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

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19 **Abstract**

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

37

38 **Keywords**

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

41 **1. Introduction**

42 Inflammatory bowel disease (IBD) is an incurable autoinflammatory disorder that affects about
43 3.7 million people in Europe (Burisch et al., 2013). While the aetiology of IBD is still unknown,
44 a combination of factors (environment, genetics, microbiota) is expected to contribute to the
45 disease (Stefanelli et al., 2008). The two main types of IBD are Crohn's disease (CD) and
46 Ulcerative colitis. CD is characterised by transmural discontinuous ulcerations that can affect
47 any part of the gastrointestinal (GI) tract. Typical symptoms that patients experience are
48 abdominal pain and cramps, fatigue, fever, weight loss and diarrhoea with passage of blood
49 and/or mucus (Baumgart and Sandborn, 2012). Within the first 20 years after CD diagnosis,
50 50% of patients present complications such as strictures, fistulas, abscesses or obstructions
51 (Baumgart and Sandborn, 2012). These complications often necessitate surgeries and bowel
52 resections (Rutgeerts, 2004). Apart from the affected gastrointestinal tract, extraintestinal
53 symptoms are also common in CD patients including inflammations of the eyes such as uveitis
54 or episcleritis, certain skin conditions such as pyoderma gangrenosum and joint diseases such
55 as ankylosing spondylitis (Hedin et al., 2019). Therefore, CD necessitates a long-term drug
56 therapy adapted to the disease localisation and disease state (relapse or remission).

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

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

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

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

91 for paediatrics or hypochlorhydric and achlorhydric people (Litou et al., 2017; Maharaj et al.,
92 2016). Since drug product performance is influenced by a multitude of factors, the results from
93 these *in vitro* studies can also be used as input in physiologically-based pharmacokinetic
94 (PBPK) models taking into account all ADME (absorption, distribution, metabolism, and
95 excretion) processes.

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

111 **2. Materials**

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

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

123 **3. Methods**

124 3.1. Media development

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

134 3.1.1. GI physiological differences in CD compared to healthy subjects

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

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

$$144 \quad x_{CD-BM} = \frac{y_{CD}}{y_H} * x_{H-BM} \quad (1)$$

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

155 3.1.1.1. Bile acid pool

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

165 58% of the size in healthy subjects as presented in Table 1 (Nishida et al., 1982; Rutgeerts et
166 al., 1979; Vantrappen et al., 1977). The disease activity has been reported in two of the
167 presented studies and the majority of CD patients (15 of 22) was in relapse (Rutgeerts et al.,
168 1979; Vantrappen et al., 1977).

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

175

176 **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		

177 3.1.1.2. pH in the stomach

178 The pH profile in the stomach of CD patients was in the range of pH 1.5 to 4.1 as investigated
 179 in two studies with the majority of patients (20 out of 27) being in an active disease state (Ewe
 180 et al., 1999; Press et al., 1998). A higher pH was also indicated by a reduced gastric acid
 181 secretion observed in CD patients, being especially strong if patients were malnourished with
 182 a mean basal acid output of 0.64 ± 0.33 mEq/h (malnourished) and 2.12 ± 0.88 mEq/h (after
 183 nutritional support) vs 3.85 ± 0.93 mEq/h in controls and a maximal acid output of
 184 7.36 ± 1.38 mEq/h (malnourished) and 12.76 ± 2.50 mEq/h (after nutritional support) vs
 185 25.53 ± 4.58 mEq/h in controls (Winter et al., 2004).

186 3.1.1.3. Osmolality in the colon

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

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

195

196 **Table 2:** Osmolality of the faecal fluids of CD patients and controls [mean values (SD or
 197 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		

198 3.1.2. Design of CD media with Design of Experiment

199 The media development for CD patients followed a DoE approach. Biorelevant media
 200 developed for healthy subjects (Table 3) were used as reference and modifications were made
 201 to reflect the changes in the composition of luminal contents in patients with CD (Section
 202 3.1.1). For the gastric medium in the fasted state, pH (*p*) and bile salt (*b*) concentration were
 203 included as factors in the DoE. As previously reported for healthy subjects, low bile salt
 204 concentrations in the stomach are expected to originate from occasional bile salt reflux from
 205 the small intestine to the stomach.¹³ For intestinal media, the bile salt (*b*) concentration was
 206 included as single factor. For colonic media, osmolality (*o*) and bile salt (*b*) concentration were
 207 included as factors. The DoE was performed using XLSTAT (Addinsoft, France) with a full
 208 factorial design in CD patients for stomach, intestine, colon in the fasted state and intestine and
 209 colon in the fed state. Each parameter changed in CD compared to healthy subjects was
 210 integrated in the DoE as factor with two levels, low (l) and high (h), resulting in 17 CD media
 211 (Figure 1):

- 212 - CD- Fasted-State Simulated Gastric Fluid (FaSSGF): changed parameters pH, bile salts
213 (*lp-lb*, *hp-lb*, *lp-hb*, *hp-hb*)
- 214 - CD- Fasted-State Simulated Intestinal Fluid (FaSSIF): changed parameter bile salts
215 (only one medium, high bile salt medium corresponds to FaSSIF-V2)
- 216 - CD- Fasted-State Simulated Colonic Fluid (FaSSCoF): changed parameters osmolality,
217 bile salts (*lb-lo*, *hb-lo*, *lb-ho*, *hb-ho*)
- 218 - CD- Fed-State Simulated Intestinal Fluid (FeSSIF): changed parameter bile salts (only
219 one medium, high bile salt medium corresponds to FeSSIF-V2)
- 220 - CD- Fed-State Simulated Colonic Fluid (FeSSCoF): changed parameters osmolality,
221 bile salts (*lb-lo*, *hb-lo*, *lb-ho*, *hb-ho*)

222 Additionally, a centre point with medium (m) levels of each parameter was included for CD-
223 FaSSGF (*mp-mb*), CD-FaSSCoF (*mb-mo*) and CD-FeSSCoF (*mb-mo*).

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

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

239

240 **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)	(Markopoulou et al., 2015; Vertzoni et al., 2010)	(Jantratid et al., 2008)	(Markopoulou et al., 2015; Vertzoni et al., 2010)

241

242 3.2. Media characterisation

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

247 3.2.1. Surface tension

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

251
$$F = w_{ring} + 2\pi * (r_i + r_a) * \gamma \quad (2)$$

252 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
253 the inner and outer radius of the ring, respectively (Butt et al., 2004).

254 3.2.2. Osmolality

255 The osmolality of the media was determined with an Advanced Instruments Inc. micro-
256 osmometer Model 3300 (Norwood, MA, US) by measuring the freezing-point depression of a
257 20 µl sample. After the supercooling of the sample, crystallisation was induced by mechanical
258 agitation and the temperature when the sample was in a solid/liquid equilibrium was measured.
259 Osmolality was subsequently calculated since freezing-point depression is a colligative
260 property (freezing point depression by 1.858 m°C corresponds to 1 mOsm/kg).

261 3.2.3. Dynamic viscosity

262 Dynamic viscosity was measured with a Bohlin Rheometer C-VOR (Malvern instruments, UK)
263 using a cone-plate system (4°,40 mm). A range of shear stresses (20 points, logarithmically
264 distributed between 0.05 and 0.15 Pa) were applied to the sample of the medium tempered at
265 37°C and the shear rate was measured. Dynamic viscosity was calculated as the ratio of shear
266 stress to shear rate.

267 3.2.4. Buffer capacity

268 Buffer capacity was measured by subsequently adding volumes of 0.5 M hydrochloric acid to
269 10 mL sample until a change of one pH unit was recorded by a Mettler Toledo SevenCompact
270 S220 pH meter (Schwerzenbach, Switzerland). The buffer capacity (β) was calculated using
271 equation (3)

272
$$\beta = \left(\frac{M_{acid} * V_{acid}}{\Delta pH} \right) * \frac{1000}{V_{sample}} \quad (3)$$

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

275 3.3. Compound selection

276 For the solubility studies, poorly soluble compounds belonging to BCS class II (low solubility,
277 high permeability) or IV (low solubility, low permeability) were selected as presented in Table
278 4. While drugs with an indication for GI diseases were preferred, the main selection criterion
279 was to cover a range of different physicochemical properties. Therefore, we included
280 moderately lipophilic drugs that varied in their ionisation properties: budesonide as neutral
281 drug, dipyridamole and loperamide as weak bases and sulfasalazine as weak acid. Additionally,
282 we included drugs that were mainly neutral over the physiological pH range but varied in their
283 lipophilicity: azathioprine with a low logP and celecoxib with a high logP. Due to the pKa of
284 7.9, azathioprine is considered as neutral drug in all media except the fasted state colonic media
285 (pH of 7.8), where it is considered as weak acid.

286 **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

287

288 3.4. Solubility studies

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

293 membrane filters with a pore size of 2.7 μm (Whatman® Puradisc, diameter 13 mm) and
294 analysed by HPLC- UV. Solubility studies were performed in triplicate in 17 CD media and
295 for comparison in 5 healthy media. Average solubility differences between CD media and
296 healthy media were expressed as a % Relative effect on solubility $[(S_{\text{CD}} - S_{\text{Healthy}}) / S_{\text{Healthy}} \times$
297 $100]$. Positive values indicate that drug solubility in CD media exceeds the solubility in healthy
298 media, whereas negative values indicate the opposite. HPLC analysis was performed with an
299 Agilent Technologies 1200 series HPLC system (Santa Clara, CA): binary pump (G1212A),
300 autosampler (G1329A), thermostatted column compartment (G1316A) and diode array
301 detector (G1315D). HPLC-UV methods used for the quantitative analysis are presented in
302 Table 5.

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

Drug	Column	Mobile phase	Flow rate [mL/min]	Temperature [°C]	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 ₂ O 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 ₂ O 70:30 v/v	1	20	50	359
Azathioprine (Fazio et al., 2007)	Phenomenex Kromasil C ₁₈ , 100 Å, 150 x 4.6 mm, 3.5 μm	MeOH: Acetic acid 1% in H ₂ O 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, 3.5 μm	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 ₂ O 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 ₂ O 30:70 v/v	1	25	50	284

305 3.5. Statistical analysis

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

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

330 over-fitting. (Eriksson et al., 2008) Models were selected based on the minimum predicted
331 residual error sum of squares (PRESS) and the highest q^2 representing optimum model
332 predictability. A q^2 higher than 0.5 generally indicates good model predictability, but it should
333 be noted that q^2 is dependent on the properties of the data set, thereby impeding the setting of
334 a general limit (Triba et al., 2015). The effect of media- and drug-dependent factors on the
335 % Relative effect on solubility is shown by their standardised coefficients with high values
336 designating a considerable influence, positive values designating a positive effect and negative
337 values a negative effect, respectively. Factors with a Variable Importance in Projection (VIP)
338 higher than or equal to 0.7 are the most influential factors in the model and were considered as
339 statistically significant (Eriksson et al., 2008).

340 **4. Results and discussion**

341 4.1. Media characterisation

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

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

355 reduced bile salt concentrations (Xie et al., 2014). Considering the surface tension of fasted
356 state colonic media, only for CD-FaSSCoF *lb-ho* the surface tension was significantly
357 decreased by 8% compared to FaSSCoF ($p<0.05$). In fed state intestinal media, the CD medium
358 showed a significantly lower surface tension (-8%) compared to FeSSIF-V2. This slight
359 decrease in surface tension with lower sodium taurocholate concentration has previously been
360 observed for fed state simulated intestinal fluids in a range of 1-7 mM (Xie et al., 2014). For
361 fed state colonic media, the surface tension of CD-FeSSCoF *mb-mo*, *lb-lo*, *lb-ho* was
362 significantly decreased by -11%, -22% and -28%, respectively compared to the corresponding
363 healthy medium ($p<0.05$).

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

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

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

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

384 4.2. Solubility of drugs in CD biorelevant media

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

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

Drug	Solubility in "healthy" biorelevant media [$\mu\text{g/mL}$], {final pH}				
	FaSSGF	FaSSIF-V2	FaSSCoF	FeSSIF-V2	FeSSCoF
Azathiopri ne	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}
Dipyridam ole	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}
Loperamid e-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}
Sulfasalazi ne	* {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}

391 *Measurement value of 1.17 $\mu\text{g/mL}$ (>LOD, <LOQ) was only used as reference for
 392 comparative purposes

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

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

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

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

423 The % Relative effect of CD on the solubility of investigated drugs in colonic biorelevant media
424 in the fasted state and fed state is shown in Figure 6. In colonic media in the fasted state, the
425 CD biorelevant medium with high bile salt concentration and low osmolality corresponds to
426 FaSSCoF. In colonic media in the fed state, the CD biorelevant medium with high bile salt
427 concentration and low osmolality corresponds to FeSSCoF. The solubility of loperamide
428 hydrochloride and budesonide was significantly decreased in all CD-FaSSCoF media
429 compared to FaSSCoF ($p<0.05$). The solubility of dipyridamole was significantly decreased in
430 CD-FaSSCoF with low bile salt concentrations and high osmolality ($p<0.05$). The solubility of
431 celecoxib was significantly lower in CD-FaSSCoF media with low bile salt concentrations
432 ($p<0.05$). As for CD-FaSSIF, the results suggest a lower solubility of moderately and highly
433 lipophilic neutral and weakly basic compounds as a result of decreased bile salt and lecithin
434 concentrations in CD fasted state colonic media. Additionally, increased osmolality had a
435 negative impact on drug solubility of loperamide hydrochloride and budesonide. For
436 loperamide, this can be attributed to a common ion effect due to the higher chloride
437 concentration. The higher osmolality of the faecal fluid of CD patients was not only
438 accompanied with a higher concentration of sodium and chloride but also with an increased
439 osmotic gap, indicating an increased concentration of insoluble carbohydrates (Vernia et al.,
440 1988). Since sodium chloride was used to change the medium's osmolality, the impact of the
441 altered osmolality on the solubility of loperamide hydrochloride could be slightly lower.

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

450 4.3. Multivariate statistical analysis

451 The PLS models for the different GI compartments and prandial states are shown in Figure 7
452 with the standardised coefficients and VIPs of the respective drug- and media-dependent
453 factors and their interactions. For the fasted state gastric media, the developed PLS model for
454 the % Relative effect of CD on drug solubility showed a good fit of the experimental data (r^2
455 0.89) and a high predictive power (q^2 0.79). The model depicted a positive effect of the
456 categorical variable weak acid, of the pH and of the interplay between pH and weak acid. In
457 contrast, the categorical variable of neutral compounds had a negative effect on drug solubility.
458 For fasted state intestinal media, the PLS model with good model quality (r^2 0.78, q^2 0.71)
459 revealed a positive effect of bile salts and of the interplay between bile salts and log P, while
460 the log P had a negative effect on the % Relative effect of CD on drug solubility. This suggests
461 that drug solubilisation of lipophilic compounds is at risk in CD patients with low intestinal
462 bile salt concentrations.

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

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

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

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

476 4.4. Drugs at risk of altered solubility in luminal fluids of CD patients

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

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

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

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

494

495 **5. Conclusion**

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

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511 **7. Declaration of interest**

512 None.

513 **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

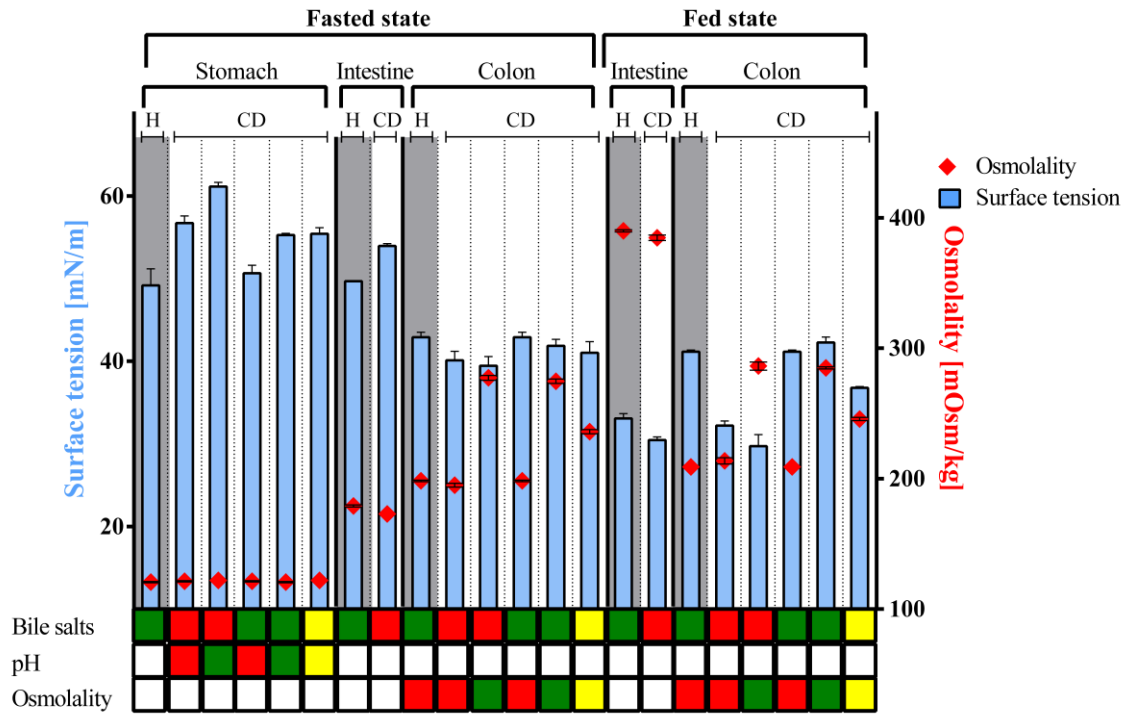
	no changes
	decrease
	increase
	value represented in healthy biorelevant media

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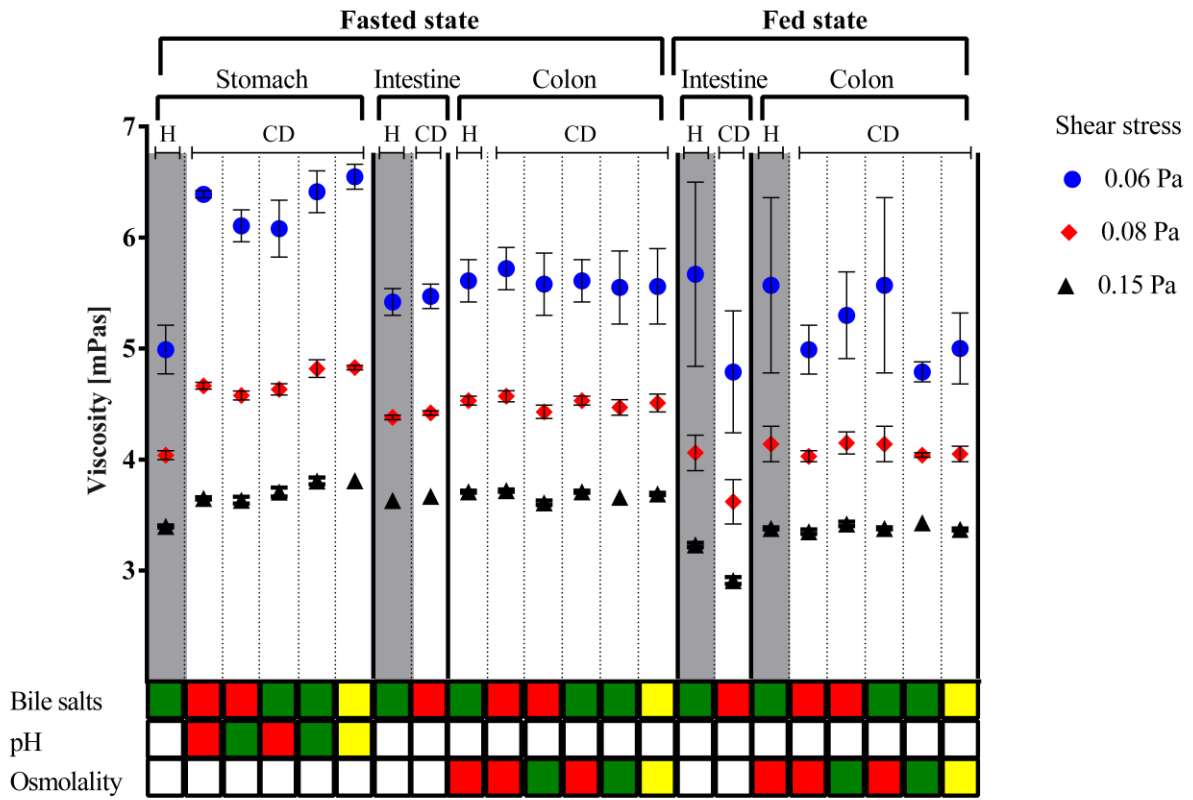


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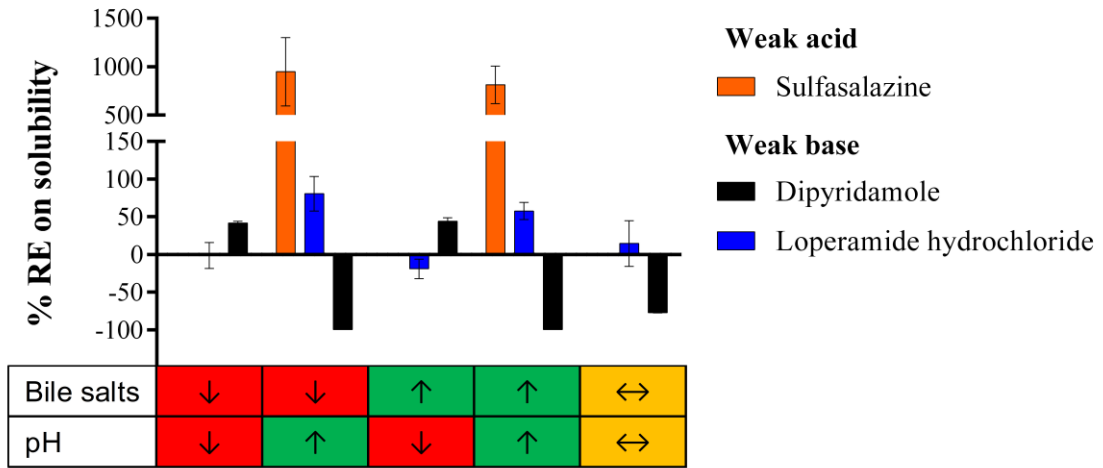


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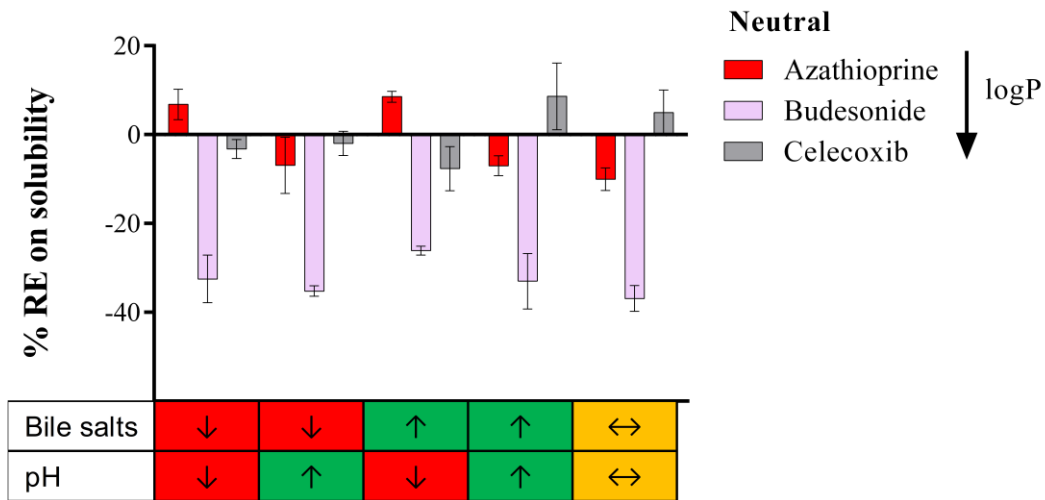
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(a)



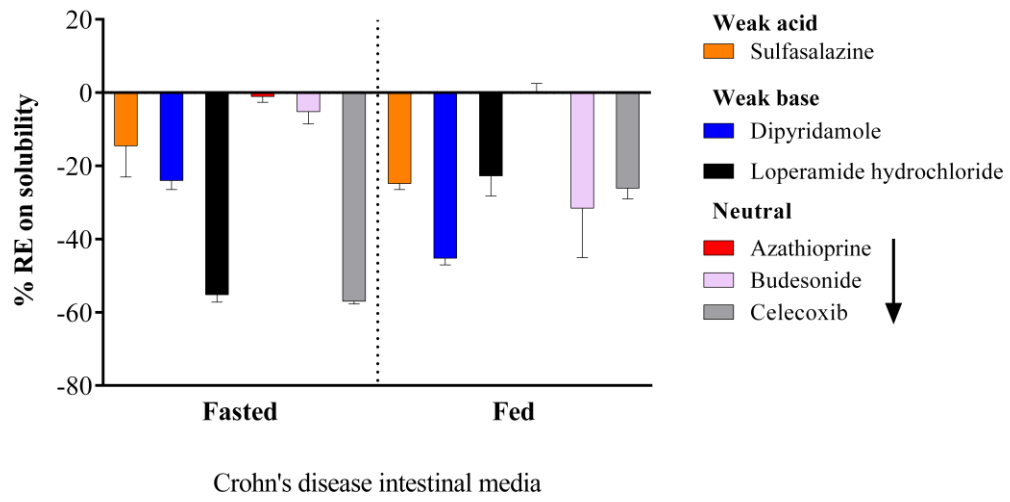
Crohn's disease fasted state gastric media

(b)



Crohn's disease fasted state gastric media

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