Use of Ordered Mesoporous Silica to Enhance the Oral Bioavailability of Ezetimibe in Dogs

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ABSTRACT: The aim of this study was to investigate the bioavailability enhancement of the biopharmaceutics classification system class II compound ezetimibe loaded in ordered mesoporous silica (OMS) in dogs. The OMS was characterized as highly ordered mesoporous material with a narrow pore size distribution. Ezetimibe was loaded in OMS via incipient wetness impregnation to obtain a 20% (w/w) drug load, characterized by nitrogen adsorption and differential scanning calorimetry, and formulated in one capsule and two tablet formulations. Physicochemical characterization of loaded OMS indicated that ezetimibe molecules were molecularly deposited on the hydrophilic surface of the OMS. Two *in vitro* dissolution experiments were performed at 37°C in simulated gastric fluid with 0.1% sodium lauryl sulfate or Tween 80 to determine the drug release. All concepts were compared *in vitro* and *in vivo* with the commercially available tablet Ezetrol®. A dog study was designed to determine the oral bioavailability of ezetimibe capsules and tablets. The tablet preparations showed similar results to that of Ezetrol®. The capsule formulation demonstrated a faster absorption into the blood circulation, including a superior metabolization of ezetimibe into the active glucuronide conjugate. *In vivo* evaluation in dogs confirmed the improvement of ezetimibe absorption with the use of OMS as drug delivery technology. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:1136–1144, 2012

Keywords: ordered mesoporous silica; poor solubility; oral bioavailability; *in vivo*; ezetimibe

INTRODUCTION

An increasing number of active pharmaceutical ingredients (APIs) under development do suffer from poor aqueous solubility and dissolution behavior associated with high lipophilicity and/or hydrophobicity, resulting in a low and variable oral bioavailability. In an attempt to overcome the solubility issue, many drug delivery technologies have been developed during the last decades. Reduction of the API particle size, by micronization or nanonization, was among the first techniques that could be applied in development, leading to the commercialization of poorly water-soluble compounds. A more recently developed series of technologies was based on the principle of converting the API from its crystalline state into a high-energy state (amorphous) using technologies such as spray drying or lyophilization. Although these types of formulations often result in increased bioavailability, the potential intrinsic instability of these high-energy-systems may limit its attractiveness toward commercialization due to limited shelf-life claims and extended development timelines. Given those potential limitations and the more than ever water-insoluble nature of the compounds in development, there is a profound need for new formulation platforms. This article describes the use of a silica carrier system, which increases the dissolution rate of APIs to the highest possible level (i.e., molecular) without the stability-related issues of amorphous dispersions or solid solutions.[1,2] The principle of dissolution enhancement originates in the adsorption of an API onto the surface of the carrier material in a molecular manner.[3] Unlike any other carrier material, which might also have a high specific surface to

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allow adsorption, the silica material described in this study has a uniquely designed, orderly mesoporous structure and an extremely high porosity. In the first step, the API is loaded onto the silica via an "incipient wetness technique" using the appropriate organic solvent.[4] Dissolution rate enhancement of the API is caused by the fact that the van der Waals forces and hydrogen bonds that keep the drug molecules adsorbed onto the surface and inside the pore system of the silica carrier are easily broken up from the contact with water. Consequently, detached drug molecules are released in a "dissolved" state and are available for absorption in the gastrointestinal tract.[5] The characteristics of the pore system in silica such as pore diameter, specific surface area, and pore volume can be tailored to accommodate the ideal environment for the drug molecules, taking into account, for example, their molecular size. The most common types of mesoporous silica particles are Mobil crystalline materials-41 and Santa Barbara amorphous-15.[6] Ezetimibe is the first agent in a new class of lipid-lowering compounds that selectively inhibit the intestinal adsorption of cholesterol across the intestinal wall. It reduces elevated total cholesterol, low-density lipoprotein cholesterol, and triglycerides and increases high-density lipoprotein cholesterol. Combination therapy with fibrates, bile acid sequestrants, and statins is often necessary. However, the use of such multidrug combinations is often undesirable because of a potential increased risk of adverse effects, many of which are dose dependent.[7] The aim of this study was to investigate the bioavailability-enhancing properties of the loaded silica in a preclinical setting using the dog as a model and correlate the findings with the *in vitro* behavior.

MATERIALS AND METHODS

Ordered Mesoporous Silica

Ordered mesoporous silica (OMS) material is synthesized according to the synthesis procedure described by Jammaer et al.[8] Typically, a citric buffered Pluronic® P123 (BASF, Ludwigshaven, Germany) solution is prepared overnight. To this surfactant solution, a sodium silicate (NaSi) solution is added and the mixture is stirred. The final synthesis mixture is kept nonstirred 24 h at room temperature followed by 48 h at 75°C. The material is then filtered, washed with deionized water, and dried. Finally the OMS material is calcined under ambient conditions at 550°C.

Nitrogen Physisorption and Calculations

Nitrogen physisorption isotherms were obtained using a Micromeritics TriStar II (Micromeritics instrument Corporation, Norcross, Georgia). The measurements were performed at–196°C and all samples were treated at 30°C for 12 h under nitrogen flow prior to analysis. The total surface area was calculated using the Brunauer, Emmet and Teller model (BET model). The total pore volume of the OMS is directly derived from the adsorption isotherm ($P/P_0 = 0.95$), in which P and P0 are the equilibrium and the saturation pressure of adsorbates at the temperature of adsorption. The mesoporous diameters (nm) were calculated from the desorption branches of the nitrogen isotherms using the Barrett–Joyner–Halenda model (d_{BJH}). This model is based on the Kelvin equation to describe the pore filling through capillary condensation.[9]

Ezetimibe Loading Procedure

The OMS was stored by conditioning in a desiccator in the presence of the dehydrating agent phosphorpentoxide (P_2O_5; VWR Prolabo, Leuven, Belgium). Next, it was dried for 12 h in an oven at 150°C with air circulation and cooled down in a desiccator in the presence of P_2O_5 at room temperature. Loading of ezetimibe onto the OMS was performed via impregnation with an automated granulator (Mi-Pro 900; ProCepT, Zelzate, Belgium). Pure API (4.69 g) was dissolved in 92.0 mL acetone (Chem-Lab, Zedelgem, Belgium) and added to 20.45 g of the OMS in a 900-mL granulation bowl in two dosing stages using an atomization nozzle. Each dosing stage was followed by a drying step (35°C) of 1 h.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) experiments were performed on a Mettler Toledo DSC 822e (Mettler–Toledo, Zaventem, Belgium). The sample was heated from 20°C to 180°C at a rate of 10°C/min. Samples were analyzed in closed aluminum pans with pierced lid prior to analysis (TA Instruments, Brussels, Belgium) under nitrogen purge. The detection limit of crystalline ezetimibe was 0.1 mg.

Drug Load Quantification

In order to determine the assay of the OMS formulations loaded with ezetimibe, approximately 7.5 mg of the formulation was weighed in a volumetric flask of 25.0 mL. Dimethyl sulfoxide (5.0 mL) was added and then filled up with MeOH. The suspension was sonicated for 30 min in a Branson 8200 ultrasonic bath and filtered afterwards using a 0.45-μm polytetrafluoroethylene (PTFE) membrane filter (VWR International, Radnor, Pennsylvania). Of a 5 mL sample, 4 mL was discarded, whereas the last milliliter was used for assay determination by high-performance liquid chromatography (HPLC).

Dosage Form

The ezetimibe (TEVA, Petah Tikva, Israel) loaded formulations were mixed with lactose (DMV-Fonterra Excipients, Tilburg, the Netherlands), microcrystalline

Table 1. Compositions of Ezetimibe-Loaded OMS Tablets and Capsules

	Tablet		Capsule
Dosage Forms	Eq. 10 mg (%)	Eq. 5 mg (%)	Eq. 5 mg (%)
Ezetimibe loaded OMS	31.00	15.50	15.50
Lactose	43.50	55.12	55.12
Microcrystalline cellulose	14.50	18.38	18.38
Sodium croscarmellose	10.00	10.00	10.00
Magnesium stearate	1.00	1.00	1.00

cellulose (MCC; Asahi Kasei Chemicals Corporation, Tokyo, Japan), croscarmellose (FMC Biopolymer, Philadelphia, Pennsylvania), and magnesium stearate (ligamed MF-2V; Peter Greven, Venlo, the Netherlands), see Table 1. Physical mixtures were prepared in an equivalent dose of 10 mg as compared with the commercial available form of ezetimibe, Ezetrol® (concept 1, eq. 10 mg), as well as in half of the prescribed dose. In the case of the latter, the quantities of lactose and MCC were adjusted to end up with the same final weight of the ezetimibe OMS tablet (eq. 10 mg). Two tablet dosage forms (concept 2, eq. 10 mg ezetimibe; concept 3, eq. 5 mg ezetimibe) prepared on an automated single-punch AC27 Courtoy tablet press (Courtoy, Halle, Belgium) with a 9 mm die and one capsule form (concept 4, eq. 5 mg) were evaluated in the *in vivo* dog study together with the commercial available Ezetrol® tablet (eq. 10 mg).

In Vitro Release Study of OMS Loaded with Ezetimibe and Pure Crystalline Ezetimibe

To study the release of ezetimibe out of the OMS carrier, tablet-loaded formulations were suspended in 900 mL 10 mM pH 7 phosphate buffer in the presence of 0.1% sodium lauryl sulfate (SLS; Merck, Darmstadt, Germany). Phosphate buffer (900 mL, 10 mM, pH 7) + 0.1% Tween 80 (Acros Organics, Geel, Belgium) was used for capsules due to the interaction of SLS with gelatin.[10] Dissolutions were performed using a Hanson Vision® Elite 8 dissolution apparatus. The dose of ezetimibe used was equivalent of 10 mg. The paddle speed was set at 50 rpm. The experiment was performed at 37°C. Samples of 1.0 mL were taken at 5, 10, 15, 30, 60, and 120 min and filtered over a 0.45-μm PTFE membrane filter (GRACE, Columbia, Maryland). The volume withdrawn was replaced by the same amount of fresh medium. The samples were analyzed using HPLC.

High-Performance Liquid Chromatography

High-performance liquid chromatography measurements of assays and *in vitro* dissolution samples were performed using an isocratic HPLC method. The HPLC system was a VWR Hitachi Elite LaChrom with a L-2200 ultraviolet detector set at 232 nm. The analytical column used was a Lichrospher 100 RP-18 (125–4.6 mm, 5 μm). An isocratic mobile phase of 34/66% (v/v) 0.05 M pH 3.7 sodium phosphate buffer/MeOH with a flow rate of 1 mL/min was used. The column temperature was 40°C. The injection volume was 20 μL.

In Vivo Studies

Pharmacokinetic (PK) parameters were evaluated after single oral administration in male Beagle dogs (8–20 months old, body weight range 9–12 kg; NOTOX B.V., 's Hertogenbosch, the Netherlands). The animals had *ad libitum* access to water. The study protocol was reviewed and agreed by the Animal Welfare Officer and the Ethical Committee of NOTOX (December 00-34) as required by the Dutch Act on Animal Experimentation (February 1997). The comparative bioavailability study was performed according to a cross-over design ($n = 4$). One group of four animals was administered with ezetimibe at a dose level of 10 mg/animal (period 1, concept 1), acting as the reference formulation. The silica concepts were evaluated in doses of 10 mg/animal (period 2, concept 2) and 5 mg/animal (period 3 and 4, concept 3 and 4, respectively). In between the consecutive periods, there was a wash out period of 1 week. From all animals, approximately 3 mL blood samples were taken from the jugular vein using vacutainers and Li–heparin (Greiner Bio-One, Bad Haller, Austria) as an anticoagulant. Blood was sampled at predose and 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after dosing. Within 30 min after sampling, blood was centrifuged at 5°C. Immediately after centrifugation, plasma was stored in labeled polypropylene tubes at–75°C prior to analysis.

The study procedures were based on the following guidelines, recommendations, and requirements:

- Workshop/conference report—Quantitative bioanalytical methods validation and implementation: Best practices for chromatographic and ligand binding assays. C.T. Viswanathan, et al. The AAPS Journal, 9, 2007, E30–E42.
- Guidance for industry: Bioanalytical method validation. US Department of Health and Human Services, Food and Drug Administration, Center

for Drug Evaluation and Research and Center for Veterinary Medicine. May 2001.

A bioanalytical method was used for the quantification of ezetimibe and ezetimibe glucuronide in dog Li–heparin plasma in the range of 20.0–5000 pg/mL. Following oral administration, ezetimibe is absorbed and extensively conjugated to its active metabolite ezetimibe glucuronide. The method was based on HPLC with tandem mass spectrometric detection. Ezetimibe glucuronide was measured by converting ezetimibe glucuronide to ezetimibe by adding β-glucuronidase to the sample. The result gave a total ezetimibe concentration. Ezetimibe glucuronide concentration was calculated by subtracting free ezetimibe concentration from the total ezetimibe concentration.

All PK parameters were calculated from the curves constructed from individual animals. Noncompartmental analysis was applied using the extravascular model. The lower limit of quantification (LLOQ) of FP-0220 and ezetimibe in plasma was 20 pg/mL. All values below the LLOQ after the maximal plasma concentration (C_{max}) were excluded from the PK evaluation. Any value below the LLOQ prior to C_{max} was set to 0 ng/mL. An intermediate value below LLOQ (FP-0220, period 2, animal 3, 8 h) was excluded from the PK evaluation. Nominal sampling times were used.

A descriptive statistical analysis was performed (mean values and standard deviations). A one-way ANOVA with a Student Newman–Keuls test was performed for the dose-normalized values of C_{max} and Area under the curve (AUC_{last}) for each compound to determine a possible statistical difference between the various compounds

Stability Program

All concepts, that is, loaded silica powders, capsules, and tablets, were stored under an extensive stability program at conditions of 25°C/60% relative humidity (RH) and 40°C/75% RH. After storage periods of 1, 3, and 6 months, the concepts were evaluated for appearance (visual inspection), water content (Karl Fischer; 870 KF Titrino plus; Mettler–Toledo, Zaventem, Belgium)), assay (HPLCVWR Hitachi Elite), *in vitro* release (Hanson Vision® Elite), and the absence of crystallinity (DSC, Mettler Toledo DSC 822e; Mettler–Toledo, Zaventem, Belgium). Powders, capsules, and tablets were stored in closed packaging (high-density polyethylene bottle with a desiccant in the stopper), whereas the loaded powders were additionally stored in open, unprotected conditions. *In vitro* release tests on the powders were carried out by evaluating equivalents of 10 mg ezetimibe in 125 mL simulated gastric fluid + 0.1% SLS ($n = 3$).

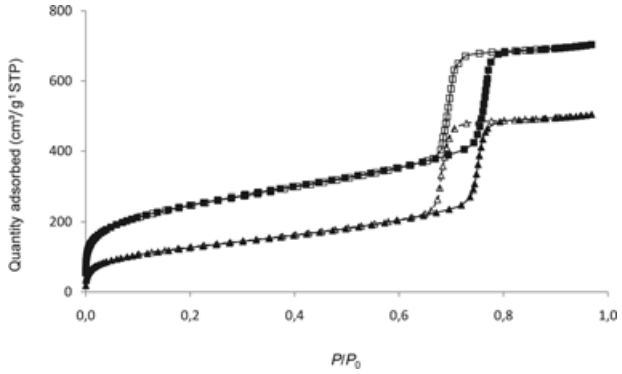

Figure 1. Adsorption (fill) and desorption (no fill) branches of the nitrogen adsorption isotherm of blank (square) and loaded (triangle) ordered mesoporous silica.

RESULTS AND DISCUSSION

Characterization of the OMS material was evaluated by nitrogen physisorption to determine the internal pore structure of the silica. Nitrogen physisorption isotherms of the OMS, before (black curve) and after (blue curve) loading with ezetimibe are presented in Figure 1. The OMS is characterized by a steep hysteresis loop, typically known for silica material with a narrow pore size distribution. The properties of the silica derived from the isotherms are shown in Table 2.

Loading the OMS with ezetimibe significantly changed the porosity of the silica by inclusion of ezetimibe into the porous structure of the carrier. The pore size diameter was calculated to be 7.0 nm, which is large enough to adsorb ezetimibe molecules ($1.52 \times 1.25\,nm^2$). A small drop in the pore size diameter to 6.7 nm was observed when 17.9% (w/w) of the API was incorporated into the pores due to capillary forces. The same change is reflected in the total pore size volume, which decreased from 1.1 to 0.8 cm^3/g. The surface area decreased almost half from 866 to 455 m^2/g. In order to maximize the dissolution rate enhancement, the API that is adsorbed onto the surface of the OMS needs to be in a molecular state. The physical

Table 2. Properties of Blank and Loaded OMS

OMS Properties	Total Pore Volume (cm^3/g)	Surface Area (m^2/g)	d_{BJH}[a] (nm)	Drug Assay (w/w%)
Blank, unloaded OMS	1.1	866	7.0	–
Ezetimibe-loaded OMS	0.8	455	6.7	17.9

[a] d_{BJH}: mesoporous diameter (nm) calculated using the Barrett–Joyner–Halenda model.

state of ezetimibe was assessed using DSC. Thermograms of pure API (heat and reheat) and loaded ezetimibe–OMS samples ($n = 3$) are presented in Figure 2. The crystalline ezetimibe exhibits a clear melting transition at 163°C, and following reheating of the cooled liquidized API, a glass transition can be observed around 70°C. The measurements of the loaded OMS samples did not show any endothermic transition at the designated temperatures of pure ezetimibe, pointing to the absence of bulk properties.

The release profiles are presented in Figure 3. The concentration of pure crystalline ezetimibe in the 900 mL 10 mM pH 7 phosphate buffer + 0.1% SLS was 0.26 mg after 5 min. After 1 h, a steady-state was reached and lasted for another hour, whereas the

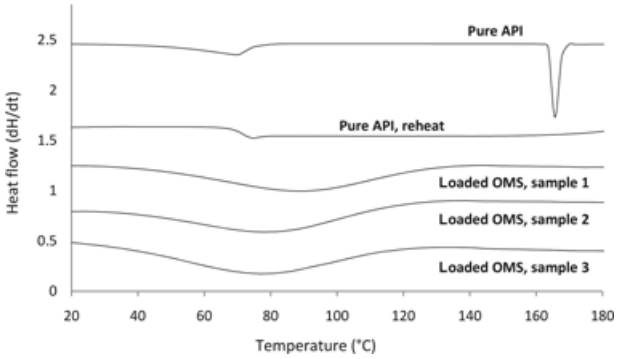

Figure 2. Differential scanning calorimetry thermograms of crystalline ezetimibe and ezetimibe-loaded ordered mesoporous silica (OMS) formulation.

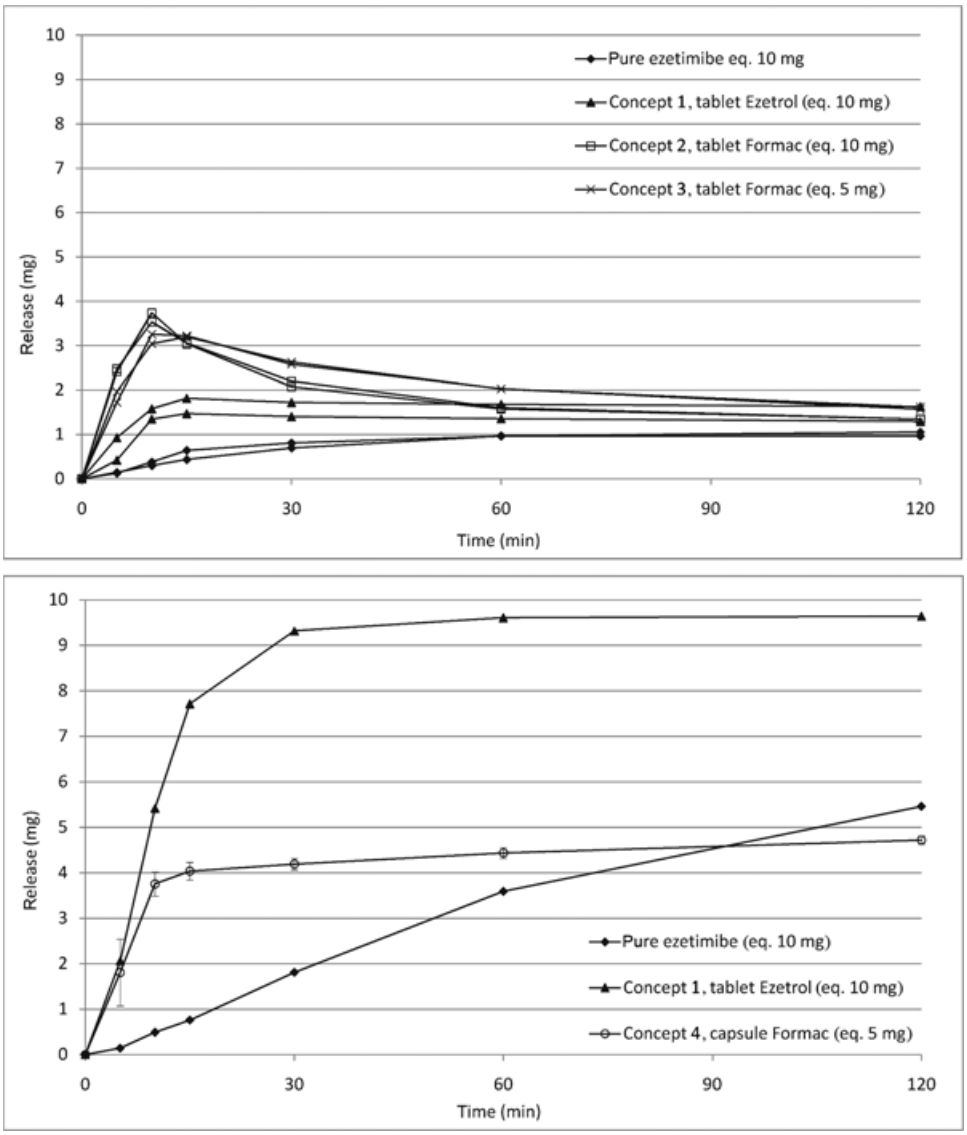

Figure 3. (a) Dissolution/release experiments of crystalline ezetimibe, Ezetrol®, and two Formac OMS tablets ($n = 2$) in 900 mL simulated gastric fluid (SGF) + 0.1% sodium lauryl sulfate. (b) Dissolution/release experiments of crystalline ezetimibe, Ezetrol®, and one Formac OMS capsules ($n = 3$) in 900 mL SGF + 0.1% Tween 80.

 DOI 10.1002/jps

Table 3. Plasma Concentrations of Ezetimibe and Ezetimibe–Glucuronide of the Different Concepts

		10 mg Ezetrol Tablet	10 mg OMS Tablet	5 mg OMS Tablet	5 mg OMS Capsule
Ezetimibe pharmacokinetic data					
Dose level	mg/kg	0.973 ± 0.122	0.965 ± 0.110	0.485 ± 0.050	0.483 ± 0.059
t_{last}	h	12.0–24.0[a]	24.0	12.0–24.0[a]	12.0–24.0[a]
T_{max}	h	4.00–6.00[a]	0.50–1.50[a]	0.50–8.00[a]	0.50–4.00[a]
C_{max}	pg/mL	396 ± 133	501 ± 384	210 ± 203	442 ± 418
C_{max}[b]	(kg pg)/(mL mg)	417 ± 177	321	428 ± 411	901 ± 845
C_{last}	pg/mL	133 ± 53.5	146 ± 162	54.6 ± 18	59.4 ± 51.4
AUC_{last}	(h pg)/mL	2650 ± 771	3080 ± 1330	1230 ± 607	2690 ± 1730
AUC_{last}[b]	(h pg/mL)/(mg/kg)	2790 ± 989	3120 ± 1080	2500 ± 1160	5540 ± 3510
F_{rel}	%	100	136 ± 90.2	112 ± 93.0	249 ± 244
Ezetimibe–glucuronide pharmacokinetic data					
Dose level	mg/kg	0.973 ± 0.122	0.965 ± 0.110	0.485 ± 0.050	0.483 ± 0.059
t_{last}	h	24.0	24.0	24.0	24.0
T_{max}	h	0.50–4.00[a]	0.50–1.50[a]	0.50–1.50[a]	0.50
C_{max}	pg/mL	15, 600 ± 7650	21, 500 ± 10, 400	11, 200 ± 6450	25, 800 ± 7660
C_{max}[b]	(kg pg)/(mL mg)	16, 600 ± 9940	22, 700 ± 11, 000	23, 300 ± 13, 300	54, 500 ± 17, 700*
C_{last}	pg/mL	3060 ± 3190	4150 ± 3600	1050 ± 1070	924 ± 466
AUC_{last}	(h pg)/mL	117, 000 ± 28, 600	128, 000 ± 37, 300	54, 200 ± 19, 800	130, 000 ± 53, 700
AUC_{last}[b]	(h pg/mL)/(mg/kg)	124, 000 ± 43, 500	136, 000 ± 50, 500	112, 000 ± 41, 200	274, 000 ± 118, 000*
F_{rel}	%	100	111 ± 22.4	96.4 ± 42.9	230 ± 106

[a] Range.
[b] Dose normalized to 1 mg/kg.
* $p < 0.05$.

Ezetrol® reached a plateau after 15 min, which is maintained until the end of the experiment. The OMS tablet (eq. 10 mg) shows an initial burst after 5 min, which reaches its maximum after 10 min. However, the ezetimibe precipitates, leading to a similar level of Ezetrol® after 120 min of dissolution testing. The progress for the OMS tablet (eq. 5 mg) is similar to the OMS tablet (eq. 10 mg), but has a less steep initial release and a slower precipitation rate of ezetimibe. The release out of OMS from n capsule (eq. 5 mg) was also compared with the pure crystalline ezetimibe and the Ezetrol® product as a reference in 900 mL 10 mM pH 7 phosphate buffer + 0.1% Tween 80. The pure drug increased linearly, whereas the capsule dosage form almost maintained a steady state after 15 min. The Ezetrol® tablet reached a maximum release after 30 min. Both formulated ezetimibe samples attained a release of almost 100% of their original dosage form. Therefore, it can be concluded that the 10 mM pH 7 phosphate buffer + 0.1% Tween 80 is not very discriminative for these concepts.

Four different formulations were evaluated—Ezetrol® and three ezetimibe-loaded OMS based formulations (Table 3). Figure 4 shows the average plasma concentrations versus time curves of ezetimibe and the active metabolite ezetimibe glucuronide after dosing. The PK profiles of ezetimibe and ezetimibe glucuronide show a biphasic pattern. This is a well-known profile for ezetimibe due to enterohepatic recirculation of the compound. Administration of Ezetrol® resulted in a dose-normalized AUC_{last} value of 2790 ± 989 (h pg/mL)/(mg/kg), with a C_{max} of 417 ± 177 pg/mL and a maximal Plasmaconcentration (T_{max}) of 4.5 ± 1 h. When compared with the metabolite, the dose-normalized AUC_{last} value was 124,000 ± 43,500 (h pg/mL)/(mg/kg), with a C_{max} of 16,600 ± 9940 pg/mL and a T_{max} of 1.9 ± 1.5 h. When the same dose of ezetimibe was formulated into an OMS-formulated tablet, the systemic exposure resulted in a dose-normalized AUC_{last} value of 3120 ± 1080 (h pg/mL)/(mg/kg), with a C_{max} of 492 ± 321 pg/mL and a T_{max} of 0.8 ± 0.5 h. When compared with the metabolite, the dose-normalized AUC_{last} value was 136,000 ± 50,500 (h pg/mL)/(mg/kg), with a C_{max} of 22,700 ± 11,000 pg/mL and a same T_{max} of 0.8 ± 0.5 h. The systemic exposure of a dose-normalized Ezetrol® and loaded OMS tablet was comparable, except for the T_{max}. All the releases from loaded OMS were characterized by a fast initial burst. The *in vivo* dog study was designed as a cross-over study with a lower-dosed, ezetimibe-loaded OMS-formulated tablet and capsule (eq. 5 mg). The average plasma profile for the OMS-formulated tablet was lower than expected, given the *in vitro* experimental data. After oral dosing, the dose-normalized AUC_{last} value was 2500 ± 1160 (h pg/mL)/(mg/kg), with a C_{max} of 428 ± 411 pg/mL and a T_{max} of 3.3 ± 3.6 h. When compared with the metabolite, the dose-normalized AUC_{last} value was 112,000 ± 41,200 (h pg/mL)/(mg/kg), with a C_{max} of 23,300 ± 13,300 pg/mL and a faster T_{max} of 0.8 ± 0.5 h. The average plasma profile for the OMS capsule was remarkably higher than the average plasma profile of Ezetrol®. After administration of

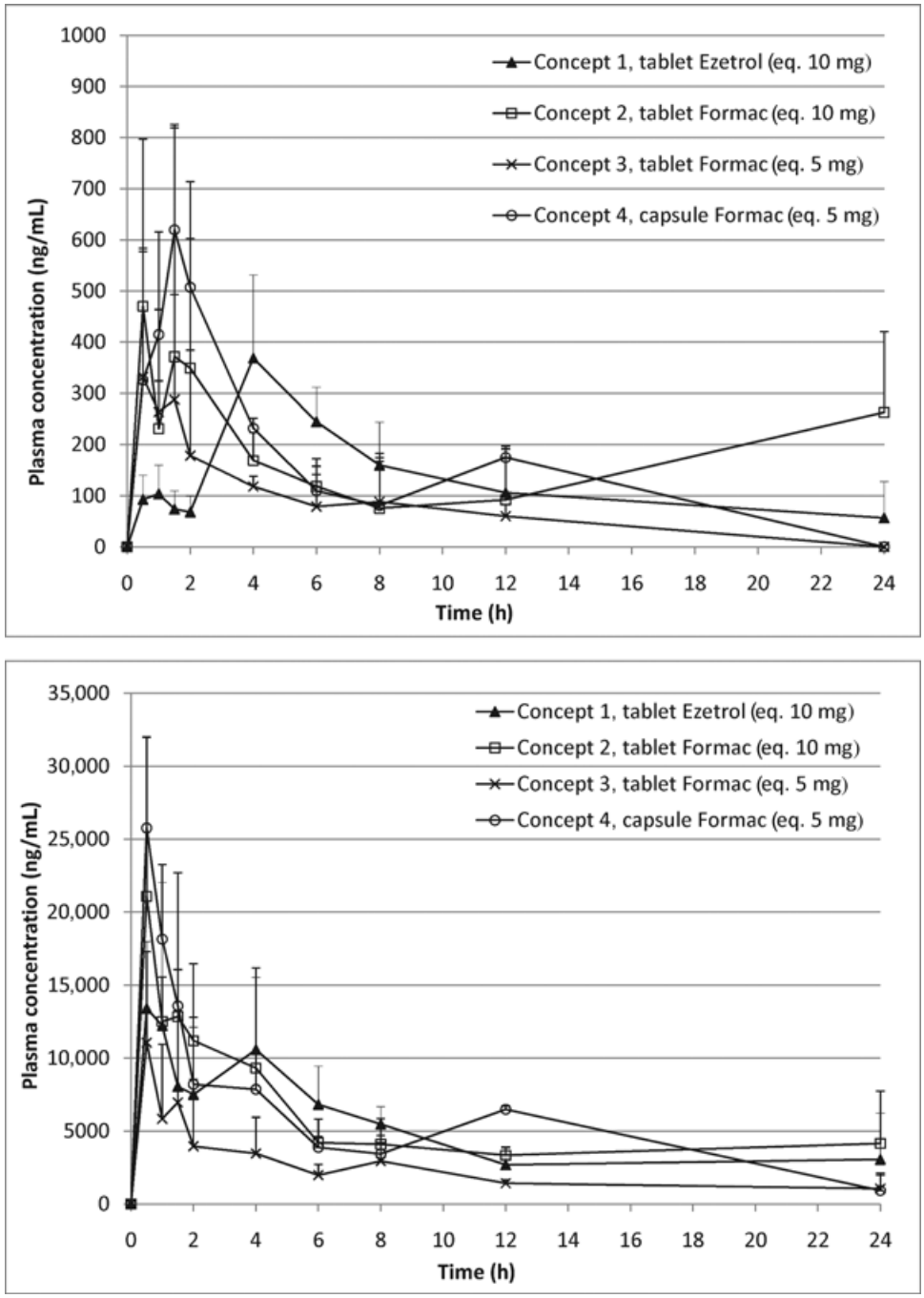

Figure 4. (a) Average plasma concentration versus time curves of ezetimibe after single dosing in male Beagle dog. (b) Average plasma concentration versus time curves of ezetimibe–glucuronide after metabolization of ezetimibe single dosing in male Beagle dog.

the capsule, the dose-normalized AUC_{last} value was 5540 ± 3510 (h pg/mL)/(mg/kg), with a C_{max} of 901 ± 845 pg/mL and a T_{max} of 2.5 ± 1.8 h. When compared with the metabolite, the dose-normalized AUC_{last} value was $274{,}000 \pm 118{,}000$ (h pg/mL)/(mg/kg), with a C_{max} of $54{,}500 \pm 17{,}700$ pg/mL and a faster T_{max} of 0.5 ± 0.0 h. The formulation of ezetimibe-loaded OMS (eq. 5 mg) into a capsule lead to a faster absorption into the blood circulation, whereby the prodrug could be metabolized sooner to its active conjugate. The amount of ezetimibe absorption out of the 5 mg OMS-formulated capsule was more than double than that out of the commercially available 10 mg Ezetrol®.

The results from the stability study (Table 4) showed that the water content in the capsules and the tablets (closed condition) increased slightly (less than 1% increase over the storage time of 6 months)

Table 4. Stability Results (Water Content and Assay) of All Concepts and Conditions

		Powder		5 mg Tablet		5 mg Capsule		10 mg Tablet	
Time Point	Storage Condition	Water Content % (w/w)	Assay % (w/w)	Water Content % (w/w)	Assay % (w/w)	Water Content % (w/w)	Assay % (w/w)	Water Content % (w/w)	Assay % (w/w)
$T = 0$	NA	3.16 ± 0.12	18.46 ± 0.05	2.27 ± 0.08	16.51 ± 0.81	NM	NM	2.98 ± 0.04	18.65
$t = 1$ min	25°C/60%RH; open	8.32 ± 0.06	17.68 ± 0.13						
	25°C/60%RH; closed	2.11 ± 0.08	18.66 ± 0.29	1.79 ± 0.22	17.73 ± 0.18	NM	NM	2.45 ± 0.11	17.86 ± 0.28
$t = 3$ min	25°C/60%RH; open	5.99 ± 0.25	17.38 ± 0.48						
	25°C/60%RH; closed	1.97 ± 0.29	18.39 ± 0.59	2.59 ± 1.05	16.56 ± 0.39	1.56 ± 0.34	NM	2.49 ± 0.51	17.44 ± 0.26
	40°C/75%RH; open	5.77 ± 0.38	16.18 ± 0.42						
	40°C/75%RH; closed	2.33 ± 0.12	17.68 ± 0.25	2.02 ± 0.30	15.92 ± 0.22	1.55 ± 0.63	NM	2.10 ± 0.47	16.57 ± 0.49
$t = 6$ min	25°C/60%RH; open	5.56 ± 0.26	18.64 ± 0.17						
	25°C/60%RH; closed	2.50 ± 0.25	20.34 ± 0.25	2.80 ± 0.39	18.64 ± 0.21	1.75 ± 0.13	NM	3.11 ± 0.32	19.27 ± 0.15
	40°C/75%RH; open	3.98 ± 0.18	16.40 ± 0.29						
	40°C/75%RH; closed	3.14 ± 0.08	18.89 ± 0.02	3.21 ± 0.29	17.55 ± 0.39	2.13 ± 0.14	NM	3.62 ± 0.10	18.14 ± 0.22

NA, not applicable; NM, not measured.

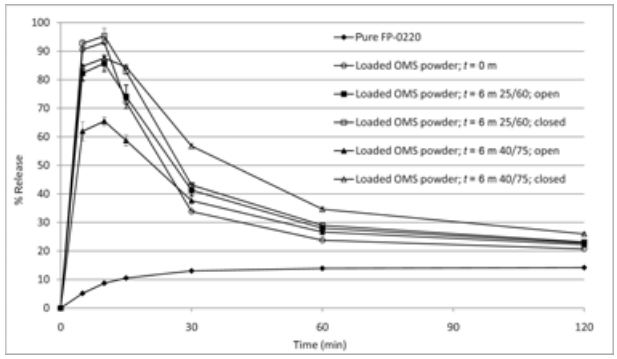

Figure 5. Dissolution/release experiments of stability samples of loaded ordered mesoporous silica (OMS) powder ($n = 3$; eq. 10 mg ezetimibe) in 125 mL simulated gastric fluid + 0.1% sodium lauryl sulfate.

due to protection by the packaging. The water content in the open-stored loaded powder was significantly higher than that in its closed packaging; however, after a first rapid absorption period of moisture (high water content after 1 month), the moisture content decreased again with storage time.

The assay values remained quite constant throughout storage for all concepts, and differences observed in the values were due to natural variation. However, a decrease in the assay value was observed for the powder samples stored in open conditions, but this is most likely caused by the moisture absorption and concurrent weighing bias on the sample.

The most important results are those from the DSC measurements because none of the concepts showed any crystallinity during the whole stability period. Even the powder samples that were stored in extreme stress conditions (open, unprotected conditions at 40°C/75% RH) did not show crystallinity. This advocates the unique stability properties of the silica technology as compared with other solubility-enhancing technologies such as amorphous solid dispersions.

From Figure 5, in which the release rate is illustrated of loaded OMS powder during stability (all conditions, 6 month time point), it could be concluded that this release was similar in all conditions, except for the 40°/75 RH closed stress condition in which the "supersaturation" peak was lower than the other conditions. Part of this decrease could be explained by the fact that the weighed samples were not corrected for water content, which could be significant when stored at 40°/75 RH in open conditions.

CONCLUSION

The bioavailability-enhancing properties of the use of silica were confirmed *in vitro* and *in vivo*. This silica technology, in which poorly water-soluble drugs are adsorbed on OMS materials, can be defined as an important new formulation development tool with

unique stability properties as compared with other formulation technologies.

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