molecular pharmaceutics

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Dry Hybrid Lipid−Silica Microcapsules Engineered from Submicron Lipid Droplets and Nanoparticles as a Novel Delivery System for Poorly Soluble Drugs

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Received September 16, 2008; Revised Manuscript Received April 9, 2009; Accepted April 9, 2009

Abstract: We report on the fabrication and characterization of dry hybrid lipid−silica nanoparticle based microcapsules with an internal porous matrix structure for encapsulation of poorly soluble drugs, and their delivery properties (*in vitro* release and lipolysis and *in vivo* pharmacokinetics demonstrated for indomethacin as a model drug). Microcapsules were prepared by spray drying of Pickering o/w emulsions containing either negatively or positively charged lipophilic surfactant in the oil phase and hydrophilic silica nanoparticles in the aqueous phase. Effective microcapsule formation is critically dependent on the interfacial structure of the nanoparticle containing emulsions, which are in turn controlled by the surfactant charge and the nanoparticle to lipid ratio. Microcapsules (containing 50−85% oil) can be prepared with 10 times fewer silica nanoparticles when a droplet−nanoparticle charge neutralizing mechanism is operative. Cross-sectional SEM imaging has confirmed the internal porous matrix structure and identified pore sizes in the range 20−100 nm, which is in agreement with BET average pore diameters determined from gas adsorption experiments. Differential scanning calorimetry and X-ray diffraction analysis have confirmed that the model drug indomethacin remains in a noncrystalline form during storage under accelerated conditions (40 °C, 75% RH). Dissolution studies revealed a 2−5-fold increase in dissolution efficiency and significantly reduced the time taken to achieve 50% of drug dissolution values (≥2- or 10-fold) for indomethacin formulated as microcapsules in comparison to o/w submicron emulsions and pure drug, respectively. Orally dosed *in vivo* studies in rats have confirmed superior pharmacokinetics for the microcapsules. Specifically, the fasted state absolute bioavailability (F) was statistically higher ($93.07 \pm 5.09\%$) ($p < 0.05$) than for aqueous suspension ($53.54 \pm 2.91\%$) and o/w submicron emulsion ($64.57 \pm 2.11\%$). The microcapsules also showed the highest maximum plasma concentration (C_{max}) among the investigated formulations ($p < 0.05$). *In vitro* lipolysis showed statistically higher ($p < 0.05$) fasted digestion (75.8% after 5 min) and drug solubilization (98% after 5 min) in digestive products for microcapsules than o/w emulsions. The hybrid lipid−silica microcapsules improve oral absorption by enhancing lipolysis and drug dissolution.

Keywords: Pickering emulsions; particulate emulsifiers; microcapsules; porous materials; poorly water-soluble drugs

Introduction

Various approaches have been used for improving the solubility of poorly soluble drugs. A significant effort has been directed toward optimizing the therapeutic application of drug delivery systems based on lipids. Lipid-based formulations have evolved from simple lipid solutions of drugs to more advanced

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10.1021/mp900063t CCC: $40.75
Published on Web 04/09/2009

oral delivery systems, including self-microemulsifying delivery systems (SEDDS/SMEDDS),[1] solid lipid nanoparticles (SLNs),[2] lipid-based liquid crystalline systems,[3] and dry emulsions.[4−7] Recent reports[8−10] demonstrate that the dissolution rate is not a sufficient parameter for assessment of lipid based formulations. It has been shown that colloid structures formed during the digestion of lipids provide a series of lipophilic phases within which a lipophilic drug may reside during intestinal transit, thereby preventing precipitation and promoting absorption.[8] As digestion products can decisively influence the rate and extent of absorption and hence bioavailability, it is critically important to consider the rate and extent of lipid digestion when formulating lipid based delivery systems for poorly soluble drugs.

Dry emulsions are a new lipid delivery form under exploration for their biopharmaceutical prospects. The procedure for their preparation includes spray drying, lyophilization or rotary evaporation to remove the water phase from liquid o/w emulsion containing solid carriers. Dry emulsions have the ability to (i) increase the dissolution rates and bioavailability of poorly water-soluble drug compounds, (ii) protect the drug against light[7,11] or oxidation[12] and (iii) eliminate the shortcomings of conventional liquid emulsions, in particular physical instability.[4−7,11,12] Published work to date defines dry emulsions as powdery, lipid based formulations from which an o/w emulsion can readily be reconstituted *in vitro* and *in vivo*.[4−7,11,12] Consequently, the stability of dry emulsions is improved while the delivery performance for poorly soluble drugs is regarded as equivalent to emulsion oil droplets.

In this article, we demonstrate that dry lipid capsules stabilized by silica nanoparticles can be engineered not only as a delivery system with properties equivalent to emulsion droplets reconstituted *in vitro* and *in vivo*, but rather as a complex porous nanostructured material with specific properties that enhance the delivery of poorly soluble drugs in comparison with equivalent emulsion droplets. The aim of the study was 2-fold: (i) to present a general strategy for the preparation of dry hybrid lipid−silica microcapsules with a highly porous internal matrix structure from nanoparticle stabilized Pickering emulsions, and (ii) to demonstrate the advantages of such microcapsules and the mechanisms whereby the delivery of poorly soluble drugs are enhanced, namely, optimized encapsulation/loading, the effect of lipolysis on the rate and extent of drug release and *in vivo* bioavailability using a rat model.

Pickering emulsions have attracted extensive attention, not only because of their greater stability in comparison to surfactant-stabilized analogues[13] but also as templates for fabrication of new encapsulation materials with complex hierarchical structures, e.g. filters and catalysts,[14] colloidosomes[15] and hollow spheres.[16] Porous silica materials have a long history of successful use as catalysts,[14] so it is reasonable to propose that similar biocompatible materials will catalyze lipolysis of oils and improve bioavailability of poorly soluble drugs. Furthermore, in recent years nanostructured mesoporous amorphous silica materials have been intensively investigated for biomedical applications and controlled release.[17] Such materials are derived from supramolecular assemblies of surfactants which template amorphous silica during synthesis. Upon removal of surfactants by pyrolysis or dissolution, silica mesoporous matrices are formed and are effective drug carriers due to a pore

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network with high surface area that controls drug loading and release kinetics.[17]

In this work we argue that the high surface area of amorphous silica can be beneficial not only for drug release but also for the catalytic effect of silica matrices on the lipolysis of adsorbed triglycerides and phospholipids. It is also important that our novel lipid based microcapsule delivery system for poorly soluble drugs does not include a high concentration of surfactant(s). Current SEDDS/SMEDDS delivery systems that improve bioavailability by enhancing release and subsequent lipolysis contain at least 25% surfactants.[1,9,18] This raises health safety issues since they act as irritation and sensitizing agents and are clearly not recommended for chronic therapies.[19,20] It is therefore of interest to develop alternative, surfactant-free systems with equivalent efficiency and greater economical viability.

Experimental Section

Materials. High-purity water (Milli-Q) was used throughout the study. Lecithin and oleylamine were supplied by Aldrich (Milwaukee, WI). The oil phase used was fractionated triglyceride coconut oil (Miglyol 812 N) supplied by Hamilton Laboratory (Australia). Pancreatin (porcine) and bile extract (porcine) were purchased from Sigma-Aldrich, USA. 4-Bromobenzeneboronic acid (BBBA) was purchased from Lancaster, Germany. Phosphatidylcholine Epikuron 200 (purity, minimum 92%) was purchased from Degussa, Germany. Fumed silica nanoparticles Aerosil 380 (Degussa, Germany) have a BET surface area of 380 ± 30 m^2 g^{-1}, 2.5 Si-OH groups nm^{-2} (determined from Li-Al-hydride method) and an average primary particle size of 7 nm. Contact angles estimated from enthalpy of immersion data are reported[21] to be 14° (water/air) and 0° (toluene/water). Indomethacin (Sigma Aldrich) was used as a model lipophilic drug; it has a water solubility (pH = 7.2) of 0.768 mg/mL, pK_a of 4.5 and log P of 3.6.

Preparation and Characterization of Pickering Emulsions. Lecithin or oleylamine (0.6 or 1 wt %, respectively) was dissolved in the oil phase (with or without 10 wt % indomethacin), added into the silica nanoparticle aqueous dispersion prepared using an ultrasonic bath (Labec, model J-LTB3) (300W for 2 h) under hand mixing and passed through a high pressure homogenizer (Avestin EmulsiFlex, C50 high pressure homogenizer) for 5 cycles under pressure 5 mbar. Diluted Pickering emulsions were analyzed for size and zeta potential using a Malvern Zetasizer Nano ZS. A freeze−fracture SEM technique (Philips XL 30 FEG scanning electron microscope with Oxford CT 1500 cryotransfer system) was used to image the nanoparticle coated emulsions prior to drying. The methodology, which has been reported previously[22,23] consisted of emulsion cryofixation, fracturing, etching, platinum coating and imaging. Emulsion samples (50 μL) were deposited on a flat copper substrate holder and cryofixed by rapid cooling with liquid nitrogen (−196 °C) in order to reach the vitreous state. Frozen samples were then mounted on a cold table under liquid nitrogen and then inserted into the freeze−fracture equipment at −150 °C and 10^{-6} Torr. A single-edge scalpel blade precooled to −150 °C was then used to induce the fracture. Surface ice was removed during a sublimation step, i.e. by increasing the sample temperature to either −92 or −100 °C for a period of 2−5 min. Great care is necessary during this step in order to avoid droplet disintegration. The fractured and etched sample was then sputter-coated with platinum (∼2 nm), prior to SEM imaging. Energy dispersive analysis of X-rays (EDAX) was used during imaging to elucidate the chemical nature of the observed features.

Preparation and Characterization of Dry Hybrid Lipid−Silica Microcapsules. Nanoparticle containing emulsions (in the absence and presence of encapsulated indomethacin (10 wt % in the oil phase) were spray-dried (Buchi 190 mini spray dryer) at a flow rate of 5 mL/min, aspirator setting 10, air flow 0.6 m^3/min, inlet temperature 160 °C and outlet temperature 85 °C. The XPS spectra of dry microcapsules were recorded using a Kratos AXIS Ultra DLD spectrometer with a monochromated Al Kα radiation source ($h\nu$ = 1486.7 eV) operating at 15 kV and 10 mA. The pressure in the vacuum chamber during the analysis was less than 10^{-10} bar, and the takeoff angle of the photoelectrons was perpendicular to the sample. The areas under selected photoelectron peaks in the spectrum were used to calculate the atomic concentrations. High-resolution (0.1 eV) spectra were then recorded for pertinent photoelectron peaks at a pass energy of 20 eV to identify the chemical state of each element. All the binding energies (BEs) were referenced to the C1s neutral carbon peak at 285 eV, to compensate for the effect of surface charging. The processing and curve-fitting of the high resolution spectra was performed using CasaXPS software. The analysis area was 700 × 300 μm. The oil encapsulation, i.e. oil level within the microcapsules, was determined using thermogravimetric analysis (TA Instruments). Dry microcapsules were heated from 20 to 600 °C at a scanning rate of 10 °C min^{-1} in nitrogen. The oil

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evaporated at 346 °C, and the silica remained thermally stable. After correction for the water content of silica and spray dried silica, the observed weight loss corresponded to the oil content of the dry microcapsules. Oil entrapment efficiency was calculated based on the initial oil:silica weight ratio. The surface structure of the hybrid lipid−silica microcapsules was examined by scanning electron microscopy (JMS-5310LV, JEOL) at an accelerating voltage of 15 kV. The samples were mounted on a double-faced adhesive tape, and sputtered with gold before imaging. An FEI FIB201 focused ion beam instrument was used for sectioning and high-resolution imaging. The instrument is capable of producing a gallium ion beam of between 7 nm (at 1 pA beam current) and 300 nm (at 12 nA) in diameter at 30 keV energy. A platinum organometallic gas injector allows ion beam assisted deposition of platinum over selected areas of the sample, and this facility was used prior to the sectioning in order to alleviate sample charging from the insulating specimens. For sample sectioning, a large ion current (12 nA) was used initially to remove material by sputtering. A finer beam of lower current was then used to "polish" the vertical face of the sample by scanning the beam in a line and moving it progressively up to remove further material. The sample was then tilted to 45° and the polished face imaged using the same ion beam, at a much lower beam current to achieve the high-resolution ion beam induced secondary electron images shown here. BET surface area and pore volume of spray dried microcapsules after oil removal by hexane extraction were determined by N_2 adsorption using a Micrometrics ASAP 2010 porosimeter.

Drug Encapsulation, *in Vitro* Dissolution Tests and Digestion Experiments. The degree of crystallinity of indomethacin after preparation and 6 months storage under accelerated conditions (40 °C and 75% RH) was determined by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRD). DSC was performed using a TA Instruments Q100 differential scanning calorimeter. A 15 mg sample was heated in an aluminum pan at a rate of 278.15 K/min over a temperature range of 25−200 °C, under a flow of dry nitrogen gas (80 mL/min). Instrument calibration was undertaken using an indium standard. Powder XRD patterns were obtained using a Philips (PW 1050/25) X-ray diffractometer with Co Kα radiation (45 kV, 35 mA). The samples were scanned between 10 and 50° (2θ) at a rate of 1.0 °/min. The amount of indomethacin in the microcapsules was determined using a HPLC method (based on a modified previous study[24]). The HPLC system (Hewlett-Packard 1100) consisted of a series G1310A isopump, G1313A auto sampler and G1314A variable UV detector (Shimadzu Corporation, Japan). Separation was achieved using a LiChrospher 100 RP-18 analytical column (5 μm, 100 mm × 4.6 mm i.d.). The isocratic mobile phase consisted of acetonitrile:water (65:35, v/v) containing 0.1% acetic acid. All chromatographic separations were performed at room temperature at a flow rate of 1 mL/min. The column eluent was monitored at a wavelength of 320 nm. The run time of each injection was set at 10 min, and the injection volume was 50 μL. The dissolution tests under sink conditions were performed using a USP paddle system (Vankel) and kept at a controlled 37 °C. Twenty-five milligrams of pure indomethacin powder and dry microcapsules containing 25 mg of pure indomethacin were used for dissolution tests. The dissolution vessel contained 900 mL of phosphate buffer at pH 7.2 to simulate intestinal fluid. The paddle was rotated at 50 rpm. Samples (5 mL) were taken every 15 min for 3 h. The 5 mL removed was replaced using fresh phosphate buffer at 37 °C. Five hundred microliters of the sample was added to an Eppendorf tube and centrifuged (Sigma 1-15 centrifuge, Quantum Scientific) at 8000 rpm for two minutes. Supernatant (200 μL) was removed and placed in another tube. 200 μL of HPLC grade acetonitrile was added. The samples were measured using HPLC.

In vitro digestion experiments were performed as previously described.[25,26] Briefly, 0.1 g of lipid (indomethacin solution in miglyol containing 0.6% lecithin, equivalent submicron emulsion and microcapsule) was dispersed in 20 mL of digestion buffer (50 mM TRIS maleate, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5) containing 5 mM NaTDC and 1.25 mM PC (conditions broadly representative of fasted state intestinal conditions[26,27]). Experiments were performed at 37 °C in a stirred and thermostatted glass vessel and were initiated by the addition of 3 mL of pancreatin extract containing 40000 tributyrin units (TBU) of pancreatic lipase (final lipase concentration of 1000 TBU per mL digest). Lipolysis was followed over 60 min using a pH-stat titration unit (Radiometer, Copenhagen, Denmark), which maintained the pH at 7.5. The fatty acids produced on lipolysis were titrated with 0.6 M NaOH. Aliquots (1.4 mL) were taken from the digestion medium at 5, 10, 15, 30, 45, and 60 min and a lipolysis inhibitor (0.5 M 4-BPB in methanol, 9 μL/mL digestion medium) immediately added to each sample to prevent further lipolysis. Samples were subsequently ultracentrifuged for 30 min at 37 °C and 334000*g* (Optima XL-100K centrifuge, SW-60 rotor, Beckman, Palo Alto, CA) in order to separate the digests into an aqueous phase and a pellet phase. Samples obtained from each separated phase

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were assayed for indomethacin content by HPLC as described previously. Blank digestion experiments were also performed to account for the fatty acids produced on digestion of the lecithin present in the digestion media. Lecithin samples typically contained small quantities of glycerides (1% w/w triglyceride; 6% w/w diglyceride, <1% w/w fatty acids) and PC which is hydrolyzed by phospholipase A_2 (present in pancreatin) to produce fatty acids and lyso-PC. Blank digestion experiments were therefore performed in the same manner as that described above, but in the absence of the added formulation. The digestion data obtained for the experimental formulations were subsequently corrected for background fatty acid production (i.e., fatty acids derived from the lecithin present in the digestion media) by subtraction of the fatty acids produced during blank digestion experiments.

After dissolution tests for 4 h and lipolysis for 1 h, filtered and air-dried microcapsules were imaged by SEM method described in the previous section. The oil content in dried and filtered macrocapsules after 10 min was determined by TGA (see previous section).

***In Vivo* Bioavailability Studies.** All animal experiments were approved by the Animal Ethics Committee, Institute of Medical and Veterinary Science (Australia). Groups of 5 male Sprague−Dawley rats weighing 320 ± 20 g were used for the absorption study. One group was dosed intravenously with 1 mL of 1.78 mg/kg indomethacin in PEG 400/saline (2:1 v/v) solution, while the other groups were administered orally with one of the following formulations at the same dose by oral gavage (1 mL): indomethacin aqueous suspension, indomethacin o/w liquid lecithin based submicron emulsion and indomethacin microcapsules. Pure indomethacin powder was suspended in 0.25% (w/v) sodium carboxymethylcellulose, while microcapsules were redispersed in Milli-Q water at a suitable concentration. The cannulated rats were fasted overnight (14 ± 1 h) prior to each oral dosing and were given access to food 4 h postdose, with water accessible at all times. Blood samples (0.2 mL) were collected from the jugular vein at designated time intervals 0.083, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 24 h postdose, and the cannula was flushed with an equal volume of heparinized normal saline (50 units/5 mL) to prevent blood clotting. Blood samples (about 200 μL) were collected in heparinized 1.5 mL polythene tubes immediately at different times after dosing, and centrifuged at 800 rpm for 10 min at 4 °C. An aliquot of 100 μL of plasma sample, 10 μL of acemetacin as an internal standard (IS, 20 μg/mL) was mixed for 30 s. After addition of 200 μL of acetonitrile with 0.1% acetic acid, the mixture was vortex-mixed for 1min and centrifuged at 10000*g* for 10 min to remove proteins. Indomethacin content in the supernatant was determined by HPLC. The chromatographic separation was performed using an Alltech Lichrospher 100 RP-18 (4.6 mm × 250 mm, 5 μm) analytical column. The mobile phase consisted of a mixture of 0.1% (v/v) acetic acid in methanol: acetonitrile:distilled water (60:20:20 v/v), ultrasonically degassed prior to use. The mobile phase was delivered at a flow-rate of 1.0 mL/min, the detection wavelength was 320 nm, the attenuation was 0.001 and the injection volume was 20 μL. The pharmacokinetic parameters were determined using the PC software, WinNonlin Standard Edition Version 4.1 (Pharsight Corp.), employing a noncompartmental model. The maximum plasma concentration (C_{max}) and the time at which C_{max} is reached (t_{max}) were obtained from the individual plasma concentration−time curves. The area under the plasma concentration−time curve from time zero to infinity ($AUC_{0\rightarrow\infty}$) was calculated using the linear trapezoidal rule. The values of $AUC_{0\rightarrow\infty}$ obtained were used to estimate the absolute bioavailability (F) according to eq 1:

$$F = \frac{AUC_{oral}(h \times mg/mL)}{AUC_{IV}(h \times mg/mL)} \times \frac{dose_{IV}(mg)}{dose_{oral}(mg)} \quad (1)$$

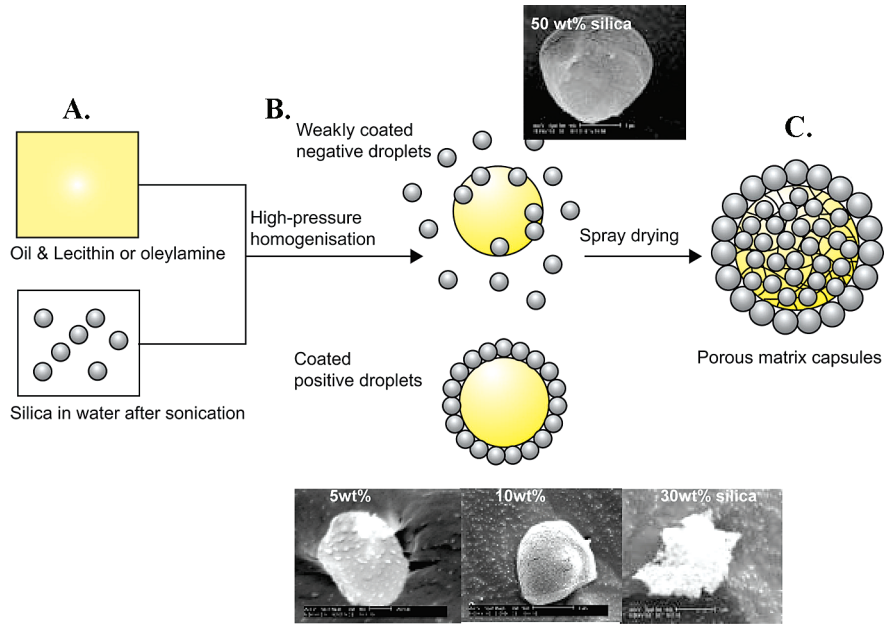
The experimental data from different formulations were analyzed statistically by one-way analysis of variance (ANOVA) coupled with the least significant difference (LSD) t test using the statistical package for social sciences (SPSS version 15.0) software, with the level of significance set at $p < 0.05$.

Results and Discussion

Formation of Hybrid Lipid−Silica Microcapsules. A schematic for the fabrication of hybrid lipid−silica microcapsules is shown in Scheme 1. First, the oil/lipid phase (e.g., a medium chain triglyceride) containing either a negatively or positively charged lipophilic surfactant with HLB < 10 (e.g., lecithin or oleylamine, respectively) is emulsified with an aqueous dispersion of hydrophilic silica nanoparticles (average diameter of 50 nm). The nanoparticle containing emulsions are then spray dried to obtain micron-sized microcapsules with a porous matrix structure and a number of novel pharmaceutical properties.

Effective microcapsule formation is critically dependent on the interfacial structure of the nanoparticle containing emulsions, which is in turn controlled by the surfactant charge and nanoparticle to lipid ratio. Lecithin confers negative charge to the emulsion droplets, and the presence of silica nanoparticles (zeta potential, $\zeta = -27 \pm 2$ mV) has no significant effect on the droplet size and ζ (Figure 1). Investigations[23] on droplet−nanoparticle attachment indicate the presence of bare and partially coated negative droplets even if the silica nanoparticles are present in amounts that are well above the level for monolayer formation. A typical image of a negative droplet in the presence of 50 wt % silica is included in Scheme 1. By contrast, positive droplets (oleylamine containing) are electrostatically coated by oppositely charged silica nanoparticles (Figure 1). The interaction is dependent on the nanoparticle-to-lipid ratio: (i) droplets are positively charged up to 5 wt % silica, (ii) droplets are neutralized and aggregated (to ~4.5 μm) at 6−10 wt % silica and (iii) droplets are charge reversed and slightly aggregated (to ~1.5 μm) at ≥10 wt % nanoparticles. Freeze−fracture SEM images (Scheme 1) reveal partial, monolayer and multilayer coatings on the droplets at 5, 10,

Scheme 1. Schematic Illustration of the Fabrication of Hybrid Lipid−Silica Microcapsules Using Pickering Emulsions[a]



[a] (A) Oil phase: meduim chain tryglicerides (10 wt %) with dissolved lipophilic emulsifiers, lecithin (0.5 wt %) or oleylamine (1 wt %) and silica nanoparticles (5−50 wt % relative to oil) are dispersed in water by using sonication. (B) Silica stabilized Pickering emulsions are formed by using Avestin high pressure homogenizer 5 cycles at 5 mbar; negatively charged emulsion droplets are weakly covered by silica and positively charged droplets undergo coating by electrostatics. (C) Lipid hybrid dry microcapsules with internal porous matrix structure are obtained.

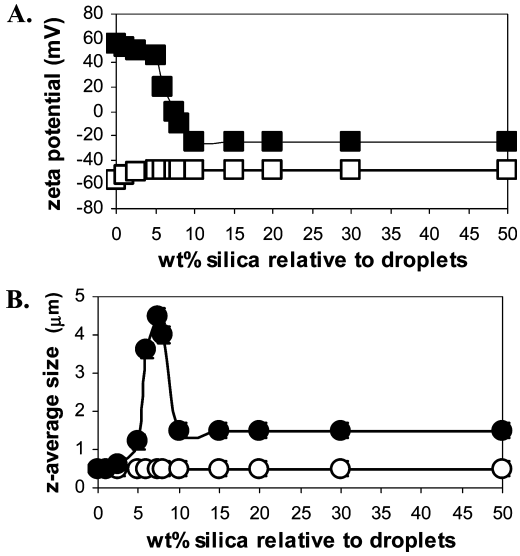


Figure 1. (A) ζ potentials and (B) *z*-average size of negative (open symbols) and positive (closed symbols) Pickering emulsion droplets as a function of silica nanoparticle addition.

and 30 wt % silica, respectively. Theoretically, 40 wt % of silica nanoparticles (~50 nm) is required for a hexagonally close packed monolayer at the interface of ~0.5 μm droplets.[22] Droplet flocculation occurs in the neutralized region due to the bridging between oppositely charged patches on partially coated droplets. Multilayer coatings in the charge reversed region reduce the level of size enlargement.

It is significant that free-flowing dry microcapsules with good redispersibility properties can be prepared from both negative and positive droplets. However, positive droplets form microcapsules at 10 times lower levels of silica, i.e. positive droplets require at least 5 wt % silica relative to droplets, whereas negative droplets require a minimum of 50 wt % silica. This difference in behavior is a consequence of the different nanoparticle coating levels of negative and positive droplets. The exact boundary between barrier-controlled and barrierless nanoparticle adsorption (and hence three phase wetting) depends not only on particle and droplet size, surface potentials, Hamaker constant, and hydration forces but also on the hydrodynamic force applied during mixing. For instance, the intensity of stirring has a significant impact on the concentration of particles required to obtain stable emulsions,[27] i.e. higher particle concentrations are required to obtain stable emulsions by mild stirring in comparison with jet-homogenization, where hydrodynamic forces are sufficient for particles to scale the energy barrier to adsorption. In the current study the hydrodynamic forces experienced by droplet−nanoparticle mixtures during high pressure homogenization and spray drying are considered to promote adsorption and facilitate silica nanoparticle transport into the oil phase and hence the establishment of a three-dimensional matrix structure.

Microcapsules from both negative and positive droplets (see SEM images in Figure 2) have diameters in the range 1 to 5 μm. However, the external texture of the microcapsule is dependent on the droplet type and mirrors the emulsion's interfacial structure. That is, negative droplets form microcapsules with smooth surfaces and positive droplets and silica form microcapsules with rough surfaces (i.e., structured nanoparticle surface layers are visible); this is considered a

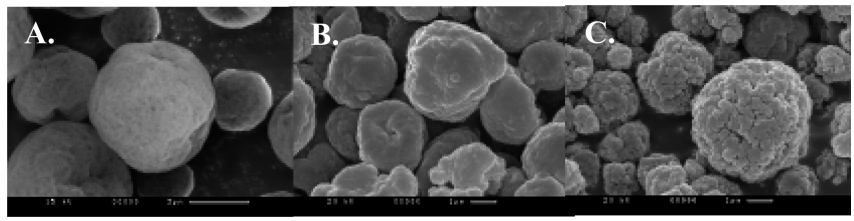


Figure 2. SEM images of hybrid lipid−silica microcapsule prepared by spray drying from (A) negative droplets and 50 wt % silica; (B) positive droplets with 5 wt % silica; (C) charge reversed droplets with 10 wt % silica.

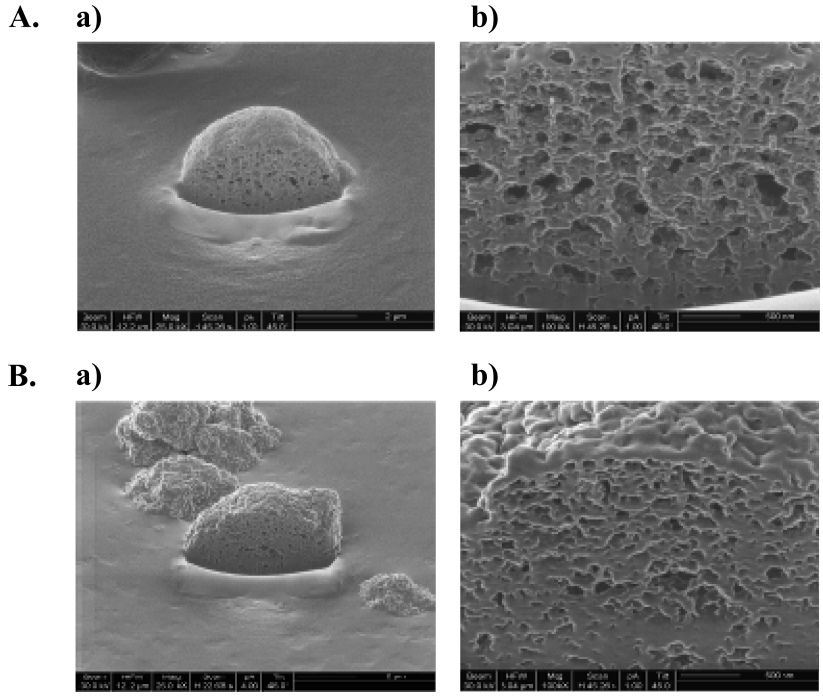


Figure 3. Ion beam induced secondary electron images of hybrid lipid−silica microcapsule cross sections: (A) lecithin based microcapsules with 50 wt % silica relative to oil and (B) oleylamine based microcapsules with 10 wt % silica relative to oil.

consequence of the strong electrostatic attraction between the positive droplets and nanoparticles, hence greater stability of aggregate structures. Cross-sectional SEM imaging (Figure 3) has confirmed the internal porous matrix structure for microcapsules prepared from both negative and positive droplets. In general, the pore size range for microcapsules from negative droplets is in the range from 100 to 500 nm (Figure 3Ab), whereas microcapsules from positive droplets have a higher proportion of 25−100 nm pores (Figure 3Bb). Average pore diameter determined by BET gas adsorption was 20 nm for both spray dried silica nanoparticles and microcapsules after oil extraction. BET surface area for spray dried silica nanoparticles without oil is 311 m^2/g and for microcapsules after oil extraction with hexane 184 m^2/g; the difference in surface areas may be regarded as a consequence of oil entrapment. Considering our previous work[28] which demonstrated a significantly increased contact angle for hydrophilic nanoparticles at the triglyceride−water interface in the presence of lecithin or oleylamine, it is reasonable to propose that the porous structures are formed during spray drying as a result of silica nanoparticles penetrating into the droplets or, alternatively, lipid adsorption by the silica. This is considered to be a consequence of the combination of an increased contact angle at the interface due to the lipophilic surfactant and hydrodynamic forces introduced by spray drying. The three phase contact angle for Miglyol812−water−silica increases from ∼110° to 159° ± 1° and 162° ± 3.4° in the presence of lecithin and oleylamine, respectively.[28] In addition, we also prepared microcapsules from Pickering emulsions that contain 5 wt % silica nanoparticles in the oil phase, and such microcapsules have equivalent internal structures.

Hydrophilic nanoparticles do not generally adsorb at the surface of emulsion droplets and consequently do not stabilize emulsions.[29−32] Hydrophilic silica nanoparticles are an attractive encapsulating material especially for drug delivery purposes due to their high level of biocompatibility;

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Table 1. Characteristics of Dry Hybrid Lipid Microcapsules

					redispersibility			
					pH = 2		pH = 7	
sample[a]	Si2p (%)[b]	N1s (%) 398.59 eV	C1s (%) 283.39 eV	oil loading[c]	*z*-average size (μm)	zeta potential (mV)	*z*-average size (μm)	zeta potential (mV)
L50%	11.35							
	100 0	0	53.45	50.67	1.6	-18.3 ± 8.76	3.05	-28.7 ± 10.7
OA5%	5.19							
	91.9 8.03	0.16	72.69	80.44	1.74	$+72.5 \pm 9.8$	3.71	$+11.6 \pm 14.1$
OA10%	11.14							
	96.8 3.12	0.082	56.76	78	0.603	$+71.7 \pm 15.4$	2.15	-8.11 ± 6.26
OA15%	11.53							
	100 0	0	52.49	74.5	1.2	$+68.8 \pm 15$	2.3	5 ± 6.2
OA20%	15.5							
	100 0	0	48.85	84.27	1.47	$+65.9 \pm 27.4$	3.14	6.02 ± 7.44
SDS	27							
	100 0	0	4.87		1.5	-5.42 ± 3.28	2.5	-25 ± 8.6
HS	27.33							
	100 0	0	3.67					

[a] Sample labels: L, lecithin based; OA, oleylamine based; SDS, spray dried silica; HS, heated silica to 160 °C and cooled to room temperature; numbers denote wt % silica relative to droplets in the aqueous phase. [b] Numbers on left-hand side, SiO_2 matrix at 102.92 eV; right-hand side, silicates at 101.63 eV. [c] Determined by thermogravimetric analysis.

however they cannot be used for encapsulation of emulsion droplets unless an additional driving force for attachment is provided. The reduction of the electrostatic repulsion and hydration interactions between the particles and droplets has been reported as one of the attachment strategies.[33,34] Electrostatic droplet coatings have been explored by Bausch et al.[35] Addition of oppositely charged hydrophilic surfactant may promote particle adsorption at the surface of the oil droplets, but only in the narrow region where droplets and particles are oppositely charged. A surplus of hydrophilic surfactant causes its adsorption at the surface of both droplets and particles and, consequently, diminishes electrostatic droplet−particle electrostatic attraction. The strategy employed here for nanoparticle coating of droplets based on electrostatics uses lipophilic molecules, initially added in the oil phase and located exclusively at the interface[36] so that competitive adsorption at oil−water and nanoparticle−water interfaces is avoided. It should be emphasizes that in our previous work[22] we found that negatively charged silica nanoparticles ~20−50 nm do not spontaneously self-assemble onto the surface of lecithin stabilized droplets due to electrostatic and hydration forces, whereas positively charged droplets are strongly electrostatically coated by oppositely charged nanoparticles.

X-ray photoelectron spectroscopy (Table 1) shows that the microcapsule surfaces are composed of both silica and carbon from the oil phase and emulsifiers. Thus, the microcapsule surface has a dual hydrophilic−lipophilic character. The carbon surface coverage is dependent on the oil:silica ratio for microcapsules based on positive droplets (Table 1). Furthermore, the positions of the Si 2p photoelectron peaks (Table 1) show chemical changes in the surface silica form, i.e. both SiO_2 and silicate are present for microcapsules composed of 5−10 wt % silica, whereas for >10 wt % silica only SiO_2 is observed; these differences are considered to be due to a greater proportion of charge neutralization between silica and the amino groups from oleylamine at lower silica levels (small surface nitrogen XPS signal are also observed).

The oil content (from thermogravimetric analysis) is ~50 wt % for negative and significantly greater (75−85 wt %) for microcapsules prepared from positive droplets (in comparison the spray dried product from hydrophilic silica nanoparticles and oil without emulsifiers which contained ~5 wt % oil). The microcapsules show excellent redispersibility in acidic (pH = 2) and neutral (pH = 7) aqueous media (Table 1); this is equivalent to the redispersion behavior of spray dried silica. In general, the microcapsules redisperse to smaller sizes at pH 2 in comparison to pH 7. The redispersibility of dry microcapsules prepared from

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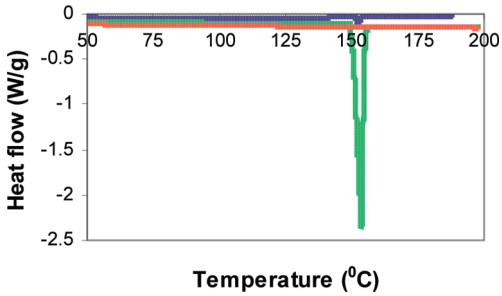

Figure 4. DSC thermograms for pure and encapsulated drug (crystalline drug peak disappears in hybrid lipid−silica microcapsules and is absent during storage under accelerated conditions).

positive droplets mirrors their coating level in the initial Pickering emulsions, i.e. due to surface expression of amino groups. At pH 7 microcapsules prepared from partially neutralized emulsions remain positively charged, whereas those prepared from charge reversed Pickering emulsions are negatively charged when redispersed (Table 1). In acidic media all oleylamine microcapsules are positively charged, with the highest positive value for microcapsules prepared from partially neutralized microcapsules.

Pharmaceutical Properties of Hybrid Lipid−Silica Microcapsules. *Solid State Stability.* The hybrid lipid−silica microcapsules show many properties that are attractive for pharmaceutical application. First, they can encapsulate lipophilic compounds in amounts well above their solubility in the pure oil phase, e.g. the lipophilic drug compound indomethacin has been encapsulated into microcapsules at concentrations several times higher than its solubility in the oil phase. Figure 4 shows the DSC thermograms of the pure drug and encapsulated indomethacin. Indomethacin exhibited a sharp endothermic peak at 159.4°C for the melting of the stable γ-form.[37] The absence of an endothermic peak for microcapsules suggested that the encapsulated drug was molecularly dispersed in the lipid matrices, and no crystalline drug was detected even after 6 months storage under accelerated conditions (75% RH and 40 °C). Considering the limitations of DSC to detect small crystals, we also conducted XRD analysis (Figure 5), which is considered to be more sensitive to the presence of small crystals.[38] XRD analysis clarified the presence of the stable γ-form in physical mixtures[39] and no crystalline drug in the microcapsules after accelerated storage conditions (Figure 5). It is well documented that indomethacin in the stable γ-form is partially

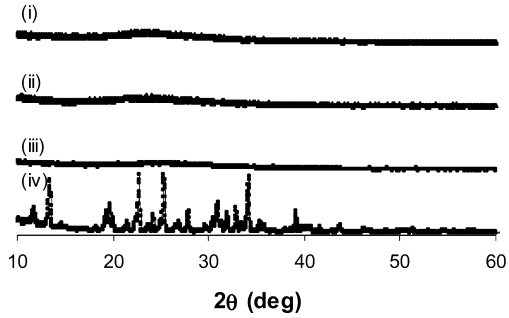

Figure 5. XRD patterns of indomethacin in (i) indomethacin containing hybrid lipid−silica microcapsules after 6 months storage; (ii) indomethacin microcapsules after preparation; (iii) drug free microcapsules; (iv) physical mixture indomethacin:microcapsules 0.01:1.

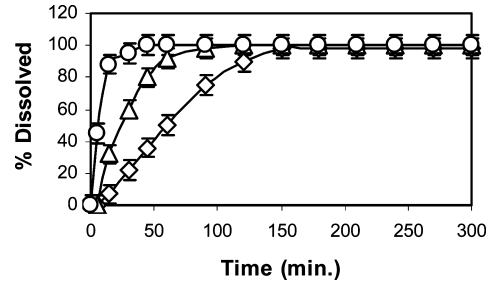

Figure 6. Mean dissolution profiles of indomethacin (25 mg) in phosphate buffer (0.05 M, pH 7.2) under sink conditions: (◇) pure indomethacin; (Δ) o/w lecithin stabilized submicron emulsion; (○) hybrid lipid−silica microcapsules.

transformed into the metastable α-form upon spray drying and is then recrystallized back into the stable γ-form upon storage.[39]

Drug Dissolution in Vitro. The dissolution profiles of indomethacin from o/w emulsions and hybrid lipid−silica microcapsules are illustrated in Figure 6. The pattern of dissolution was found to be consistent for microcapsules with an initial fast release and complete dissolution after 15 min. Pure indomethacin demonstrated the lowest rate of dissolution at all times over a 4 h period. Two parameters were evaluated: the dissolution efficiency (% DE) and the time taken to achieve 50% of drug dissolution ($t_{50\%}$). % DE is the area under the dissolution curve between two specified time points expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time intervals, which can be calculated using eq 2:[40]

$$\%\ \mathrm{DE} = \frac{\int_{t_1}^{t_2} y\ \mathrm{d}t}{y_{100}(t_2 - t_1)} \times 100\% \qquad (2)$$

where y is the percentage of drug dissolved at time t. Taking pure indomethacin as the reference, microcapsules produced a 2−5-fold improvement in % DE at the initial stage (i.e., the first 5−15 min). The value of $t_{50\%}$ was substantially

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reduced by microcapsules (5.0 ± 0.5 min) compared to pure indomethacin (58.8 ± 0.5 min) and o/w emulsion (12 ± 1 min). With the conventional assumption that the rate and extent of drug dissolution directly reflects the concentration of readily absorbable drug in the intestinal lumen, it was proposed that microcapsules have the potential to enhance the oral absorption process. Thus, microcapsules attained more than 85% of drug dissolution within 30 min under appropriate sink conditions, hence meeting the FDA criteria for classification as immediate-release dosage forms.[41] It should be emphasized that the surface area of o/w submicron emulsions, spray dried silica and microcapsules is in good agreement with dissolution profiles (Figure 6). The dissolution kinetics can be fitted into Peppas model:[42]

$$M_t/M_0 = at^n \quad (3)$$

where M_0 is the initial amount of drug in the pharmaceutical dosage form, M_t is the amount of drug at time t, a is a constant incorporating the structural and geometric characteristics of the drug dosage form and the n value is 0.5 for Fick diffusion, i.e. the Higuchi drug release mechanism is applicable if (i) the initial concentration of drug in a molecular form in the system is much higher than the matrix solubility; (ii) perfect sink conditions are maintained; (iii) the diffusivity of the drug is constant and (iv) the swelling of the matrices is negligible. The sink conditions are achieved by ensuring the concentration of the released drug in the release medium reaches no more than 10% of its saturation solubility.[42] Release behavior presented in Figure 6 can be well fitted into diffusion model with $n = 0.5$ ($r = 0.99$). Higuchi release rate constants were calculated to be 12.4, 23.7, and 43.8 min^{-1} for oil/phospholipid solution, o/w submicron emulsion and microcapsules, respectively. BET surface area of the microcapsules after oil extraction (184 m^2/g) and interfacial area of submicron droplets (5.68 m^2/g) can be directly correlated to the release kinetic data.

In Vivo Pharmacokinetics. The oral absorption of indomethacin from the aqueous suspension, o/w submicron emulsion and silica lipid hybrid microcapsules was studied in a fasted rat model, and the corresponding pharmacokinetic data is summarized in Table 2. Indomethacin aqueous suspension gave the lowest bioavailability in fasted rats (53.5%). The pharmacokinetic profile for the o/w submicron emulsion (64.6 ± 2.1%) was not significantly different from that of the aqueous suspension (53.5 ± 2.9%) (Table 2). The microcapsules demonstrated a significant increase in the fasted state bioavailability (93.1 ± 05.1%) as compared to indomethacin aqueous suspension and o/w emulsion ($p < 0.05$). This is different from previous reports[43,44] showing insignificant differences in the AUC, C_{max} and t_{max} of vitamin E acetate formulated in lipid solution, o/w emulsion and a dry emulsion system[43] and nimodipine formulated in liquid and solid dry emulsions.[44]

Table 2. Pharmacokinetic Data for Rats after Administration of 1.78 mg/kg Indomethacin

indomethacin formulations	PK parameters			
	t_{max} (min)	C_{max} (μg/mL)	$AUC_{0\rightarrow}$ (min·μg/mL)	F (%)
iv injection			66.92 ± 6.74	
oral control systems				
aqueous suspension	2.75 ± 0.65	2.48 ± 0.38	35.83 ± 1.95	53.54 ± 2.91
o/w emulsion	1.13 ± 0.25	3.16 ± 1.03	43.22 ± 1.41	64.57 ± 2.11
oral microcapsules	1.83 ± 0.29	4.17 ± 0.75[a]	62.29 ± 3.40[a]	93.07 ± 5.09[a]

[a] Statistically higher than aqueous suspension and o/w emulsion ($p < 0.05$).

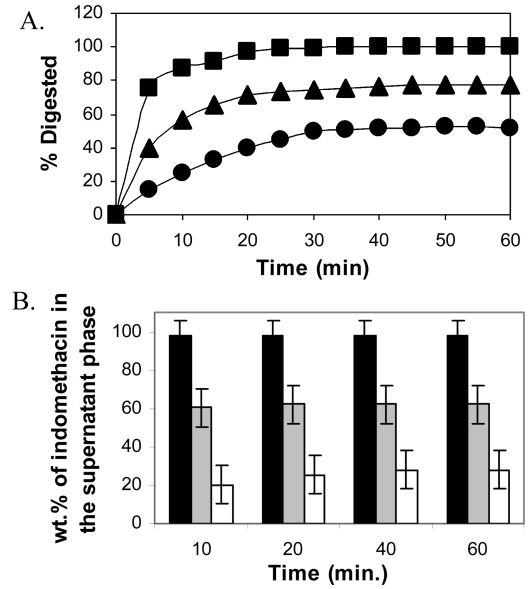

Figure 7. (A) Lipid degradation of ■ lecithin stabilized hybrid lipid−silica microcapsules; ▲ equivalent o/w submicron emulsions; ● lipid solutions. (B) Indomethacin content in the supernatant during lipolysis: black bar, lecithin stabilized microcapsules; gray bar, equivalent o/w submicron emulsions; white bar, lipid solutions.

Statistical analysis showed that microcapsules resulted in a superior C_{max} compared to the aqueous suspensions and o/w submicron emulsions ($p < 0.05$). It is apparent that differences in the size (submicron emulsion vs microcapsules) did not have a pronounced effect on the extent of indomethacin absorption, although some previous studies[45,46] have reported that reduced droplet sizes can lead to increased bioavailability. It is clear

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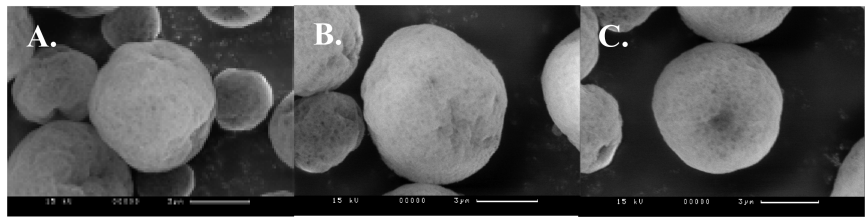

Figure 8. SEM images of dry lipid−silica microcapsules A. before dissolution and lipolysis; B. after dissolution for 4 h; C. after lipolysis for 1 h.

that a novel delivery mechanism is operative for the hybrid lipid−silica microcapsules.

Microcapsules were shown to exhibit a statistically higher C_{max} (4.17 ± 0.75 μg/mL) in comparison to all other tested formulations ($p < 0.05$) (2.48 ± 0.38 μg/mL for aqueous suspension and 3.16 ± 1.03 μg/mL for o/w submicron emulsion). The higher C_{max} and bioavailability provide an opportunity to lower the dose of drugs such as indomethacin that can cause local irritation in the GIT at high doses, hence the incidence of GI bleeding and ulceration would be reduced.[47,48] Our additional studies have shown improved *in vivo* oral bioavailability of celecoxib[49] in comparison to an o/w emulsion and dry emulsions containing maltodextrin as a solid carrier.

Drug Delivery Mechanism of Hybrid Lipid−Silica Microcapsules. It is well established that the *in vitro* dissolution rate is not a sufficient parameter for assessment of lipid based formulations.[8−10] Lipolysis experiments have enhanced the understanding of the changes to solubilization capacity that might occur on lipid digestion, with a series of colloidal structures, including multilamellar and unilamellar vesicles, mixed micelles and micelles. Recent data[25] suggest that lipolysis is a dynamic process of exchange between a lamellar phase formed immediately after initiation of lipolysis and hexagonal phase. The formation of liquid crystalline phases is governed by hydrolytic degradation of oil droplets to mono- and diglycerides and fatty acids. Moreover, it is suggested[8,50,51] that the *in vivo* performance of lipid-based systems is more dependent on the interaction between formulation lipids and lipid digestion products with the secreted bile contents in the intestinal lumen. Three major mechanisms have been described to rationalize how lipid−bile interaction can facilitate drug absorption.[50,51] First, the formation of colloidal and liquid crystalline structures resulting from bile, lipids and other endogenous secretions keeps the poorly soluble drug solubilized in the intestinal fluid with reduced precipitation. Drugs solubilized in the mixed micellar phases can then diffuse easily through the pre-epithelial unstirred aqueous layer to the absorptive site. The third mechanism involves an alteration in the intestinal permeability induced by the lipid−bile interaction in which the solubilized drug molecules can be absorbed via the paracellular or the transcellular routes. Some highly lipophilic drugs (i.e., log $P \geq 5$ or long chain triglyceride solubility ≥ 50 mg/g) were proposed to be transported into the blood-stream via the lymphatic pathway, hence increasing the oral bioavailability by avoiding the hepatic first pass metabolism.[8] It should be noted that rats do not possess a gallbladder as do humans, therefore there is always a continuous secretion of bile salts into the gastrointestinal tract even without stimulation by lipids or food.[52] This may explain the negligible effect of o/w emulsions in improving the bio-availability of indomethacin in rats.

To further explore the mechanisms of lipid−silica hybrid microcapsule drug delivery, we performed *in vitro* lipolysis in conjuction with drug content analysis. Our data suggest that lipolysis rate and extent of lipolysis are much higher for microcapsules than equivalent submicron droplets or lipid solutions (Figure 7A). Partial lipolysis of submicron lecithin stabilized droplets and lipid lecithin solutions may be explained by formation of viscous liquid crystalline phases at the droplet surfaces that prevent further lipolysis. Moreover, indomethacin content in supernatant soluble phases is significantly higher for microcapsules in comparison with o/w submicron droplets and lipid solutions (Figure 7B). Microcapsules were imaged after 4 h of dissolution and 1 h of lipolysis (Figure 8). It can be seen that capsules retain their structural integrity, so it is reasonable to assume silica nanoparticles do not penetrate into enterocites, i.e. they are considered inert porous structures. Furthermore, thermogravimetric lipid content measurements showed that microcapsules completely lose their lipid content after 10 min of dissolution and lipolysis. This is a confirmation that hybrid

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lipid−silica microcapsules represent a novel delivery system for poorly soluble drugs and that they are not equivalent to simple mixtures of submicron droplets and silica matrices. If this were the case, the lipolytic profile would be the same for submicron droplets and microcapsules. Based on the presented results we may hypothesize that oil and phospholipid are adsorbed within the silica amorphous matrix structure and upon contact with dissolution/digestion medium nanodroplets are released and undergo faster lipolytic degradation than submicron droplets, and hence improved adsorption *in vivo*.

Conclusion

A new class of hybrid lipid−silica microcapsules based on Pickering emulsions as the initial templates is reported. The microcapsules exhibit an internal porous matrix structure composed of oil attached to a silica matrix by lipophilic negatively or positively charged surfactants. Surfactant charge has a profound effect on microcapsule formation, as microcapsules can be fabricated with ten times fewer nanoparticles when a charge neutralization mechanism is operative. The model drug indomethacin exhibited excellent storage stability under accelerated storage conditions of 6 months. *In vivo* studies in orally dosed rats confirmed statistically higher ($p < 0.05$) bioavailability and C_{max} in comparison with o/w submicron emulsions and an aqueous drug suspension. Enhanced drug release due to the high surface area of amorphous silica matrices and enhanced lipolysis are the principal drug delivery mechanisms.

Acknowledgment. Australian Research Council's Discovery grant scheme (Project DP0558920) is acknowledged for funding.

MP900063T