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Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum

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Abstract

The solubility of drugs in the gastrointestinal tract is very challenging to simulate with artificial media due to the high complexity of human intestinal fluid (HIF). In particular, bile salt composition, pH and buffer capacity are very important characteristics of HIF, since they determine the solubility of drugs in-vivo. In this study, we have measured the concentrations of individual bile salts in human intestinal fluids ($n = 6$) collected from two different locations (duodenum and jejunum) in the fasted state. Total bile salt concentrations ranged from 570 to 5137 μM in the duodenum and from 829 to 5470 μM in the jejunum. The following rank order of relative bile salt concentration in duodenum was observed: taurocholic acid > glycocholate ≥ glycochenodeoxycholate > glycodeoxycholate > taurochenodeoxycholate > taurodeoxycholate. Cholic acid, tauroursodeoxycholate, chenodeoxycholic acid, and deoxycholic acid represented less than 1% of bile salts present in the samples. Ursodeoxycholate could not be detected in HIF. No statistically significant difference between bile salt composition of duodenal and jejunal aspirates was observed. The buffer capacity of HIF was compared with other media commonly used for solubility/dissolution determinations, indicating a relatively low buffer capacity of HIF (4–13 mmol L^{-1}/pH). This low buffer capacity was reflected in the change in pH (between 4 and 9.5) that occurred in HIF after addition of model compounds covering a broad pK_a range. Interindividual variability in pH, buffer capacity and bile salt contents of HIF will contribute to differences in the rate and extent of absorption of compounds for which dissolution/solubility is the rate limiting step. The variability observed warrants further research to explore the impact of intraluminal conditions on drug solubility.

Introduction

Along with stability, permeability and first-pass metabolism, solubility in gastrointestinal fluid is considered to be a key parameter for the prediction of oral drug bioavailability. For the prediction of in-vivo dissolution and solubility, a broad range of media is currently being used, including fasted-state simulated intestinal fluid (FaSSIF), fed-state simulated intestinal fluid (FeSSIF) (Galia et al 1998) and dog intestinal fluid (Kostewiz et al 2002; Persson et al 2005). Bile salt composition and pH are considered to be important parameters that affect drug solubility in the gastrointestinal (GI) tract. The bile salt concentration and composition has been shown to enhance solubility of various drugs. For instance, Bates et al (1966a, b) examined the ability of bile salts to increase the solubility of glutethimide, griseofulvin and hexestrol and recognized the importance of biological factors for the intestinal absorption of water-insoluble drugs. Enhancement of solubility by bile salts has also been discussed when comparing the oral bioavailability of drugs in the fasted and fed state. For instance, the area under the concentration–time curve (AUC) and the maximal concentration (C_{max}) were twofold higher when phenytoin was administered in the fed vs the fasted state, probably due to a solubilization effect of the bile salts (Hamaguchi et al 1993); a fourfold food-induced increase in bioavailability was observed for danazol (Charman et al 1993). The mixed micelle formation of sodium glycocholate with

testosterone was also reported to increase its solubility (Martis et al 1972). Testosterone solubility increased even before reaching the critical micellar concentration (CMC) value of the bile salt and the increase in solubility was higher at concentrations in excess of the CMC value due to micellar encapsulation. Steroidal compounds as well as β-blockers have extensively been used to demonstrate the effects of bile salts on solubilization (Bakatselou et al 1991; Grosvenor & Löfroth 1994; de Castro et al 2001b; Wiedmann et al 2002). Mithani et al (1996) attempted to build a model to predict the extent of enhancement of solubility with bile salts for six steroidal compounds (triamcinolone, hydrocortisone, dexamethasone, betamethasone, betamethasone 17-valerate and danazol). In addition to an effect on solubility, bile salts (taurochenodeoxycholate) have been shown to alter the integrity of tight-junctional complexes between epithelial cells of the rabbit colon (Freel et al 1983). Deferme and coworkers also hypothesized that compounds present in human intestinal fluids, most probably bile salts, may attenuate the modulatory effect of P-glycoprotein along the GI tract (Deferme et al 2003). Moreover, bile salts have been shown to amend gastrointestinal epithelial restitution through increased cell migration after injury (Yamaguchi et al 2004).

All bile salt effects described over the years have been related to a specific concentration range of selected bile salts. For some bile salts, the concentration used may be of biological relevance. However, for other bile salts studied, the intraluminal concentrations have not yet been accurately established in human intestinal fluids. Therefore, we determined not only the total bile salt concentration but also the composition of bile acid and salts in human intestinal fluids in the fasted state.

Variation of pH along the GI tract has been observed in many studies in the fasted (Dressman et al 1990) and the fed (Russell et al 1993) state. In several studies, a variation in pH has been reported to alter absorption through changes in permeability (Merfeld et al 1986) and solubility (Youngberg et al 1987; Henderson et al 1995). However, accurate determination of the capacity of the human intestinal fluid to correct for changes in pH (i.e. buffer capacity) has received little or no attention in the literature. One of the objectives of this study was therefore to assess the pH and buffer capacity of human intestinal fluid aspirated from healthy volunteers in the fasted state.

Additionally, the buffer capacity of the fasted-state human intestinal fluids was compared with fasted-state simulated intestinal fluid (FaSSIF), which is commonly used as a dissolution/solubility medium to simulate in-vivo behaviour (Galia et al 1998).

Materials and Methods

Materials

The bile acids and bile salts taurocholic acid (TC), chenodeoxycholic acid (CDC), deoxycholic acid (DOC), sodium glycocholate (GC), sodium glycochenodeoxycholate (GCDC), sodium glycodeoxycholate (GDC), sodium taurochenodeoxycholate (TCDC), sodium taurodeoxycholate (TDC), sodium ursodeoxycholate (UDC), sodium tauroursodeoxycholate (TUDC) and cholic acid (C) were purchased from Sigma (St Louis, MO) and were used as standards to determine the individual and total bile acid/salt contents of the intestinal fluids.

Aciclovir, azithromycin, bepridil hydrochloride, buspirone hydrochloride, captopril, carbamazepine, citalopram hydrobromide, clomipramine HCl, felodipine, furosemide, glibenclamide, haloperidol, itraconazole, levothyroxine, meclozine HCl, metoprolol tartrate, moclobemide, norfloxacin, perphenazine, phenytoin, propranolol, ranitidine HCl, risperidone, sulfasalazine, terbinafine HCl, venlafaxine HCl and ziprasidone were used to evaluate the buffer capacity of human intestinal fluid (HIF) and other media. All these drugs except for sulfasalazine and propranolol (Sigma, St Louis, MO, USA) were synthesized by the chemical department of Eli Lilly (Indianapolis, IN, USA) and had purity values higher than 95%.

Sodium taurocholate (NaTC) (Fluka, Basel, Switzerland), lecithin (Phospholipon 90G, Nattermann Phospholipid GmbH), 1 M HCl, 1 M NaOH, glacial acetic acid, sodium chloride, ortophosphoric acid, NaH_2PO_4, sodium lauryl sulfate (Sigma, St Louis, MO, USA), and Triton (Merck, Belgium) were used for buffer preparation and buffer capacity determinations.

Collection of human intestinal fluids (HIF)

HIF was collected from six healthy volunteers (two men and four women, aged 22–35 years) after an overnight fast by simultaneous duodenal and jejunal aspiration with two catheters. The catheters were introduced orally and positioned under fluoroscopic control such that the proximal suction site was located in the duodenum (5–10 cm from the pylorus) and the second site 90 cm distal to the duodenal site (i.e. in the jejunum). The catheter consisted of two channels, each with a lateral opening at the end. Luminal content was aspirated through one opening, while the other opening allowed equilibration with the atmosphere, thus avoiding generation of negative pressure during sampling. The sampling procedure took 2 h, with an aspirate collection every 30 min. The volunteers were informed of the sampling procedure, the purpose of the study and the possible risks by a written informed consent form. The study was approved by the Committee of Medical Ethics and Clinical Research of the University Hospital in Leuven (Belgium) (approval number ML1819). The volume collected was very variable and ranged from 40 to 480 mL. The pH value of the samples was determined immediately after collection. Samples were centrifuged for 10 min at 3000 rev min^{-1} using an ALC PJ180R centrifuge (Winchester, VA, USA) and the supernatants were stored at −30°C until analysis and/or use in experiments.

Determination of osmolality and pH

Osmolality and pH of each intestinal fluid sample were assessed. Osmolality values were measured with a Model 3D3 osmometer (The Advanced Instruments, MA, USA) by the determination of the freezing point depression. The pH value was measured with a pH Boy from Camlab Ltd (Cambridge, UK).

Quantification of total and individual bile salts

Individual bile salts were determined as follows: after alkaline hydrolysis of bile salt–protein conjugates in HIF (mixed with dehydrocholic acid as internal standard) and solid-phase extraction with methanol, bile acid/salt species were separated by HPLC using 75% (v/v) methanol and 10 mM phosphate buffer on a reversed-phase C18 column (Chromolith Speed ROD C18, Merck) at a flow rate of $0.7\,mL\,min^{-1}$. The bile salts were detected by electrospray ionization tandem mass spectrometry (Kirchherr & Kuhn-Velten 2003).

Determination of buffer capacity

Buffer capacity (ability of the medium to resist changes in pH) was measured by titrating 25 mL (HIF) or 50 mL (FaSSIF) samples with 1 M HCl or 1 M NaOH in samples of 50 μL using an auto-titrator, under constant agitation (DL58 titrator, Mettler Toledo, Zaventem, Belgium). The pH of the solution was recorded after the addition of each portion and the buffering capacity (β) was calculated using the following equation:

$$\beta = \Delta AB/\Delta pH \tag{1}$$

where ΔAB is the amount of acid or base added and ΔpH is the change in pH induced by the acid or base added (Butler 1984; Levis et al 2003).

Additionally, the buffering capacity of HIF was qualitatively compared with eleven other media and buffer solutions by determining the shift in pH induced by adding a drug ($2\,mg\,mL^{-1}$) to the medium; the effect of the previously mentioned 27 structurally diverse drugs with pK_a values ranging from 1.36 to 10.5 was tested. Vials containing both the compound ($2\,mg\,mL^{-1}$) and the media were mixed with a Gilson Automix 818 (Gilson, Middleton) for 3 h at 37°C (blank FaSSIF; FaSSIF; FeSSIF; simulated gastric fluids (SGF) modified; FaSSGF) (Galia et al 1998, 1999; USP XXIII) or 16 h at room temperature (water; 50 mM phosphate buffer at pH 2, 4, 6 and 8, and HCl 0.1 M). After this process, the samples were filtered (Gelman Acrodisc filter, 13 mm PTFE, 0.2 μm) and the pH of the resulting solutions was measured. Due to the limited availability of HIF, these experiments could not be performed in triplicate. To test the reproducibility of the pH methodology, 9 of the 27 compounds (azithromycin, captopril, clomipramine HCl, furosemide, glibenclamide, itraconazole, norfloxacin, perphenazine and phenytoin) were tested in triplicate in blank FaSSIF, FaSSIF, FeSSIF, SGF modified and FaSSGF. Two compounds (clomipramine HCl and norfloxacin) were selected for reproducibility experiments (n = 3) in water, 50 mM phosphate buffer at pH 2, 4, 6 and 8, and HCl 0.1 M. An analysis of variance with appropriate random effect(s) was used to estimate the variance and account for the differences in replicates. Fourteen compounds (aciclovir, azithromycin, captopril, carbamazepine, felodipine, furosemide, glibenclamide, levothyroxine, meclozine HCl, norfloxacin, perphenazine, phenytoin, sulfasalazine, and terbinafine HCl) were selected to measure the pH change in HIF from different volunteers and locations.

Calculation of hydrophobic index (HI)

Hydrophobic index (HI) values for the different media obtained from the volunteers were calculated according to Heuman's quantitative estimations (Heuman 1989) using the following equation:

$$HI = \sum_{x=1}^{n} HI_x F_x \tag{2}$$

where HI_x is the hydrophobic index of the pure bile salts for each of the eleven bile salts found in our study and F_x is the obtained molar fraction of bile salt x in a mixture of n different salts.

Data analysis

Values were expressed as mean ± s.d. An analysis of variance test was performed to compare the pH, osmolality, total bile salt composition and percentage for each of the individual bile salts between duodenum and jejunum. The level of statistical significance was set at 0.05 (JMP software 4.0.2, SAS institute, Cary, NC, USA).

A principal component analysis (PCA) (Eriksson et al 2001) was conducted to evaluate and visualize similarities between volunteers and locations (by score plots) using the composition of the individual bile salts (as a percentage). The PCA representation of the score plot and the loading plot was performed using the software SIMCA-P version 10.0 (Umetrics AB, Umea, Sweden). Before PCA analysis, data were centered and scaled to unit variance.

The variance of the final pH determined for the different solubility media was estimated taking into account the differences in replicates using an analysis of variance model with appropriate random effects (JMP software 4.0.2, SAS Institute, Cary, NC, USA).

Results and Discussion

Characterization of the HIF, pH and osmolality

A very critical aspect of the aspiration of HIF from human volunteers is the exact positioning of the tube within the intestine. The fluids retrieved must provide an accurate representation of the intestinal content in the fasted state. To achieve representative samples, we

selected two positions that could be reproduced among subjects with respect to distance from the pylorus: duodenum at 5–10 cm from the pylorus and jejunum 90-cm distal to the duodenal part. A summary of the characteristics (pH, osmolality, volume of aspirate) of HIF collected from duodenum (location 1) and jejunum (location 2) for six volunteers is shown in Table 1. The pH values obtained in the duodenum and jejunum immediately after collection were very similar: 7.0 ± 0.4 vs 6.8 ± 0.4, respectively. Although the osmolality appeared to be higher for aspirates collected from the jejunum as compared with duodenum (200 mOsm kg^{-1} (range 107–275) vs 137 mOsm kg^{-1} (range 85–185)), no statistically significant difference was observed due to a large inter-subject variability. Both pH and osmolality values were comparable with other values reported for fasted conditions: typical reported pH values range from 5.3 to 8.1 in duodenal aspirates (Dressman et al 1990; Lindahl et al 1997; Deferme et al 2003) and 7.1 ± 0.6 in jejunal fluids (Lindahl et al 1997). Typical osmolality values at different sites of the intestine have been reported to range from 218 to 292 mOsm kg^{-1} (Lindahl et al 1997) and 236–296 mOsm kg^{-1} (Pedersen et al 2000) for jejunum, and from 193 to 301 mOsm kg^{-1} for duodenum (Deferme et al 2003).

Total intraluminal bile concentration

The total bile acid/salt concentrations (reported in Table 2) were determined by HPLC-MS/MS. Total bile acid/salt concentrations in duodenum (location 1) fell in a similar range (570–5137 μM) as reported by Deferme et al (2003) (800–2760 μM, eight fasted subjects) using the same methodology. Total bile acid/salts concentration in jejunum (829–5470 μM) was in agreement with data from Pedersen et al (2000) (360–5570 μM, nine fasted subjects). Other authors employing different methods reported very broad ranges of total bile acid/salt levels. Tangerman et al (1986) obtained values ranging from 100 to 14000 μM in aspirates from upper and lower jejunum from 16 volunteers (in fasted conditions). Lindahl et al (1997) reported values between 100 and 13000 μM in jejunum (60 cm from pylorus) in 11 fasted subjects, thus covering a much larger range. When comparing total bile acid/salt concentrations in HIF collected from duodenum and jejunum (Table 2), the values in jejunal aspirates in all but one volunteer (V5) were higher than in duodenum. However, it has to be mentioned that, in contrast to the other volunteers, a larger volume could be aspirated from jejunum than from duodenum in volunteer 5 (Table 1).

Individual bile salts

Bile acids (free or conjugated with glycine and taurine) and bile salts were determined by HPLC-MS/MS. The structures are shown in Figure 1. Table 2 displays the distribution of the individual bile acids/salts for each volunteer. The average values show the following rank order for percentage of bile acids/salts present in duodenum: TC ($42\% \pm 17$) > GC ($16\% \pm 4$) ≥ GCDC ($16\% \pm 10$) > GDC ($12\% \pm 8$) > TCDC ($8\% \pm 2$) > TDC ($5\% \pm 3$). C ($0.5\% \pm 0.4$), TUDC ($0.3\% \pm 0.2$), CDC ($0.3\% \pm 0.4$), and DOC ($0.3\% \pm 0.2$) each comprised less than 1% of total bile acids/salts. UDC was not detected. A similar rank order was obtained for jejunal aspirates: TC ($48\% \pm 18$) > GC ($19\% \pm 7$) > GCDC ($12\% \pm 8$) > GDC ($8\% \pm 5$) > TCDC ($8\% \pm 2$) > TDC ($4\% \pm 2$). C ($0.3\% \pm 0.2$), TUDC ($0.3\% \pm 0.2$), CDC ($0.1\% \pm 0.06$) and DOC ($0.08\% \pm 0.03$) accounted for less than 1% of total bile acids/salts and no UDC was detected. The relative contribution of GC was consistently higher in jejunum than duodenum for all volunteers. On the other hand, the relative contribution of CDC, GCDC and GDC was consistently higher in duodenum vs

Table 1 Summary of volunteers' characteristics and human intestinal fluid (HIF) collection from duodenum (location 1) and jejunum (location 2)

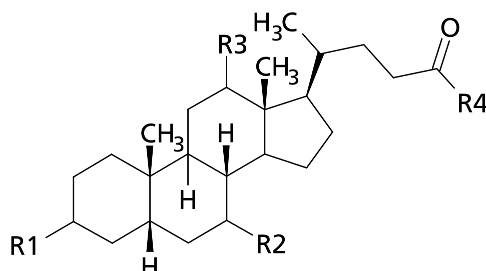
Volunteer identification	Gender	Age	Duodenum			Jejunum			Water consumption during experiment
			mL	pH	Osmolality (mOsm kg^{-1})	mL	pH	Osmolality (mOsm kg^{-1})	
V1	Female	22	50	7.1	220	15	7.3	N.D	No
V2	Female	28	135	7.4	185	34	6.6	259	No
V2b	Female	28	480	6.8	116	40	7.1	275	Yes
V3	Female	24	160	7.5	85	60	7.2	189	Yes
V4	Male	35	355	7.2	91	10	6.5	172	Yes
V5	Female	22	40	7	127	267	6.5	107	Yes
V6	Male	26	70	6.3	N.D.	16	6.5	N.D.	Yes
Mean ± s.d.		26 ± 4.5	184 ± 169	7.0 ± 0.4	137 ± 54	63 ± 92	6.8 ± 0.4	200 ± 68	

V2b refers to the volunteer V2 from whom fluids were obtained on two separate occasions (one month interval). V1 and V2 did not drink water during the 120-min HIF aspiration. V2b, V3, V4, V5 and V6 drank one glass of water (220 mL) 60 min after initiating HIF aspiration. V6 catheter blocked after 100 min and received an extra glass of water. (N.D. = not determined).

Table 2 Relative amount of bile salts in aspirates from six volunteers (two different locations: L1 refers to the human intestinal fluid (HIF) aspirate from the duodenum; L2 refers to the HIF aspirates from the jejunum). UDC could not be detected

Volunteer	Gender	Location	Bile salts total (μM)	% C	% CDC	% DOC	% GC	% GCDC	% GDC	% TCDC	% TDC	% TC	% TUDC
V1	Female	1	3721	0.23	0.08	0.16	13.81	6.59	7.85	8.32	6.61	56.07	0.27
V1	Female	2	4630	0.24	0.04	0.08	15.37	3.61	3.66	6.47	4.23	66.12	0.17
V2	Female	1	3599	0.28	0.11	0.19	17.92	16.60	17.10	5.42	4.28	37.65	0.44
V2	Female	2	4000	0.23	0.09	0.12	24.99	13.55	16.44	7.44	5.85	30.66	0.63
V2b	Female	1	1723	0.36	0.19	0.50	14.85	15.28	27.19	7.00	9.40	24.56	0.66
V3	Female	1	2239	0.39	0.15	0.04	14.31	11.25	2.29	11.13	2.23	58.08	0.13
V3	Female	2	5470	0.72	0.13	0.05	14.39	9.09	1.92	11.24	2.05	60.25	0.16
V4	Male	1	570	1.46	1.19	0.69	21.89	37.18	15.66	4.94	3.55	13.01	0.45
V4	Male	2	3655	0.39	0.22	0.10	30.64	28.61	14.34	5.22	1.86	18.24	0.37
V5	Female	1	5137	0.14	0.06	0.07	7.57	6.51	7.86	10.06	7.98	59.59	0.16
V5	Female	2	829	0.19	0.06	0.10	8.30	5.92	6.19	9.94	7.24	61.89	0.17
V6	Male	1	1486	0.46	0.12	0.13	19.09	15.90	8.70	7.98	2.96	44.60	0.06
V6	Male	2	2715	0.20	0.04	0.04	20.59	8.95	6.00	7.48	3.02	53.64	0.05
Mean			3060	0.41	0.19	0.18	17.21	13.77	10.40	7.89	4.71	44.95	0.29
s.d.			1602	0.35	0.31	0.20	6.37	9.59	7.32	2.16	2.47	18.17	0.21

Cholic acid, C; taurocholic acid, TC; glycocholate, GC; deoxycholic acid, DOC; taurodeoxycholate, TDC; glycodeoxycholate, GDC; chenodeoxycholate, CDC; taurochenodeoxycholate, TCDC; glycochenodeoxycholate, GCDC; ursodeoxycholate, UDC; tauroursodeoxycholate, TUDC.



Bile acid	R1	R2	R3	R4
Cholic acid (C)	OH	OH	OH	OH
Taurocholic acid (TC)	OH	OH	OH	$NHCH_2CH_2SO_3H$
Chenodeoxycholic acid (CDC)	OH	OH	H	OH
Deoxycholic acid (DOC)	OH	H	OH	OH
Glycocholate (GC)	OH	OH	OH	$NHCH_2COO^-$
Glycochenodeoxycholate (GCDC)	OH	OH	H	$NHCH_2COO^-$
Taurochenodeoxycholate (TCDC)	OH	OH	H	$NHCH_2CH_2SO_3^-$
Taurodeoxycholate (TDC)	OH	H	OH	$NHCH_2CH_2SO_3^-$
Ursodeoxycholate (UDC)	OH	OH (β)	H	OH
Glicodeoxycholate (GDC)	OH	H	OH	$NHCH_2COO^-$
Tauroursodeoxycholate (TUDC)	OH	OH (β)	H	$NHCH_2CH_2SO_3^-$

Figure 1 Chemical structure of the bile acids and salts determined in this work.

jejunum. Total bile salt concentration and osmolality were higher in jejunum than duodenum for all volunteers except for V5, from whom a larger volume was collected from jejunum as compared with the other subjects.

The principal outcome of the PCA analysis was the identification of clusters. This was illustrated by the finding that the qualitative composition of the jejunal and duodenal aspirates of volunteers V1, V2, V3, V5, and V6 was very similar (Figure 2). However, for V4 a different

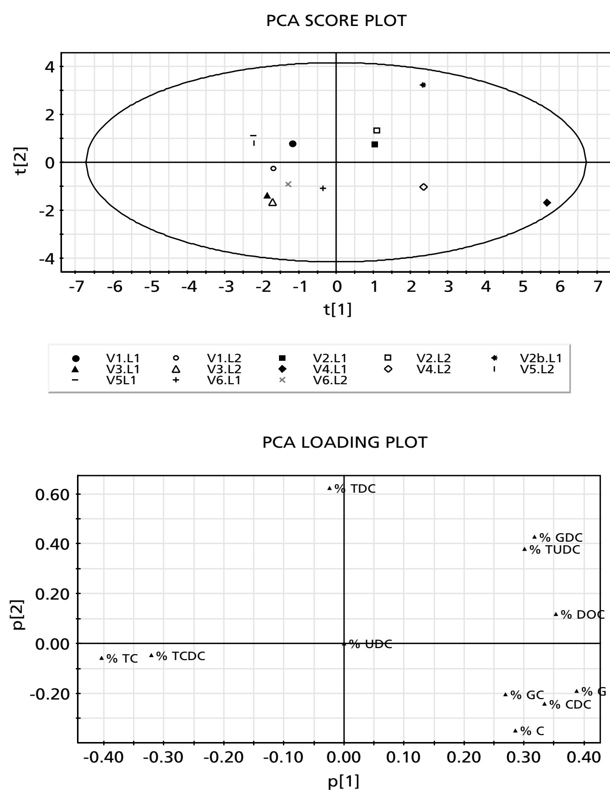


Figure 2 Score plot and loading plot of the two first principal components of the multivariate analysis of the percentage of individual bile salt for different volunteers and locations. The score plot shows how the volunteers and locations group together.

qualitative composition of the jejunal and duodenal aspirates was observed.

Very few data have been published on the analysis of individual bile salts in individual aspirates from duodenum and jejunum of healthy volunteers. Most of the data available refer to: the gallbladder; bile from patients suffering from gallstones or hepatobiliary diseases; aspirations obtained after stimulation with cholecystokin or ceruletid administered before sample collection; and individual bile salts in pooled aspirates. When comparing the data obtained in this study to those obtained in these particular conditions or after gallbladder stimulation (Nakayama & Nakagaki 1980; Ruben & Van Berge-Henegouwen 1982; Swobodnik et al 1985; Rossi et al 1987; Janowitz et al 1990), the percentages of individual bile salts were very similar for GC, GCDC, GDC, TCDC, TDC, UDC and TUDC (even though total bile salt concentrations differed significantly). However, TC levels appeared to be lower in the previously mentioned studies (6.6–13% vs 13–66% in this study), while the percentage of C was higher (8.8–52% vs 0.14–1.46% in this study). CDC and DOC contents were also lower in this study, as compared with previous reports (Rossi et al 1987; Janowitz et al 1990) of gallstone patients who are well known to display a more lipophilic bile acid pool (Carulli et al 2000). In contrast to a previously reported study in which GC was found to be the most abundant bile salt in fasted state HIF (Persson et al 2005), TC was observed to be the major bile salt present in fasted state HIF in this study. This difference could probably be attributed to interindividual differences (Brouwers et al 2006).

Physicochemical characteristics of the bile acid/salts studied influencing solubilization: CMC values, pK_a and hydrophilic–hydrophobic balance (HHB)

Enhanced solubility of poorly soluble drugs by incorporation into micelles is well known to occur in the presence of artificial bile. The solubilization capacity depends on the physiochemical characteristics of bile salts, more specifically the CMC, pK_a, HHB and the number and disposition of the OH groups in the molecule (Ninomiya et al 2003).

The CMC values for the different bile salts have been extensively studied by many authors using a broad range of techniques (particularly surface tension and light scattering). Typical CMC values for some of the bile acids/salts in different conditions are summarized in Table 3. Compared with pure water, the CMC value of the bile acids was considerably lower as a result of: conjugation; ionic strength (Kratohvil & Delli Colli 1968; Long et al 1994; Roda et al 1998); and the presence of phospholipids and lecithin (Carey & Small 1970; Imai et al 1983; de Castro et al 2001a, b).

The bile salts conjugated with glycine and taurine, which were most abundant in intestinal fluids, had lower CMC values as compared with the unconjugated forms (see Table 3). As their pK_a values are two or four units lower, respectively, than those of the corresponding unconjugated bile salts, these conjugates formed more hydrogen bonds with water and were ionized to a greater extent (Roda et al 1983).

The hydrophilic–hydrophobic balance of the bile acid pool influences biliary lipid secretion, cholesterol synthesis, gallbladder emptying rate, cytotoxicity and apoptosis effect, and also affects the capacity to solubilize lipids and lipophilic drugs (Carey 1984; Carulli et al 2000). Taking into account the hydrophobic index (HI) values for the eleven different bile acid species studied, the HI for the different volunteers ranged from 0.14 to 1.08 (Table 4). Although a relatively high variability was observed, these values indicated that the human bile salt pool was more hydrophobic than for other species including rat ($HI = -0.31$) and mice or dog ($HI = 0.11$) (Heuman 1989). Rodents display very hydrophilic bile salt pools (Wang et al 1997), which are expected to result in lower solubilization of lipophilic drugs compared with man. The main bile salt present in intestinal fluid obtained from dogs, a medium sometimes used for solubility determination, was shown to be taurocholate (Heuman 1989; Kostewicz et al 2002). Interspecies differences in HI and types of

Table 3 Summary of physicochemical characteristics of bile acids and salt found in the luminal content of duodenum and jejunum as reported in the literature

Bile acid/salts	Reported CMC value in water (mM)	Relative reduction of CMC value		pK_a	HI[f]
		by ionic strength	by lecithin (750 μM)		
Cholic acid (C)	13[a]	1.2[a]	6.25[c]	4.75[e], 5.00[g]	0.13
Taurocholic acid (TC)	10[a]	1.7[a]	—	—	0.00
Glycocholate (GC)	12	1.2[a]		3.67[e], 3.88[g]	0.07
Deoxycholic acid (DOC)	10[a]	3.5[a]	4[c]	5.10[c], 5.02[g]	0.72
Taurodeoxycholate (TDC)	2.8–6[a–b]	2.5[b]	—	—	0.59
Glycodeoxycholate (GDC)	2.1–6[a–b]	3.5[b]	—	3.75[e], 3.88[g]	0.65
Chenodeoxycholate (CDC)	9[a]	2.1–2.25[a,d]	—	4.78[e], 4.98[g]	0.59
Taurochenodeoxycholate (TCDC)	7[a]	2.3[a]	—	—	0.46
Glycochenodeoxycholate (GCDC)	6[a]	3.4[a]	—	3.82[e], 3.87[g]	0.51
Ursodeoxycholate (UDC)	19[a]	2.7[a]	—	4.8[f], 5.02[g]	−0.31
Tauroursodeoxycholate (TUDC)	8[a]	3.6[a]	—	—	−0.47

[a]Roda et al (1983) (ionic strength: 0.15 M Na^+). [b]Kratohvil & Delli Colli (1968) (ionic strength: 0.5 M NaCl). [c]de Castro et al (2001a,b). [d]Roe & Barry (1985). [e]de Castro et al (1994). [f]Heuman (1989). [g]Fini & Roda (1987).

Table 4 Hydrophobic index (HI) of the bile content for the different volunteers (duodenum and jejunum). The values were calculated taking into account the HI for each bile acid/salt at physiological pH according to the bile salts content for each volunteer

Volunteer	HI duodenum	HI jejunum
V1	0.64	0.50
V2	0.93	1.04
V2b	0.61	–
V3	0.33	0.73
V4	0.21	1.08
V5	0.94	0.14
V6	0.31	0.41
Mean ± s.d.	0.57 ± 0.30	0.65 ± 0.37

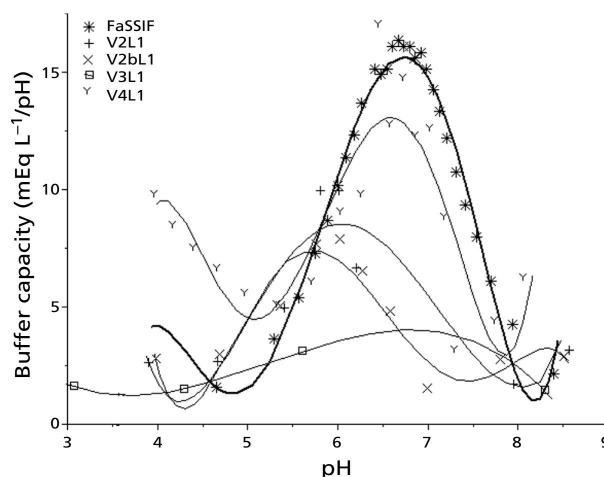


Figure 3 Buffer capacity versus pH. Experimental data for fasted-state simulated intestinal fluid (FaSSIF), human intestinal fluid (HIF) from V4L1 (volunteer 4, duodenum), V2L1 (volunteer 2, duodenum), V2bL1 (volunteer 2, second visit, duodenum) and V3L1 (volunteer 3, duodenum) are displayed.

bile salts will probably lead to differences in solubility for poorly soluble, lipophilic drugs. An extensive examination of the solubilization of drugs by bile salt micelles with more than 50 different drugs determined the following rank order of solubilization by bile salts: C ≈ TDC > GC ≈ TC ≈ GDC (Wiedmann & Kamel 2002). However, this order did not correlate with hydrophobicity or CMC value, indicating that multiple factors play a role in the solubilization properties of bile salts.

Buffer capacity

Figure 3 shows the experimental values of buffer capacity vs pH profile for FaSSIF and HIF obtained from four volunteers (V2L1, V2bL1, V3L1, V4L1; the buffer capacity of medium obtained from the other volunteers was not assessed due to the limited volume of the aspirates). Maximal buffer capacity values obtained for fasted-state human intestinal fluid in our study varied between 4–13 mmol L^{-1}/pH, which was in line with the value of 5.6 mmol L^{-1}/pH that was reported as a median value by Kalantzi et al (2006). However, the values obtained for the buffer capacity were higher than those obtained by Persson et al (2005), who reported an average buffer capacity value for pooled fasted HIF from 12 volunteers of 2.8 mmol L^{-1}/pH. The buffer capacity values reported for Labrador dogs (Kostewicz et al 2002) were in a similar range as the HIF values obtained in this study ($\beta = 5.8 \pm 2$ vs 4–13 mmol L^{-1}/pH, respectively), indicating that fasted-state dog intestinal fluid had a similar buffer capacity

as fasted-state human intestinal fluids. The buffer capacity values obtained for FaSSIF were in good agreement with previously reported data. The maximum buffer capacity of FaSSIF in this study was 16.4 mEq L^{-1}/pH, which was similar to the value reported by Levis et al (2003). The inter-subject differences in pH at which the maximum buffer capacity was observed should also be mentioned: for the aspirates of volunteers V2 and V2b, the maximum buffer capacity was at pH 5.5, while for FaSSIF and aspirates of volunteer V4 a value of 6.5 was observed. For volunteer V3, the buffer capacity was very limited. Results obtained for volunteer 2 (V2 and V2b) suggested that the buffer capacity showed little intra-subject variability.

In summary, HIF buffer capacity appeared to be relatively low and in the same range as dog intestinal fluid. The buffer capacity of FaSSIF was slightly higher than the values obtained in this study and the study of Persson et al (2005).

Resistance to changes in pH of different media (water, 50 mM phosphate buffer at different pH values, HCl 0.1 M and several simulated intestinal and gastric fluids including FaSSIF, FeSSIF, FaSSGF, and modified SGF) were also determined by addition of 2 mg mL^{-1} of 27 different drugs (specifically selected to cover a wide range of pK_a values). The results (Figure 4) confirmed that HIF aspirates had a relatively low buffer capacity. The pH change upon inclusion of test compounds (2 mg mL^{-1}) in HIF was approximately five-times higher as compared with FaSSIF. The change in pH of commonly used phosphate buffers (50 mM at pH 2, 6, 8) was lower as compared with HIF (Figure 4). The change in pH after addition of

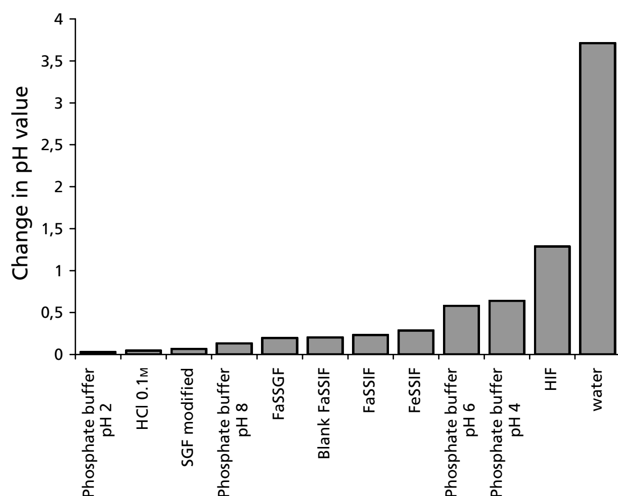


Figure 4 Change in the pH after addition of 2 mg mL^{-1} of 27 drugs in a series of solubility media (buffers, intestine and gastric simulated media and human intestinal fluid (HIF)). Every bar represents the change in pH measured for the 27 drugs in single (n = 1) or triplicate measurements (n = 3) as described in Materials and Methods. FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; FaSSGF, fasted-state simulated gastric fluid; SGF modified, simulated gastric fluids modified.

selected drugs is illustrated in Figure 5. The pH of HIF (initially 6.5 to 7.5) shifted, for example, to values as low as 4 when in contact with meclozine HCl and as high as 9.5 when mixing the HIF with drugs such as azithromycin. These variations resulted in a shift of pH up to 2–3 units depending on the physicochemical properties of the drug considered. A potential artefact of this experiment could be the loss of CO_2 when HIF was exposed to the atmosphere. Bardow et al (2000) observed that the change in partial pressure of CO_2 in saliva vs the atmosphere resulted in an increase in pH in the alkaline direction due to loss of CO_2 and consequently of HCO_3^- (bicarbonate) believed to be the principal buffer of saliva. Buffer capacity of human saliva depleted of CO_2/HCO_3^- was significantly reduced at pH above 5.25 as compared with CO_2/HCO_3^- containing stimulated saliva. As bicarbonate is also believed to be the principal buffer of the fasted-state small intestine (Dressman et al 1998), the same could happen in HIF not sparged with carbon dioxide, thus pH and buffer capacity in HIF are not maintained (Vertzoni et al 2004).

The observed limited buffer capacity of HIF could potentially have a big impact on both the solubility and permeability of drugs. The change in pH in the intestinal fluids after drug administration could affect the permeability of highly dosed ionizable compounds (Neuhoff et al 2003), and the functional activity of P-glycoprotein; therefore it could contribute to drug–drug and food–drug interactions (Varma et al 2005).

It is obvious that interindividual variability in pH, buffer capacity and bile salt contents of HIF could lead to differences in the rate and extent of absorption of compounds for which dissolution/solubility is the rate limiting step of absorption.

Conclusions

A better understanding of the role of bile salt composition, pH and buffer capacity in the solubilization of drugs can be achieved by careful analysis of the intraluminal contents. We have characterized human intestinal aspirates and determined the percentage of 11 relevant individual bile salts with an HPLC-MS/MS method. The total bile salts concentration corresponded well with published data and the percentages of individual bile salts were qualitatively similar to the bile salts reported for hepatic and gallbladder bile. In addition, the buffer capacity of HIF was determined. It was shown that HIF had a lower buffer capacity than the other media investigated (except for water).

More comprehensive research on the mechanism underlying intraluminal solubilization of drugs could lead to the development of more accurate artificial media to mimic the behaviour of the HIF. FaSSIF is already a widely accepted medium for simulating the intraluminal content in the fasted state. Further study is necessary to compare solubility in HIF with solubility in FaSSIF (and other

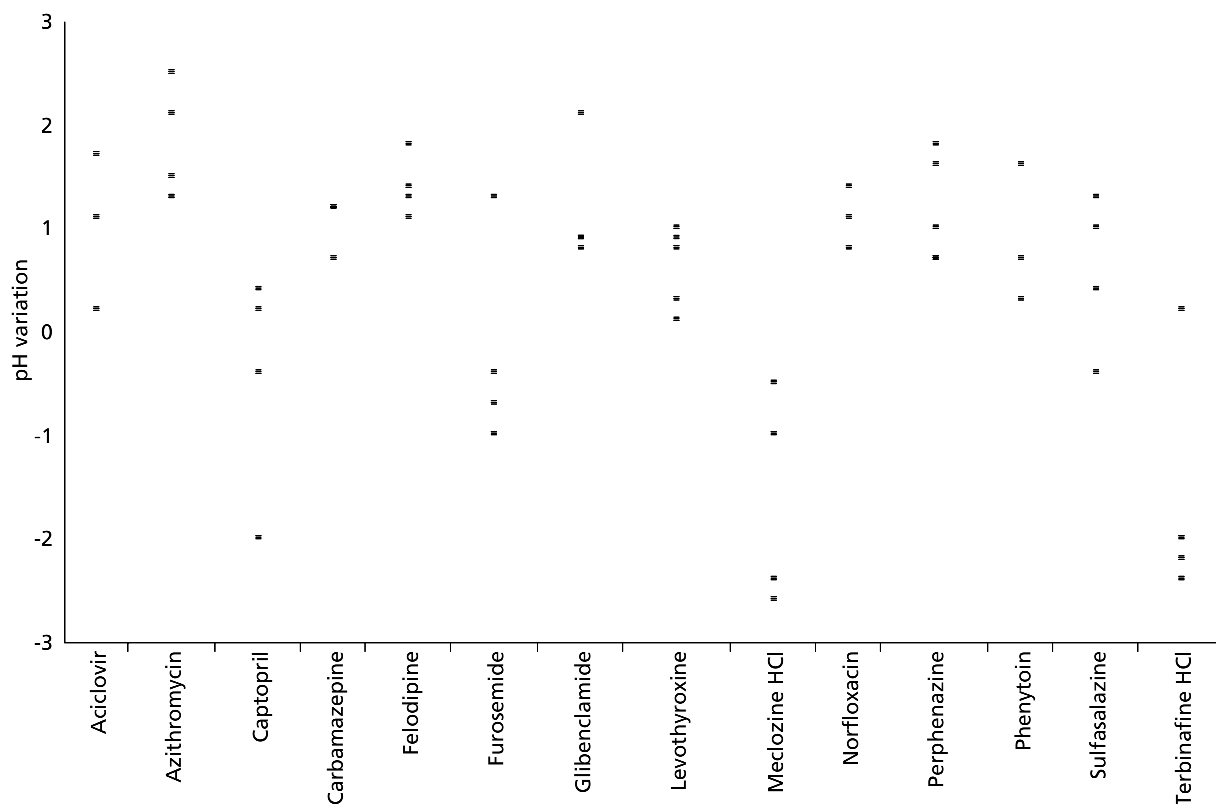


Figure 5 Change in pH (difference between the initial pH of the human intestinal fluid (HIF) and the final measured pH of the solution) after 3-h agitation of various drugs in HIF for the different volunteers and locations. Each point represents one pH determination in one of the HIF samples.

artificial media) for a broad range of compounds to determine which media best simulate the luminal content for this purpose.

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