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Silica Nanoparticles To Control the Lipase-Mediated Digestion of Lipid-Based Oral Delivery Systems

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Abstract: We investigate the role of hydrophilic fumed silica in controlling the digestion kinetics of lipid emulsions, hence further exploring the mechanisms behind the improved oral absorption of poorly soluble drugs promoted by silica−lipid hybrid (SLH) microcapsules. An *in vitro* lipolysis model was used to quantify the lipase-mediated digestion kinetics of a series of lipid vehicles formulated with caprylic/capric triglycerides: lipid solution, submicrometer lipid emulsions (in the presence and absence of silica), and SLH microcapsules. The importance of emulsification on lipid digestibility is evidenced by the significantly higher initial digestion rate constants for SLH microcapsules and lipid emulsions (>15-fold) in comparison with that of the lipid solution. Silica particles exerted an inhibitory effect on the digestion of submicrometer lipid emulsions regardless of their initial location, i.e., aqueous or lipid phases. This inhibitory effect, however, was not observed for SLH microcapsules. This highlights the importance of the matrix structure and porosity of the hybrid microcapsule system in enhancing lipid digestibility as compared to submicrometer lipid emulsions stabilized by silica. For each studied formulation, the digestion kinetics is well correlated to the corresponding *in vivo* plasma concentrations of a model drug, celecoxib, via multiple-point correlations ($R^2 > 0.97$). This supports the use of the lipid digestion model for predicting the *in vivo* outcome of an orally dosed lipid formulation. SLH microcapsules offer the potential to enhance the oral absorption of poorly soluble drugs via increased lipid digestibility in conjunction with improved drug dissolution/dispersion.

Keywords: Lipid-based formulations; poorly soluble drugs; hydrophilic fumed silica; *in vitro* lipolysis; *in vitro*−*in vivo* correlations.

1. Introduction

The versatility of lipid-based vehicles for oral delivery of poorly soluble drugs is evidenced by the commercialization of a number of therapeutic products, namely Sandimmune/Neoral (cyclosporine), Norvir (ritonavir) and Fortovase (saquinavir). In 2007, oral lipid-based formulations were reported to contribute up to 4% of the pharmaceutical market worldwide.[1] However, research in this area is significantly behind the rate of discovery of new chemical entities exhibiting poor aqueous solubility. Potential barriers that impede further clinical studies and regulatory approval of many lipid-based drug delivery systems are the lack of

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complete mechanistic understanding of the behavior of the dosage forms, and the shortage of suitable *in vitro* tests that are predictive of the *in vivo* performance.[2,3]

Conventionally, dissolution tests have been regarded as the most appropriate *in vitro* tool for predicting the absorption profiles of Biopharmaceutical Classification System (BCS) class II drugs (low solubility, high permeability).[4,5] However, the complexity of the intraluminal processes has compromised the utility of standard pharmacopeial dissolution models for developing *in vitro*−*in vivo* correlation (IVIVC) of modified formulations of class II drugs.[6−8] This leads to further exploration of a myriad of biorelevant dissolution media;[8−10] out of all these methods only one was investigated for a lipid suspension.[10] Some of these modified dissolution methods have successfully improved the prediction of drug bioavailability. However, their applicability to more complex lipid dosage forms is somewhat limited, because drug release (and hence intestinal absorption) is not simply governed by the dispersion/miscibility of the lipid phase with the aqueous medium but also by endogenous processing of the lipid excipients that enhances solubilization of the encapsulated drugs.[2,3] Therefore, studies on the kinetics and dynamics of lipid digestion have become increasingly important for characterizing the state of drug solubilization resulting from lipid-based dosage forms.

The development of various *in vitro* lipolysis models[11−13] has fostered more thorough investigations into the physical and chemical changes of various formulation lipids digested under simulated gastrointestinal conditions. The intestinal phase behavior of lipid digests is well understood: the biliary secretions interact with the lipid digestion products to generate a series of colloidal species (lamellar/hexagonal/cubic liquid crystals, uni-/multilamellar vesicles, simple/mixed micelles) that provide varying degrees of solubilizing capacity to drugs according to their lipophilicity.[14] These colloidal reservoirs also facilitate drug diffusion across the nonstirred aqueous layer[15] and create a concentration gradient at the absorptive site to drive drug absorption.[2] The influence of the lipid properties (i.e., digestibility, acyl chain length, degree of unsaturation, lipid class), solubilizing excipients (e.g., surfactants, cosolvents), and the formulation type (e.g., solution, suspension, emulsion, self-emulsifying system) on drug absorption have been investigated using lipolysis approaches, as recently reviewed by Porter et al.[2] Due to the diversity of lipid excipients and their specific and differential effects on gastric emptying, drug solubilization and absorption pathways, it is difficult to establish distinct criteria for the use of certain lipid formulations for a given drug. Thus far, the use of *in vitro* lipolysis models to predict *in vivo* absorption of poorly soluble drugs from different formulations has been attempted with some degree of success.[16−19] However, there is little evidence that lipolysis models can be used to establish higher levels of *in vitro*−*in vivo* correlations (IVIVC) in comparison with dissolution approaches.

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The need to employ surfactants to facilitate the dispersibility of lipid-based formulations often poses issues with toxicity.[20] The development of formulation approaches that take advantage of the solubilization and bioavailability enhancement from dispersed lipid formulations, but do not require the addition of potentially toxic surfactants, is therefore highly desirable. Recently, nanoparticulate vehicles have received considerable interest for controlled and/or targeted drug delivery via various administration routes.[21,22] Silica-based materials have been demonstrated to be excellent particulate carriers for a variety of poorly soluble drugs, mainly via preservation of the drug amorphous form and increased wettability, which lead to enhanced dissolution kinetics.[23−25] By coupling the solubilizing effect of lipids and stabilizing/protective effect of inorganic nanoparticles, we developed a novel silica−lipid hybrid (SLH) microcapsule system for oral delivery of poorly soluble drugs. SLH microcapsules are composed of nanostructured internal porous matrices, with pore sizes ranging from 50 to 500 nm.[26,27] In previous work, we have demonstrated improved oral absorption of a model poorly soluble drug, celecoxib (aqueous solubility 3−7 μg/mL at pH 7.0, 40 °C), when formulated with SLH microcapsules as compared to aqueous suspension, lipid solution, submicrometer emulsion and a dry emulsion stabilized by maltodextrin.[26] Dissolution characteristics were examined and it was proposed that enhanced drug dissolution or dispersion was one of the underlying mechanisms for the improved absorption. However, a limited IVIVC was obtained from the simple dissolution method, in which a synthetic surface active agent (sodium lauryl sulfate) was employed to mimic *in vivo* sink conditions. This suggests the need to adopt an alternative *in vitro* model to further explore the mechanistic aspect of the SLH formulations, and to better predict the corresponding *in vivo* performance.

In this work, the role of an inorganic solid carrier, namely hydrophilic fumed silica, in controlling the digestion kinetics of emulsified lipid systems was explored. The digestion kinetics of a series of medium-chain lipid-based systems (i.e., lipid solution, submicrometer lipid emulsion, and two types of SLH microcapsules prepared using different precursors) was investigated using a well-established lipolysis model. Silica particles are known to be chemically stable and "stealthy" in biological environments due to their intrinsic hydrophilicity.[21] Mechanisms of emulsion stabilization by colloidal silica particles have been well documented.[28−31] Yet the question remains whether the presence of silica particles would inhibit the digestion of lipid emulsions/redispersed lipid-containing microcapsules due to physical blockade of the oil−water interface. Such control is essential in manipulating the release of drugs into the solubilized aqueous environment with minimized precipitation. The current investigation also provides insight into the utility of the selected lipolysis model in establishing a higher level of IVIVC for these novel nanoparticle-stabilized lipid formulations.

2. Materials and Methods

2.1. Materials. Celecoxib (CEL, 99.0%) was purchased from ChemPacific (USA). Materials used for formulation development include caprylic/capric triglyceride (Miglyol

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812), soybean lecithin (containing >94% phosphatidylcholine and <2% triglycerides), and hydrophilic fumed silica (Aerosil 380), which were obtained from Hamilton Laboratories (Australia), BDH Merck (Australia), and Evonik Degussa (Germany), respectively. The fumed silica has an average primary particle diameter of 7 nm, BET surface area of 380 m^2/g and 2.5 silanol groups per nm^2. Chemicals used for the lipolysis study include sodium taurodeoxycholate (NaTDC) 99%, 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 dihydrate and sodium hydroxide pellets, which were purchased from Sigma Chemical Co. (USA). The titration solution of 0.6 M NaOH was diluted from a 1 M NaOH stock solution (Titrisol, Merck, Germany). 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 Lipid-Based Formulations. Two types of SLH microcapsules, designated as SLH-A and SLH-B, were prepared according to the previous method.[26] Briefly, o/w emulsions containing the following compositions (% w/w) were homogenized under a pressure of 1000 bar for 5−6 cycles (Avestin EmulsiFlex-C5 Homogenizer): 1% CEL, 0.6% soybean lecithin, and 10% Miglyol oil. In the case of SLH-B, 0.6% silica (the maximum miscible amount in Miglyol oil) was predispersed in the lipid phase by sonication prior to homogenization of the emulsion. Dispersion of hydrophilic silica in the oil phase has proved to be enhanced by the presence of lecithin, which is an amphiphilic surface active agent.[30] Subsequently, an aqueous dispersion of hydrophilic fumed silica was added to the homogenized emulsions and tumbled for 12 h to produce emulsion-A (containing 5% silica in the aqueous phase) and emulsion-B (containing 5% silica in the aqueous phase and 0.6% silica in the lipid phase), respectively. The silica-stabilized emulsions were spray-dried (Mini Spray Dryer B-290, BÜCHI Labortechnik AG) to form powdery SLH microcapsules under the following conditions: emulsion flow rate 5 mL/min, aspirator setting 10, air flow rate 0.6 m^3/min, inlet and outlet temperature 160 and 65 °C. In parallel to this, liquid submicrometer emulsions containing the same lipid and lecithin compositions, with fumed silica dispersed in the aqueous (0−50% relative to oil content) or lipid (0−6% relative to oil content) phases were prepared using the same homogenization method without spray-drying.

2.3. Physical Characterization. The particle sizes and zeta potential of liquid emulsions and SLH microcapsules redispersed in phosphate buffer (0.05 M, pH 7.2) were analyzed by using dynamic light scattering (DLS) and phase analysis light scattering (PALS), respectively (Malvern Zetasizer Nano). Additional particle sizing by laser diffraction analysis was undertaken to confirm the absence of larger particles. The cross-sectional structure of the SLH microcapsules was examined by a FEI FIB201 focused ion beam instrument, as described previously.[27] The BET surface area and pore diameters of the spray-dried silica and oil-free microcapsules (in which the oil content was extracted with hexane prior to analysis) were examined by nitrogen gas adsorption (Micrometrics ASAP 2010 porosimeter).

2.4. *In Vitro* Lipolysis Study. *2.4.1. Preparation of Digestion Medium.* The lipid digestion medium was prepared as previously described.[12] The fasted state phospholipid/bile salt (1.25 mM PC/5 mM NaTDC in the reaction mixture after enzyme addition) mixed micelles were prepared in the following sequence: egg lecithin was dissolved in chloroform, and the chloroform was evaporated under vacuum (Rotavapor-RE, Buchi, Switzerland) to form a thin film of lecithin on the walls of a 50 mL round-bottom flask; NaTDC and digestion buffer (50 mM Trizma maleate (pH 7.5), 150 mM NaCl, and 5 mM $CaCl_2 \cdot 2H_2O$) were added, and the mixture was stirred at room temperature, using a magnetic stirrer, for ∼12 h to produce a transparent (light yellow) micellar solution. Pancreatin extracts (containing pancreatic lipase, colipase and other nonspecific 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 ∼1600*g*, 5 °C) for 15 min. The supernatant was collected and stored on ice until use.

2.4.2. Digestion Experimental Procedure. The progress of lipid digestion was monitored for 60 min by using a pH-stat titration unit (Radiometer, Copenhagen, Denmark), according to the lipolysis protocol as described by Sek et al.[12] Briefly, a known quantity of formulation (equivalent to ∼100 mg lipid in all cases) was dispersed in 9 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 readjusted with 0.1 M NaOH or HCl to 7.500 ± 0.002. Lipolysis was initiated by addition of 1 mL of pancreatin extract (containing ∼1000 TBU of pancreatic lipase activity) into the digestion medium. Free fatty acids (FFA) produced in the reaction vessel were immediately titrated with 0.6 M NaOH via an autoburet to maintain a constant pH in the digestion medium throughout the experiment. The consumption of NaOH was used to quantify the amount of FFA liberated based on the 1:1 stoichiometric reaction ratio. Blank experiments (for micellar solutions) were performed in the same way without the addition of lipid formulations to compensate for the background fatty acid produced by components other than the studied lipid vehicles, which was subtracted from the digestion data of the studied lipids.

2.5. Statistical Analysis. All values are expressed as the mean ± standard error of the mean (SEM). The experimental data from different lipid formulations were evaluated by one-way analysis of variance (ANOVA) with a least significant difference (LSD) post hoc test using the statistical package for social sciences (SPSS version 15.0) software, with the level of significance set at $p < 0.05$.

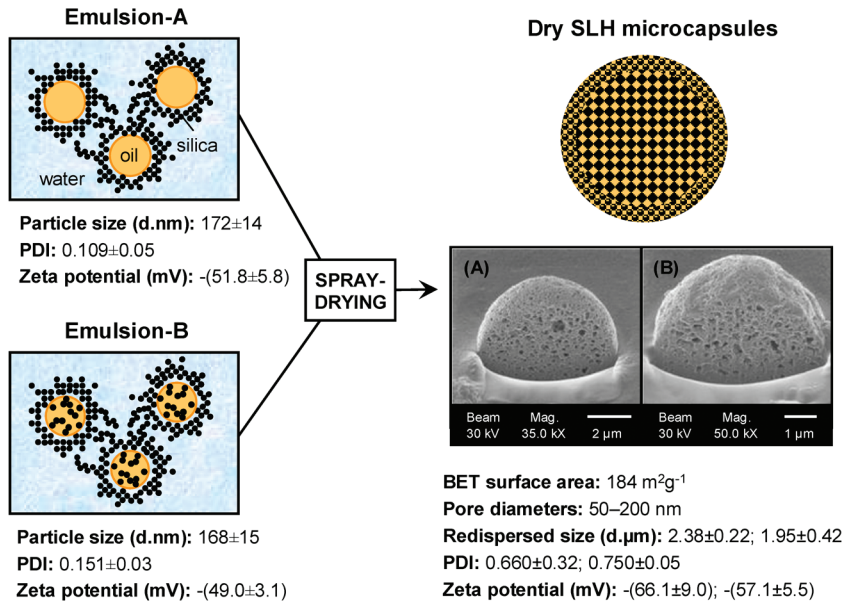

Figure 1. Schematic of SLH microcapsule formation from two different precursor emulsion systems (A, silica in aqueous phase, and B, silica in both aqueous and oil phases) and the colloidal properties of the emulsified systems before/after spray-drying ($n = 3$).

3. Results

3.1. Physical Properties of SLH Microcapsules. The formation of SLH microcapsules from two different precursors, i.e., emulsion-A (containing silica in the aqueous phase) and emulsion-B (containing silica in both aqueous and lipid phases), is schematically depicted in Figure 1. As shown by the ion beam induced secondary electron images of the microcapsule cross section, the internal and external morphology of the SLH microcapsules formed is not affected by the initial location of the silica particles in the precursor emulsions. In both cases, water removal during the process of spray-drying has induced agglomeration of silica into larger units, producing highly porous "spongelike" microcapsules (2−5 μm) cross-linked with or adsorbed by lipid phases. The adsorption or encapsulation of lipid oil onto/within the silica cross-linked network is evidenced by a reduction in the BET surface area from 311 $m^2\ g^{-1}$ (for the spray-dried silica) to 184 $m^2\ g^{-1}$ (for the spray-dried microcapsules). From the SEM images we can visualize pores of sizes in the range 50 to 200 nm, but it is also noted that the silica is microporous and contains pores less than 2 nm. The oil loading levels for SLH-A and SLH-B, as determined by thermogravimetric analysis, were 51% (w/w) and 42% (w/w), respectively, with corresponding oil entrapment efficiencies of 100% and 92%.[26]

In contrast to the precursor submicrometer emulsions (<0.2 μm), the SLH microcapsules redispersed to form micrometer size heterogeneous dispersions: average diameters measured using DLS were 2.38 ± 0.22 μm (polydispersity index, PI 0.66 ± 0.32) and 1.95 ± 0.42 μm (PI 0.75 ± 0.05) for SLH-A and SLH-B, respectively. The presence of particles bigger than 10 μm was negligible, as confirmed by laser diffraction analysis.

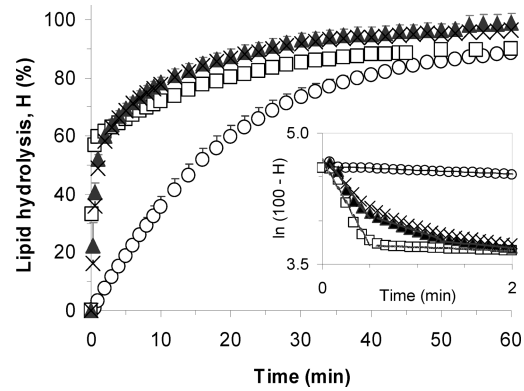

Figure 2. Time dependence of the lipase-mediated digestion for various lipid-based formulations under simulated fasted intestinal conditions ($n = 3$): lipid-lecithin solution (○), lipid emulsion (□), SLH-A (▲) and SLH-B (∗). The inset graph represents the pseudo-first-order descriptions of the lipolysis process for each formulation. Data points were collected for every 5 s; for clarity, only a subset of data points is shown in each case.

3.2. *In Vitro* Lipolysis. *3.2.1. Digestion Kinetics of Various Lipid-Based Vehicles.* Figure 2 depicts the percentage of lipid digestion as a function of time for samples of lipid solution (in which lecithin was predissolved at a lipid:lecithin mass ratio of 1:0.06, similar to that of emulsions and SLH microcapsules), lipid emulsion and SLH microcapsules. The inset graph shows the pseudo-first-order descriptions of the lipid hydrolysis process. Under simulated fasted intestinal conditions, lipolysis of the lipid solution occurred at a relatively slow and constant manner and the entire process can be well-described by a first-order kinetic model ($R^2 > 0.99$). In contrast, the studied emulsified systems

Table 1. Lipolysis Characteristics of Various Medium-Chain Lipid-Based Formulations: Lag Phase Duration, Initial Hydrolysis Rate Constant, and the Maximum Percentage of Hydrolysis (at 60 min) ($n = 3$)

		hydrolysis rate constant, k ($min^{-1} \times 10^{-1}$)			
lipid formulations	lag phase duration (s)[a]	phase I	phase II	phase III	max hydrolysis, H_{max} (%)[a]
lipid solution (no lecithin)	17.5 ± 7.5	0.75 ($R^2 = 0.996$)			97 ± 0.8
lipid solution (lipid:lecithin 1:0.06)	22.5 ± 7.5	0.45 ($R^2 = 0.998$)			89 ± 0.4
lipid emulsion (170 ± 15 nm)	5.1 ± 0	19.3 ($R^2 = 0.960$)	0.41 ($R^2 = 0.999$)		90 ± 0.2
SLH-A (2384 ± 222 nm)	10.0 ± 0	14.8 ($R^2 = 0.963$)	3.61 ($R^2 = 0.988$)	0.78 ($R^2 = 0.992$)	99 ± 3.3
SLH-B (1955 ± 422 nm)	6.7 ± 1.7	11.9 ($R^2 = 0.995$)	4.7 ($R^2 = 0.984$)	0.81 ($R^2 = 0.982$)	96 ± 1.5

[a] $p < 0.03$.

demonstrated a typical time-dependent reduction in the hydrolysis rate during digestion, in which biphasic kinetics was observed for lipid emulsion, and triphasic kinetics for SLH microcapsules. The plots clearly revealed relatively faster initial rates of lipolysis for both lipid emulsion and SLH microcapsules as compared to the lipid solution. In all cases, the lipids were hydrolyzed near to completion (>89%) after 1 h of reaction, regardless of the lipid droplet sizes and homogeneity.

The lipolysis characteristics of each formulation are evaluated based on (i) the lag time, i.e., the period of time elapsed between the time of enzyme addition and the time of detecting lipid hydrolysis; (ii) the pseudo-first-order hydrolysis rate constants (k), which were calculated employing data points of every 5 s up to 10 min; and (iii) the maximum percentage of lipid hydrolysis (H_{max}) obtained at the end of each experiment (i.e., at 60 min). These parameters are used as an indirect measure of the susceptibility of each formulation to enzymatic degradation. The results are summarized in Table 1.

The detection of a lag phase is common for lipase-mediated hydrolytic reactions, mainly due to slow penetration/adsorption of the enzymes from the aqueous continuous phase before they become catalytically active at the interface.[32] The digestion kinetics of lipid solutions in the presence and absence of soybean lecithin was studied. The presence of lecithin does not alter significantly the digestion profile of a lipid solution, but a reduction in the rate constant (from 0.075 min^{-1} to 0.045 min^{-1}) and the extent of lipolysis (from 97% to 89%) was observed. The lag phase duration for lipid emulsion and SLH microcapsules was 2−4-fold lower than that observed for the lipid solutions ($p < 0.03$), suggesting a faster initiation of digestion for the emulsified systems, presumably due to the greater interfacial area available for lipase/colipase binding. Upon lipolysis initiation, the lipid emulsion was digested at the highest rate (1.93 min^{-1}) compared to other formulations, but the reaction was quickly retarded to a rate resembling that of a lipid−lecithin solution after 50−60% of lipolysis. In contrast, both SLH microcapsules were digested in a three-phase manner: an intermediate early digestion rate relative to that of the lipid solution and emulsion was observed (described as phase I), followed by a short "transition rate" when lipolysis reached ~50% (phase II), and the reaction ended with a rate similar to that of a pure lipid solution (phase III). It is clear that SLH-A and SLH-B were not significantly different from one another in their lipolysis behavior despite the different initial silica composition in the lipid and aqueous phases. This might be explained by their similar internal and surface morphology after the spray-drying process, which also rendered a consistent pattern in their dissolution profiles and *in vivo* absorption parameters for the model poorly soluble drug, celecoxib (CEL).[26]

3.2.2. Assessing the Inhibitory Effect of Hydrophilic Fumed Silica on Lipid Digestion. The adsorption behavior of the same type of silica at the oil−water interface has been well-characterized and visualized previously: silica nanoparticles are partially/weakly adsorbed at the interface but randomly dispersed in the aqueous phase when added from water; but due to reduced droplet interfacial tension and "hydrophobization" by lecithin, accommodation of silica in the oil bulk phase/interface was enhanced, which resulted in improved thermodynamic stability of the emulsions.[29,30] In the present study, the digestion kinetics of lipid emulsions with similar lipid and lecithin contents, but different concentrations of hydrophilic fumed silica dispersed either in the aqueous or lipid phase, was determined. Figure 3 reveals that the presence of silica in either phase reduced the extent of lipolysis (H_{45min}) of an emulsion by 5−10% ($p < 0.01$), which suggests a significant degree of physical shielding effect. With increasing concentration of silica in the aqueous phase from 10 to 50% (mass ratio relative to the lipid content), there is a small but systematic reduction in the H_{45min} values. Theoretically, the amount of silica particles adsorbed at the oil−water interface is restricted by the electrostatic repulsion between the negatively charged silica (at ~pH 7) and the anionic lecithin-based droplets. As a result, the water-soluble enzymes probably gain access to the lipid interface relatively easily at the beginning regardless of the [silica] present around the droplets, but as lipolysis proceeds, the droplet sizes become smaller and hence the shielding effect of silica particles becomes more noticeable in the latter phase of lipolysis. A similar shielding effect can be achieved when silica particles are dispersed in the lipid phase, but at ~10 times lower silica concentrations (with a miscible concentration limit of 6% relative to oil in the

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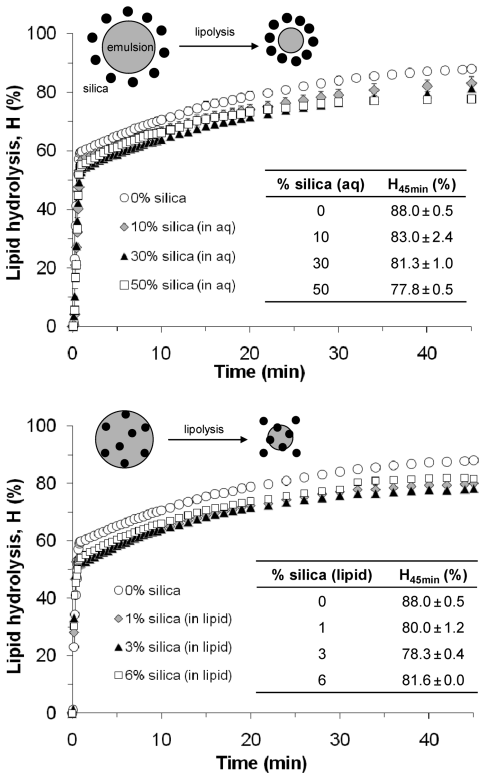

Figure 3. The effect of hydrophilic fumed silica on the digestion profiles of lipid emulsions when silica particles were added into the aqueous phase (upper graph) or into the lipid phase (lower graph) ($n = 3$, $p < 0.01$). The concentration of silica is expressed as percentage value relative to the lipid content in the emulsion systems.

current systems). Curiously in the latter case, the systematic trend of enhanced inhibition with increasing [silica] is less clear; this may be attributed to redistribution of silica into the aqueous phase during digestion since accommodation of silica in the oil bulk phase/interface is highly dependent on lecithin.[30] It is worthy of note that a reduced variation in the H_{45min} values (indicated by smaller SEM values) was observed in both cases when the silica content is increased.

3.3. *In Vitro−in Vivo* Correlation (IVIVC). To assess the *in vivo* predictability of the current lipolysis model, lipid digestion data obtained in this work is correlated with the *in vivo* data for CEL obtained from an orally dosed rat absorption study.[26] The pharmacokinetic data for CEL is summarized in Figure 4. Both types of SLH microcapsules dramatically increased the fasted-state absorption of CEL, giving a superior bioavailability (represented by the area under the curve, $AUC_{0\to\infty}$) and maximum plasma drug concentration (C_{max}) in comparison to lipid solution and submicrometer emulsion. Apparently, differences in the particle sizes of the studied systems did not have a pronounced effect on the extent of CEL absorption, i.e., submicrometer emulsions, though having smaller particle sizes, did not produce better absorption than SLH microcapsules. Previously, linear single-point correlations based on % dissolution efficiencies and absolute bioavailability (F) or C_{max} were successfully established for pure CEL, the

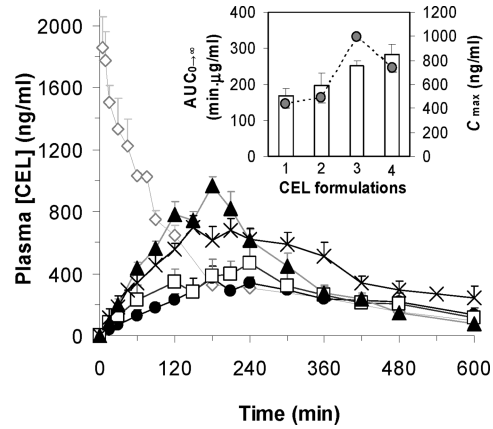

Figure 4. The plasma concentration−time curves and the summarized pharmacokinetic data (inset) of celecoxib in rats following an intravenous dose (◇) and a single oral administration of various lipid-based formulations equivalent to 5 mg/kg celecoxib: lipid solution (●, 1), lipid emulsion (□, 2), SLH-A (▲, 3) and SLH-B (∗, 4) ($n = 5$). In the inset, the bar graphs represent the AUC values, whereas dot points correspond to the C_{max} values.

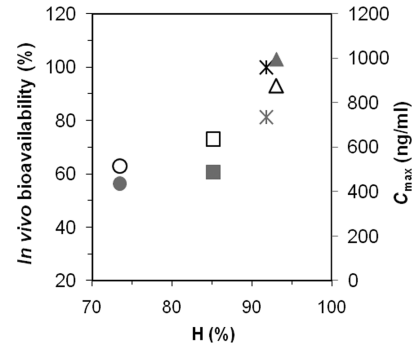

Figure 5. Percentage of lipid hydrolysis (H) at 30 min correlated with the *in vivo* bioavailability (black open markers) and C_{max} values (gray solid markers) of celecoxib following oral administration of the corresponding lipid formulations: lipid solution (circles), lipid emulsion (squares), SLH-A (triangles) and SLH-B (asterisks).

marketed product Celebrex, and SLH microcapsules. Similarly, the percentages of lipid hydrolysis (up to 40 min) from Figure 2 are well-correlated with the F and C_{max} values for the studied lipid systems; representative correlation data employing the middle phase of digestion is presented in Figure 5. There is a strong agreement in the trends of lipid digestibility and absorption improvement for the studied formulations: SLH-A ≈ SLH-B > lipid emulsion > lipid solution ($R^2 > 0.8$).

The lipolysis-based correlation is extended to a multiple-point correlation for individual lipid formulations. Specifically, the percentage of lipid digestion at the early, middle and late stages ($t = 1$, 5, 15, 30, and 60 min) is correlated with the plasma concentration (C_p) of CEL when the absorption rate dominated over the elimination rate, i.e., time prior to reaching C_{max} ($t = 15$, 30, 60, 120, and 180 min)

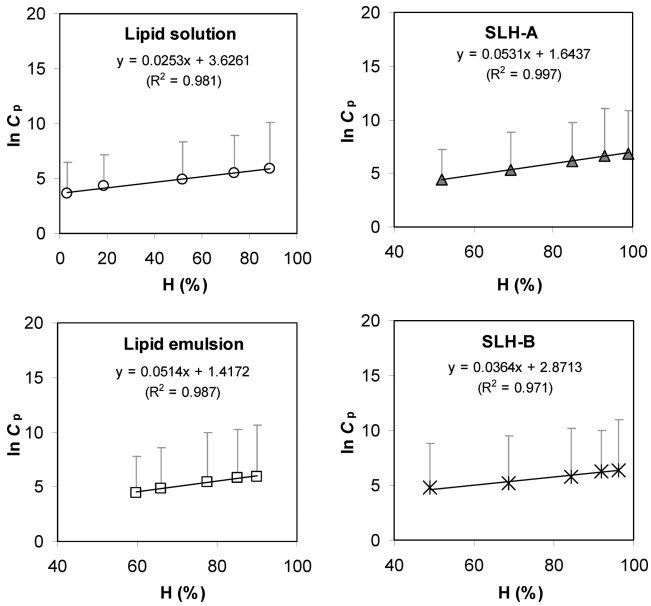

Figure 6. Multiple-point correlation between the percentage of *in vitro* lipid hydrolysis (*H*) obtained at $t =$ 1, 5, 15, 30, and 60 min, and the *in vivo* plasma concentration of celecoxib at a logarithmic scale (ln C_p) at $t =$ 15, 30, 60, 120, and 180 min resulted from oral administration of the corresponding dosage forms.

(Figure 6). There is a log−linear relationship between the percentage of lipid digested and the C_p data obtained at the specified time points. A strong multiple-point correlation was obtained for each of the tested formulations ($R^2 > 0.97$).

4. Discussion

4.1. Factors Affecting Lipid Digestion Kinetics. Lipase-mediated digestion is known to be self-catalytic due to continuous interfacial binding of lipase promoted by the paired amphiphilic cofactor (colipase), despite the desorption effect caused by bile salts.[2] Theoretically, both the rate and extent of lipid digestion are greatly dependent on the accessibility of the enzymes to the substrates, or the binding affinity of a lipid substrate to the enzymes at the open active or catalytic site.[32,33] The susceptibility to digestion is therefore significantly affected by the physicochemical/structural properties of a lipid substrate. Here we assessed the influence of three variables engineered into the current formulations: the significance of emulsification, the effect of a lipidic (lecithin) and nonlipidic (silica) excipient. Out of these variables, our major interest is to investigate the role of hydrophilic fumed silica in influencing the digestibility of lipid formulations. This information provides further mechanistic knowledge concerning SLH microcapsules in enhancing drug release and absorption. Silica nanoparticles, being a relatively "inert" (or stable) solid material, have been shown to provide some extent of physical protection to submicrometer lipid emulsions from enzymatic attack, as shown in Figure 3. This small but systematic inhibition is significantly different from the enhancing effect of SLH microcapsules prepared using silica-stabilized emulsions as the precursors. Despite the relatively large particle sizes (~3 μm cf. ~170 nm), lipids from SLH microcapsules were digested rather quickly at all time points, with complete hydrolysis within 1 h. This does not support our initial hypothesis that the silica nanoparticles may inhibit lipolysis through the formation of a physical barrier at the interface. Apparently, the matrix porosity of the hybrid microcapsule system exerts a positive effect toward lipid digestibility. Specific investigation on the influence of different porosities and internal structures on the lipolysis of SLH microcapsules would be of further interest. Methods or factors controlling the porosity of silica materials have been discussed recently,[34] but the effectiveness of such controls when apply in spray-drying technology has yet to be explored.

Further insight into the current results comes from the finding that emulsification plays an important role in determining the rate of lipid digestion. It is evident that homogenized emulsions have a higher susceptibility to enzymatic degradation as compared to a lipid solution, especially in the early phase of digestion (Figure 2). A markedly reduced lag time, together with a higher initial hydrolysis rate constant, was observed for lipid emulsion (Table 1). This suggested the ease of lipase penetration into the o/w interface when a higher surface area is presented for lipase−colipase binding.[32] However, earlier reports including our previous work[26,35] have shown that medium-chain lipid emulsions, although showing improved *in vitro* dispersion and digestibility compared to crude oils, do not necessarily lead to enhanced bioavailability. Therefore it is important to consider the influence of added nonlipidic excipients on the lipid digestion process (such as silica in our case), as well as the capacity of the digestion products (e.g., free fatty acids, monoglycerides) to solubilize the drug upon release from the digesting vehicles. The presence of lecithin did not favor the digestion of a lipid solution (Table 1). It has been reported previously that lipase−colipase complexes demonstrate a greater selectivity toward simple and mixed micelles formed from lipids and bile salts, but less activity toward liquid crystalline bilayers or vesicles formed by pure lecithin in solution.[33]

4.2. Mechanisms of Digestion of SLH Microcapsules. It is now recognized that formulation of lipids as SLH microcapsules leads to enhanced lipid digestibility, in conjunction with increased drug dispersion and dissolution

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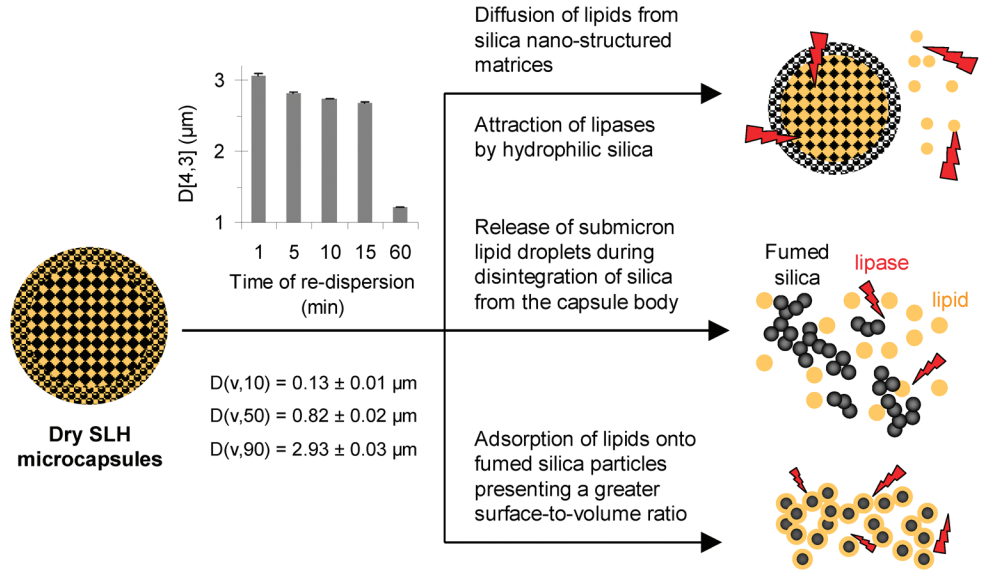
Scheme 1. Schematic Representation of the Possible Fate of SLH Microcapsules under Simulated Intestinal Digesting Conditions (Drawing Not According to Scale)[a]



[a] $D[4,3]$ is the volume-weighted mean diameter; D(v,10), D(v,50) and D(v,90) are the particle diameters at 10, 50 and 90% cumulative size, respectively, after 60 min of redispersion.

as reported previously.[26] The SLH microcapsules were prepared from homogenized submicrometer emulsions (<0.2 μm), but they do not redisperse to form back the original emulsions. The spray-drying process has resulted in hybridization of the lipids with fumed silica, forming a micrometer-sized (2−5 μm) capsule with an internal nanostructured network. The increased digestibility of SLH microcapsules is accredited to the specific porous structure which presents an extremely high surface-to-volume ratio that facilitates lipid vehicle digestion and drug release/solubilization. The three-phase kinetics observed for the SLH microcapsules suggests that there are multiple mechanisms that govern the digestion process. The lipid components could have been presented in different ways for the enzymes to bind and digest, as illustrated in Scheme 1. The high polydispersity of the redispersed SLH microcapsules determined by laser diffraction, i.e., size span >3 and uniformity >3, proposes the possibility of a combination of the following events.

Fumed silica particles readily form a strong cross-link network via the silanol groups after spray-drying; therefore the microcapsules potentially retain the porous structure after being redispersed in the aqueous medium. In this case, the enzymatic reaction would depend on the rate of lipid leakage from the nanopores. Furthermore, silica nanoparticles have been extensively studied for surface immobilization of enzymes for improved catalytic activities.[36−40] Considering that silica may have specific adhesive properties toward cellular surfaces and protein macromolecules through the hydroxyl groups, the cross-linked silica components have the potential to act as a strong adsorption template for lipase−colipase complexes. This would reduce the penetration barrier of the enzymes to the substrate interfaces, and provide control over the propagation of enzymes from one substrate to another along/within the microcapsule network; this renders the microcapsule a digestion pattern different from that of the lipid solution and lipid emulsions stabilized by fumed silica. A previous report[27] has provided a freeze-fracture scanning electron micrograph showing that a majority of the equivalent microcapsules retained their structural integrity after 1 h of lipolysis.

There is also the likelihood that fumed silica, by virtue of its hydrophilic character, becomes partially disintegrated from the microcapsule body when subjected to constant stirring in the aqueous medium. This is evidenced by the presence of smaller particle size fractions, i.e., <0.2 μm (comparable to the original emulsion droplet sizes), and a reduction in particle sizes of the microcapsules as a function of redispersion time (Scheme 1). The disintegrated fumed silica fragments, known to possess extremely high specific surface area capable of adsorbing organic molecules,[40] may serve as an adsorbent for lipids, hence presenting a greater surface-to-volume ratio for substrate−enzyme interaction.

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4.3. Lipolysis-Based IVIVC. The use of lipolysis models to characterize the postdigestive state of the formulation lipids and encapsulated drugs has become increasingly well-recognized and effectively employed to assess or rank the *in vivo* performance of many lipid-based formulations.[16–18] In these previous publications, a single-point correlation was reported between the concentrations of drug solubilized in the postdigestive aqueous phase and the *in vivo* area under the curve (AUC) values. Yet, no report has been presented on correlations based directly on the lipid hydrolysis profiles and drug absorption data. Bearing in mind that drug release takes place immediately following digestion of the lipid vehicles, some extent of correlation should exist between % lipid digestion and % drug absorption especially for drugs demonstrating high intestinal permeability.

Support for this argument is provided in this study by a comparison of % lipolysis (H) with the corresponding CEL in plasma profiles (C_p) at more than one time point. A semilogarithmic plot (Figure 6) evidences a linear relationship between these two parameters at multiple time points. This makes possible the estimation of CEL absorption based solely on the extent of lipid digestion for formulations ranging from a simple lipid solution and emulsions to the more complex microcapsule systems. The nonzero intercepts in each linear curve in Figure 6 suggest that drug release via diffusion can occur before the lipid vehicles are digested. The logarithmic relationship observed between C_p and H can be attributed to a number of time-dependent *in vivo* events. First, in the current *in vitro* test, the % digestion is calculated based on the titratable [free fatty acids], with the assumption that one triglyceride molecule is completely hydrolyzed to produce 1 glycerol and 3 fatty acid chains. This does not take into account the real physiological situation in which triglycerides do not have to undergo a complete hydrolysis for a full absorption to occur. Digested triglycerides can be fully absorbed through the intestinal membrane in the forms of monoglycerides and fatty acids at only 66% hydrolysis.[41] Second, the current method also does not consider the lipolytic effect contributed by other nonspecific catalysts or "minor" enzymes, specifically gastric lipase, which becomes increasingly important at the late phase of *in vivo* intraduodenal lipolysis. Third, although the lymphatic transport pathway capacity for CEL is less clear from the literature, lymphatic transport (~2%) of medium-chain fatty acids has been reported.[42] Essentially, this correlation confirms the significance of lipid carrier digestibility to drug absorption, in which the generated lipid digestion products have crucial influence on the physiological conditions (i.e., formation of highly solubilizing vesicular and micellar phases in the intestinal milieu) and biochemical changes (e.g., increased intestinal permeability).[14,43] Thus far, this correlation method has been attempted for CEL with one selected dose, lipid type and composition, enzyme level, and bile salt concentration, all of which, if modified, would change the result of % lipolysis, hence the correlation pattern. Therefore, further evaluation is required to clearly establish the general applicability of the current correlation approach.

According to the U.S. Food and Drug Administration (FDA) guidance, a biowaiver can be justified only if a correlation covering at least three data points from the *in vitro* profiles with any relevant pharmacokinetic parameters is obtained at the same time points.[44] The limitation for the current correlation is the difference in the time scale of the *in vitro* and *in vivo* studies. Since drug absorption does not necessarily occur immediately or as rapidly as the lipid digestion process, an important consideration would be the ability of the resultant digestion products to keep the released drugs solubilized during intestinal transit. Based on previous case studies,[2,45] long-chain triglycerides (LCT) which are digested more slowly may be more appropriate for encapsulating drugs with higher lipophilicity ($\log P > 5$) due to slower drug transfer into the aqueous environment and potential stimulation of lymphatic absorption. For less lipophilic drugs ($2 < \log P < 4$), medium-chain triglycerides (MCT) may be more suitable due to a quicker formation of highly solubilizing species which can support the released drugs in a supersaturated state. Considering that CEL is an intermediate lipophilic compound ($\log P = 3.5$), digestion products from MCT are expected to provide a highly solubilizing environment with the potential to prevent drug precipitation.[46] This aspect will be further elucidated by the study of phase partition of drugs during or on completion of lipolysis experiments, a current focus of studies in our laboratory.

5. Conclusions

A dynamic *in vitro* lipolysis model was effectively employed to highlight the either inhibitory or enhancing effect of hydrophilic fumed silica in lipase-mediated digestion of medium-chain lipid formulations. Fumed silica, when used as a stabilizer for liquid emulsion systems, produced a physical shield around the lipid droplets to retard lipolysis. Such retardation was not observed for SLH microcapsules, but rather an enhancement in lipid digestibility is evident.

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This suggests the significance of the microcapsule porous structure in determining the digestion kinetics. The lipolysis kinetics is well-correlated to the corresponding *in vivo* parameters via multiple-point correlations for formulations ranging from simple lipid solution and emulsion, to solid dosage lipid vehicles such as SLH microcapsules. Thus, the current findings emphasize the reliability of the lipolysis method to correctly interpret the *in vivo* drug absorption profiles, and its applicability to mechanistically characterize the next generation of lipid dosage forms.

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