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Research Paper

Postprandial Changes in Solubilizing Capacity of Human Intestinal Fluids for BCS Class II Drugs

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Purpose. To explore the effect of the nutritional state on the solubilizing properties of human intestinal fluids (HIF) on a time-after-food administration basis.
Methods. HIF were collected in fractions of 30 min from five volunteers in the fasted, fed and fat-enriched fed state. *In vitro* solubility of five BCS class II drugs (danazol, diazepam, nifedipine, ketoconazole, indomethacin) was assessed in the intestinal fractions and simulated intestinal fluids.
Results. Solubilities in intestinal fractions were characterized by high time- and subject-dependent variability. For the non-ionized drugs, solubility in early intestinal fractions was higher in both fed states compared to the fasted state, and in the fat-enriched fed state compared to the fed state. Solubility in simulated intestinal fluids did not sufficiently predict the solubilizing capacity of the early postprandial phase. Solubility in HIF was shown to be determined by a complex interplay of various intraluminal parameters. For the ionized drugs, pH played a significant role for indomethacin (R^2=0.86); for the partly ionized ketoconazole other intraluminal parameters were also important.
Conclusions. Solubilizing capacity of HIF in the fed state is strongly time-dependent. Intraluminal dissolution may, therefore, vary with drug arrival time in the small intestine and constitute a source of variability in intestinal drug absorption.

KEY WORDS: human intestinal fluids; poorly water soluble drugs; postprandial; simulated intestinal fluid; solubilizing capacity.

INTRODUCTION

Co-administration of a lipophilic drug with a meal often results in increased bioavailability compared to administration in the fasted state. For danazol for example, it was demonstrated that the oral bioavailability was threefold higher when a single dose was taken together with a lipid-rich meal compared to the conventional intake with 200 mL of water (1). Enhanced bioavailability may be attributed to a number of potential mechanisms including increased intraluminal solubility, stimulated lymphatic transport, prolonged gastrointestinal residence time and motility, increased permeability across the luminal wall, and reduced metabolism and efflux activity (2). An increased solubility in the postprandial intestinal environment is postulated as the major cause of the observed rise in oral bioavailability. Mainly poorly water soluble drugs suffer from poor clinical outcome due to low intraluminal solubility. Consequently, drugs classified as class II and IV in the Biopharmaceutics Classification System (BCS) will benefit from enhanced intraluminal solubility due to food intake. Changes in the composition of the intraluminal environment caused by bile and pancreatic secretions along with the presence of exogenous lipid products are thought to be the major contributors to the enhanced solubilizing capacity of fed state intestinal fluids. A prerequisite for the intestinal absorption of a lipophilic drug is its solubilization in the aqueous phase of the intestinal contents; therefore, micellar phases, constituted of bile salts, phospholipids, lipid degradation products and cholesterol are essential.

Information on the importance of food on intraluminal drug solubilization can be obtained by directly sampling from the intestinal contents. Although it has been demonstrated that direct measurement of intraluminal drug concentrations is possible (3–5), this procedure is complex, labor intensive and not feasible to be performed on a large scale. In order to overcome these limitations, it is desirable to have a surrogate intestinal medium that can be used in *in vitro* solubility studies allowing a reliable estimation of the amount of solubilized drug *in vivo*. Dressman and coworkers were the

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ABBREVIATIONS: BCS, Biopharmaceutics Classification System; FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; FeSSIF v2, fed state simulated intestinal fluid version 2; HIF, human intestinal fluids; MLR, multiple linear regression.

first to address this problem and proposed a simulated medium for the fed state (fed state simulated intestinal fluid, FeSSIF) (6). FeSSIF consists of a phosphate buffer adjusted to pH 5.0, supplemented with 15 mM taurocholic acid and 3.75 mM lecithin. To establish the predictive power of FeSSIF for *in vivo* solubility estimation, a limited number of studies have compared the solubility obtained in this simulated medium with the solubility obtained in fed state human aspirates. For ketoconazole, it was found that the use of FeSSIF as solubility medium underestimated the solubilizing capacity of aspirated fed state human intestinal fluids (HIF); in contrast, for the drug dipyridamole, a higher solubility was found in FeSSIF (7). Based on pooled aspirates, Persson *et al.* reported a much lower solubility in FeSSIF as compared to HIF for the poorly water soluble drugs cyclosporine, danazol, griseofulvin and felodipine (8). The authors attributed this to the lack of dietary lipids in FeSSIF in spite of the high bile salt concentration. It has been suggested that the impact of food on drug solubility is probably dependent on the log*P* value of the drug, although it is possible that all poorly soluble drugs can benefit from the occurrence of a postprandial intestinal state (8).

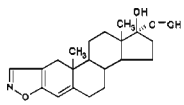
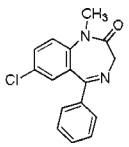
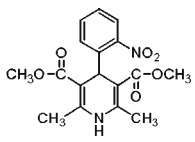
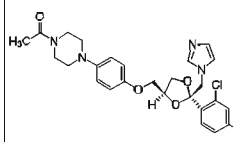
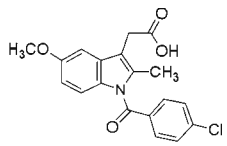
Besides the lack of lipid degradation products, FeSSIF is a homogenous medium which does not simulate the dynamic gastrointestinal environment. After the intake of food, the intestinal contents are changing continuously due to the digestion process; therefore, the solubilizing capacity of the intestinal fluids may be altered concomitantly. Very recently, Dressman and coworkers proposed three snapshot media that should better mimic the changing postprandial contents (early, middle and late FeSSIF) (9). The composition of these media is based on the characterization of HIF aspirated every 20 min for a period of 210 min after the ingestion of 500 mL Ensure Plus® (10). In addition to bile salts and phospholipids, the snapshot media also contain monoolein and oleic acid. On the basis of these newly proposed compositions, a second generation biorelevant medium (FeSSIF v2) that could be used for global assessment of drug solubilization capacity of the fed small intestinal contents was introduced as alternative to FeSSIF. However, as the evolving solubilizing capacity of the postprandial contents has not been examined yet, there is no evidence that the *in vitro* solubility data in the recently proposed snapshot media or FeSSIF v2 reflect the true *in vivo* solubility. Despite their design to simulate the intestinal composition, simulated *in vitro* media and real HIF may have different functional characteristics. Therefore, it is the purpose of this study to elucidate the solubilizing capacity of human postprandial duodenal fluids. Human duodenal fluids were aspirated at different time points after water/meal intake and were used for *in vitro* solubility studies. Different liquid meals were administered to explore any effect of meal composition on solubilizing capacity. Together with data available from a thorough characterization of the intestinal fractions used in this study (11), this strategy will allow determining the predictive power of the existing simulated intestinal media for *in vivo* solubility estimation by comparing the obtained solubility profiles with solubility data resulting from using simulated intestinal fluids as solubilizing media. These data may assist in the improvement of the composition of biorelevant media for *in vivo* solubility estimation. To achieve the aim, five poorly water soluble drugs with different ionization behavior [danazol, diazepam, nifedipine, ketoconazole and indomethacin (Table I)] were used as model drugs in this study.

MATERIALS AND METHODS

Materials

Ketoconazole and indomethacin were purchased from Alpha Pharma (Waregem, Belgium). Danazol was obtained

Table I. Physicochemical Parameters and Solubility Data in (Blank) FaSSIF and FeSSIF for the Five Model Drugs Used in the Present Study

	Danazol	Diazepam	Nifedipine	Ketoconazole	Indomethacin
Structure					
Ionization behavior	Neutral	Weak base[a]	Neutral	Dibasic	Weak acid
pK_a[b]		3.4		6.5 and 2.9[c]	4.5
xlogP[d]	4.2	2.9	2	4	3.4
Added amount (mg) per milliliter of medium	1	6	5	6	5
Equilibrium time fasted/fed (h)	4/4	2/2	2/2	4/2	2/4
Solubility in blank FaSSIF (μM)	0.9 ± 0.1	272 ± 6	38 ± 4	18.8 ± 0.2	1000 ± 10
Solubility in FaSSIF (μM)	16 ± 1	355 ± 4	68 ± 2	73 ± 5	1376 ± 8
Solubility in FeSSIF (μM)	52 ± 1	909 ± 4	173 ± 7	1420 ± 30	402 ± 8

Solubility data are presented as mean values of three experiments±standard deviations
[a] Not ionizable at the pH of the aspirated fasted, fed or fat-enriched fed state HIF
[b] The Merck Index, tenth edition, Windholz, M. (Ed.), Merck & Co., Inc, Rahway, N.J., USA
[c] (7)
[d] http://pubchem.ncbi.nlm.nih.gov/

from Indis NV (Aartselaar, Belgium) and diazepam from Fagron (Waregem, Belgium). Nifedipine, orlistat, glucose and sodium oleate (purum) were purchased from Sigma-Aldrich (St. Louis, MO). Glyceryl monooleate (GMO, Rylo MG 19 Pharma®) was kindly donated by Danisco Specialties (Grindsted, Denmark). Sodium taurocholate (practical grade) was purchased from ICN Biomedicals, Inc (Eschwege, Germany). D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) was provided by Eastman Chemical Company (Kingsport, TN) and Phospholipon 90G (lecithin) by Nattermann Phospholipid Gmbh (Köln, Germany). Acros Organics (Geel, Belgium) supplied methanol, sodium acetate trihydrate and $NaH_2PO_4 \cdot H_2O$. NaCl and acetonitrile were provided by Fisher Scientific (Leicestershire, UK). Chloroform and acetic acid were obtained from Chemlab NV (Zedelgem, Belgium); NaOH pellets were obtained from BDH Laboratory Supplies (Poole, UK). Hanks' Balanced Salt Solution (HBSS) and 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) were purchased from Lonza (Verviers, Belgium); maleic acid was obtained from VWR International (Leuven, Belgium). Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

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%, respectively; the osmolality amounts to 670 mOsm/kg; the pH is 6.6. Scandishake Mix® (Nutricia, Liverpool, UK) was used to create fat-enriched fed state intestinal conditions. After preparation as instructed by the manufacturers, the volume of one portion amounts to 300 mL with a total energy of 2,505 kJ, consisting of 46% lipids, 46% carbohydrates and 8% proteins on energy basis; the osmolality is 1,031 mOsm/kg and the pH is 6.7.

Solubility Media

Duodenal fluids of five healthy volunteers (two female, three male), collected every 30 min for a total period of 2 h (fasted state) or 5 h (fed state) in three different nutritional states, were used as solubility media in the *in vitro* solubility experiments. 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 30 min from the duodenum (D2–D3) after the intake of water (fasted state) or after the intake of a liquid meal (fed state). Two liquid meals, Ensure Plus® (400 mL+ 200 mL water) and Scandishake Mix® (300 mL+300 mL water), respectively, with equal caloric content but different energy values from fat (29% and 46%, respectively), were used in order to create a standard fed state (further referred to as fed state) and a fat-enriched fed state, respectively. The first sample was collected immediately after the water/liquid meal was ingested (t=0). We refer to a previous study for a more detailed description of the aspiration protocol of HIF (11). In order to inhibit further *in vitro* lipolysis, the general lipase inhibitor orlistat, dissolved in ethanol, was immediately added to the collected fractions in a final concentration of 1 μM (IC_{50}=11 nM, Unilever Research and Development, Vlaardingen, data not published) such that the ethanol concentration in the HIF sample was only 0.1% (*v*/*v*). For each volunteer separately, one pooled sample per nutritional state was made by adding equal volumes of all corresponding fractions, except for the fasted state for subject 2 and subject 5 (not enough volume available). All 30-min fractions and pooled samples were stored at −30°C until the assessment of their solubilizing capacity, and characteristics with respect to pH, triglycerides and lipid degradation products (diglycerides, monoglycerides and free fatty acids), bile salts, phospholipids, osmolality and surface tension. Protocol details and results of the characterization are discussed in a previously published paper (11).

Solubility experiments were also performed using the administered liquid meals (Ensure Plus® and Scandishake Mix®) and using simulated intestinal media for the fasted (FaSSIF) and the fed (FeSSIF) state. 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) (12). Blank FaSSIF (FaSSIF without bile salts and lecithin) was used to determine the solubility of each of the drugs in the absence of any solubilizing agent. In addition, recently proposed media simulating the early, middle and late postprandial phase as well as a second generation FeSSIF (FeSSIF v2) were used (9).

Solubility Studies

The solubilizing capacity of various media and of collected and pooled HIF was studied for five low aqueous solubility drugs (BCS class II) with different ionization behavior: danazol, diazepam, nifedipine, ketoconazole and indomethacin (Table I). All solubility experiments were performed in triplicate. An excess amount of solid drug substance was added to Eppendorf tubes containing 500 μL of the medium. Reliability of the protocol was confirmed as similar solubilities (RSD<5%) were obtained in 500 μL medium (Eppendorf tubes) and 5 mL medium (test tubes) (data not shown). The Eppendorf tubes were placed at 37°C in a prewarmed shaking incubator (Incubator shaker series 25D, New Brunswick Scientific Co., Inc, Edison, NJ). After incubation, samples were centrifuged for 15 min at 10,000×*g* and 37°C. This allowed separating the undissolved drug substance without phase separation of the medium. For samples containing HIF, 100 μL of the supernatant was mixed with 100 μL of methanol (protein precipitation) and centrifuged for 5 min at 14,000×*g* and 37°C. The supernatant was diluted into the mobile phase used during the subsequent high-performance liquid chromatography (HPLC) analysis. Stability of the model drugs in HIF was confirmed by adding known amounts of the drug to selected HIF fractions from the fasted and fed state (data not shown). The presence of orlistat and 0.1% (*v*/*v*) ethanol in the samples did not alter the solubilizing capacity as was indicated from solubility results obtained in FaSSIF with and without 1 μM orlistat and 0.1% ethanol as solubilizing medium (data not shown).

For samples containing the liquid meals, an extra centrifugation step (5 min at 14,000×*g*, 37°C) was required after dilution into the mobile phase to remove all the proteins. The resulting supernatant was analyzed by HPLC. For samples containing simulated intestinal fluid or blank FaSSIF, the supernatant resulting from separating the solubi-

lized drug from the undissolved drug substance was analyzed by HPLC without methanol dilution. In contrast to the other simulated media used in this study, middle FeSSIF was not a homogenous solution. In addition, this medium was not stable and lipid droplets were separated from an underlying turbid phase in which the solubility was assessed. Preliminary data showed, though, that the drug was also dissolved in the lipid phase (data not shown).

For danazol and ketoconazole in blank FaSSIF, the undissolved drug was separated from the drug in solution by filtration (PTFE syringe filter, pore size 0.1 μm, Whatman, Inc, Clifton, NJ). Due to poor wetting, the excess drug formed a film on the liquid surface which could not be removed by centrifugation (in contrast to the other model drugs). After the first 5 mL were discarded, 1 g of filtrate was retained. In order to inhibit drug adsorption to the glass recipient after filtration, TPGS, in a final concentration of 0.1% (*w*/*w*), was added after which the samples were diluted in the HPLC mobile phase and subjected to HPLC analysis.

Equilibrium time for the five model drugs in fasted, fed and fat-enriched fed state HIF was based on solubility measurements in FaSSIF and FeSSIF (Table I). After 1, 2, 4, 6, 8 and 24 h of incubation, samples were taken and analyzed. Equilibrium was considered to be reached when consecutive measurements showed less than 5% difference. Equilibration time for FaSSIF was considered representative for the fasted state intestinal samples; equilibration time for FeSSIF was considered representative for the intestinal samples collected after intake of Ensure Plus® or Scandishake Mix®.

Analytical Methods

All drug concentrations were determined by reversed-phase HPLC analysis with UV or fluorescence detection. An aliquot of the diluted supernatants, obtained as described above, was injected into a Waters HPLC system consisting of a 600E controller and pump, a 717plus autosampler and a Novapak C-18 column (Waters, Milford, MA). UV signals were detected by a Waters UV detector (W2487); fluorescence signals were detected by a Jasco FP-1520 fluorescence detector (Tokyo, Japan). All chromatographic methods were run in the isocratic mode. Details of the chromatographic methods are listed in Table II. The observed peaks were integrated using Empower Pro (Empower 2) software.

Calibration curves were made in HBSS (supplemented with glucose to a final concentration of 25 mM and 10 mM HEPES) adjusted to a pH of 7.4. To prevent adsorption, calibration curves for danazol and ketoconazole were prepared in HBSS supplemented with 0.1% (*v*/*v*) TPGS. 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 below 5.0% at all concentrations and for all model drugs. The relative error remained below ±6.3%.

Data Analysis

All solubility data presented in this article are mean values of triplicate experiments. In the mean solubility–time profiles, the standard deviations of the replicate experiments were omitted as the latter were negligible compared to the inter-subject variations. The presented boxplots are constructed corresponding to the whisker Tukey type. Data were compared using repeated measures ANOVA combined with Tukey multiple comparison test or using a paired *t*-test. Differences were considered statistically significant at $p<0.05$. The correlation between solubility data and intraluminal parameters was investigated by multiple linear regression (MLR) analysis (XLSTAT add-in for Microsoft Excel) based on the least squares principle.

RESULTS AND DISCUSSION

Solubility–Time Profiles in HIF

Fig. 1 illustrates the solubility of the five model drugs in the aspirated human intestinal 30-min fractions as a function of collection time in fasted, fed and fat-enriched fed state conditions. The solubility of the drugs in FaSSIF, FeSSIF and FeSSIF v2 indicated on the graphs will be discussed in the "FaSSIF, FeSSIF, FeSSIF v2" section.

The solubility–time profiles illustrated time-dependent variability in solubilizing capacity of intestinal fluids. This was more obvious in the fed and fat-enriched fed state although in the fasted state some time-dependent variability was noticeable as well. Fig. 1 also displays significant differences in solubilizing capacity of HIF aspirated from different subjects

Table II. Parameters for the HPLC Analysis for the Five Model Drugs Used in the Present Study

	Danazol	Diazepam	Nifedipine	Ketoconazole	Indomethacin
Mobile phase	85:15 MeOH[a]/water	50:50 ACN[b]/buffer	60:40 ACN[b]/buffer	80:20 MeOH[a]/buffer	50:50 ACN[b]/buffer
Buffer	–	25 mM sodium acetate, pH 3.5	25 mM sodium acetate, pH 3.5	25 mM sodium acetate, pH 3.3	25 mM sodium acetate, pH 3.5
Flow rate (mL/min)	1	1.5	1	0.8	1.5
Retention time (min)	6.5	7.2	5.5	6.7	9.5
Detection	UV, 288 nm	UV, 228 nm	UV, 340 nm	Fluo[c], ex=260nm, em=375nm	UV, 266 nm
Quantification limit (μM)	0.04	0.8	0.4	0.02	0.5

[a] Methanol
[b] Acetonitrile
[c] Fluorescence, excitation and emission wavelength

as was demonstrated by the high inter-subject standard deviations. The time- and subject-dependent variability in solubilizing capacity corresponds with the varying time-profiles of the intraluminal parameters which were determined for these 30-min intestinal fractions (11). This is, to the best of our knowledge, the first study in which both inter-subject and time-dependent variability in solubilizing capacity of intestinal fluids are taken into account. Inter-subject variability in solubility of danazol in pooled fasted and fed state intestinal samples has been demonstrated by others (8, 13): a sixfold difference was found between the highest and lowest solubility obtained in fasted state intestinal fluids (13). This agreed relatively well with the eightfold difference between the solubility values in the pooled fasted state samples of subject 1, 3 and 4. However, a much higher difference (36-fold) between the highest and lowest solubility in all individual fractions was found. This illustrates the importance of the time factor even in fasted state conditions.

The mean solubilities obtained in pooled fed and fat-enriched fed state HIF were 99.3, 1,160, 122, 1,140, 1,020 μM (fed state), and 142, 1,530, 172, 1,350, 1,620 μM (fat-enriched fed state) for danazol, diazepam, nifedipine, ketoconazole and indomethacin, respectively. No statistically significant differences were found between the two fed states. In general, these solubility values for the pooled samples underestimated the solubility obtained during the first 60 min postprandially which may be attributed to the fact that the pooled samples were obtained by pooling from 0 until 5 h after food intake. Consequently, the first postprandial samples are diluted such that the resulting solubilizing capacity may be decreased. This illustrates that pooling of aspirates collected at various times after meal administration may influence the solubilizing capacity of HIF; therefore, the use of such pooled aspirates is questionable in estimating intraluminal solubility.

For the non-ionized drugs (danazol, diazepam, nifedipine), there was a trend for higher solubilities in the fed state HIF compared to the fasted state intestinal samples during the first 60 min after food intake. After 60 min, solubility values decreased and a difference with the fasted state was no longer obvious. Similarly, the only difference in solubilizing capacity that could be observed between the fed and fat-enriched fed state was also within the first 30 min postprandially (not statistically significant).

Also for the partly ionized model drug ketoconazole, solubility values at 0 and 30 min post food intake tended to be higher than in the fasted state. This contrasts with the solubility–time profiles obtained for indomethacin where solubility in the fasted state was sometimes higher than solubilities in fed and/or fat-enriched fed state fractions. The specific food effect for indomethacin may be limited due to its relatively high aqueous solubility and the pH–pK_a interplay which is more important (further clarified in the "Solubility in Simulated Intestinal Fluids and in Administered Drinks" and "Relationship Between Solubility and Intraluminal Parameters" sections).

Kalantzi *et al*. performed a similar solubility study with HIF aspirated from the distal duodenum at 30, 60, 120 and 180 min after administration of 500 mL Ensure plus® and with two weak bases as model drugs (7). One sample was created per time point by pooling the corresponding fractions of 12 subjects. For ketoconazole, the authors found a solubility of about 1.8 mM at 30 min after intake of Ensure Plus®. This value stayed relatively constant until a significant decrease was noticed 180 min postprandially. The solubility of 1.8 mM approaches the mean fed state value of 1.98 mM obtained for ketoconazole in the current HIF aspirated 30 min postprandially. In contrast with the findings of Kalantzi and coworkers who did not see any effect of aspiration time on the solubility for times up to 120 min after administration of Ensure Plus® (7), a maximum solubility was reached in the 30-min fraction (although not statistically significant) (Fig. 1). The difference between both studies might be explained by protocol differences (coadministration of water, total volume administered, volume of aspiration).

Solubility in Simulated Intestinal Fluids and in Administered Drinks

Comparison of solubility in different media revealed a broad range in solubilities for the model drugs tested (Fig. 2, Table I). In blank FaSSIF, the highest solubility was observed for indomethacin (pK_a 4.5) while the lowest solubility was found for danazol which also has the highest log$P$ value of the model drugs used in this study. The solubility of danazol (0.85 μM) falls in the range of aqueous solubilities reported in literature (0.3–3 μM) (13–15). Fig. 2 illustrates that, by keeping pH and buffer species constant, the solubility increased by including bile salts and lecithin. The highest impact of these amphiphilic compounds on solubility was observed for danazol and ketoconazole for which solubility was enhanced 18-fold and fourfold, respectively. This suggests that an increase in solubility induced by bile salts and phospholipids was more pronounced for the most lipophilic drugs (Table I). A correlation between enhanced solubility elicited by amphiphiles, and drug lipophilicity has also been reported before (14,16).

It has been proposed that the total amphiphile concentration is more important in determining the solubility of a drug, rather than the individual components of the solubilizing medium (17,18). This has been illustrated for the solubilization of danazol (18) and estradiol (17) for which solubilization showed an improved correlation with the cumulative concentration of bile salts, (phospholipids), monoglycerides and free fatty acids. This was explored in the present study with simulated media representative of the fed state intestine. The correlation was confirmed for danazol, but not for the other non-ionized drugs (Fig. 3). Total concentration of amphiphiles corresponding to the presented media increases in the following order: late FeSSIF (6.8 mM), FeSSIF v2 (17.8 mM), FeSSIF (18.75 mM), middle FeSSIF (44.5 mM) and early FeSSIF (59.5 mM). Solubility in middle FeSSIF was higher than in FeSSIF and FeSSIF v2 for danazol; in contrast, FeSSIF and FeSSIF v2 appeared to have a higher solubilizing capacity towards diazepam and nifedipine than middle FeSSIF. At this point, it is important to mention that middle FeSSIF has two major practical drawbacks. Firstly, middle FeSSIF did not give a clear solvent system; working with a turbid system is not practical for performing solubility experiments. Secondly, the medium was found to be unstable. In contrast to the other media used in this study, small lipid droplets were separated from an underlying turbid phase in which the solubility was assessed.

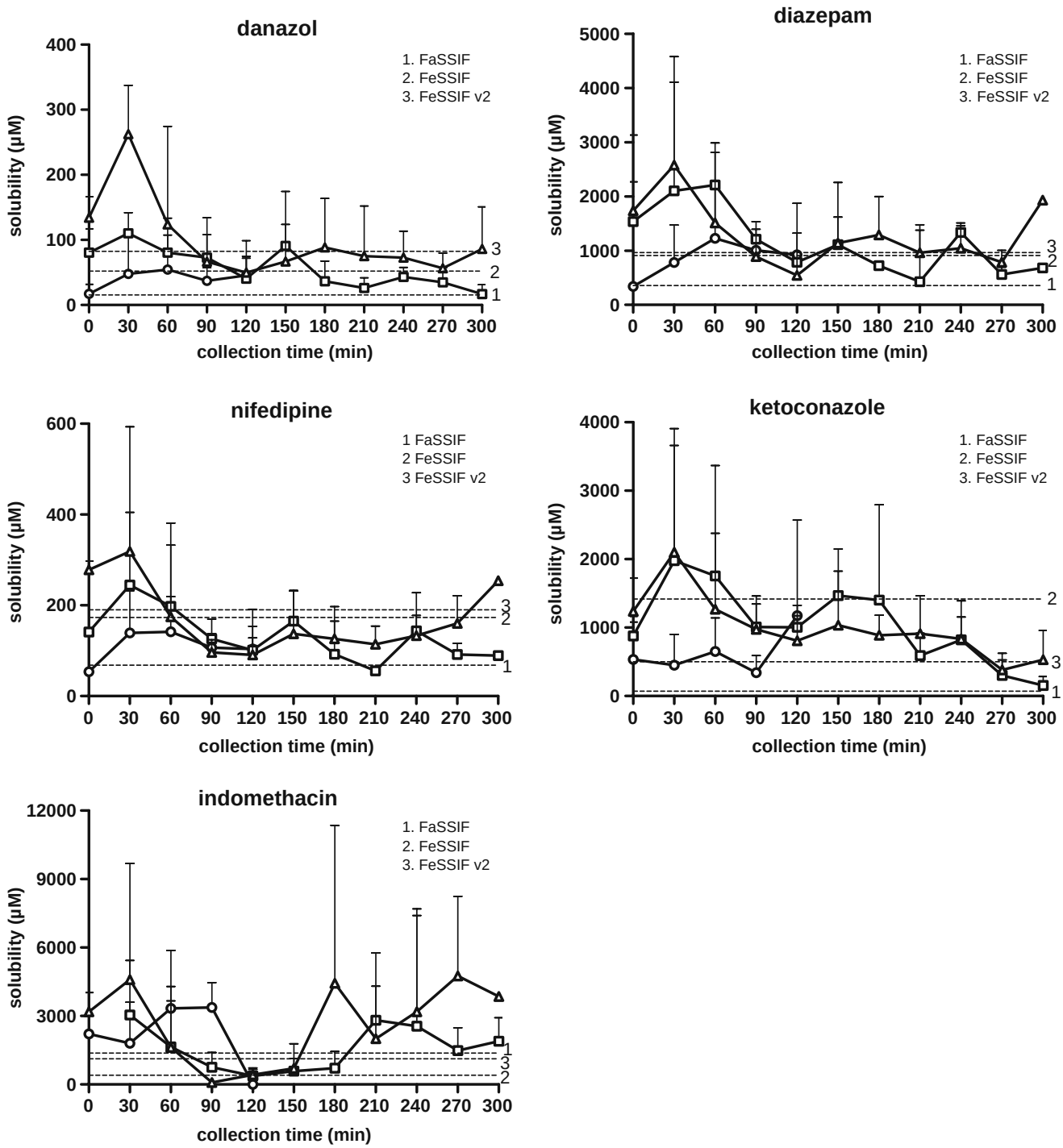


Fig. 1. Mean (±inter-subject SD) solubility–time profiles in fasted state (*open circles*), fed state (*open squares*) and fat-enriched fed state (*open triangles*) HIF for the five model drugs. Solubility of the model drugs was assessed in each HIF fraction of the five subjects unless not enough volume was available ($n \leq 5$). Measurements were performed in triplicate but corresponding variability was much lower than the inter-subject variability shown on the graphs. For each drug, solubility in FaSSIF (*1*), FeSSIF (*2*) and FeSSIF v2 (*3*) is indicated by *broken lines*.

The partitioning of drugs between different phases will be drug dependent and therefore influence the results.

In Fig. 2, it was demonstrated that solubility for the non-ionized drugs was further increased with complexity of the medium. In contrast, for the ionized model drugs, pH of the medium primarily determined the rank order. Nevertheless, the increase in solubility of ketoconazole in Ensure Plus® and Scandishake Mix® compared to FaSSIF could probably be attributed to the lipid products present in the administered drinks.

Several studies have indicated the usefulness of adding lipolytic products to an *in vitro* medium in order to enhance the solubility of a drug. The solubility of seocalcitol (log*P* 4.8), for instance, was increased by adding either long-chain or short-chain monoglycerides and fatty acids. This increase was higher when the concentration of bile salts in the medium

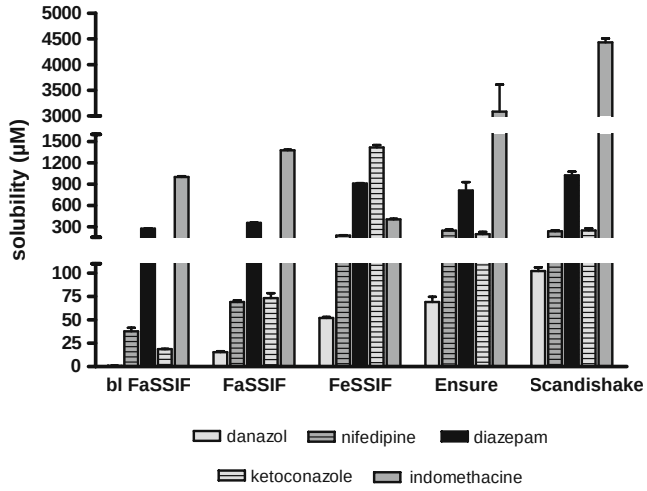


Fig. 2. Mean±SD (n=3) solubility data of the five model drugs in buffer [blank FaSSIF (bl FaSSIF)], in simulated intestinal fluids (FaSSIF and FeSSIF) and in the administered drinks [Ensure Plus® (Ensure) and Scandishake Mix® (Scandishake)]. The solubility of danazol in blank FaSSIF (0.85 µM) was too low to be distinguished from the x-axis.

was low (19). Kaukonen and coworkers examined the *in vitro* postdigestion solubilization of a range of poorly water soluble drugs (20). It was demonstrated that a substantial improvement in solubility could be attained by inclusion of a digested lipid: solubility increased with a factor up to 7 and 11 for diazepam and danazol, respectively. The enhancing effect of digested lipids on solubility was more pronounced for lower concentrations of bile salts and phospholipids. The positive effect of including lipolysis products to a simulated medium to enhance drug solubility was clearly shown for the solubilizing capacity of early FeSSIF towards non-ionized and ionized drugs. Early FeSSIF appeared to have the highest solubilizing capacity for all model drugs (Figs. 3 and 4). This snapshot medium consists of a high concentration of monoglycerides (6.5 mM) and free fatty acids (40 mM). Middle and late FeSSIF showed a further gradual decrease in solubilizing capacity (Figs. 3 and 4). Following this observation for the basic drug ketoconazole and taking into account the pH of the media relative to the pK_a of the drug, it can be stated that, in addition to pH, solubility of ketoconazole is also highly dependent on other intraluminal parameters.

Simulated *In Vitro* Media *Versus* Real *In Vivo* Media

FaSSIF, FeSSIF, FeSSIF v2

Fig. 1 allows comparing solubility data obtained in HIF with those obtained in simulated intestinal fluids which do not discriminate between different postprandial phases. For the non-ionized drugs it was observed that FaSSIF resulted in solubility underestimation compared to the fasted state intestinal samples. As the concentration of bile salts and phospholipids in the simulated (3 and 0.75 mM, respectively) and the *in vivo* media (mean 3.17 and 0.86 mM, respectively) are comparable (11), other factors are involved to explain the observation. The absence of proteins in FaSSIF might be a possible explanation: *in vivo*, binding to proteins may result in increased apparent solubility. Another explanation might be related to the colloidal phases that are present in simulated and *in vivo* intestinal fluids. These colloidal phases may have different structures and, therefore, their solubilizing capacity may be different. For the solubilization of the non-ionized drugs in FeSSIF, Fig. 1 also demonstrates a lower solubilizing capacity compared to HIF aspirated the first 30 to 60 min after intake of Ensure Plus® or Scandishake Mix®. The lack of lipid degradation products in FeSSIF is most likely the most important reason for the observed underestimation. Persson *et al.* and Kalantzi *et al.* came to the same conclusion in their solubility study with (pooled) fed state intestinal fluids and ketoconazole, cyclosporine, danazol, griseofulvine or felodipine (8, 10). Probably due to the presence of oleic acid and monoolein in FeSSIF v2, the solubilizing capacity for the non-ionized drugs was higher than for FeSSIF, although the difference was not very extensive. For danazol, diazepam and nifedipine, FeSSIF v2 seemed to be a relatively good predictor for solubilities obtained in HIF aspirated later than 60 min after food intake. Nevertheless, this was more obvious for danazol and diazepam than for nifedipine (Fig. 1). For ketoconazole (pK_a 6.5) on the other hand, the solubility obtained in FeSSIF and FeSSIF v2 was quite different. The pH of the media relatively to the pK_a may explain this observation. For indomethacin, an underestimation was found for all simulated media presented in Fig. 1. Solubility increased with increasing pH; this is in the following order: FeSSIF (pH 5.0), FeSSIF v2 (pH 5.8), FaSSIF (pH 6.5). Based on the previous observations, a slight pH adjustment may thus already improve the predictive power of the simulated intestinal media for the ionized drugs. Also for the non-ionized drugs, it was proven that, especially for the early postprandial phase, the above simulated intestinal media were inadequate in predicting the *in vivo* solubility. Because this phase probably contributes most to the solubilization of orally administered drugs, there is a need for an *in vitro* medium that better simulates the solubilizing capacity of the early postprandial intestinal fluids. Due to the fluctuating solubility profile, one medium will not suffice in predicting the whole profile. In line with this thought, Dressman and coworkers have recently proposed three snapshot media that should be representative of the early, middle and late postprandial phase. The usefulness of these simulated media in predicting the solubilizing capacity of HIF will be discussed in the next paragraphs.

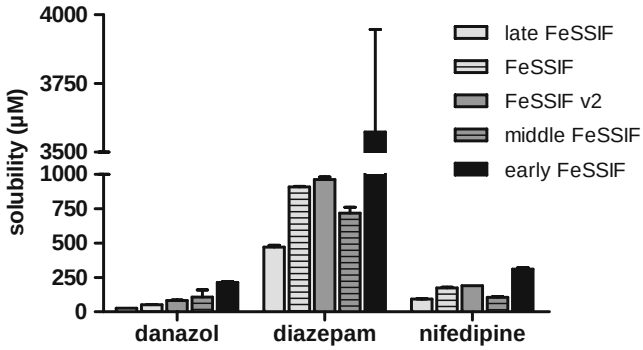


Fig. 3. Solubility of the three non-ionized model drugs (danazol, diazepam, nifedipine) in different simulated intestinal media. Early FeSSIF, middle FeSSIF, late FeSSIF and FeSSIF v2 refer to the snapshot media proposed by Dressman and coworkers (9).

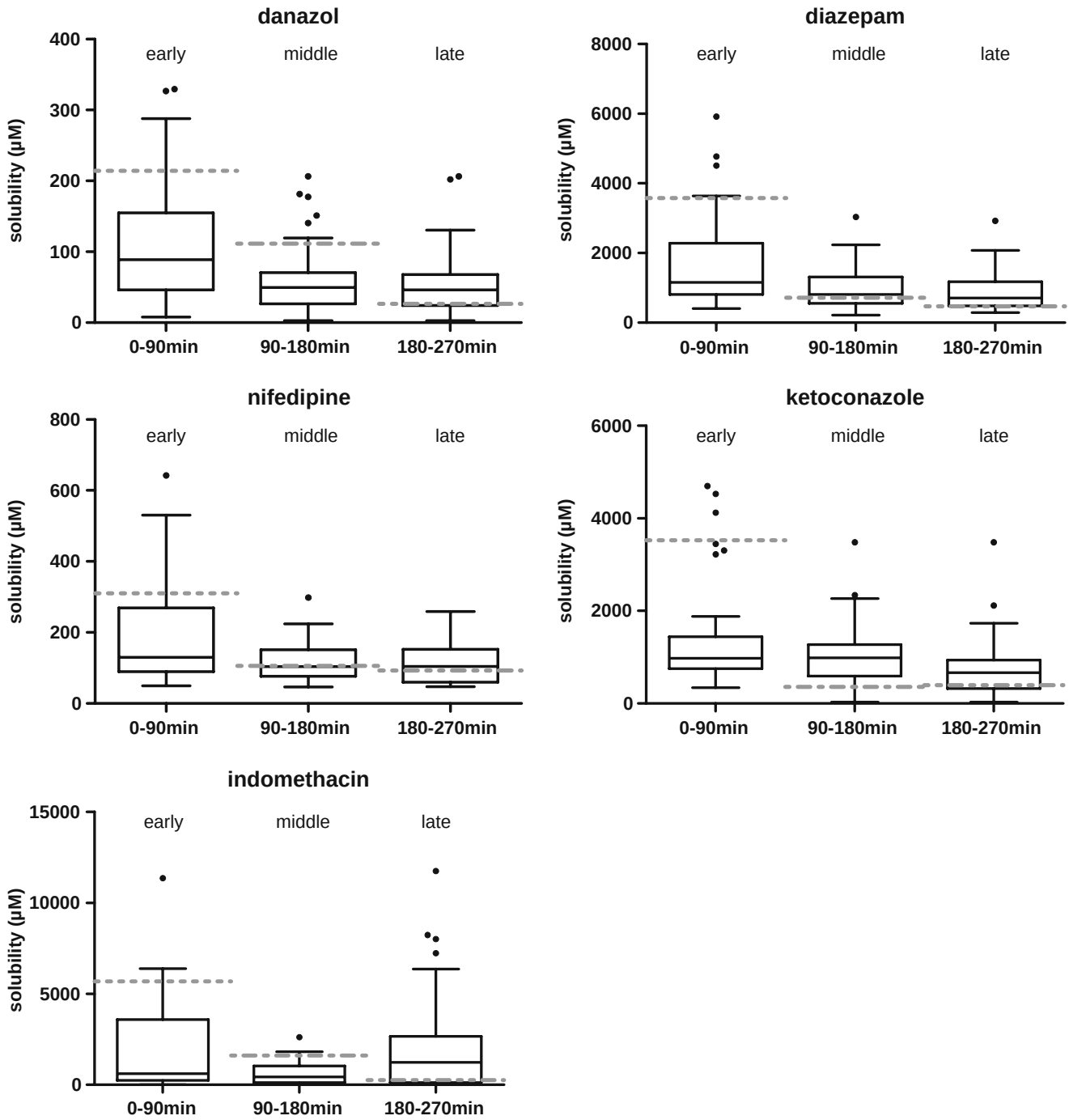


Fig. 4. Whisker Tukey-type box plots of the solubility data of the five model drugs in individual 30-min fractions aspirated in the fed and fat-enriched fed state, divided in an early, middle and late postprandial phase corresponding to the design of early FeSSIF, middle FeSSIF and late FeSSIF (9). The solubility in early, middle, and late FeSSIF is indicated by a *broken line* in the corresponding box plot.

Early, Middle and Late Postprandial Media

In order to better compare solubility data obtained in individual HIF fractions with solubility data resulting from the snapshot media, box plots were created (Fig. 4). The composition of early, middle and late FeSSIF is based on the composition of HIF aspirated from 0 to 75, 75 to 165 and 165 to 240 min after the intake of Ensure Plus®, respectively (9). Box plots that matched these time periods were created. The box plot indicated as 'early' contains all the solubility data resulting from using individual HIF fractions aspirated at 0, 30, 60 and 90 min postprandially. For the construction of the box plots indicated as 'middle' and 'late', a similar approach was followed. As a difference between the fed and fat-enriched fed state was only detected for the first 30 min after food intake, the box plots were constructed by combining both fed states. No significantly different conclusions evolve by splitting the fed and the fat-enriched fed state data (data not shown). For all drugs, early FeSSIF seemed to be indicative of the maximum solubility that was obtained

during the early postprandial phase. However, it can be seen from the graphs in Fig. 4 that the solubility in early FeSSIF was slightly to a lot higher than the majority of the solubilities in HIF. This indicated that, although early FeSSIF was designed on the basis of the composition of HIF aspirated during the first 75 min after the intake of Ensure Plus® (10), the solubilizing capacity of the simulated medium towards different drugs was not similar to the solubilizing capacity of the intestinal media aspirated in this study. This discrepancy could in the first place be explained by differences in composition of the aspirated fluids used to perform the current solubility study (11) and the composition of the snapshot medium. Secondly, as was mentioned before, the kind and shape of colloidal structures in the simulated medium and in the *in vivo* media may be different and therefore also the solubilizing capacity. Simulated media are created on the basis of one type of bile salt, phospholipid, monoglyceride and free fatty acid compared to *in vivo* samples which, for example, contain more types of lipid products than those with a mono-unsaturated C18 chain. It has been demonstrated that the micellar size increased upon increasing concentration of lipid degradation products for long-chain lipid products. On the contrary, for medium-chain lipid products micellar size was not altered during an *in vitro* lipolysis study (21). Furthermore, a very distinct difference in degree of solubilization when long-chain lipolytic products were used to when medium-chain lipolytic products were used, has been demonstrated for the solubility of very lipophilic drugs. The use of long-chain lipolytic products was beneficial for an enhanced solubility (22). This may partly explain the high solubility found for all model drugs in early FeSSIF (6.5 mM monoolein, 40 mM oleate). It has also been substantiated that the equilibrium of several colloidal components is dependent on the composition of the medium. In addition, the proportional contribution of each colloidal component to overall drug solubility is different (23). To further examine the relative contribution of the types of colloidal phases on the solubility of poorly water soluble drugs, the colloidal phases in both HIF as well as in the simulated media should be characterized. The simplest method to perform these experiments would be using the dynamic light scattering technique. This is, though, not applicable to (semi-) turbid systems like aspirated fed state HIF.

In general, the solubility obtained in middle and late FeSSIF was more in line with the solubility reached in HIF aspirated after 90 min postprandially (Fig. 4). Unlike for early FeSSIF, the relative position of middle and late FeSSIF to the box plot is more dependent on the drug tested. For danazol for example, middle FeSSIF overestimated the solubilizing capacity of HIF aspirated in the mid-postprandial phase whereas the same snapshot medium gave reliable results for the solubility of diazepam. Although the proposition of the recent snapshot media was a step ahead, the results of this study illustrate that further fine-tuning is required.

Relationship Between Solubility and Intraluminal Parameters

MLR was performed to detect any correlation between the solubilizing capacity of HIF fractions towards poorly water soluble drugs, described in this article, and the intraluminal parameters of these intestinal samples, reported in a previously published paper (11) (Table III). Solubility, without discrimination of the nutritional state, was taken as the dependent variable; the characterized parameters (pH, total bile salts, total phospholipids, free fatty acids, monoglycerides, diglycerides, triglycerides, surface tension, osmolality) were considered the independent variables. The log(solubility) was taken for MLR analysis in the case of ketoconazole and indomethacin to minimize the trend that was detected in the residual plot. In order to eliminate multi colinearity (R^2=0.76), bile salts and phospholipids were considered as one independent variable (total bile salts+total phospholipids). The presented R^2 corresponds to the model including all independent variables. For indomethacin, it was clear that pH was by far the dominating parameter (Table III). Log(solubility) was linearly correlated with pH expressed by a R^2 of 0.86 (Fig. 5). The sum of bile salts and phospholipids only added a small additional value to the model. Also for ketoconazole, solubility in HIF was significantly affected by pH, although to a lesser extent. pH, in combination with other intraluminal parameters, mainly free fatty acids, interplay to determine the solubility. The findings based on MLR concerning these ionized drugs

Table III. Multiple Linear Regression (MLR) of the Solubility of Five Model Drugs in Human Intestinal Fluids as a Function of Intraluminal Parameters

	Danazol	Diazepam	Nifedipine	Ketoconazole	Indomethacin
Dependent variable	Solubility	Solubility	Solubility	Log(solubility)	Log(solubility)
R^{2a}	0.68	0.74	0.76	0.73	0.93
Intraluminal parameters (standardized coefficient)[b]	BS+PL (0.469), fatty acids (0.420)	Fatty acids (0.477), monoglycerides (0.377)	BS+PL (0.492), fatty acids (0.292), monoglycerides (0.257), pH (0.154)	pH (−0.439), fatty acids (0.369), surface tension (−0.299), BS+PL (0.275), monoglycerides (0.241), diglycerides (−0.202)	pH (0.907), BS+PL (0.102)

MLR was performed for each drug, using the solubility or the log(solubility) as the dependent variable and pH, osmolality, surface tension, the total concentration of endogenous amphiphilic components [bile salts+phospholipids (BS+PL)] and the concentrations of lipids (fatty acids, mono-, di- and triglycerides) as the independent variables

[a] For all drugs, the multiple regression was statistically significant ($p<0.0001$)

[b] Only the parameters with a regression coefficient significantly differing from zero ($p<0.05$) are mentioned. These parameters are listed by their relative importance in the model as indicated by the standardized regression coefficients

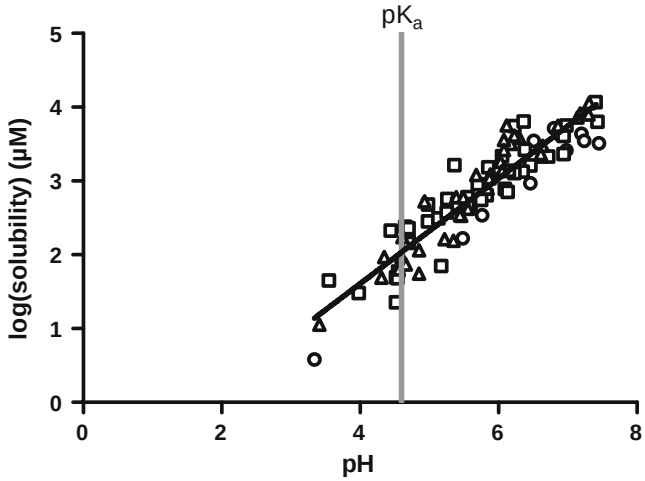


Fig. 5. Log(solubility) of indomethacin in HIF fractions in the fasted (*open circles*), fed (*open squares*) and fat-enriched fed (*open triangles*) state as a function of pH. Coefficient of determination is 0.86.

were also quoted during the discussion above. For the non-ionized drugs, the MLR results define free fatty acids, sum of bile salts and phospholipids, and monoglycerides as the important variables. The existence of a correlation between these parameters and solubility of poorly water soluble drugs has been illustrated before.

The performed MLR analysis provided us with an idea of the general correlation between solubility in HIF and the corresponding intraluminal parameters. This study protocol did not allow examining the specific contribution of one parameter to the overall solubility. To do so, all possible combinations of the independent variables should be included in the analysis; for this study, only combinations occurring in real duodenal samples (11) were available. If one is interested in the exact contribution of a specific parameter, an extensive experimental design would probably give more detailed information.

CONCLUSIONS

The aim of this study was to examine the solubilizing capacity of HIF that were aspirated from five subjects as a function of time after water or food intake. In order to detect the possible contribution of meal composition on the solubilizing capacity of the intraluminal environment, a standard and a fat-enriched fed postprandial state were created. Therefore, liquid meals with equal caloric content but different energy percentages coming from the fat portion were ingested by the volunteers. As HIF were collected in fractions without any pooling step, it was possible to take into account the effect of both the time-dependent as well as the inter-subject variability.

The resulting solubility–time profiles illustrated a high time-dependency as well as a high inter-subject variability in all nutritional states and for all model drugs. As a result, the usefulness of pooled intestinal fluids obtained by combining aspirates at various times after meal administration in intraluminal solubility estimation is questionable. Only during the first 30 min after water/food intake, a significant difference between the three nutritional states could be identified.

Simulated intestinal fluids representative of the fasted or fed intestinal contents did not match the solubility profiles well. The least predictive power was obtained for the solubility in the early postprandial phase. FeSSIF and FeSSIF v2 underestimated the solubilizing capacity of this phase; in contrast, the solubility in early FeSSIF was only indicative for the maximum solubility that could be reached in HIF. This might be explained by a difference in kind and structure of colloidal phases in simulated and *in vivo* media which can have its influence on the solubilizing capacity. To the best of our knowledge, studies that compare the colloidal phases in intestinal samples and simulated intestinal media have not been performed. MLR analysis demonstrated that, except for indomethacin, the solubilizing capacity of HIF for the model drugs was determined by a complex interplay of intraluminal parameters.

The latter may contribute to the observed variability in the solubility-profiles, suggesting important changes in dissolution depending on the arrival time in the duodenum, possibly creating an important source for variability in intestinal absorption under fed state conditions.

It was illustrated that, when simulated fluids are intended to be used in predictive solubility studies, improvement is necessary. Certainly media that simulate the solubilizing capacity of the early postprandial intestinal contents need further attention. Aspects that should be taken into account include the user-friendliness and stability of the medium. Therefore, when defining simulated media representative of the solubilizing capacity of the HIF, it is important to consider functionality rather than creating a perfectly simulated match of the intestinal composition.

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