Gastrointestinal diseases and their impact on drug solubility: Crohn's disease

Angela Effinger[1], Caitriona M O'Driscoll[2], Mark McAllister[3], Nikoletta Fotaki[1]*

[1] Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

[2] School of Pharmacy, University College Cork, Cork, Ireland

[3] Pfizer Drug Product Design, Sandwich, UK

Address for correspondence:

Dr Nikoletta Fotaki

Department of Pharmacy and Pharmacology

University of Bath

Claverton Down

Bath, BA2 7AY

United Kingdom

Tel. +44 1225 386728

Fax: +44 1225 386114

E-mail: n.fotaki@bath.ac.uk

Abstract

In order to investigate differences in drug solubilisation and dissolution in luminal fluids of Crohn's disease (CD) patients and healthy subjects, biorelevant media representative of CD patients were developed using information from literature and a Design of Experiment (DoE) approach. The CD media were characterised in terms of surface tension, osmolality, dynamic viscosity and buffer capacity and compared to healthy biorelevant media. To identify which drug characteristics are likely to present a high risk of altered drug solubility in CD, the solubility of six drugs was assessed in CD media and solubility differences were related to drug properties. Identified differences in CD patients compared to healthy subjects were a reduced concentration of bile salts, a higher gastric pH and a higher colonic osmolality. Differences in the properties of CD compared to healthy biorelevant media were mainly observed for surface tension and osmolality. Drug solubility of ionisable compounds was altered in gastric CD media compared to healthy biorelevant media. For drugs with moderate to high lipophilicity, a high risk of altered drug solubilisation in CD is expected, since a significant negative effect of log P and a positive effect of bile salts on drug solubility in colonic and fasted state intestinal CD media was observed. Simulating the conditions in CD patients *in vitro* offers the possibility to identify relevant differences in drug solubilisation without conducting expensive clinical trials.

Keywords

Gastrointestinal diseases; Crohn's Disease; Inflammatory Bowel Disease; Biorelevant media; Physicochemical properties; Solubility

1. Introduction

Inflammatory bowel disease (IBD) is an incurable autoinflammatory disorder that affects about 3.7 million people in Europe (Burisch et al., 2013). While the aetiology of IBD is still unknown, a combination of factors (environment, genetics, microbiota) is expected to contribute to the disease (Stefanelli et al., 2008). The two main types of IBD are Crohn's disease (CD) and Ulcerative colitis. CD is characterised by transmural discontinuous ulcerations that can affect any part of the gastrointestinal (GI) tract. Typical symptoms that patients experience are abdominal pain and cramps, fatigue, fever, weight loss and diarrhoea with passage of blood and/or mucus (Baumgart and Sandborn, 2012). Within the first 20 years after CD diagnosis, 50% of patients present complications such as strictures, fistulas, abscesses or obstructions (Baumgart and Sandborn, 2012). These complications often necessitate surgeries and bowel resections (Rutgeerts, 2004). Apart from the affected gastrointestinal tract, extraintestinal symptoms are also common in CD patients including inflammations of the eyes such as uveitis or episcleritis, certain skin conditions such as pyoderma gangrenosum and joint diseases such as ankylosing spondylitis (Hedin et al., 2019). Therefore, CD necessitates a long-term drug therapy adapted to the disease localisation and disease state (relapse or remission).

The oral route of drug administration is still the mainstay for patients with CD. Biological medicines (e.g., anti-tumor necrosis factor α and anti-integrin agents) with subcutaneous or intravenous administration are only indicated when other treatment options failed. Recommended oral therapies for CD patients include 5-aminosalicylates (e.g., sulfasalazine, mesalamine), traditional corticosteroids (e.g., prednisone), budesonide, antibiotics (e.g., metronidazole) and immunosuppressive agents (e.g., azathioprine) (Talley et al., 2011). To locally treat the disease in the GI tract, special drug delivery systems have been developed to deliver the drug to the affected GI compartment (Ma et al., 2019). Apart from medication for the GI condition, IBD patients also used other drug classes such as antidepressants, antibiotics

and nonsteroidal anti-inflammatory analgesics more frequently compared to the general population (Haapamaki et al., 2013).

For concomitant medications, the GI environment of CD patients may impact drug delivery and absorption. To reach the systemic circulation, orally administered drugs must be released from the pharmaceutical formulation, dissolve in the GI fluids, permeate the GI membrane and escape luminal degradation, gut wall and hepatic metabolism. These processes depend on the physiological conditions in the GI tract. Alterations of the physiological conditions due to disease states, can impact on drug product performance, which was observed for several drugs in GI disease patients with local and systemic action (Bai et al., 2016; Effinger et al., 2019; Hatton et al., 2018, 2019). For poorly soluble compounds, classified according to the Biopharmaceutics Classification System (BCS) in class II or IV, drug absorption can be solubility- or dissolution rate-limited (Amidon et al., 1995). Differences in the composition of the GI fluids such as pH, osmolality, bile salt and lecithin concentrations can impact on these rate-limiting steps and thus, affect drug absorption (Khadra et al., 2015; Zhou et al., 2017). Pathophysiological changes in CD may alter the composition of the luminal fluids in the GI tract of CD patients and therefore, potentially result in altered drug product performance. Differences in drug product performance in GI disease patients compared to healthy subjects are rarely assessed in clinical trials due to high costs and small patient populations. The development of *in vitro* tools to assess the impact of CD on drug absorption could thus, improve the drug therapy of CD patients.

For healthy subjects, biorelevant media closely simulating GI fluids of different GI compartments and prandial states have been developed to evaluate drug product performance *in vitro* using solubility or dissolution studies (Galia et al., 1998; Jantratid et al., 2008; Markopoulos et al., 2015; Vertzoni et al., 2010; Vertzoni et al., 2005). This approach has previously been extended to special populations and biorelevant media have been developed

for paediatrics or hypochlorhydric and achlorhydric people (Litou et al., 2017; Maharaj et al., 2016). Since drug product performance is influenced by a multitude of factors, the results from these *in vitro* studies can also be used as input in physiologically-based pharmacokinetic (PBPK) models taking into account all ADME (absorption, distribution, metabolism, and excretion) processes.

The aim of this study was to develop a cost- and labour-effective tool to assess the risk of altered luminal drug solubility in patients with GI diseases *in vitro*. Biorelevant media representative of the stomach, intestine and colon of CD patients were developed based on literature data and biorelevant media describing GI conditions in healthy subjects. Fasted and fed state conditions were considered for the intestine and also for the colon, where the different prandial states represent the extreme conditions expected in a clinical study setting. To take into account the interindividual variability in CD patients, a Design of Experiment (DoE) approach was followed. The simulated GI fluids representing patients with CD were characterised according to their surface tension, osmolality, buffer capacity and dynamic viscosity. The solubility of six drugs, belonging to BCS class II or IV and possessing different physicochemical characteristics, was assessed in CD biorelevant media. The investigated drugs were azathioprine, budesonide, celecoxib, dipyridamole, loperamide and sulfasalazine. The results of the solubility studies were analysed with partial least squares (PLS) regression to identify the impact of media-dependent factors (e.g, bile salt concentration) on the solubility of the drugs according to their physicochemical characteristics.

2. Materials

Acetic acid HPLC grade, methanol, pepsin from porcine gastric mucosa, sodium oleate, α-D-glucose, budesonide, phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich Company Ltd., Dorset, England. Sulfasalazine, loperamide hydrochloride, dipyridamole, celecoxib, azathioprine, methanol HPLC grade, acetonitrile HPLC grade and

cholic acid sodium salt were purchased from VWR International Ltd, Lutterworth, UK. Tris(hydroxymethyl)aminomethane, hydrochloric acid 36.5–38%, sodium chloride, trifluoroacetic acid (TFA), potassium dihydrogen orthophosphate and maleic acid were used from Fisher Scientific UK Ltd., Loughborough, England. Other chemicals used included sodium taurocholate (Prodotti Chimici Alimentari S.P.A., Basaluzzo, Italy), egg lecithin–Lipoid EPCS (Lipoid GmbH, Ludwigshafen, Germany) and glyceryl monooleate–Rylo Mg 19 (Danisco, Brabrand, Denmark). Water was ultra-pure (Milli-Q) laboratory grade.

3. Methods

3.1. Media development

For the development of biorelevant media for patients with CD, a DoE approach (Section 3.1.2) was followed to reflect interpatient variability. Briefly, relevant differences in CD patients compared to healthy subjects were identified in literature, a low and a high concentration level was defined based on the available data and the differences were integrated as factors with two levels in the DoE. Biorelevant media based on healthy subjects were used as reference for all media properties and components that were not used as factors in the DoE. These biorelevant media reflect an average healthy subject. Since variability in the gastrointestinal fluid composition of healthy subjects has previously been reported, only parameters with an altered mean value in CD patients compared to healthy subjects were changed (Khadra et al., 2015).

3.1.1. GI physiological differences in CD compared to healthy subjects

A literature search was performed to identify differences in the GI fluid composition of CD patients compared to healthy subjects. Due to the low number of studies investigating the concentration of GI fluid components in CD, studies investigating parameters that are likely to impact on GI fluids were also considered e.g., bile acid pool. For parameters that were directly measured in the GI fluids, the observed range was included in the experimental design with the minimum value observed representing the low level of the factor and the maximum value

representing the high level of the factor, respectively. For parameters that were not directly measured in the GI fluids, an indirect percental approach was followed to determine the level of the corresponding factor according to

$$x_{CD-BM} = \frac{y_{CD}}{y_H} * x_{H-BM} \quad (1)$$

where x_{CD-BM} is the high or low level of the factor in CD media, y_{CD} and y_H are the median of the corresponding parameter observed in studies of CD patients and healthy subjects, respectively and x_{H-BM} is the level of the factor in biorelevant media based on healthy subjects. In the case of a decrease of the factor in CD patients compared to healthy subjects, Equation 1 was used to set the low level and the high level was set to the level in biorelevant media based on healthy subjects. In the case of an increase of the factor in CD patients compared to healthy subjects, Equation 1 was used to set the high level and the low level was set to the level in biorelevant media based on healthy subjects. For the factor bile salt concentration, the bile acid pool was the corresponding parameter and for the factor colonic osmolality, the osmolality of the faecal fluid was the corresponding parameter.

3.1.1.1. Bile acid pool

Bile acids, after being synthesised in the liver, are secreted into bile and further undergo a process of enterohepatic recirculation including reabsorption from the terminal ileum, return to the liver and again secretion into bile (Hofmann, 1999). The physiological function of bile salts includes e.g., the elimination of cholesterol, lipid transport due to micellar solubilisation and the stimulation of bile flow and biliary phospholipid secretion (Hofmann, 1999). The bile acid pool is the total amount of bile acids circulating in the enterohepatic circulation. CD can affect any part of the gastrointestinal tract but most frequently the inflammation is localized in the terminal ileum, the main reabsorption area of bile salts. Several studies investigated the size of the bile acid pool in CD patients compared to healthy subjects, revealing a reduction to 38-

58% of the size in healthy subjects as presented in Table 1 (Nishida et al., 1982; Rutgeerts et al., 1979; Vantrappen et al., 1977). The disease activity has been reported in two of the presented studies and the majority of CD patients (15 of 22) was in relapse (Rutgeerts et al., 1979; Vantrappen et al., 1977).

An increased loss of bile salts can be compensated by higher production. However, the constant loss of bile salt during the day, when bile salts are released in response to meals, is expected to lower the bile salt concentrations in gastrointestinal fluids. This is in line with a study by Lenz et al. (1976) revealing reduced postprandial duodenal bile acid concentrations in 9 out of 19 CD patients. Bile salts are present in the luminal fluids of all gastrointestinal compartments and thus, lower bile acid concentrations were integrated in the DoE of all CD media.

Table 1: Bile acid pool in CD patients and controls [mean (SD)].

	Bile acid pool healthy [g]	**Bile acid pool CD [g]**	**Number of subjects (CD/controls)**	**Reference**
	2.29 (0.33)	1.32 (0.17)	8/4	(Nishida et al., 1982)
	3.09 (0.27)	1.48 (0.16)	10/14	(Vantrappen et al., 1977)
	3.10 (0.27)	1.18 (0.20)	13/10	(Rutgeerts et al., 1979)
Median	**3.09**	**1.32**		

3.1.1.2. pH in the stomach

The pH profile in the stomach of CD patients was in the range of pH 1.5 to 4.1 as investigated in two studies with the majority of patients (20 out of 27) being in an active disease state (Ewe et al., 1999; Press et al., 1998). A higher pH was also indicated by a reduced gastric acid secretion observed in CD patients, being especially strong if patients were malnourished with a mean basal acid output of 0.64 ± 0.33 mEq/h (malnourished) and 2.12 ± 0.88 mEq/h (after nutritional support) vs 3.85 ± 0.93 mEq/h in controls and a maximal acid output of 7.36 ± 1.38 mEq/h (malnourished) and 12.76 ± 2.50 mEq/h (after nutritional support) vs 25.53 ± 4.58 mEq/h in controls (Winter et al., 2004).

3.1.1.3. Osmolality in the colon

The faecal osmolality in CD patients was increased by 32% to 52% as observed in two studies and presented in Table 2 (Schilli et al., 1982; Vernia et al., 1988). Apart from higher sodium and chloride concentrations, this observation was also accompanied with a large osmotic gap indicating osmotic diarrhoea in CD patients from osmotic active agents other than electrolytes such as undigested carbohydrates. Since these undigested components are already present in the large intestine, an increased osmolality in the colon is expected for patients with CD. A

higher osmolality in colonic luminal fluids was reflected by integrating the osmolality as factor in the DoE of colonic CD media.

Table 2: Osmolality of the faecal fluids of CD patients and controls [mean values (SD or range)].

	Osmolality in CD [mOsm/kg]	**Osmolality in controls [mOsm/kg]**	**Number of subjects (CD/Controls)**	**Reference**
	487 (SD 87)	321 (range 254-464)	13/11	(Schilli et al., 1982)
	463 (SD 21)	350 (SD 20)	20/16	(Vernia et al., 1988)
Median	**475**	**336**		

3.1.2. Design of CD media with Design of Experiment

The media development for CD patients followed a DoE approach. Biorelevant media developed for healthy subjects (Table 3) were used as reference and modifications were made to reflect the changes in the composition of luminal contents in patients with CD (Section 3.1.1). For the gastric medium in the fasted state, pH (*p*) and bile salt (*b*) concentration were included as factors in the DoE. As previously reported for healthy subjects, low bile salt concentrations in the stomach are expected to originate from occasional bile salt reflux from the small intestine to the stomach.[13] For intestinal media, the bile salt (*b*) concentration was included as single factor. For colonic media, osmolality (*o*) and bile salt (*b*) concentration were included as factors. The DoE was performed using XLSTAT (Addinsoft, France) with a full factorial design in CD patients for stomach, intestine, colon in the fasted state and intestine and colon in the fed state. Each parameter changed in CD compared to healthy subjects was integrated in the DoE as factor with two levels, low (l) and high (h), resulting in 17 CD media (Figure 1):

- CD- Fasted-State Simulated Gastric Fluid (FaSSGF): changed parameters pH, bile salts (l*p*-l*b*, h*p*-l*b*, l*p*-h*b*, h*p*-h*b*)
- CD- Fasted-State Simulated Intestinal Fluid (FaSSIF): changed parameter bile salts (only one medium, high bile salt medium corresponds to FaSSIF-V2)
- CD- Fasted-State Simulated Colonic Fluid (FaSSCoF): changed parameters osmolality, bile salts (l*b*-l*o*, h*b*-l*o*, l*b*-h*o*, h*b*-h*o*)
- CD- Fed-State Simulated Intestinal Fluid (FeSSIF): changed parameter bile salts (only one medium, high bile salt medium corresponds to FeSSIF-V2)
- CD- Fed-State Simulated Colonic Fluid (FeSSCoF): changed parameters osmolality, bile salts (l*b*-l*o*, h*b*-l*o*, l*b*-h*o*, h*b*-h*o*)

Additionally, a centre point with medium (m) levels of each parameter was included for CD-FaSSGF (m*p*-m*b*), CD-FaSSCoF (m*b*-m*o*) and CD-FeSSCoF (m*b*-m*o*).

In terms of the levels set for the factors in the DoE, the pH range observed in the stomach of CD patients was included with 1.5 as low level and 4.1 as high level for fasted state gastric CD media (Section 3.1.1.2). For the bile salt concentrations in all CD media, the low level was set based on the percental approach described in Section 3.1.1 corresponding to 43% of the concentration in the corresponding healthy biorelevant media. The ratio of bile salts to lecithin was kept constant in all CD media and set according to the ratio in healthy biorelevant media (Table 3), in order to reflect the mixed micelles in GI fluids. For the osmolality in the colonic CD media, the high level was based on the percental difference (Section 3.1.1) with 142% of the osmolality in corresponding healthy biorelevant media. Sodium chloride was used to adjust the osmolality in the respective colonic CD media. For all other CD media (osmolality not included as factor in the DoE), the osmolality was adjusted to the value of the corresponding healthy biorelevant medium.

The method described by Jantratid et al. (2008) was followed for the preparation of gastric and intestinal biorelevant media. Colonic biorelevant media were prepared according to Vertzoni et al. (2010).

Table 3: Biorelevant media representing conditions in healthy subjects.

Medium	FaSSGF	FaSSIF-V2	FaSSCoF	FeSSIF-V2	FeSSCoF
Sodium chloride [mM]	34.20	68.60		125.50	34.00
1M HCl	qs pH 1.60				
Sodium taurocholate [mM]	0.08	3.00		10.00	
Lecithin [mM]	0.02	0.20	0.36	2.00	0.50
Pepsin [mg/mL]	0.10				
Maleic acid [mM]		19.10	75.80	71.90	30.15
NaOH [mM]		34.80	120.00	102.40	16.50
Sodium cholate [mM]			0.15		0.60
Tris [mM]			45.40		30.50
Sodium oleate [mM]			0.10	0.80	0.20
Glycerol monooleate [mM]				5.00	
Glucose [mg/ml]					14.00
Osmolality [mOsm/kg]	121	180	196	390	207
pH	1.6	6.5	7.8	5.8	6.0
Reference	(Vertzoni et al., 2005)	(Jantratid et al., 2008)	(Markopoulos et al., 2015; Vertzoni et al., 2010)	(Jantratid et al., 2008)	(Markopoulos et al., 2015; Vertzoni et al., 2010)

3.2. Media characterisation

Healthy biorelevant media and biorelevant media developed for CD were characterised according to their surface tension, osmolality, dynamic viscosity and buffer capacity. All experiments were performed in triplicate and results are presented as mean with standard deviation.

3.2.1. Surface tension

A Du Noüy ring tensiometer (Sigma 700 Force tensiometer, Attension, UK) was used to measure the surface tension of biorelevant media at room temperature. The surface tension of the medium can be related to the measured force according to equation (2) with

$$F = w_{ring} + 2\pi * (r_i + r_a) * \gamma \quad (2)$$

where F is the force, γ is the surface tension, w_{ring} is the weight of the ring and r_i and r_a are the inner and outer radius of the ring, respectively (Butt et al., 2004).

3.2.2. Osmolality

The osmolality of the media was determined with an Advanced Instruments Inc. micro-osmometer Model 3300 (Norwood, MA, US) by measuring the freezing-point depression of a 20 µl sample. After the supercooling of the sample, crystallisation was induced by mechanical agitation and the temperature when the sample was in a solid/liquid equilibrium was measured. Osmolality was subsequently calculated since freezing-point depression is a colligative property (freezing point depression by 1.858 m°C corresponds to 1 mOsm/kg).

3.2.3. Dynamic viscosity

Dynamic viscosity was measured with a Bohlin Rheometer C-VOR (Malvern instruments, UK) using a cone-plate system (4°,40 mm). A range of shear stresses (20 points, logarithmically distributed between 0.05 and 0.15 Pa) were applied to the sample of the medium tempered at 37ºC and the shear rate was measured. Dynamic viscosity was calculated as the ratio of shear stress to shear rate.

3.2.4. Buffer capacity

Buffer capacity was measured by subsequently adding volumes of 0.5 M hydrochloric acid to 10 mL sample until a change of one pH unit was recorded by a Mettler Toledo SevenCompact S220 pH meter (Schwerzenbach, Switzerland). The buffer capacity (β) was calculated using equation (3)

$$\beta = \left(\frac{M_{acid} * V_{acid}}{\Delta pH}\right) * \frac{1000}{V_{sample}} \quad (3)$$

where M_{acid} is the molarity of the acid used, V_{acid} is the added volume of the acid, V_{sample} is the volume of the sample and ΔpH corresponds to the change in pH (Rabbie et al., 2015).

3.3. Compound selection

For the solubility studies, poorly soluble compounds belonging to BCS class II (low solubility, high permeability) or IV (low solubility, low permeability) were selected as presented in Table 4. While drugs with an indication for GI diseases were preferred, the main selection criterion was to cover a range of different physicochemical properties. Therefore, we included moderately lipophilic drugs that varied in their ionisation properties: budesonide as neutral drug, dipyridamole and loperamide as weak bases and sulfasalazine as weak acid. Additionally, we included drugs that were mainly neutral over the physiological pH range but varied in their lipophilicity: azathioprine with a low logP and celecoxib with a high logP. Due to the pKa of 7.9, azathioprine is considered as neutral drug in all media except the fasted state colonic media (pH of 7.8), where it is considered as weak acid.

Table 4: Properties and indication of selected compounds for solubility studies.

Drug	**Molecular weight [g/mol]**	**pKa (acid/base)**	**logP**	**BCS class**	**Indication**
Azathioprine	277.3	7.9 (acid) (Mitra and Narurkar, 1987)	0.1 (Hansch et al., 1995)	IV (Lindenberg et al., 2004)	Immunosuppressive
Budesonide	430.5	12.0 (acid) (Corey and Fossel, 2016)	2.6 (Bharate et al., 2016)	II (Bhatt et al., 2014)	Locally acting corticosteroid in IBD
Celecoxib	381.4	11.1 (acid) (G.D. Searle LLC Division of Pfizer Inc, 2019)	3.5 (G.D. Searle LLC Division of Pfizer Inc, 2019)	II (Paulson et al., 2001)	Nonsteroidal anti-inflammatory drug
Dipyridamole	504.6	6.4 (base) (Pedersen, 1979)	2.2 (Betageri and Dipali, 1993)	II (Zaki et al., 2010)	Platelet aggregation inhibitor
Loperamide	477.0	8.6 (base) (Manallack, 2007)	5.5 (Dickson et al., 2017)	II (Zaki et al., 2010)	Anti-diarrheal agent
Sulfasalazine	398.4	2.3, 7.9 (acid) (Shalaeva et al., 2008)	2.9 (Graham and Pile, 2015)	II/IV (Lindenberg et al., 2004)	Anti-inflammatory agent in IBD

3.4. Solubility studies

The solubility studies of the investigated drugs were performed using the shake-flask method (Baka et al., 2008). Therefore, 5 mL of medium were transferred to a glass tube with an excess amount of drug. The glass tube was placed for 24 h in a shaking water bath (Grant instruments, Royston, UK) (37°C, 200 strokes/min). Subsequently, the sample was filtered with GF/D

membrane filters with a pore size of 2.7 μm (Whatman® Puradisc, diameter 13 mm) and analysed by HPLC- UV. Solubility studies were performed in triplicate in 17 CD media and for comparison in 5 healthy media. Average solubility differences between CD media and healthy media were expressed as a % Relative effect on solubility [((S_{CD}-$S_{Healthy}$)/ $S_{Healthy}$) x 100)]. Positive values indicate that drug solubility in CD media exceeds the solubility in healthy media, whereas negative values indicate the opposite. HPLC analysis was performed with an Agilent Technologies 1200 series HPLC system (Santa Clara, CA): binary pump (G1212A), autosampler (G1329A), thermostatted column compartment (G1316A) and diode array detector (G1315D). HPLC-UV methods used for the quantitative analysis are presented in Table 5.

Table 5: HPLC/UV analytical methods used for the quantification of the investigated drugs.

Drug	Column	Mobile phase	Flow rate [mL/min]	Temperature [[ºC]	Inj. Volume [µL]	UV detection [nm]
Budesonide (Faouzi et al., 1995)	Waters Spherisorb ODS2 C_{18}, 80 Å, 250 x 4.6 mm, 5 µm	MeOH: Acetic acid 0.1% in H_2O 75:25 v/v	1	25	100	245
Sulfasalazine (Elmasry et al., 2011)	Phenomenex Synergi Max-RP C_{12}, 80 Å, 150 x 4.6 mm, 4 µm	MeOH: Acetic acid 3.3% in H_2O 70:30 v/v	1	20	50	359
Azathioprine (Fazio et al., 2007)	Phenomenex Kromasil C_{18}, 100 Å, 150 x 4.6 mm, 3.5µm	MeOH: Acetic acid 1% in H_2O 65:35 v/v	0.8	30	20	279
Loperamide (Crowe and Wong, 2004)	Phenomenex Kromasil C_{18}, 100 Å, 150 x 4.6 mm, 3.5µm	MeOH: Phosphate buffer pH 2.8 70:30 v/v	0.8	30	20	219
Celecoxib (Dhabu and Akamanchi, 2002)	Waters Spherisorb ODS2 C_{18}, 80 Å, 250 x 4.6 mm, 5 µm	MeOH: H_2O 75:25 v/v	1	25	50	251
Dipyridamole	Waters Xbridge Shield C_{18}, 130 Å, 150 x 4.6 mm, 3.5 µm	ACN: TFA 0.1% in H_2O 30:70 v/v	1	25	50	284

3.5. Statistical analysis

One-way analysis of variance (ANOVA) with a post-hoc Tukey's test was applied to identify statistically significant differences of media properties and drug solubility between biorelevant media based on healthy subjects and various biorelevant media of CD patients. Therefore, the software XLSTAT (Addinsoft, France) was used with a significance level of $p \leq 0.05$.

Multivariate statistical analysis was used to identify drugs at risk of altered drug solubilisation in CD according to the physicochemical properties of the drug. Therefore, the % Relative effect on drug solubility ($(S_{CD}-S_{Healthy})/ S_{Healthy}) \times 100$) was correlated with media-dependent factors of the DoE and drug physicochemical properties by Partial Least Squares (PLS) regression using the software XLSTAT (Addinsoft, France). Media-dependent factors were for gastric fasted state CD media the bile salt concentration and pH, for intestinal CD media in the fasted and fed state only the bile salt concentration and for colonic CD media in both prandial states the bile salt concentration and osmolality. In terms of drug-dependent parameters, the partition coefficient, log P, derived from literature (Table 4) was included for all CD media. For media with pH as media-dependent factor (CD-FaSSGF), a categorical variable discriminating between weak acids, weak bases and neutral compounds was introduced. For the remaining CD media (CD-FaSSIF, CD-FaSSCoF, CD-FeSSIF, CD-FeSSCoF), the % Fraction ionised (calculated using Advanced Chemistry Development, Inc. (ACD/Labs) Software V11.02, Toronto, On, Canada and defined for anionic species as negative and cationic species as positive), was integrated as additional drug-dependent factor (Advanced Chemistry Development Inc., 2019). Interactions between media-dependent and drug-dependent factors were included in the model. The quality of the obtained models was evaluated based on the square of coefficient of determination (r^2) and goodness of prediction (q^2), indicating when close to 1 a good fit of the data and a good predictive ability of the model, respectively. Highly disparate r^2 and q^2 (difference higher than 0.3) indicate inappropriate models due to model

over-fitting. (Eriksson et al., 2008) Models were selected based on the minimum predicted residual error sum of squares (PRESS) and the highest q^2 representing optimum model predictability. A q^2 higher than 0.5 generally indicates good model predictability, but it should be noted that q^2 is dependent on the properties of the data set, thereby impeding the setting of a general limit (Triba et al., 2015). The effect of media- and drug-dependent factors on the % Relative effect on solubility is shown by their standardised coefficients with high values designating a considerable influence, positive values designating a positive effect and negative values a negative effect, respectively. Factors with a Variable Importance in Projection (VIP) higher than or equal to 0.7 are the most influential factors in the model and were considered as statistically significant (Eriksson et al., 2008).

4. Results and discussion

4.1. Media characterisation

Surface tension of biorelevant media based on CD patients and healthy subjects is presented in Figure 2. In gastric media, the surface tension was significantly higher in all CD-FaSSGF media (h*p*-h*b* +12%, m*p*-m*b* +13%, l*p*-l*b* +15%, h*p*-l*b* +24%,) except CD-FaSSGF l*p*-h*b* compared to FaSSGF ($p<0.05$). A higher surface tension of CD-FaSSGF media with low and medium bile salt and lecithin concentrations could be due to bile salt and lecithin concentrations being below the critical micellar concentration (CMC). The higher surface tension of CD-FaSSGF h*p*-h*b* could be related to the different salt composition, since less hydrochloric acid and a higher concentration of sodium chloride was used compared to the healthy medium. The surface tension has been reported to increase with a higher salt concentration due to solute depletion at the interface (Hsin et al., 2004).

For fasted state intestinal media, the surface tension of the CD medium was significantly increased by 9% compared to the corresponding healthy medium ($p<0.05$). This is in agreement with a previous study showing a higher surface tension for fasted state simulating fluids with

reduced bile salt concentrations (Xie et al., 2014). Considering the surface tension of fasted state colonic media, only for CD-FaSSCoF l*b*-h*o* the surface tension was significantly decreased by 8% compared to FaSSCoF ($p<0.05$). In fed state intestinal media, the CD medium showed a significantly lower surface tension (-8%) compared to FeSSIF-V2. This slight decrease in surface tension with lower sodium taurocholate concentration has previously been observed for fed state simulated intestinal fluids in a range of 1-7 mM (Xie et al., 2014). For fed state colonic media, the surface tension of CD-FeSSCoF m*b*-m*o*, l*b*-l*o*, l*b*-h*o* was significantly decreased by -11%, -22% and -28%, respectively compared to the corresponding healthy medium ($p<0.05$).

Osmolality in CD fasted state gastric and intestinal media and fed state intestinal media was similar to the corresponding healthy biorelevant media as presented in Figure 2. Differences in osmolality were observed when osmolality was integrated as factor in the DoE according to the specified levels, which was the case for fasted and fed state colonic CD media. The altered osmolality in the colonic media can have an impact on the dissolution rate of certain drugs due to a common ion effect and therefore, the conversion of the drug to another salt.[55] Additionally, osmolality can affect the swelling behaviour of polymers possibly due to ion exchange and thus, drug release can be slowed down with increased osmolality (Jantratid et al., 2008; Wagner and McGinity, 2002).

The dynamic viscosity of CD biorelevant media at three different shear stresses is presented in Figure 3. All investigated biorelevant media showed pseudoplastic behaviour. With an applied shear stress of 0.06 Pa, the dynamic viscosity of CD biorelevant media was in the range of 4.23 mPas to 6.67 mPas. An increase of the shear stress to 0.08 Pa and 0.15 Pa, resulted in a reduced viscosity in the range of 3.36 mPas to 4.92 mPas and 2.86 mPas to 3.85 mPas, respectively. Significant differences with application of the three different shear stresses were only observed

for all CD-FaSSGF media, which possessed a significantly higher viscosity compared to FaSSGF ($p<0.05$).

Buffer capacity was not altered in intestinal and colonic CD media compared to the corresponding media based on healthy subjects due to the use of the same buffer system and no changes in pH value (data not shown).

4.2. Solubility of drugs in CD biorelevant media

The solubility of six different drugs was investigated biorelevant media based on CD patients and healthy subjects simulating stomach, small intestine and colon in the fasted state and small intestine and colon in the fed state. Drug solubility of all investigated drugs in biorelevant media based on healthy subjects is presented in Table 6.

Table 6: Mean drug solubility (SD) of investigated drugs in biorelevant media developed to represent the GI conditions in healthy subjects (the final medium pH at 24 h is reported).

	Solubility in "healthy" biorelevant media [µg/mL], {final pH}				
Drug	**FaSSGF**	**FaSSIF-V2**	**FaSSCoF**	**FeSSIF-V2**	**FeSSCoF**
Azathioprine	242.90 (7.97) {1.6}	242.53 (6.82) {6.5}	316.27 (11.09) {7.8}	254.33 (1.14) {5.8}	252.82 (8.41) {6.0}
Budesonide	17.83 (0.19) {1.6}	22.72 (0.64) {6.5}	18.43 (0.15) {7.8}	43.75 (4.68) {5.8}	17.48 (0.40) {6.0}
Celecoxib	2.94 (0.05) {1.6}	14.77 (0.44) {6.5}	12.34 (0.95) {7.8}	97.98 (0.81) {5.8}	22.50 (0.88) {6.0}
Dipyridamole	13.1 (4.40) x 10^3 {3.0}	11.91 (0.46) {6.5}	7.10 (0.33) {7.8}	80.02 (5.72) {5.8}	18.91 (0.58) {6.0}
Loperamide-HCl	266.74 (0.84) {1.6}	204.69 (13.76) {6.5}	29.31 (2.87) {7.8}	241.13 (7.43) {5.8}	231.19 (30.06) {6.0}
Sulfasalazine	* {1.6}	1.28 (0.03) x 10^3 {6.2}	7.34 (0.11) x 10^3 {6.7}	1.07 (0.02) x 10^3 {5.7}	561.71 (2.75) {5.8}

*Measurement value of 1.17 ug/mL (>LOD, <LOQ) was only used as reference for comparative purposes

In fasted state gastric media, differences in drug solubility between biorelevant media based on CD patients and healthy subjects were observed (Figure 4). The solubility of the weak acid sulfasalazine was significantly increased in CD gastric media with high pH ($p<0.05$) as a higher fraction of the drug was ionised. For the weak base dipyridamole, the solubility was significantly decreased in CD gastric media with high and medium pH and increased in CD gastric media with low pH ($p<0.05$), indicating also a higher solubility with increasing ionisation of the drug. The solubility of loperamide hydrochloride, another weak base, was significantly increased in CD gastric media with high pH and low bile salt concentrations, most probably due to the common ion effect since less chloride ions are present in the gastric CD media with high pH (less hydrochloric acid), and decreased in CD gastric media with low pH and high bile salt concentrations ($p<0.05$). For neutral compounds, significant differences in drug solubility in CD gastric media were only observed for budesonide with a lower solubility in all CD gastric media compared to FaSSGF ($p<0.05$).

The % Relative effect of CD on drug solubility in fasted and fed state intestinal media is shown in Figure 5. In fasted state intestinal media, the solubility of celecoxib and the weak bases, loperamide hydrochloride and dipyridamole, was significantly lower in CD intestinal media ($p<0.05$). This is in accordance with another study showing an impact of bile salt and lecithin concentration on the solubility of four weak bases and four neutral compounds in fasted state simulated intestinal fluids (Khadra et al., 2015). Therefore, relevant differences in drug solubilisation in CD are expected for neutral lipophilic compounds and moderately lipophilic weak bases. The higher impact of reduced bile salt concentrations on weak bases could be explained by an interaction of the protonated drug with the charged head group of sodium taurocholate (Niederquell and Kuentz, 2018).

In fed state intestinal media, the solubility of sulfasalazine, dipyridamole, celecoxib and loperamide hydrochloride was significantly decreased in CD media ($p<0.05$). The solubility of

budesonide was lower in CD-FeSSIF but the difference was not statistically significant ($p=0.06$). Drug solubilisation of hydrophilic drugs, such as azathioprine, is not expected to be altered in CD-FeSSIF. For moderately to highly lipophilic drugs, a decrease in drug solubilisation is expected in fed state intestinal CD media, irrespective of their ionisation properties.

The % Relative effect of CD on the solubility of investigated drugs in colonic biorelevant media in the fasted state and fed state is shown in Figure 6. In colonic media in the fasted state, the CD biorelevant medium with high bile salt concentration and low osmolality corresponds to FaSSCoF. In colonic media in the fed state, the CD biorelevant medium with high bile salt concentration and low osmolality corresponds to FeSSCoF. The solubility of loperamide hydrochloride and budesonide was significantly decreased in all CD-FaSSCoF media compared to FaSSCoF ($p<0.05$). The solubility of dipyridamole was significantly decreased in CD-FaSSCoF with low bile salt concentrations and high osmolality ($p<0.05$). The solubility of celecoxib was significantly lower in CD-FaSSCoF media with low bile salt concentrations ($p<0.05$). As for CD-FaSSIF, the results suggest a lower solubility of moderately and highly lipophilic neutral and weakly basic compounds as a result of decreased bile salt and lecithin concentrations in CD fasted state colonic media. Additionally, increased osmolality had a negative impact on drug solubility of loperamide hydrochloride and budesonide. For loperamide, this can be attributed to a common ion effect due to the higher chloride concentration. The higher osmolality of the faecal fluid of CD patients was not only accompanied with a higher concentration of sodium and chloride but also with an increased osmotic gap, indicating an increased concentration of insoluble carbohydrates (Vernia et al., 1988). Since sodium chloride was used to change the medium's osmolality, the impact of the altered osmolality on the solubility of loperamide hydrochloride could be slightly lower.

In fed state colonic media, the solubility of sulfasalazine was decreased in all CD media ($p<0.5$) suggesting a negative impact of decreased bile salt and lecithin concentration and increased osmolality on the solubility of sulfasalazine. The solubility of loperamide hydrochloride and celecoxib was decreased in CD media with low or medium bile salt concentrations ($p<0.5$). The solubility of dipyridamole was decreased in CD-FeSSCoF with low bile salt concentration and low osmolality ($p<0.5$). The results suggest a decreased solubility for neutral and weakly acidic drugs with high lipophilicity in media with lower bile salt and lecithin concentrations also in CD-FeSSCoF media.

4.3. Multivariate statistical analysis

The PLS models for the different GI compartments and prandial states are shown in Figure 7 with the standardised coefficients and VIPs of the respective drug- and media-dependent factors and their interactions. For the fasted state gastric media, the developed PLS model for the % Relative effect of CD on drug solubility showed a good fit of the experimental data (r^2 0.89) and a high predictive power (q^2 0.79). The model depicted a positive effect of the categorical variable weak acid, of the pH and of the interplay between pH and weak acid. In contrast, the categorical variable of neutral compounds had a negative effect on drug solubility. For fasted state intestinal media, the PLS model with good model quality (r^2 0.78, q^2 0.71) revealed a positive effect of bile salts and of the interplay between bile salts and log P, while the log P had a negative effect on the % Relative effect of CD on drug solubility. This suggests that drug solubilisation of lipophilic compounds is at risk in CD patients with low intestinal bile salt concentrations.

For fasted state colonic media, a predictive PLS model was developed (r^2 0.57, q^2 0.50). According to the model, the % Relative effect of CD on drug solubility was negatively influenced by % Fraction ionised and log P, while bile salts and the interplay between bile salts and % Fraction ionised showed a positive influence. The positive influence of the interplay

between bile salts and % Fraction ionised can be explained by the interaction between the cationic fraction of the weak bases and the headgroup of sodium taurocholate.

For fed state intestinal media, the PLS model (r^2 0.60, q^2 0.51) showed that bile salts had a positive effect on drug solubility.

For fed state colonic media, the predictive power of the developed PLS model was low (q^2 0.37) and the model could only account for a low percentage of variability in the dependent variable (r^2 0.42). Important variables of the model were bile salts and the interplay of bile salts and log P with a positive effect and log P with a negative effect on the % Relative effect of CD on drug solubility.

4.4. Drugs at risk of altered solubility in luminal fluids of CD patients

In simulated gastric fluids of CD patients compared to biorelevant media based on healthy subjects, differences of drug solubility were observed for a weak acid and weak bases. Therefore, an altered gastric pH in CD is expected to pose a risk for ionisable drugs. For weak acids, an increased gastric pH in CD patients is expected to result in a higher drug solubility.

For drugs with moderate to high lipophilicity, a high risk of altered drug solubilisation is expected in the fasted state intestinal fluids of CD patients with low bile salt and lecithin concentrations. In contrast, hydrophilic drugs have a low risk of altered drug solubility in intestinal fluids of CD patients as shown by a similar drug solubility of azathioprine in intestinal biorelevant based on CD patients and healthy subjects.

Considering colonic fluids of CD patients, a reduced drug solubility is expected with an increased log P in the fasted and fed state as indicated by the PLS models (Section 4.3), especially when low bile salt and lecithin concentrations are present in the colonic fluids of CD patients. Drugs that are at the same time also weak bases possess a higher risk for a reduced drug solubility in the fasted state colonic fluids as indicated by the negative effect of the % Fraction ionised in the respective PLS model.

Given the high number of CD media, solubility studies with six compounds were performed and resulted in appropriate statistical models.

5. Conclusion

Simulating the conditions in CD patients *in vitro* offers the possibility to identify relevant differences in drug solubilisation without conducting clinical trials. Especially for drugs for concomitant diseases, drug product performance is rarely investigated in CD patients due to the high costs associated with clinical trials. For the local treatment of CD in the GI tract, drug release/ dissolution and solubility are particularly relevant since high drug concentrations need to be achieved at the target site. The presented simulated media for CD patients can further be used for drug release/dissolution studies and results can be integrated in mechanistic PBPK models to consider additional pathophysiological differences (e.g., permeability, distribution, gut wall/hepatic metabolism and elimination) regarding all ADME processes in order to predict a drug's plasma concentration profile *in vivo*.

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7. Declaration of interest

None.

8. References

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678 **Figure Legends**

679 Figure 1: Design of Experiment for the development of biorelevant media for CD patients.

680 Figure 2: Surface tension (blue, left y-axis) and osmolality (red, right y-axis) of CD
681 biorelevant media according to the Design of Experiments (green: high level, yellow:
682 medium level, red: low level, white: healthy) and biorelevant media based on healthy
683 subjects.

684 Figure 3: Dynamic viscosity of CD biorelevant media according to the Design of
685 Experiments (green: high level, yellow: medium level, red: low level, white: healthy) and the
686 corresponding biorelevant media based on healthy subjects at different shear stress (0.06 Pa:
687 blue, 0.08 Pa: red, 0.15 Pa: black).

688 Figure 4: % Relative effect (RE) on solubility of investigated drugs in CD gastric biorelevant
689 media according to the Design of Experiments (green: high level, yellow: medium level, red:
690 low level) in the fasted state compared to the corresponding medium based on healthy
691 subjects.

692 Figure 5: % Relative effect (RE) on solubility of investigated drugs in CD intestinal
693 biorelevant media in the fasted state and fed state compared to the corresponding media based
694 on healthy subjects.

695 Figure 6: % Relative effect (RE) on solubility of investigated drugs in CD colonic biorelevant
696 media in the fasted state (top) and fed state (bottom) according to the Design of Experiments
697 (green: high level, yellow: medium level, red: low level) compared to the corresponding
698 media based on healthy subjects.

699 Figure 7: Standardised coefficients of the PLS regression of drug solubility in CD simulated
700 gastrointestinal fluids in the fasted state (left) and fed state (right) and different compartments

701 of the GI tract (top: stomach, middle: small intestine, bottom: colon). Red colour denotes
702 coefficients of VIP values > 1, green > 0.7 and blue < 0.7.

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	Crohn's disease											
Prandial state	**Fasted state**						**Fed state**					
Compartment	**stomach**		**intestine**		**colon**		**stomach**		**intestine**		**colon**	
Level	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**
Bile salts [mM]	0.035	0.08	1.29	3.00	0.07	0.15			4.30	10.00	0.26	0.60
Lecithin[mM]	0.008	0.02	0.09	0.20	0.13	0.30			0.86	2.00	0.22	0.50
BS/Lecithin	4:1		15:1		1:2				5:1		6:5	
pH	1.5	4.1										
Osmolality [mOsm/kg]					196	278					207	294

	no changes
	decrease
	increase
	value represented in healthy biorelevant media

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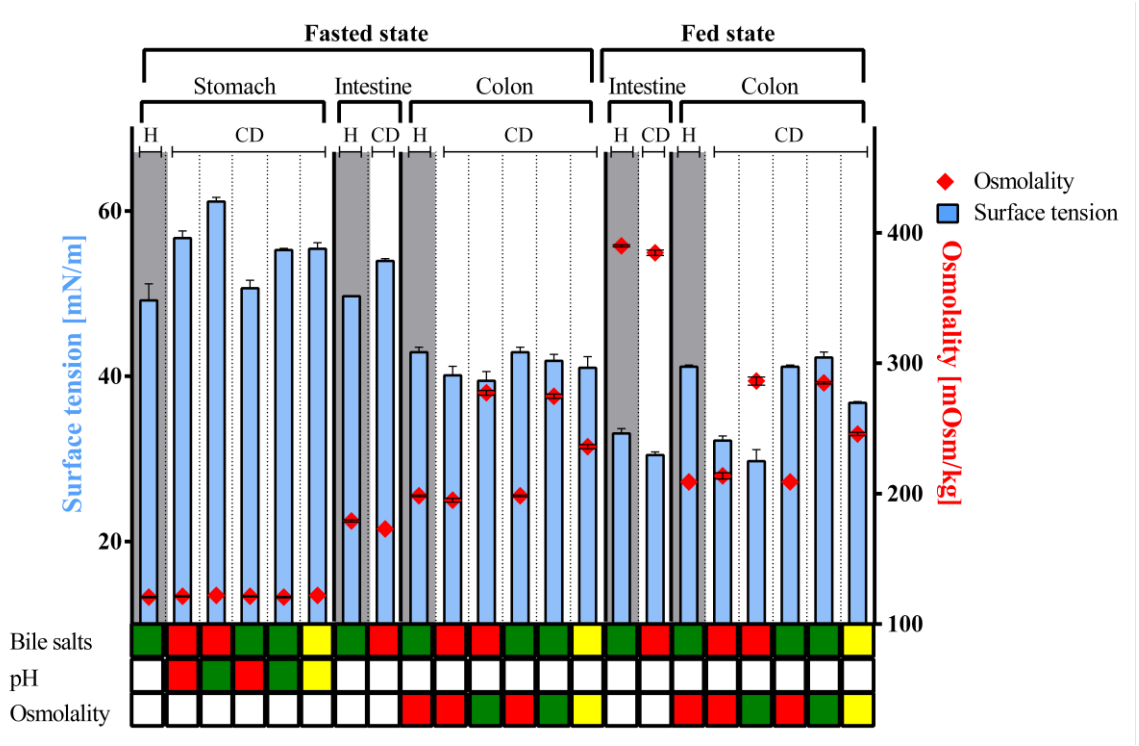

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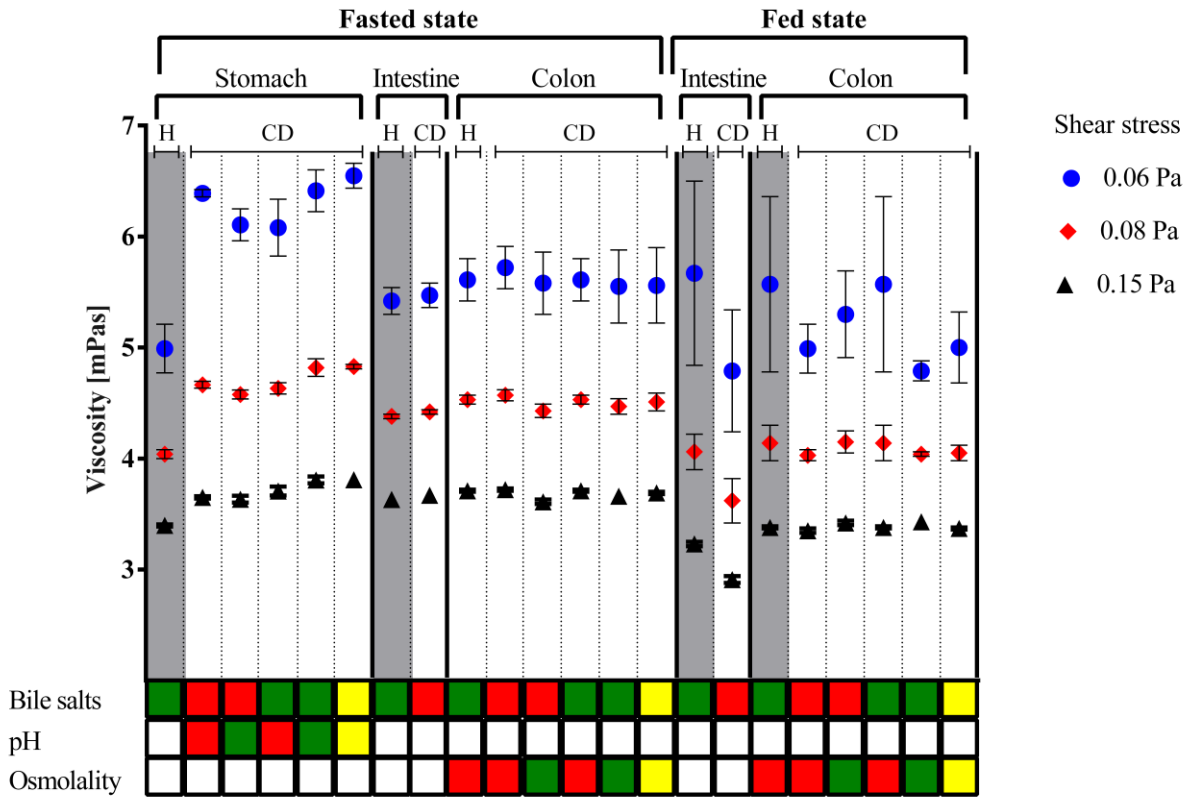

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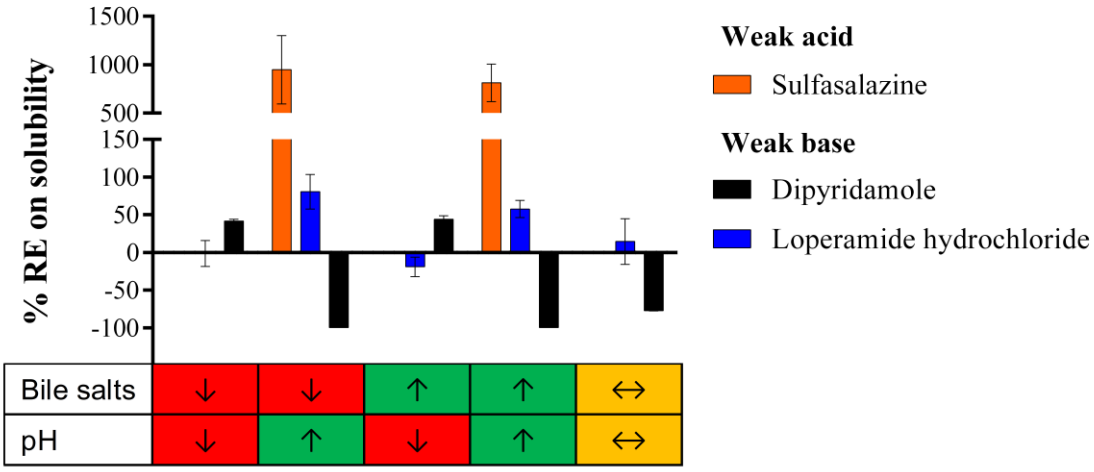
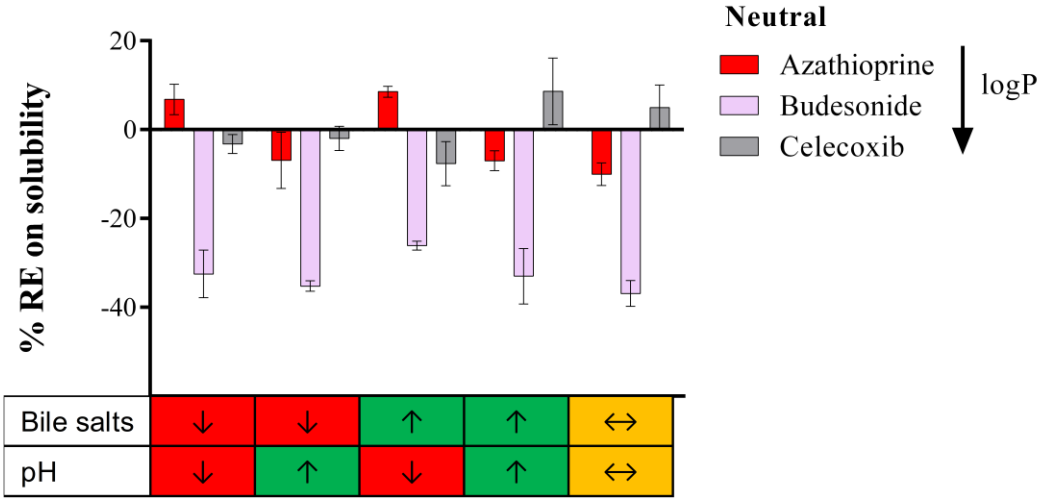
(a)



Crohn's disease fasted state gastric media

(b)



Crohn's disease fasted state gastric media

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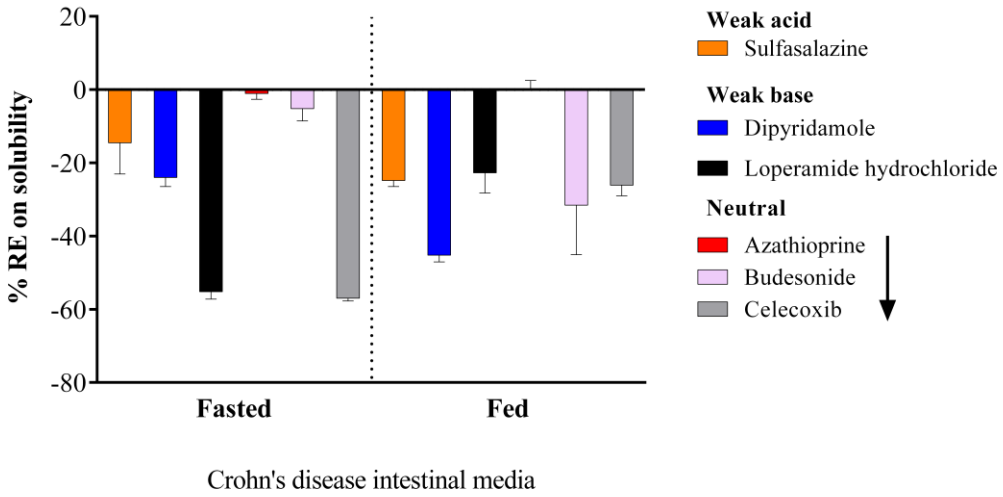

Crohn's disease intestinal media

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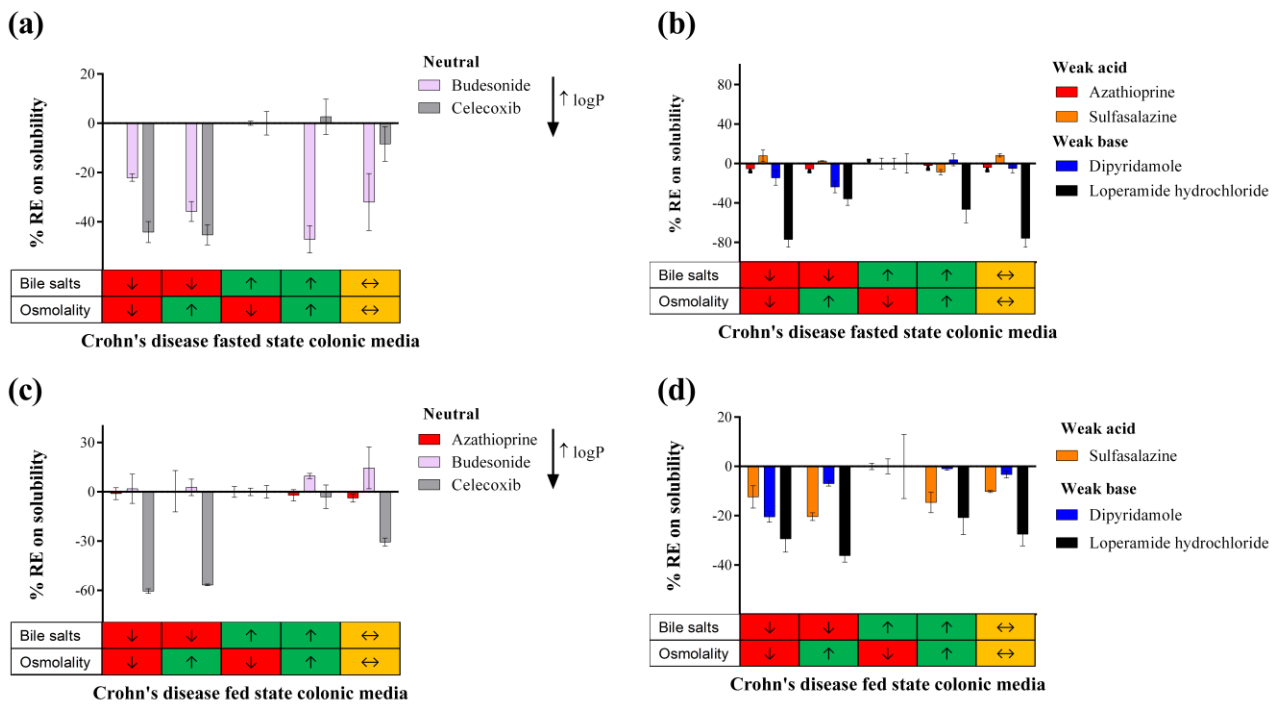

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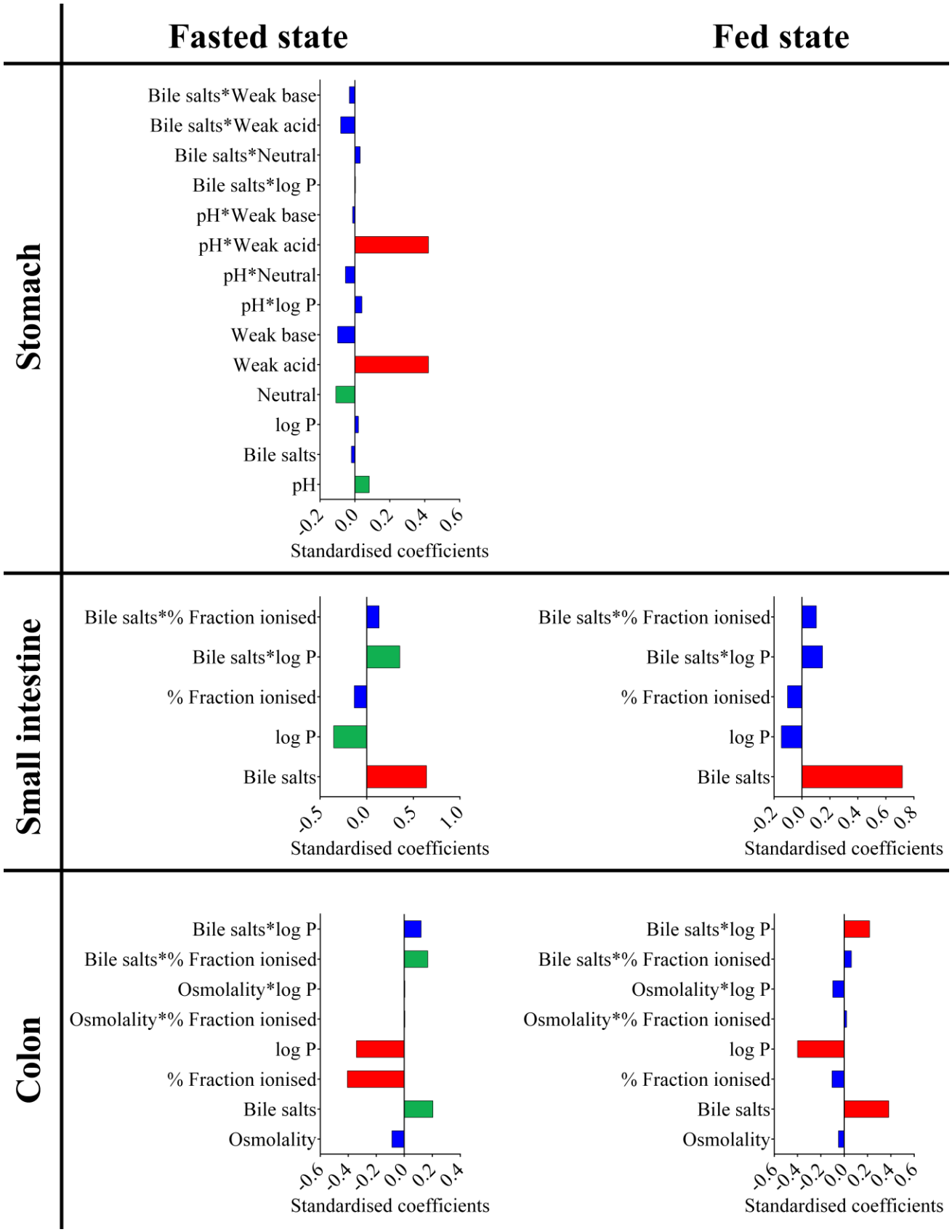
Fasted state
Fed state
Stomach
Bile salts*Weak base
Bile salts*Weak acid
Bile salts*Neutral
Bile salts*log P
pH*Weak base
pH*Weak acid
pH*Neutral
pH*log P
Weak base
Weak acid
Neutral
log P
Bile salts
pH
-0.2 0.0 0.2 0.4 0.6
Standardised coefficients
Small intestine
Bile salts*% Fraction ionised
Bile salts*log P
% Fraction ionised
log P
Bile salts
-0.5 0.0 0.5 1.0
Standardised coefficients
Bile salts*% Fraction ionised
Bile salts*log P
% Fraction ionised
log P
Bile salts
-0.2 0.0 0.2 0.4 0.6 0.8
Standardised coefficients
Colon
Bile salts*log P
Bile salts*% Fraction ionised
Osmolality*log P
Osmolality*% Fraction ionised
log P
% Fraction ionised
Bile salts
Osmolality
-0.6 -0.4 -0.2 0.0 0.2 0.4
Standardised coefficients
Bile salts*log P
Bile salts*% Fraction ionised
Osmolality*log P
Osmolality*% Fraction ionised
log P
% Fraction ionised
Bile salts
Osmolality
-0.6 -0.4 -0.2 0.0 0.2 0.4 0.6
Standardised coefficients

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