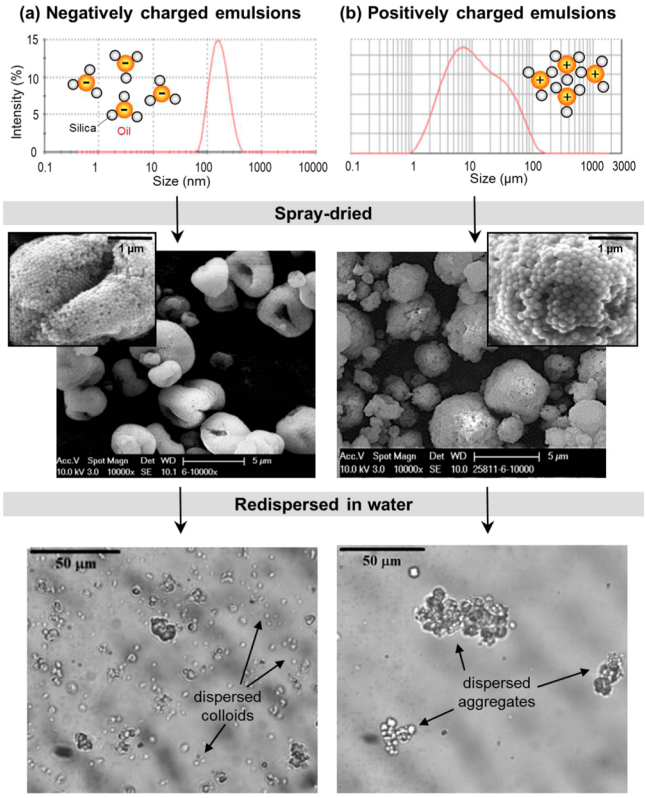


Figure 2. Representative particle size distribution (top figures), scanning electron micrographs (middle), and optical microscopy images (bottom) demonstrating the effect of emulsifier charge on the precursor droplet sizes, spray-dried solid structures, and redispersibility of triglyceride emulsions stabilized by Si-100 nm based on a lipid-to-silica mass ratio of 1 to 3: (a) lecithin-based negatively charged emulsions and (b) oleylamine-based positively charged emulsions.

silica-stabilized droplets (which had zeta potentials of −51.8 ± 5.8 mV) were well separated in the presence of electrostatic repulsion forces.[22] In contrast, the Si 100 nm−positive emulsions (net zeta potentials −23.6 ± 3.3 mV) exhibited a broad size distribution within the range of 1 to 100 μm, indicating massive droplet-nanoparticle aggregation prior to spray drying (Figure 2b, top figure). The formation of such emulsion-particle clusters is resulted from electrostatic attractions between the adjacent lipid droplets (+52.6 ± 4.5 mV) and silica nanoparticles (−48.7 ± 5.8 mV).

When subjected to spray drying, the Si 100 nm−negative emulsions effectively formed well-enclosed, mostly toroidal particles of ≤5 μm (Figure 2a, middle figure). On the other hand, the Si 100 nm−positive emulsion clusters formed relatively compact and spherical microparticles different from the toroidal shaped lecithin-based systems (Figure 2b, middle figure). When visualized under a light microscope, the toroidal composites easily redispersed in water into separated micro-meter-sized structures (Figure 2a, bottom figure); the compact aggregation induced by attractive droplet-nanoparticle interactions was presumably irreversible so that the positive emulsion hybrids remained clustered when redispersed in water (Figure 2b, bottom figure). The formation of toroidal versus spherical spray dried structures was attributed to the binding force or clustering behavior of the droplets and nanoparticles in the precursor, wet state (i.e., the state of dispersion and sedimentation stability).[46] The well-separated, negatively charged emulsions were prone to rearrangement of the neighboring nanoparticles during evaporative drying, leading to the formation of buckled silica shells. In contrast, the positively charged emulsions clustered into compact, micrometer-sized structures in the wet state. These tightly jammed droplet-nanoparticle structures resisted morphological buckling during drying and thereby, retaining their spherically aggregated structures.

The lipid loading capacity of the Si-100 nm hybrids at varying lipid-to-silica ratio is presented in Table 1. For both lecithin and oleylamine-based hybrids, the lipid encapsulation efficiency (or lipid recovery in the spray dried mass) is reasonably constant across the investigated lipid-to-silica levels. The lecithin-based hybrids produced almost 2-fold higher maximum lipid loading (43% w/w) than that of the oleylamine-based hybrids (i.e., 19% w/w). The poor lipid recovery of the latter system is related to the coagulating behavior of the precursor emulsions. This hindered oil encapsulation that is typically induced by diffusion and collapsing of the surrounding particles during the rapid evaporation step of spray drying. It is also possible that the emulsion droplets were not completely "loaded" into the aggregated precursor structures in the wet state, leading to substantial loss of the "uncoated droplets" when fed into the spray drying hot chamber. One potential strategy to minimize droplet-nanoparticle aggregation in the wet state is to employ a much lower amount of silica nanoparticles as the solid carrier; it has previously been shown possible to obtain relatively stable oleylamine-based emulsions at a lipid:silica mass ratio of 20:1 (where the emulsions retain a positive surface charge of approximately +50 mV). These emulsions were successfully spray dried to form powdery spherical microparticles with >80% of lipid encapsulation efficiency.[27] The current findings essentially show that massive aggregation in the precursor emulsions has a negative impact on the lipid loading efficiency.

3.1.2. Influence of Particle-Droplet Size Ratio on Composite Structures and Lipid Load. Varying particle-droplet size ratio was investigated for the negative emulsions (~200 nm) at a fixed lipid-to-silica mass ratio of 1:3. Similar to the Si 100 nm-emulsions, the Si 200 nm-emulsions also showed a monodisperse size distribution of approximately 200 nm with a PDI < 0.100 (Figure 3a, b, top figures). Because the expected size range for Si 200 nm emulsions (in the vicinity of 400 nm) was not observed, this size distribution is more likely to correspond to that of the individual lipid droplets and silica nanoparticles. For Si 1000 nm emulsions, the size distribution profile clearly reveals the presence of two populations that are well correlated to the original sizes of the lipid droplets and silica particles, respectively (Figure 3c, top figure). Such correlation was corroborated by the fact that the magnitude of the peak attributed to the lipid droplets increased relative to the other when the lipid-to-silica mass ratio was increased. Therefore, adsorption between the submicron negative droplets

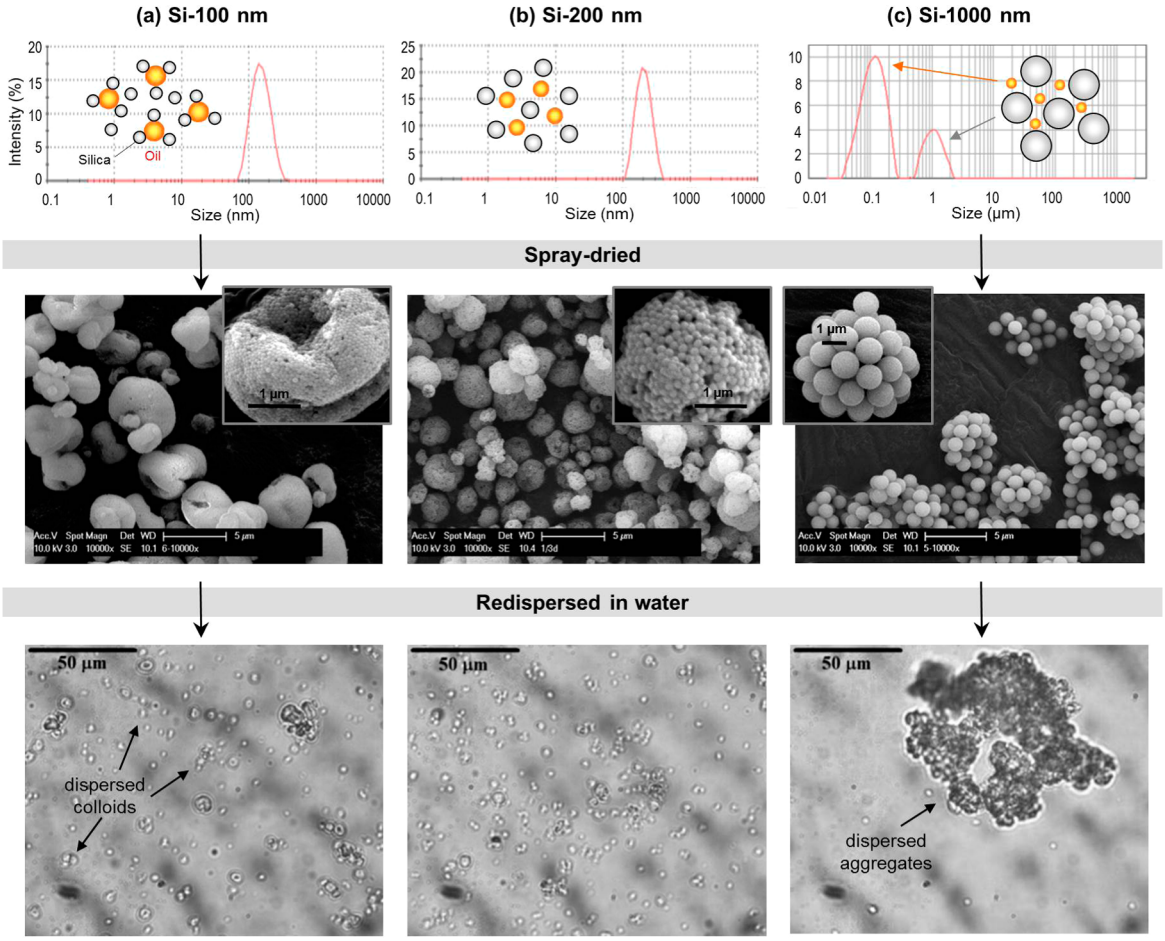


Figure 3. Representative particle size distribution (top figures), scanning electron micrographs (middle), and optical microscopy images (bottom) demonstrating the effect of silica size on precursor droplet sizes, spray-dried solid structures, and redispersibility of negatively charged triglyceride emulsions based on a lipid-to-silica mass ratio of 1 to 3: (a) Si-100 nm, (b) Si-200 nm, and (c) Si-1000 nm.

and the nonporous silica particles was considered minimal in the wet state.

The weak interfacial adsorption is influenced by the electrostatic repulsive interactions, which are expected to be dependent on the radii of the coating particles.[37] According to the Derjaguin approximation, the force between two colloidal particles "$F(h)$" as a function of the surface separation "h" can be expressed as

$$F(h) = 2\pi R_{\text{eff}} W(h) \tag{2}$$

where $W(h)$ is the interaction energy per unit area between the two planar walls (which obviously does not depend on the radii), and R_{eff} is the effective radius. The effective radius between two spheres of radii R_{oil} (i.e., radius of oil droplets) and R_{particle} (i.e., radius of the coating particles) is given by

$$\frac{1}{R_{\text{eff}}} = \frac{1}{R_{\text{oil}}} + \frac{1}{R_{\text{particle}}} \tag{3}$$

although R_{oil} is fixed (i.e., similar droplet size is assumed), the value of $1/R_{\text{eff}}$ in the case of a smaller particle (e.g., Si-100 nm) is larger than that of a relatively bigger particle (e.g., Si-200 nm). Therefore, R_{eff} and the value of the repulsive electrostatic force, $F(h)$, is effectively larger in the case of the bigger particles. This means that a longer equilibrating time is needed for the interfacial adsorption to occur, if at all.

Regardless of the weak interfacial adsorption capacity, both Si-200 nm and Si-1000 nm systems effectively formed reasonably spherical and well-enclosed aggregates in the solid state (Figure 3b and c, middle figures). Upon redispersion in water, both hybrids of Si-100 nm and Si-200 nm were visualized as separated dispersed colloids, whereas the larger Si-1000 nm hybrid was seen as large aggregates in the dispersed state (Figure 3, bottom figures). Fundamentally, the formation of buckled or spherical structures from colloidal droplets through spray drying is influenced by two categories of parameters: (i) the physicochemical properties of the colloidal dispersion (e.g., solid concentration, particle size, interparticle interactions, viscosity, and surface tension), and (ii) the drying conditions (e.g., inlet/outlet temperature, drying time and atomization pressure).[38−40] Given that the drying condition is consistent in this study, the morphological buckling for the Si-100 nm hybrid is considered to be related to its greater silica particle number (and therefore, relatively higher dispersion viscosity) at the selected lipid:silica mass ratio. As illustrated by Bahadur et al.[38] and Sen et al.,[39] the diffusion coefficient of colloidal particles is reduced at higher viscosity, meaning that the particles have limited time to move from the surface to the center of the atomized droplet, thus leading to the formation of "buckled shell" structures. While this remains a hypothesized explanation, it certainly warrants an in-depth study to specifically address the effect of silica particle size and dispersion viscosity

dx.doi.org/10.1021/am5038577 | ACS Appl. Mater. Interfaces 2014, 6, 15363−15371

on the structural changes of various spray dried colloidal droplets.

The dimensional impact of silica particles on the lipid loading capacity of spray dried hybrid structures was compared for the negative emulsions. Similar to the Si-100 nm hybrids, both Si-200 nm and Si-1000 nm hybrids produced a relatively high lipid encapsulation efficiency of at least 88%, leading to a final lipid content of 40% and 22% w/w, respectively (Table 1). The SEM images in Figure 4 illustrate that the lipid feed level imparts

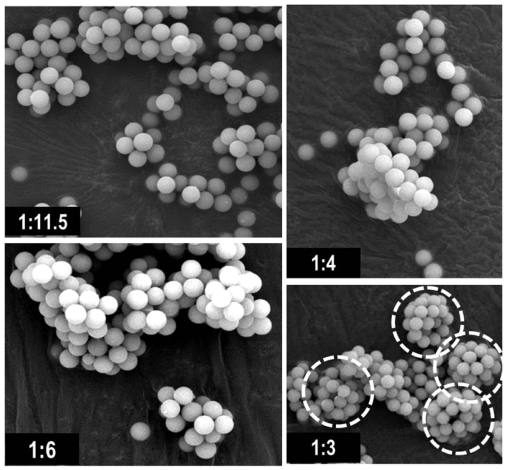


Figure 4. Scanning electron micrographs of spray dried hybrids prepared from Si-1000 nm at varying lipid-to-silica mass ratio.

significant influence on the solid-state structures of the Si-1000 nm hybrids. It is inferred that the lipid component plays a critical role for its "adhesive" or "void-filling" function to confer close-packing of the silica particles. This shows the feasibility to control the resultant morphology of the hybrid materials via tuning of the lipid-to-silica ratio during the bottom-up fabrication of the Si-1000 nm systems.

3.2. Nanostructure Effect for Controlling the Gastrointestinal Enzymatic Action. The influence of substrate surface charge and nanostructure confinement on the enzymatic digestion of lipids was investigated under simulated human gastrointestinal conditions. Two parameters are used to measure the susceptibility of each silica-lipid hybrid system to enzymatic degradation, i.e., $t_{60\%}$ (time taken to reach 60% lipolysis) and $L_{60\ min}$ (the maximum percentage of lipolysis achieved at 60 min). In terms of structural integrity of the various silica-droplet composites, it was confirmed through laser diffraction analysis that the composites undergo negligible changes in their particle sizes under the mild stirring conditions used in the digestion study. Therefore, the effect of the nanostructure in each composite system on the enzymatic activity could be reliably followed.

In the presence of excess enzymes, the coarse oil droplets were digested near to completion (88.5 ± 0.5%) in 60 min (Figure 5). When the lipid droplets were homogenized and their sizes reduced to approximately 200 nm, the lecithin-based (negatively charged) submicron emulsions demonstrated a $t_{60\%}$ (1.2 min) of 17-times faster than the coarse droplets ($t_{60\%}$ = 20.6 min) (Figure 5a). In contrast, the oleylamine-based (positively charged) submicron emulsions underwent a fast initial lipolysis before the reaction was markedly impeded at 2 min, reaching only approximately 20% of lipolysis (Figure 5b). This clearly shows the potent inhibitory role of a cationic

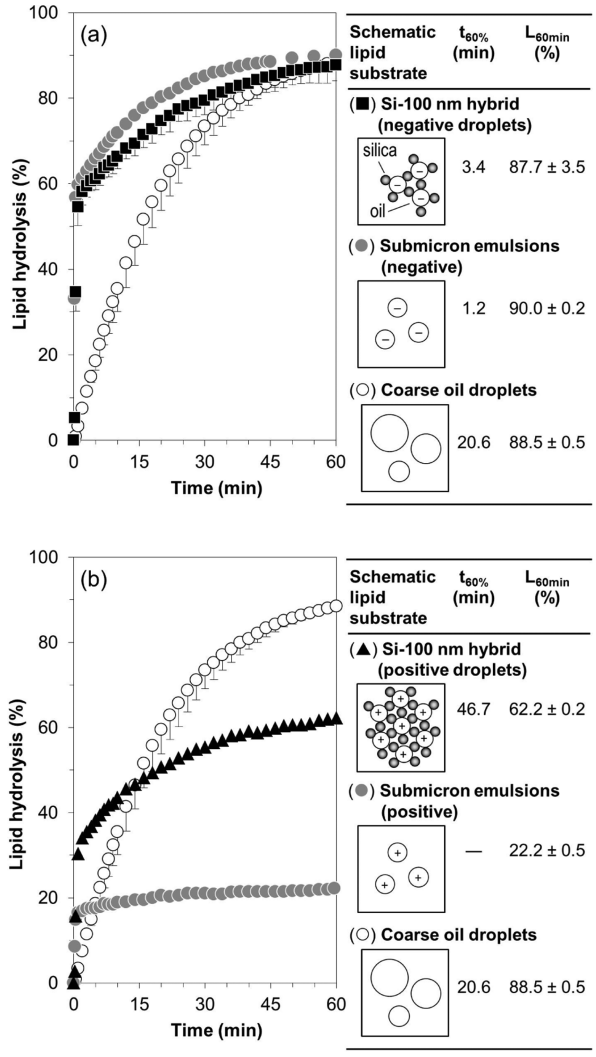


Figure 5. Lipolysis profiles of spray dried triglyceride emulsions stabilized by Si-100 nm based on a lipid-to-silica mass ratio of 1:3: (a) lecithin-based negatively charged emulsions and (b) oleylamine-based positively charged emulsions.

emulsifier in the lipolysis process, despite the enhancing effect of droplet emulsification which theoretically should render higher substrate surface area accessible to lipase.[41] Because of potential interactions between the cationic oleylamine and various anionic components such as bile salts and digestion breakdown molecules, the lipolytic products may not be efficiently removed from the substrate interface.[25] This in turn led to the formation of a penetration barrier to the digesting enzymes where their subsequent propagation along the substrate interface and hydrolytic action were dramatically hindered. This inhibitory effect resembles that of a number of basic or cationic polymers (e.g., chitosan, modified polydextrose, and polylysine) which have been shown to inhibit lipase interfacial activities in a dose-dependent manner.[7,42,43]

Interestingly, the hybrid nanostructure, which is controlled by the voids between closed-packed silica particles in the aggregated structures, plays a critical role in either retaining or enhancing the lipid digestion profiles. When the emulsified droplets were confined within the Si-100 nm aggregates, the fast and complete lipolysis pattern was almost retained for the lecithin-based (negative droplets) hybrid (Figure 5a). For the oleylamine-based (positive droplets) hybrid, the progress of

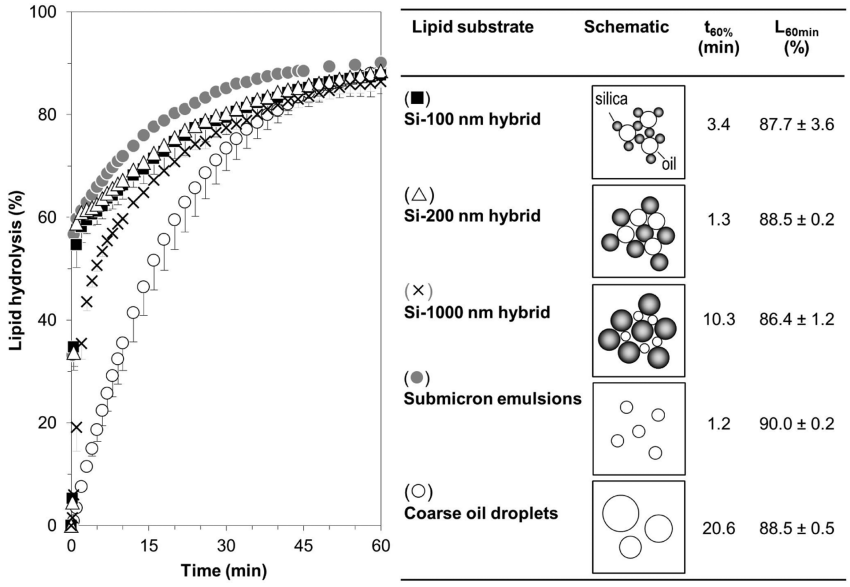


Figure 6. Lipolysis profiles of spray dried triglyceride emulsions (200 nm) stabilized by silica particles of different sizes (100, 200, and 1000 nm) based on a lipid-to-silica mass ratio of 1:3. Lecithin was used as an emulsifier for the submicrometer emulsions and the hybrid microparticles.

lipolysis was relatively more sustained; the $L_{60\ min}$ successfully reached more than 60%, that is 3-times higher compared to the submicron emulsions (Figure 5b). The delayed but continuously progressing lipolysis profile could be related to the irreversible aggregation of the oppositely charged droplets and nanoparticles, where geometrical restriction played a role in influencing the access of digesting enzymes from the aqueous phase and/or diffusion of the lipid droplets and lipolytic products. Such inhibitory digestion control is exploitable to facilitate gastrointestinal solubilization of a number of highly lipophilic drugs such as danazol and halofantrine; prolonging the gastrointestinal residence of various lipid entities is useful to sustain the accommodation of active compounds that are susceptible to precipitation and/or degradation upon breakdown of the lipid cores.[44,45]

The effect of silica particle size and pore confinement on the lipolysis of negatively charged droplets is shown in Figure 6. Altering the sizes of silica particles essentially produced internal nanostructures of varying specific surface area (Table 1). While there was minute difference in the values of $L_{60\ min}$ among the three investigated hybrid microparticles (i.e., >85% lipid digested), lipid digestibility in term of $t_{60\%}$ was relatively shorter for hybrids constructed using silica particles of smaller sizes (i.e., 100 and 200 nm). The trend of $t_{60\%}$ could be ranked in the following increasing order: submicron emulsions (1.2 min) ≈ Si-200 nm hybrid (1.3 min) ≥ Si-100 nm hybrid (3.4 min) > Si-1000 nm hybrid (10.3 min) > coarse oil droplets (20.6 min). Despite the chemically inert and "stealthy" properties of silica particles, confinement of lipid substrates in these differently sized nanostructured matrices led to different enhancement pattern in the lipid digestibility. The void sizes of these hydrophilic silica aggregates impart significant influence on the accessibility and hydrolytic action of lipase enzymes.

Collectively, the current findings highlight the prime importance of several parameters that critically determine the substrate digestibility and enzyme action, specifically lipid preemulsification (i.e., enhanced specific surface area of substrates), the ease of carrier redispersion (i.e., controlled geometrical constraint), and the potential interactions between enzymes and carrier materials (i.e., interfacial enzyme anchoring effect and efficient removal of lipolytic products via electrostatic repulsion).[23] The types of emulsifiers and sizes of silica particles as the solid carrier are important considerations in the design of nanostructured delivery systems; appropriate interplay between these two components is predicted as a viable approach to manipulate the enzymatic breakdown of the confined substrates and applicable for enhancement of controlled delivery for food and drug substances.

4. CONCLUSION

Hybrid materials with three-dimensional nanostructured network composed of lipid emulsions and silica particles were successfully engineered based on (i) three types of monodisperse silica particles (in the size range of 100 to 1000 nm) and (ii) two types of lipophilic emulsifiers (negatively charged lecithin and positively charged oleylamine). Specific parameters including the droplet-nanoparticle size and mass ratios, as well as attractive and repulsive droplet-nanoparticle interactions were shown to influence the pattern of particle packing and the resultant spray dried solid-state structures (e.g., toroidal particles, spherical aggregates, or irregularly shaped structures). Confinement of lipid droplets in the nanostructured matrices produced significant enhancement in the lipolysis kinetics as compared to dispersed coarse oil droplets. It was shown possible to manipulate the gastrointestinal enzymatic action by integrating silica particles of different sizes and emulsifiers of different charges (i.e., anionic or cationic) into a unique hybrid structure. The versatile fabrication of the silica–lipid hybrid microparticles emerges as a promising approach for the control of lipolysis reactions that is significant toward optimizing lipid-based drug and nutrient delivery.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The Australian Research Council (ARC) is acknowledged for research funding and support (Discovery Project, DP120101065).

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