molecular pharmaceutics

ARTICLE

pubs.acs.org/molecularpharmaceutics

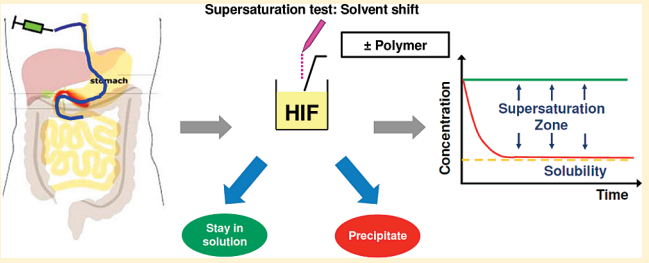
Excipient-Mediated Supersaturation Stabilization in Human Intestinal Fluids

Jan Bevernage,[†] Thomas Forier,[†] Joachim Brouwers,[†] Jan Tack,[‡] Pieter Annaert,[†] and Patrick Augustijns*[,†]

[†]Laboratory for Pharmacotechnology and Biopharmacy, O&N 2, Herestraat 49—Box 921, 3000 Leuven, Katholieke Universiteit Leuven, Belgium

[‡]Department of Gastroenterology, University Hospitals Leuven, 3000 Leuven, Belgium

ABSTRACT: It was the purpose of this study to investigate excipient-mediated precipitation inhibition upon induction of supersaturation of poorly water-soluble drugs in aspirated human intestinal fluids (HIF) representing both the fasted and fed state. Etravirine, ritonavir, loviride, danazol and fenofibrate were selected as model compounds. For comparative purposes, precipitation inhibition was also evaluated in simple aqueous buffer, and in intestinal simulation media representative for the fasted and fed state (FaSSIF and FeSSIF, respectively). Supersaturation was induced in the test media containing predissolved excipient (HPMC-AS, HPMC-E5, HPMC-E50, HPMC-E4M, HPMC-P and PVP) at a defined degree of supersaturation (DS = 20) using the solvent shift method. The results illustrate that cellulosic polymers can reduce the precipitation rate and stabilize supersaturation in HIF. The extent of stabilization was compound and excipient dependent but independent of the nutritional state. Whenever excipient effects were observed, the predictive value of simple buffer or FaSSIF/FeSSIF was rather limited. In general, excipient-mediated precipitation inhibition was less pronounced in HIF compared to simple aqueous buffer or FaSSIF/FeSSIF. However, excipients showing no effect in simple aqueous buffer or FaSSIF/FeSSIF also proved to be ineffective in HIF, indicating the value of these simulation media in the elimination of excipients during formulation development.



KEYWORDS: supersaturation, solubility, precipitation inhibition, biorelevant

INTRODUCTION

With the increasing number of poorly water-soluble compounds in contemporary drug discovery pipelines,[1,2] the concept of supersaturation as a tool for enhancing bioavailability is gaining momentum, especially in the field of oral drug delivery where increased intraluminal concentrations through supersaturation are expected to enhance the intestinal absorption. For this enhanced intestinal absorption to take place, supersaturation must be induced and maintained in the gastrointestinal environment. In vivo, induction of supersaturation can be achieved through various formulation approaches (supersaturating drug delivery systems) or, in the specific case of a basic drug, upon transfer from the stomach to the intestine (pH-shift); different approaches to induce supersaturation have recently been reviewed by Brouwers et al.[3] Upon induction, the thermodynamically unstable state of supersaturation has to be maintained for a time period long enough to allow intestinal absorption. Maintenance of the supersaturated state has been the subject of research of various academic and industrial laboratories. It has been demonstrated that inclusion of excipients that delay precipitation may stabilize supersaturation in vitro; pharmaceutical excipients which have been applied to serve this purpose include polymers,[3−9] surfactants[4,10,11] and cyclodextrins.[5,12] The awareness that supersaturation in the intraluminal environment could enhance intestinal absorption has urged pharmaceutical companies to implement high throughput precipitation screening systems to evaluate the supersaturation potential during lead selection/optimization and to assist in excipient selection during formulation development. As it can be expected that the gastrointestinal environment affects drug precipitation in vivo, a biorelevant in vitro evaluation of supersaturation requires careful selection of the test medium. Whereas most publications report on the use of simple aqueous buffer based supersaturation assays,[4,5] also more biorelevant media [simulated intestinal fluids (SIF)] have been used during precipitation screening in an attempt to improve the in vitro−in vivo correlation.[6,13] Recent research performed in our laboratory has shown that supersaturation can be maintained up to a certain degree in human intestinal fluids (HIF) in the absence of pharmaceutical excipients;[14] however, it remains unclear whether supersaturation in HIF can be further stabilized by the use of excipients and whether components present in the intestinal environment (bile salts, lecithin, food components) affect

Received: November 8, 2010
Accepted: January 26, 2011
Revised: January 23, 2011
Published: January 26, 2011

Table 1. Physicochemical Parameters of the Five Model Drugs Used in the Present Study

compound	ionization behavior	mol wt (g/mol)	no. of H-donors	no. of H-acceptors	cLogP
etravirine	weakly basic (pK_a 4.5)	435	2	6	5.54
ritonavir	weakly basic (pK_a 2.98)	721	4	11	5.22
loviride	neutral	351	2	6	3.98
danazol	neutral	337	1	3	3.38
fenofibrate	neutral	360	0	4	5.28

excipient-mediated precipitation inhibition. This lack of knowledge hampers extrapolation of the results from excipient screening using in vitro buffer or SIF based precipitation assays to the in vivo situation.

To gain more insight into the impact of the human intestinal environment on excipient-mediated precipitation inhibition, supersaturation studies were performed in aqueous buffers, SIF and HIF, representing both fasted and fed state, using 5 poorly soluble drugs classified as class 2 (etravirine, loviride, danazol, fenofibrate) or class 4 (ritonavir) compounds in the biopharmaceutics classification system (BCS). The model compounds were selected to cover a broad range of low solubilities in FaSSIF and FeSSIF which implies that, in theory, all compounds could benefit from supersaturation to enhance intestinal absorption. Physicochemmical properties of the respective model compounds are summarized in Table 1. Based on literature research[3,6,15] and preliminary experiments, which did not show any beneficial effects of surfactants or cyclodextrins on precipitation inhibition (data not shown), only polymers (cellulosics and PVP) were considered as stabilizing excipients in this study. The cellulosic polymers included hydroxypropylmethylcellulose (HPMC) (grade E5, E50 and E4M), hydroxypropylmethylcellulose acetate succinate (HPMC-AS) (grade LF) and hydroxypropylmethylcellulose phthalate (HPMC-P) (grade HP50). Using combinations of these polymers and drugs, it was the goal of this study (1) to investigate the extent of precipitation inhibition in SIF and simple buffer solutions following the inclusion of different excipients, (2) to compare the obtained results with results obtained in human intestinal fluids (HIF) ex vivo, and (3) to identify potential food effects on the precipitation inhibition performance of excipients.

MATERIALS

Loviride was kindly donated by Johnson & Johnson Pharmaceutical Research and Development (Beerse, Belgium); etravirine was provided by Tibotec-Virco Virology bvba (Beerse, Belgium); ritonavir was received from APL Healthcare Limited (Ameerpet, India); danazol and fenofibrate were obtained from Indis (Aartselaar, Belgium). Hydroxypropylmethylcellulose acetate succinate (HPMC-AS) (grade LF), hydroxypropylmethylcellulose phthalate (HPMC-P) (Grade HP50) (Shin Etsu Chemical Co., Ltd., Tokyo, Japan), hydroxypropylmethylcellulose (HPMC) (Grade E5, E50 and E4M) (Colorcon Ltd., Kent, U.K.) and polyvinylpyrrolidone K25 (PVP K25) (BASF, Ludwigshafen, Germany) were received as free samples. Orlistat [Sigma-Aldrich (St. Louis, MO, USA)], sodium taurocholate (practical grade) [ICN Biomedicals, Inc. (Eschwege, Germany)] and Phospholipon 90G (lecithin) [Nattermann Phospholipid Gmbh (Köln, Germany)] were used as received. Acros Organics (Geel, Belgium) supplied methanol (MeOH), dimethyl sulfoxide (DMSO), sodium acetate trihydrate and $NaH_2PO_4 \cdot H_2O$, while NaCl and acetonitrile were provided by Fisher Scientific (Leicestershire, U.K.). Chloroform and acetic acid were from Chemlab NV (Zedelgem, Belgium); NaOH pellets were obtained from BDH Laboratory Supplies (Poole, U.K.). Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, U.K.). Ensure Plus (Abbott Laboratories B.V., Zwolle, The Netherlands) was used to simulate a standard meal. One portion of 200 mL has an energy content of 1.263 kJ of which lipids, carbohydrates and proteins constitute 29%, 54% and 17% on energy basis, respectively; the osmolality amounts to 670 mOsm/kg; the pH is 6.6. Double-lumen polyvinyl catheters [Salem Sump Tube 14 Ch (external diameter 4.7 mm), Sherwood Medical, Petit Rechain, Belgium] were used for the aspiration of human intestinal fluids.

METHODS

Human Intestinal Fluids (HIF) Sampling. Duodenal fluids of four healthy volunteers (two female, two male, between 19 and 35 years old), collected in two different nutritional states, were used for solubility measurements and supersaturation assays. The procedure for collecting HIF followed the tenets of the Declaration of Helsinki and was approved by the Committee of Medical Ethics of the University Hospitals Leuven, Belgium (ML3242). All volunteers provided written informed consent to participate in this study. The HIF were collected every 15 min for up to 120 min from the duodenum (D2−D3) after the intake of 200 mL of water (fasted state) or a liquid meal (Ensure Plus 400 mL) + 200 mL of water (fed state). We refer to a previous study for a more detailed description of the HIF aspiration protocol.[16] In order to inhibit further in vitro lipolysis after sampling, the general lipase inhibitor orlistat, dissolved in ethanol, was immediately added to the collected fractions in a final concentration of 1 μM (final ethanol concentration 0.1% (v/v)). For each nutritional state, one pooled sample was made by combining the aspirates from all 4 volunteers. The pooling was performed in order to obtain media with average characteristics for each nutritional state. The pH of the pooled HIF was measured immediately after pooling of the different fractions, before and after experiments (Hamilton Slimtrode); the pooled HIF were stored at −30 °C until usage in solubility or supersaturation assays.

Preparation of FaSSIF and FeSSIF. For comparative purposes, experiments were also performed using simple aqueous buffer (i.e., blank FaSSIF; FaSSIF without biorelevant components, pH 6.5) and simulated intestinal fluid (SIF) for the fasted (FaSSIF) and the fed (FeSSIF) state. Blank FaSSIF, FaSSIF and FeSSIF were made according to the composition reported by Vertzoni et al. (revised standard FaSSIF and FeSSIF with practical grade taurocholate and soybean lecithin).[17]

Solubility Measurement. The thermodynamic solubility of the model compounds was determined using the standard shake flask method in blank FaSSIF, simulated intestinal fluids (FaSSIF and FeSSIF) and in pooled fasted and fed state HIF corresponding to the fasted and fed states. All solubility experiments were performed in triplicate. When centrifugation was used for solid

Table 2. Parameters for the HPLC Analysis of the Five Drugs Used in the Present Study

	etravirine	ritonavir	loviride	danazol	fenofibrate
mobile phase	20/80 buffer[a]/MeOH[b]	20/80 buffer[a]/MeOH[b]	50/50 buffer[a]/AcN[c]	85/15 buffer[a]/MeOH[b]	20/80 buffer[a]/MeOH[b]
flow rate (mL/min)	1.0	1.0	1.0	1.0	1.5
retention time (min)	6.2	5.5	7.7	6.0	7.6
detection UV (nm)	312	247	366	288	287
quantification limit (μM)	0.02	0.4	0.2	0.04	0.083

[a] 25 mM sodium acetate buffer, pH 3.5. [b] Methanol. [c] Acetonitrile.

phase separation, approximately 2 mg of drug compound was added to microcentrifuge tubes containing 1 mL of blank FaSSIF, FaSSIF or FeSSIF or 500 μL of HIF. Samples for filtration were prepared by adding approximately 20 mg of drug to 10 mL of blank FaSSIF in a test tube. Prior to centrifugation or filtration, the samples were allowed to equilibrate in a prewarmed shaking incubator [37 °C at 130 rpm (Incubator shaker series 25D, New Brunswick Scientific Co., Inc., Edison, NJ)]. Since previously reported equilibrium times in biorelevant media for poorly soluble drugs showed that equilibrium was always reached within 24 h,[18,19] the equilibration time was set at 24 h. The solid phase was separated from the dissolved part using centrifugation (15 min, 20817 g at 37 °C) in all cases except for ritonavir in blank FaSSIF where filtration was used (0.2 μm regenerated cellulose, Macherey Nagel, Düren, Germany). Adsorption of ritonavir to filter material was minimal, and 1 mL appeared to saturate the filter material. The filtrate/supernatant of the blank FaSSIF samples was diluted twice in MeOH. The supernatant of the FaSSIF and FeSSIF samples was diluted 1/10 with mobile phase. The diluted blank FaSSIF and SIF samples were used as such for further analysis. For HIF samples, precipitation and separation of proteins present in HIF were achieved by diluting the supernatant 1/10 in mobile phase, followed by a second centrifugation (5 min, 20817*g* at 37 °C). The resulting supernatant was used for quantification.

Supersaturation Test. Supersaturation was investigated using the solvent-shift method. The experimental setup consisted of a water bath at 37 °C with either 40 mL dissolution vessels for larger test volumes or small 4 mL vessels for smaller test volumes (HIF samples). Preliminary experiments using FaSSIF demonstrated no significant influence of the test volume on supersaturation behavior of the 5 model compounds used (data not shown). The vessels contained 20 mL of blank FaSSIF (BFA), FaSSIF or FeSSIF, or 2 mL of HIF with or without 0.05% predissolved excipient, and were equilibrated at 37 °C. Magnetic stirrer bars provided mixing of the medium (400 rpm). Stock solutions in DMSO of various drug concentrations were prepared to induce an initial degree of supersaturation (DS) equal to 20. At the final DMSO ($\leq$2%) or excipient (0.05%) concentration applied in the tested media, no significant influence on the thermodynamic solubility was noticed (data not shown). After adding the DMSO stock solutions to the test medium, samples (1 mL FaSSIF/FeSSIF, 200 μL HIF) were taken at 15, 30, 45, 60, 90, and 120 min and centrifuged (10 min, 20817 g, 37 °C), except for the samples of ritonavir in blank FaSSIF which were filtered. The supernatant of the simulating fluid samples (FaSSIF and FeSSIF) was directly diluted 1/10 in mobile phase and analyzed as such. The filtrate/supernatant of blank FaSSIF samples was directly diluted twice in MeOH before analysis. The supernatant of HIF samples was diluted 1/10 in mobile phase and centrifuged (5 min, 20817*g*, 37 °C) to precipitate and separate proteins respectively; the resulting supernatant was used for analysis. All supersaturation experiments were performed in triplicate.

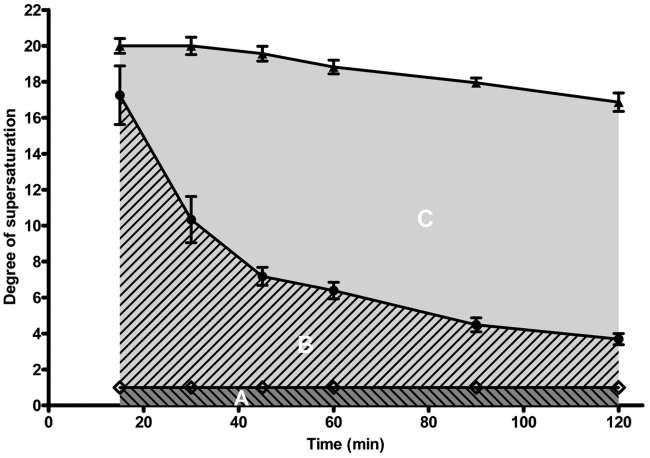


Figure 1. Degree of supersaturation−time profiles of etravirine in FaSSIF (●) and FaSSIF + 0.05% HPMC-AS (▲) upon solvent shift (initial degree of supersaturation 20). In addition, the saturation profile (DS = 1) of etravirine in FaSSIF (◇) is presented. Mean ± SD (n = 3).

Analysis. All drug concentrations were determined by reversed phase HPLC analysis with UV detection. An aliquot (50 μL) of the diluted supernatants, obtained as described above, was injected into a Waters HPLC system consisting of an Alliance 2695 separations module and a Novapak C-18 column under radial compression (Waters, Milford, MA). UV signals were detected by a Waters UV detector (W2487). All chromatographic methods were run in the isocratic mode. A detailed description of the chromatographic methods can be found in Table 2. The observed peaks were integrated using Empower Pro (Empower 2) software. Calibration curves were made in mobile phase. Samples were diluted to fit in the range of the linear calibration curve. Precision and accuracy were assessed by analyzing standard samples (n = 5) at a high and a low level. The determination of the intraday repeatability resulted in relative standard deviations and bias below 5.0% at all concentrations and for all model drugs.

Data Analysis and Presentation. *Supersaturation Profiles.* Supersaturation data were processed as degree of supersaturation−time profiles (DS−time profiles). The DS was calculated by dividing the concentration measured at a particular time point by the equilibrium solubility of the corresponding drug in exactly the same medium. For instance, a DS equal to 10 means that 10 times more drug is dissolved compared to the equilibrium solubility in the corresponding medium. Typical DS−time profiles for etravirine are given in Figure 1, showing the saturation curve (DS = 1), the DS−time profile after solvent shift into plain FaSSIF (no excipient), and the DS−time profile after solvent shift into FaSSIF including 0.05% HPMC-AS. In view of the limited time resolution of the DS−time profiles, a full kinetic analysis of the precipitation process was not feasible.

Table 3. Mean Solubility Values ± SD ($n = 3$) of the Different Model Compounds in Blank FaSSIF, FaSSIF, FeSSIF, FaHIF and FeHIF

solubility (μM)	blank FaSSIF (pH 6.5)	FaSSIF (pH 6.5)	FeSSIF (pH 5)	FaHIF (pH 6.70)	FeHIF (pH 5.88)
etravirine	na[a]	2.28 ± 0.01	14.3 ± 0.57	4.05 ± 0.39	9.60 ± 1.76
ritonavir	3.04 ± 0.30	11.7 ± 2.21	19.9 ± 0.81	24.3 ± 0.55	47.3 ± 2.68
loviride	2.12 ± 0.16	8.53 ± 0.15	26.7 ± 0.05	19.4 ± 1.13	61.6 ± 11.6
danazol	0.56 ± 0.07	19.9 ± 0.56	65.9 ± 0.25	24.5 ± 0.48	84.4 ± 4.12
fenofibrate	0.27 ± 0.03	37.7 ± 0.20	149 ± 2.81	33.1 ± 1.70	501 ± 11.8

[a] Not available.

Supersaturation Factor. To allow appreciation of the degree of supersaturation as a function of time for the different model compounds in various conditions, the supersaturation factor (SF) was calculated as follows: areas under the DS−time profiles up to 120 min (AUC_{120min}) were calculated using Graphpad Prism software (Graphpad software Inc.) and divided by the AUC_{120min} for a saturated solution (DS: 1). This is illustrated in Figure 1, where the SF for etravirine in FaSSIF is given by the following equation:

$$SF_{FaSSIF} = \frac{\text{area } A + \text{area } B}{\text{area } A} \quad (1)$$

Excipient Gain Factor. To allow quick assessment of the extent to which excipients stabilize supersaturation, the excipient gain factor (EGF) was calculated by dividing the AUC_{120min} of the DS−time profile in the presence of excipient by the AUC_{120min} of the DS−time profile in the absence of excipient. For instance, based on Figure 1, the excipient gain factor of HPMC-AS in FaSSIF for etravirine is calculated as follows:

$$EGF_{HPMC\text{-}AS} = \frac{\text{area } A + \text{area } B + \text{area } C}{\text{area } A + \text{area } B} \quad (2)$$

Statistical Analysis. To determine the significance ($p < 0.05$) of the excipient gain factor, one sided ANOVA tests were performed using the Dunnett's multiple comparison test as a post test (Graphpad software Inc.).

RESULTS AND DISCUSSION

Solubility. Knowledge of the thermodynamic solubility is a necessity when investigating supersaturation in a specific medium; therefore, thermodynamic solubilities were determined for the 5 model compounds in blank FaSSIF, FaSSIF, FeSSIF, fasted state human intestinal fluids (FaHIF) and fed state human intestinal fluids (FeHIF). Mean solubility values in the respective media are summarized in Table 3. Inclusion of the different excipients (0.05%) used did not significantly affect the solubility of the 5 model compounds in the respective media (data not shown). The solubility of etravirine in blank FaSSIF was extremely low and could not be determined in a reproducible manner; consequently, no supersaturation studies were performed for etravirine in blank FaSSIF. In line with previous reports,[14,18] food intake resulted in an increased solubilizing capacity. In general, the solubilities obtained in simulated intestinal fluids underestimated the solubility in HIF except for etravirine in FeSSIF and fenofibrate in FaSSIF. The solubilities of etravirine and loviride in the pooled HIF used in the present study are comparable to previously obtained solubilities in a different HIF-pool.[14] This indicates that the followed pooling procedure provided HIF samples representative for the average solubilizing capacity of HIF in fasted and fed state conditions.

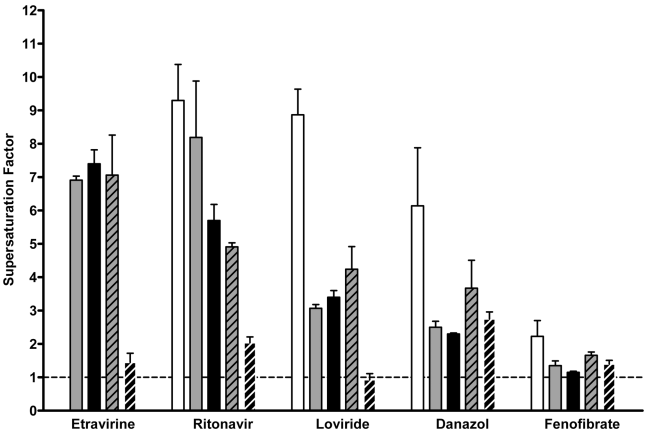


Figure 2. Supersaturation factors for the 5 model compounds in blank FaSSIF (open bars), FaSSIF (gray bars), FeSSIF (black bars), FaHIF (striped gray bars) and FeHIF (striped black bars), in absence of any pharmaceutical excipient. The supersaturation factor was calculated as the ratio of the area under the DS−time profile up to 120 min (AUC_{120min}) starting from an initial degree of supersaturation of 20 and the AUC_{120min} for a saturated solution; mean ± SD ($n = 3$); the dotted line (- - -) represents the solubility (supersaturation factor = 1).

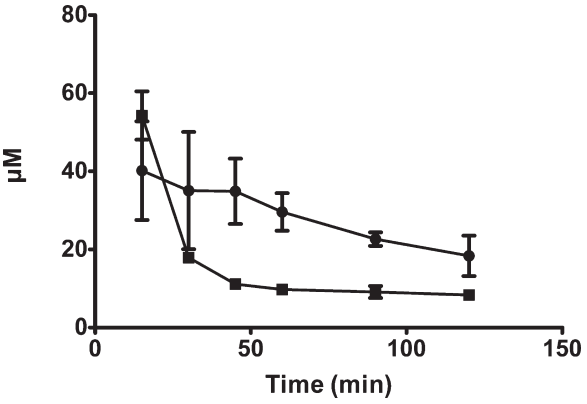


Figure 3. Concentration−time profiles of etravirine in FaHIF (●) and FeHIF (■). Mean ± SD ($n = 3$), starting from an initial degree of supersaturation of 20 after solvent shift.

Supersaturation Factor. Based on the obtained solubility values, a degree of supersaturation equal to 20 was induced in the respective media by means of a solvent shift method using DMSO as the organic phase. DS−time profiles were constructed, and the "supersaturation factor", expressed as the ratio of the area under the DS−time profiles up to 120 min (AUC_{120min}) and the AUC_{120min} for a saturated solution, was calculated for the 5 model compounds in the different media in absence of any pharmaceutical excipient (Figure 2). In line with our previous

Table 4. Mean Excipient Gain Factors of the Different Excipients in FaSSIF and FeSSIF for the Different Model Compounds[a]

Medium	FaSSIF						FeSSIF					
Compound \ Excipient	HPMC-AS	HPMC-E5	HPMC-E50	HPMC-E4M	HPMC-P	PVP	HPMC-AS	HPMC-E5	HPMC-E50	HPMC-E4M	HPMC-P	PVP
Etravirine	2,71	1,82	2,14	2,23	2,06	1,66	1,81	1,42	1,45	1,62	1,26	0,97
Ritonavir	0,95	1,05	1,03	1,04	0,76	0,74	1,10	1,07	0,96	0,98	1,25	1,22
Loviride	3,60	4,66	5,02	4,77	1,49	0,97	4,74	2,58	3,16	3,06	1,78	0,96
Danazol	2,55	2,34	2,33	2,74	1,02	0,56	2,22	2,90	3,69	3,25	2,50	1,75
Fenofibrate	1,34	0,87	0,91	1,16	1,19	0,97	1,14	0,95	0,99	0,96	1,10	1,09

x : >2,5 fold increase in AUC_{120min} compared to the no excipient condition

x : 1,3 to 2,5 fold increase in AUC_{120min} compared to the no excipient condition

x : No significant or < 1,3 fold increase in AUC_{120min} compared to the no excipient condition

[a] The excipient gain factor was calculated as the ratio of the AUC_{120min} of the DS−time profile in the presence of excipient to the AUC_{120min} of the DS−time profile in the absence of excipient.

study,[14] the results illustrate that supersaturation can occur in plain HIF; the stability appeared to be compound and medium dependent. As illustrated in Figure 2, the supersaturation stability in FaHIF was found to be different from that in FeHIF: higher mean supersaturation factors were obtained in FaHIF compared to FeHIF for all model compounds. The higher supersaturation factors in FaHIF also resulted in higher concentration−time curves in FaHIF compared to FeHIF for etravirine, ritonavir and loviride; this is illustrated for etravirine in Figure 3 where the concentration−time profiles are shown in FaHIF and FeHIF. This indicates that, for these compounds, induction of supersaturation may cause intraluminal concentrations to be lower in the fed state compared to the fasted state, despite the higher solubilizing capacity of FeHIF. In the case of danazol, the higher solubilizing capacity of FeHIF compensated for the lower supersaturation factor in FeHIF: AUC_{120min} of the concentration time curve in FeHIF was almost 3-fold higher than in FaHIF. In contrast to FaHIF and FeHIF, the supersaturation stability in FaSSIF and FeSSIF was quite comparable indicating little influence on supersaturation stability of the additional amount of taurocholate and lecithin in FeSSIF. The supersaturation factors obtained using blank FaSSIF confirmed previous observations[14] that this test medium provides a general overestimation of the supersaturation stability in HIF and FaSSIF/FeSSIF.

Excipient-Mediated Precipitation Inhibition in FaSSIF and FeSSIF. Since precipitation was observed for all model compounds, different polymers were tested for their precipitation inhibiting properties. Because of the limited availability of HIF, all polymers included in this study were first tested for their supersaturation stabilizing effects in FaSSIF and FeSSIF. Supersaturation was induced in both FaSSIF and FeSSIF in the presence of 0.05% (w/v) of excipient (HPMC-E5/E50/E4M, HPMC-AS, HPMC-P and PVP K25). The data are presented as mean excipient gain factors which indicate the fold increase in AUC_{120min} of the DS−time profile in the presence of excipient as opposed to the DS−time profile in the absence of excipient. Table 4 gives an overview of the excipient gain factors in FaSSIF and FeSSIF for the five model compounds. Any filled background represents a significant ($p < 0.05$) excipient-mediated increase in AUC_{120min} (gray background: 1.3- to 2.5-fold increase, black background: over 2.5-fold increase). Polymer effects on supersaturation stabilization appeared to be compound dependent. Etravirine, loviride and danazol were sensitive to excipient-mediated stabilization of supersaturation, whereas for ritonavir and fenofibrate, excipient inclusion elicited only a small or no effect. Among the tested excipients, the cellulosic polymers HPMC-AS and HPMC performed the best with significant increases in AUC_{120min} for at least 3 model compounds. The inclusion of different viscosity grades of HPMC (E5, E50 and E4M) resulted in comparable precipitation inhibition. Of the cellulosic polymers tested, HPMC-P was least efficient. The role of PVP as precipitation inhibiting polymer was minimal with no significant effects except for etravirine in FaSSIF and danazol in FeSSIF. Based on the results obtained in FaSSIF and FeSSIF, HPMC-AS and HPMC-E5 were selected for further experiments in HIF. The choice for HPMC-E5 was motivated by its better dissolution properties compared to HPMC-E50 and HPMC-E4M. In view of its limited effect, PVP-K25 was included as a negative control.

Excipient-Mediated Precipitation Inhibition in HIF. Following the experiments in FaSSIF/FeSSIF, the effects of the selected excipients (HPMC-AS, HPMC-E5 and PVP K25) on precipitation were further investigated in fasted and fed state human intestinal fluids (FaHIF and FeHIF, respectively). The corresponding DS−time profiles were constructed, and the excipient gain factor was calculated. In Figure 4, the excipient gain factors of the different excipients are represented in a bar chart for the different model compounds. Similar as in FaSSIF and FeSSIF, it is clear that in HIF, etravirine, loviride and danazol precipitation could be delayed through inclusion of excipients whereas, for ritonavir or fenofibrate, little or no excipient effects could be observed. In general, the extent to which excipients stabilize supersaturation in HIF was comparable between FaHIF and FeHIF indicating that excipient effects on precipitation were not altered in media representative for fed state conditions. In line with the results in FaSSIF and FeSSIF, the cellulosic polymers HPMC-AS and HPMC-E5 performed the best in HIF showing significant precipitation inhibition for 3 model compounds whereas PVP-K25 appeared to have no stabilizing effect in HIF. While in previous reports[6] HPMC-AS was selected as superior excipient for precipitation inhibition in SIF, this could not be concluded based on the results of the present study. Whenever excipient effects

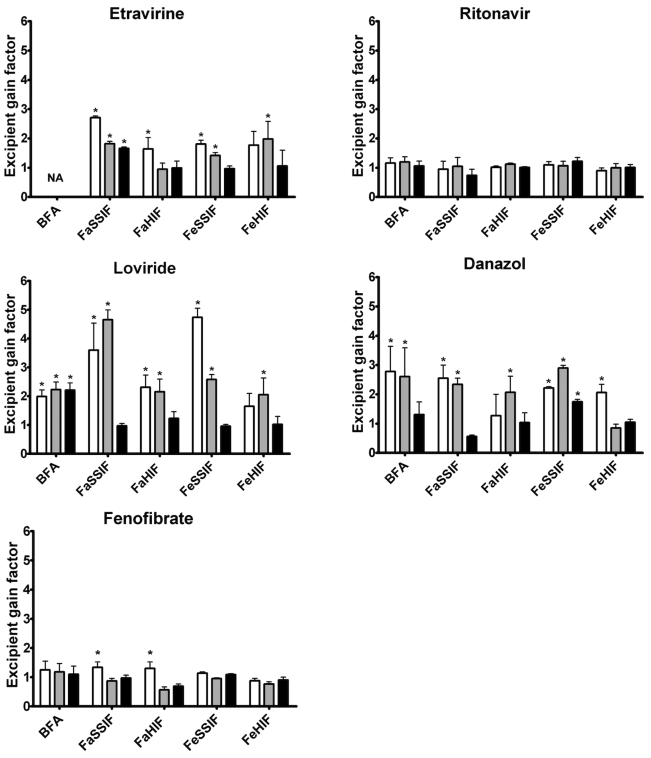


Figure 4. Excipient gain factors of HPMC-AS (open bars), HPMC-E5 (gray bars) and PVP (black bars) for etravirine, ritonavir, loviride, danazol and fenofibrate, in the different media. The excipient gain factor was calculated as the ratio of the AUC_{120min} of the DS−time profile in the presence of excipient to the $AUC_{120\ min}$ of the DS−time profile in the absence of excipient; mean ± SD (n = 3). Bars indicated with * on top represent a significant increase in AUC_{120min} ($p < 0.05$); NA, not available.

were observed in HIF, accurate prediction of the extent of precipitation inhibition using FaSSIF and FeSSIF proved to be difficult. The obtained excipient gain factors in FaSSIF and FeSSIF were often higher (i.e., up to 2.7 fold for loviride) as compared to those obtained in HIF. In some cases, this overprediction resulted in false positive results, i.e. the effect in FaSSIF of HPMC-E5 and PVP for etravirine or the effect in FeSSIF of HPMC-AS for loviride and HPMC-E5 for danazol, respectively. On the other hand, when excipients were not effective in stabilizing supersaturation in FaSSIF/FeSSIF, this also held in FaHIF/FeHIF (no false negative results), suggesting that elimination of excipients based on results from supersaturation assays in FaSSIF/FeSSIF is justified. For the two model compounds that were found to be resistant to excipient-mediated stabilization of supersaturation (ritonavir and fenofibrate), it needs to be mentioned that both compounds behaved differently with respect to precipitation. Fenofibrate supersaturation resulted in very fast precipitation toward the thermodynamic solubility (i.e., supersaturation factor close to 1, as can be seen in Figure 2). This fast precipitation of fenofibrate might hamper any stabilizing action by means of excipients. Ritonavir, however, exhibited rather atypical precipitation behavior. While the other model compounds gradually precipitated (with compound dependent kinetics) toward the thermodynamic solubility, ritonavir immediately precipitated, to remain stable at a certain degree of supersaturation depending on the medium. Since polymorphs that exhibit drastic solubility differences have been described for ritonavir,[20] further research on the physical state properties of precipitates would be very valuable in this perspective.

Excipient-Mediated Precipitation Inhibition in Blank FaSSIF. Since simple aqueous buffers are frequently used in formulation development to assess the stabilizing effect of excipients, precipitation inhibition was also investigated in blank FaSSIF (simple aqueous buffer pH 6.5) for all model compounds except etravirine. The obtained excipient gain factors are also included in Figure 4. Similar as in simulated or human intestinal fluids, excipient effects were observed for loviride and danazol, but not for ritonavir and fenofibrate. As was the case in FaSSIF/FeSSIF, the extent of excipient-mediated stabilization of supersaturation in blank FaSSIF sometimes overestimated the effects obtained in HIF with, occasionally, the generation of false positive results (i.e., the effect of PVP for loviride). However, when no effects were observed in blank FaSSIF, this was also the case in HIF. Comparing the value of blank FaSSIF versus FaSSIF/FeSSIF in predicting excipient effects in HIF, both media often overestimated the excipient gain factor generating false positive results while no false negative predictions were encountered. Therefore, as long as quantification is possible in a reproducible manner and no medium with improved predictive power has been developed, simple aqueous buffer (e.g., blank FaSSIF) may be sufficient and the preferable choice for practical and economical reasons in excipient screening protocols. It should be mentioned, though, that FaSSIF/FeSSIF perform better than blank FaSSIF in predicting precipitation kinetics in absence of excipients in HIF (Figure 2).

CONCLUSION

As it is extremely difficult to demonstrate drug supersaturation in situ in the intraluminal environment, we used human intestinal fluids as biorelevant in vitro test medium to explore the potential of supersaturation. In a previous study, we showed that drug supersaturation can be maintained up to a certain degree in human intestinal fluids. The results of the present study corroborate these observations and provide evidence that cellulosic polymers possess the potential to further increase the degree of supersaturation in human intestinal fluids, possibly resulting in enhanced absorption of low solubility compounds.

The extent to which precipitation inhibition could be obtained appeared to be compound and excipient dependent. Experiments using intestinal fluids from volunteers in the fasted or fed state evidenced that the nutritional state did not significantly affect the extent of excipient-mediated precipitation inhibition. The usefulness of simple aqueous buffers or simulated intestinal fluids representative for the fasted or fed state as solvent systems to predict excipient-mediated precipitation inhibition in human intestinal fluids appeared to be limited, illustrating the need for further evaluation of media for supersaturation screening. However, since lack of supersaturation stabilization by a given excipient in aqueous buffer or simulated intestinal fluids was always confirmed in human intestinal fluids, elimination of excipients based on screening tests in simple media appears feasible.

The mechanisms underlying the excipient-mediated supersaturation stabilization remain to be elucidated; the present study warrants further research to explore how human intestinal fluid components and food affect the performance of polymers in inhibiting precipitation, so that new test media for implementation in a drug discovery setting can be defined. As more and more poorly soluble drug candidates emerge from drug discovery pro-

grams, a better understanding of supersaturation and implementation of biorelevant supersaturation screening will allow more accurate decision making in drug and formulation development, thus assuring the successful exploitation of the supersaturation approach in vivo.

AUTHOR INFORMATION

Corresponding Author

*P.A.: Laboratory for Pharmacotechnology and Biopharmacy, O&N 2, Herestraat 49—Box 921, 3000 Leuven, Katholieke Universiteit Leuven, Belgium; e-mail, Patrick.Augustijns@pharm.kuleuven.be; tel, 32-16-330.301; fax, 32-16-330.305.

ACKNOWLEDGMENT

This research was funded by a Ph.D. grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen), as well as from grants from the "Fonds voor Wetenschappelijk Onderzoek" (FWO), Flanders and from the "Onderzoeksfonds" of the K. U. Leuven, Belgium. We also wish to thank Rita Vos (Gastroenterology, University Hospitals Leuven, Belgium) for her assistance during the in vivo studies. J.B. is a postdoctoral fellow of the Research Foundation Flanders (FWO).

REFERENCES

(1) Connors, R. D.; Elder, E. J. Using a Portfolio of Particle Growth Technologies to Enable Delivery of Drugs With Poor Water Solubility. *Drug Delivery Technol.* **2004**, *4*, 78–83.

(2) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235–249.

(3) Brouwers, J.; Brewster, M. E.; Augustijns, P. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? *J. Pharm. Sci.* **2009**, *98*, 2549–2572.

(4) Vandecruys, R.; Peeters, J.; Verreck, G.; Brewster, M. E. Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design. *Int. J. Pharm.* **2007**, *342*, 168–175.

(5) Brewster, M. E.; Vandecruys, R.; Verreck, G.; Peeters, J. Supersaturating drug delivery systems: effect of hydrophilic cyclodextrins and other excipients on the formation and stabilization of supersaturated drug solutions. *Pharmazie* **2008**, *63*, 217–220.

(6) Curatolo, W.; Nightingale, J. A.; Herbig, S. M. Utility of hydroxypropylmethylcellulose acetate succinate (HPMCAS) for initiation and maintenance of drug supersaturation in the GI milieu. *Pharm. Res.* **2009**, *26*, 1419–1431.

(7) Raghavan, S. L.; Trividic, A.; Davis, A. F.; Hadgraft, J. Crystallization of hydrocortisone acetate: influence of polymers. *Int. J. Pharm.* **2001**, *212*, 213–221.

(8) Miller, D. A.; DiNunzio, J. C.; Yang, W.; McGinity, J. W.; Williams, R. O. Enhanced in vivo absorption of itraconazole via stabilization of supersaturation following acidic-to-neutral pH transition. *Drug Dev. Ind. Pharm.* **2008**, *34*, 890–902.

(9) Gao, P.; Akrami, A.; Alvarez, F.; Hu, J.; Li, L.; Ma, C.; Surapaneni, S. Characterization and optimization of AMG 517 supersaturatable self-emulsifying drug delivery system (S-SEDDS) for improved oral absorption. *J. Pharm. Sci.* **2009**, *98*, 516–528.

(10) Dai, W.; Dong, L. C.; Li, S.; Deng, Z. Combination of Pluronic/Vitamin E TPGS as a potential inhibitor of drug precipitation. *Int. J. Pharm.* **2008**, *355*, 31–37.

(11) Guzmán, H. R.; Tawa, M.; Zhang, Z.; Ratanabanangkoon, P.; Shaw, P.; Gardner, C. R.; Chen, H.; Moreau, J.; Almarsson, O.; Remenar, J. F. Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. *J. Pharm. Sci.* **2007**, *96*, 2686–2702.

(12) Dias, M. M. R.; Raghavan, S. L.; Pellett, M. A.; Hadgraft, J. The effect of beta-cyclodextrins on the permeation of diclofenac from supersaturated solutions. *Int. J. Pharm.* **2003**, *263*, 173–181.

(13) Carlert, S.; Pålsson, A.; Hanisch, G.; von Corswant, C.; Nilsson, C.; Lindfors, L.; Lennernäs, H.; Abrahamsson, B. Predicting intestinal precipitation--a case example for a basic BCS class II drug. *Pharm. Res.* **2010**, *27*, 2119–2130.

(14) Bevernage, J.; Brouwers, J.; Clarysse, S.; Vertzoni, M.; Tack, J.; Annaert, P.; Augustijns, P. Drug supersaturation in simulated and human intestinal fluids representing different nutritional states. *J. Pharm. Sci.* **2010**, *99*, 4525–4534.

(15) Warren, D. B.; Benameur, H.; Porter, C. J. H.; Pouton, C. W. Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility. *J. Drug Targeting* **2010**, *18*, 704–731.

(16) Clarysse, S.; Tack, J.; Lammert, F.; Duchateau, G.; Reppas, C.; Augustijns, P. Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. *J. Pharm. Sci.* **2009**, *98*, 1177–1192.

(17) Vertzoni, M.; Fotaki, N.; Kostewicz, E.; Stippler, E.; Leuner, C.; Nicolaides, E.; Dressman, J.; Reppas, C. Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects. *J. Pharm. Pharmacol.* **2004**, *56*, 453–462.

(18) Clarysse, S.; Psachoulias, D.; Brouwers, J.; Tack, J.; Annaert, P.; Duchateau, G.; Reppas, C.; Augustijns, P. Postprandial changes in solubilizing capacity of human intestinal fluids for BCS class II drugs. *Pharm. Res.* **2009**, *26*, 1456–1466.

(19) Annaert, P.; Brouwers, J.; Bijnens, A.; Lammert, F.; Tack, J.; Augustijns, P. Ex vivo permeability experiments in excised rat intestinal tissue and in vitro solubility measurements in aspirated human intestinal fluids support age-dependent oral drug absorption. *Eur. J. Pharm. Sci.* **2010**, *39*, 15–22.

(20) Bauer, J.; Spanton, S.; Henry, R.; Quick, J.; Dziki, W.; Porter, W.; Morris, J. Ritonavir: an extraordinary example of conformational polymorphism. *Pharm. Res.* **2001**, *18*, 859–866.