PHD

Developing in vitro and in silico tools to predict the impact of gastrointestinal disease states on drug product performance

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Award date:
2020

Awarding institution:
University of Bath

Developing *in vitro* and *in silico* tools to predict the impact of gastrointestinal disease states on drug product performance

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

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October 2019

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Acknowledgements

First and foremost, I would like to express my deepest gratitude to my main supervisor, Dr Nikoletta Fotaki, for her feedback, mentorship and guidance during the last three years. Thank you for your invaluable support and optimism.

I would also like to thank my co-supervisors Prof Caitriona M O'Driscoll and Dr Mark McAllister for their support and involvement in the PhD project. I really enjoyed having such a great supervisory team.

I would like to thank the European Union's Horizon 2020 research and innovation programme under grant agreement No. 674909 (PEARRL) for funding this project and providing me with this great opportunity. I wish to thank all my colleagues in the PEARRL project for contributing to my scientific growth and making it an enjoyable time.

I gratefully acknowledge Prof Abdul Basit and Dr Charareh Pourzand for accepting to evaluate this work.

For my industrial secondment at Pfizer, I would like to acknowledge Dr Mark McAllister and Dr Irena Tomaszewska for their excellent organisation, help and support. Additionally, I would like to thank Dr Mark Taylor (HPLC-CAD), Mr Steve Gomersall (GC-FID), Dr James Heaton (UPLC-MS) and Mr Neil Dawson (Light microscopy) for their help with the analytics and Mr Kieran L Smith, Dr Inese Sarcevica, Ms Sudesha Wanigaratne, Mr Aidan Harper and Mr Sam Young for their help with the TIM-1 system. Thank you all for ensuring that the secondment was a success and a pleasant time for me.

I would like to thank Prof Karen Edler, Prof Roland Jones and Mr Fernando Acosta from the University of Bath for their assistance with surface tension, osmolality and viscosity measurements.

I would also like to thank my colleagues from the research group Joana Martir, Mariana Guimaraes Sa Correia, Ricardo Diaz de Leon Ortega, Nota Zarmpi, Fotios Baxevanis and John Nikolettos for their help, support and friendship. In particular, I would like to thank

Joana, Mariana and Alice for making it fun and always being a great company along the way.

I would like to express my deepest gratitude to my family, especially my parents and my siblings, for their invaluable unconditional love and support. Markus, Veronika and Matthias, I am very happy to have you as my personal consulting company, your advice and mentorship is priceless. Mama und Papa, auch über 1000 km entfernt, standet ihr mir immer zur Seite und wart bereit mir bei allem möglichen zu helfen. Ich bin euch unglaublich dankbar dafür.

Finally, I would like to thank Nils for supporting my decision to do a PhD in England. Thank you for all your love, encouragement and support during the last years.

Publications and Conference Contributions

Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Impact of gastrointestinal disease states on oral drug absorption - implications for formulation design - a PEARRL review. J Pharm Pharmacol. 2019;71(4):674-98.

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Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Towards the development of simulated gastrointestinal conditions for patients with Crohn's Disease, 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 19-22 March, 2018.

Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Investigation of solubility in simulated gastrointestinal fluids representing patients with Crohn's disease, 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 19-22 March, 2018.

Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Development and characterisation of simulated gastrointestinal fluids representing patients with Ulcerative Colitis, AAPS PharmSci 360, Washington DC, 2018.

Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Investigating the solubility of poorly soluble compounds in biorelevant media simulating patients with Ulcerative Colitis, AAPS PharmSci 360, Washington DC, 2018.

Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Investigating the impact of Crohn's disease on oral budesonide pharmacokinetics using in vitro dissolution testing and PBPK modeling, AAPS PharmSci 360, San Antonio, TX, 2019.

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List of Abbreviations

ADAM	Advanced dissolution, absorption and metabolism
ADME	Absorption, distribution, metabolism, excretion
ANOVA	Analysis of variance
AUC	Area under the curve
B	Bile salts
BCS	Biopharmaceutics Classification System
BSA	Bovine serum albumin
CAD	Charged Aerosol Detector
CD	Crohn's disease
CED	Coeliac disease
clog P	Calculated log P
C_{max}	Maximum plasma concentration
CMC	Critical micellar concentration
CRI	Clinical Rachmilewitz Index
DoE	Design of Experiment
FA	Fatty acids
FaSSCoF	Fasted-State Simulated Gastric Fluid
FaSSGF	Fasted-State Simulated Gastric Fluid
FaSSIF-V2	Fasted-State Simulated Intestinal Fluid-Version 2
FeSSCoF	Fed-State Simulated Colonic Fluid
FeSSGF	Fed-State Simulated Gastric Fluid
FeSSIF-V2	Fed-State Simulated Intestinal Fluid-Version 2
FID	Flame Ionisation Detector
GC	Glycocholic acid
GC	Gaschromatography
GCDC	Glycochenodeoxycholic acid
GES	Gastric electrolyte solution
GI	Gastrointestinal
H	High
HPLC	High Performance Liquid Chromatography
HSA	Human serum albumin

IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant irritable bowel syndrome
IBS-D	Diarrhoea predominant irritable bowel syndrome
IVIVC	*In vitro/in vivo* correlations
k_{in}	Input rate
k_{out}	Output rate
L	Low
LBF	Lipid-based formulation
log P	Decadic logarithm of the n-octanol/water partition coefficient
MG	Monoglycerides
MRI	Magnetic resonance imaging
MW	Molecular weight
NCA	Non-compartmental analysis
O	Osmolality
P	pH
PBPK	Physiologically-based pharmacokinetic
P-gp	P-glycoprotein
PK	Pharmacokinetic
pKa	Negative decadic logarithm of the acid dissociation constant
PLS	Partial Least Squares
PRESS	predicted residual error sum of squares
PSA	Parameter sensitivity analysis
PSC	Primary sclerosing cholangitis
Q	Goodness of prediction
QC	Quality control
R	Coefficient of determination
RE	Relative Effect
S	Solubility
SBS	Short bowel syndrome
SCFA	Short chain fatty acid
SIBO	Small intestinal bacterial overgrowth
SIES	Small intestinal electrolyte solution

SITT	Small intestinal transit time
TC	Taurocholic acid
TCDC	Taurochenodeoxycholic acid
TDC	Taurodeoxycholic acid
TFA	Trifluoroacetic acid
TG	Triglycerides
TGTT	Total gastrointestinal transit time
T_{max}	Time taken to reach maximum plasma concentration
UC	Ulcerative Colitis
UPLC-MS	Ultra-Performance Liquid Chromatography-Mass Spectrometry
USP	United States Pharmacopoeia
UV	Ultraviolet
VIP	Variable importance in projection
V_{SAC}	Volume of single adjusting compartment
V_{SS}	Volume of distribution at steady state

Abstract

Gastrointestinal (GI) diseases affect a large number of people and drug treatment in this patient population includes the treatment of the GI condition, of associated systemic symptoms and of concomitant conditions. Preferably, drugs are administered via the oral route and the respective pharmaceutical formulations are designed to overcome physiological challenges before reaching the systemic circulation. Pathophysiological changes in GI disease patients can affect the absorption, distribution, metabolism and excretion of drugs but clinical studies are rarely performed in patients with GI diseases. In the absence of those studies, *in vitro* and *in silico* tools can be used to identify drugs at risk of altered performance in this patient population and were developed as aim of this thesis.

To simulate the solubility and dissolution of drugs in the GI fluids of patients with Crohn's disease (CD), Ulcerative colitis (UC) and Coeliac disease (CED) compared to healthy subjects, biorelevant media were developed based on pathophysiological differences in these patient populations using a Design of Experiment (DoE) approach. The characterisation of the GI disease media revealed differences compared to healthy biorelevant media, mostly in terms of surface tension, osmolality and buffer capacity. Solubility studies in the respective media indicated that a risk of altered drug solubility in patients with GI diseases was in the majority of cases related to the drugs' lipophilicity or ionisation properties.

To predict the performance of a controlled-release budesonide formulation in healthy subjects and CD patients, *in vitro* dissolution studies representative of CD and healthy conditions were performed and integrated in a physiologically-based pharmacokinetic (PBPK) model. The developed PBPK model revealed a higher drug exposure for CD patients compared to healthy subjects as observed *in vivo*, mainly due to differences in drug metabolism and distribution.

The performance of complex formulations in GI disease patients was investigated using a complex GI simulator (TIM-1, TNO). The simulation of dynamic conditions to which a lipid-based formulation of ciprofloxacin is exposed to after oral administration in healthy subjects and CD patients revealed a similar drug performance in this specific case. A delayed and reduced lipid digestion was observed in CD, indicating a possible impact for other drugs.

Overall, this thesis provides a range of *in vitro* and *in silico* tools that can be used in combination or separately to identify drugs and formulations at risk of altered performance in patients with GI diseases.

Aims and Objectives

The overall aim of the thesis is the development of *in vitro* and *in silico* tools to predict drug product performance in patients with GI diseases as a means to improve drug therapy in patients with GI diseases in absence of clinical studies. Such tools can be used to assess which drugs are at risk of an altered performance in patients with GI diseases.

For poorly soluble drugs, *in vitro* methodologies serve to identify the risk for altered drug absorption in patients with GI diseases due to differences in drug solubility and dissolution. *In silico* methods are used to consider differences in terms of absorption, distribution, metabolism and excretion of drugs and to predict plasma concentration profiles in patients with GI diseases.

The objectives specified for each chapter of the PhD thesis are:

1. To review and describe pathophysiological differences in patients with GI diseases with possible implications on drug product performance. Investigated GI diseases comprise inflammatory bowel diseases, coeliac disease (CED), irritable bowel syndrome and short bowel syndrome. Approaches to predict drug product performance for these patient populations *in vitro* and *in silico* are discussed and the background for the development of the respective tools as part of the PhD project is set. **[Chapter 1]**

2. To develop biorelevant media representative of the GI fluid composition in patients with GI diseases as tool to investigate the risk of altered drug solubility in their GI fluids compared to healthy subjects. More specifically, the objectives are to develop biorelevant media for patients with GI diseases based on a Design of Experiment (DoE) approach in order to reflect interpatient variability in terms of the luminal fluid composition and subsequently, to characterise them according to their media properties. In order to identify the risk of altered drug solubility in respect to drug physicochemical properties, multivariate statistical analysis is used for the analysis of drug solubility in GI disease biorelevant media. This approach was followed for patients with Crohn's disease (CD) **[Chapter 2]**, patients with Ulcerative Colitis (UC) **[Chapter 3]** and patients with CED **[Chapter 4]**.

3. To combine *in vitro* and *in silico* tools to predict drug product performance in patients with CD using the model drug budesonide. The objective is to predict drug release from a budesonide formulation with *in vitro* biorelevant dissolution tests representative of conditions in healthy subjects and CD patients. Furthermore, a physiologically-based pharmacokinetic (PBPK) model for budesonide is developed and the *in vitro* release profiles are integrated with the aim to predict the plasma concentration profile after budesonide administration. By accounting for pathophysiological differences, the purpose of the PBPK model is to predict budesonide performance in healthy subjects and CD patients. **[Chapter 5]**

4. Investigating the risk of altered performance of a lipid-based formulation in CD patients using a complex dynamic GI simulator (TIM-1, TNO, Zeist, Netherlands) with ciprofloxacin as model drug. This includes the development of an *in vitro* methodology representative of conditions in CD patients and the assessment of the ciprofloxacin performance under such conditions. Additionally, the aim is to investigate if differences between CD conditions and healthy conditions are present in the luminal environment in terms of lipid and bile salt composition. **[Chapter 6]**

Title: Impact of gastrointestinal disease states on oral drug absorption – implications for formulation design – a PEARRL review

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Publication: JOURNAL OF PHARMACY AND PHARMACOLOGY

Publisher: John Wiley and Sons

Date: May 16, 2018

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Licensed Content Author	Angela Effinger, Caitriona M. O'Driscoll, Mark McAllister, et al
Licensed Content Date	May 16, 2018
Licensed Content Volume	71
Licensed Content Issue	4
Licensed Content Pages	25
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	Developing in vitro and in silico tools to predict drug product performance in patients with gastrointestinal diseases
Expected completion date	Oct 2019
Expected size (number of pages)	200
Requestor Location	University of Bath Claverton Down Bath, Somerset BA2 7AY United Kingdom Attn: University of Bath
Publisher Tax ID	EU826007151
Total	0.00 GBP

Chapter 1

Impact of Gastrointestinal Disease States on Oral Drug Absorption – implications for formulation design – a PEARRL review

Abstract

Objectives

Drug product performance in patients with gastrointestinal (GI) diseases can be altered compared to healthy subjects due to pathophysiological changes. In this review relevant differences in patients with inflammatory bowel diseases, coeliac disease, irritable bowel syndrome and short bowel syndrome are discussed and possible *in vitro* and *in silico* tools to predict drug product performance in this patient population are assessed.

Key findings

Drug product performance was altered in patients with GI diseases compared to healthy subjects, as assessed in a limited number of studies for some drugs. Underlying causes can be observed pathophysiological alterations such as the differences in GI transit time, the composition of the GI fluids and GI permeability. Additionally, alterations in the abundance of metabolising enzymes and transporter systems were observed. The effect of the GI diseases on each parameter is not always evident as it may depend on the location and the state of the disease. The impact of the pathophysiological change on drug bioavailability depends on the physicochemical characteristics of the drug, the pharmaceutical formulation and drug metabolism. *In vitro* and *in silico* methods to predict drug product performance in patients with GI diseases are currently limited but could be a useful tool to improve drug therapy.

Conclusions

Development of suitable *in vitro* dissolution and *in silico* models for patients with GI diseases can improve their drug therapy. The likeliness of the models to provide accurate predictions depends on the knowledge of pathophysiological alterations and thus, further assessment of physiological differences is essential.

1.1. Introduction

Oral drug absorption is a very complex process which is dependent on the physiological conditions in the gastrointestinal (GI) tract, the pharmaceutical formulation and the physicochemical characteristics of the drug.[1] Pharmacokinetic properties of drugs often display high variability in a healthy population group and pathophysiological changes in patients with GI diseases can further intensify this variability and affect drug product performance.[2]

Patients suffering from GI diseases take a variety of medicines not only for the GI condition but also for concomitant conditions. Differences in the bioavailability of drugs due to the GI disease state can provoke sub-therapeutic or toxic levels of drugs and therefore, have an impact on the safety and efficacy of drug therapy.[3]

Differences in the pharmacokinetics of orally administered drugs between healthy subjects (controls) and patients with GI diseases have frequently been observed.[4, 5] Careful interpretation is needed, as some of these studies are poorly controlled, include only a small patient population and study findings are conflicting. Numerous physiological factors affecting drug absorption can be altered in GI disease states. Differences in GI transit time and hydrodynamics influence the passage of the drug and formulation through the GI compartments.[6, 7] Changes in the composition and characteristics of GI fluids such as bile salt concentrations, pH and osmolality can affect the drug release from formulations and the solubilisation of the drug.[8] Alterations of the GI membranes and dissimilar expression of transporter systems can affect drug permeability.[9] Differences in the expression pattern of metabolic enzymes in the GI membrane can influence the intestinal first pass metabolism.[8] Alterations in the composition and the location of the GI microbiota can change the exposure of drugs and formulations to bacterial enzymes and may therefore change the metabolism or release of the drug, respectively.[10, 11]

To enable prediction of the *in vivo* performance of drug products in healthy adults, the use of *in vitro* dissolution methods and *in silico* models has been established.[12, 13] Knowledge of the pathophysiological GI conditions can improve the design of *in vitro* and *in silico* models, improve the ability to predict the drug product performance in patients with GI diseases and facilitate the development of suitable formulations to enhance drug efficacy.

The current review gives an overview of altered GI conditions in patients with inflammatory bowel disease (IBD), coeliac disease, irritable bowel syndrome (IBS) and short bowel syndrome (SBS). The consequences of these disease states on drug absorption are analysed. Finally, the suitability of existing *in vitro* dissolution and *in silico* models to predict the drug product performance in patients with GI diseases is critically discussed.

1.2. Physiological alterations in GI diseases affecting absorption

1.2.1. Inflammatory bowel diseases

1.2.1.1. General information

IBD is a recurrent or continuous inflammation of the bowel. Numerous factors (environmental, microbial and genetic) contribute to IBD while its aetiology remains still unknown.[14] In the US 1.4 million people suffer from IBD and 396 per 100 000 persons worldwide.[8] The prevalence of IBD is constantly rising. It is higher in northern, industrialized countries and emerges in newly industrialized countries.[15, 16] The two main forms of IBD are Crohn's disease (CD) and Ulcerative Colitis (UC). Numerous alterations in the GI physiology of IBD patients (e.g. mucosal lesions, thickened bowel wall and strictures) may influence drug absorption.[17]

1.2.1.1.1. Ulcerative Colitis

UC is a continuous uniform inflammation of the colon and rectum with periods of relapse and remission. Typically, the inflammation spreads from the rectum/descending colon to the ascending colon. Depending on the affected area and extent of the disease, it can be grouped into ulcerative proctitis, left-side colitis, sub-total colitis and pancolitis.[18] The diffuse inflammation involves only the mucosa and submucosa which appear granular and haemorrhagic. During active disease UC histology reveals neutrophil-mediated damaged epithelium.[19] This includes cryptitis, crypt abscesses where the lumen is filled with neutrophils and debris, and mucosal ulceration.[19] As the disease progresses, neutrophils infiltrate the lamina propria, crypts get shorter and branched and Paneth cells occur in the left colon.[19] The typical clinical manifestation of UC includes chronic diarrhoea with blood in the stool.[20]

1.2.1.1.2. Crohn's disease

The second type of IBD is CD. CD can affect the entire GI tract from mouth to anus, often discontinously, but is most likely to occur in the terminal ileum or ascending colon.[21]

Initially the disease is limited to the submucosa which appears red and swollen due to lymphoid hyperplasia and lymphedema.[22] In a later stage, the disease extends transmurally and involves the full thickness of the GI wall.[21, 22] Endoscopic examination of CD patients reveals cobble-stoning mucosa and linear or aphthous ulcers with a haemorrhagic rim form. Radiological findings in CD typically illustrate ileac involvement, fistulas and asymmetric manifestation. The typical clinical presentation of CD involves diarrhoea and recurrent abdominal pain. Other symptoms include abdominal cramps, fever, malaise and weight loss. CD complications include malabsorption, bowel obstruction, strictures, crypt abscesses and fistulae.[22]

1.2.1.2. Gastrointestinal transit time/motility and pH

1.2.1.2.1. Ulcerative Colitis

GI transit time varies between healthy adults and patients with UC (Table 1.1). Different results considering the total GI transit time (TGTT) have been published. TGTT was strongly increased in patients with UC, and this finding was even more pronounced in patients in remission compared to patients with severe disease.[23, 24] Similar TGTT to controls has been observed in one study possibly attributed to the methodology (large size of the telemetery capsule).[25] UC patients with severe disease have shown high variability in TGTT.[26]

Gastric residence time in the fed state was slightly prolonged in patients with UC, but this was not statistically significant.[23, 27] In the fasted state, patients with UC have shown similar gastric residence times as controls.[26] Small intestinal transit times were slightly prolonged (0.2-1.3 h) in patients with UC compared to controls as confirmed by a prolonged orocecal transit time as monitored using the lactulose breath test.[23, 24, 27-30]

Colonic transit times measured with a telemetry capsule were increased in patients with UC, mainly due to a prolonged residence time in the middle and distal colon.[23, 28] However, decreased colonic transit times were also observed which could be attributed to the mild disease state.[27] The range of colonic transit times in healthy volunteers is 7-20 h, whereas a much wider range (2-97.7 h) was observed for patients with very active UC consistent with high variability in the disease state.[13, 26]

Table 1.1: Gastrointestinal transit times in Ulcerative Colitis.

Total gastrointestinal transit	Gastric emptying time	Small intestinal transit time	Colorectal transit time	Proximal colon	Middle and distal colon	Orocecal transit time	Meal	Number of study subjects	Method	Reference
sUC: 44.5 h rUC: 51.8 h Controls: 27.6 h	sUC: 4.1 h rUC: 3.4 h Controls: 3.2 h	sUC: 5.9 h rUC: 6.2 h Controls: 4.9 h	sUC: 34.9 h rUC: 43.3 h Controls: 18.2 h	sUC: 9.7 h rUC: 7.0 h Controls: 2.1 h	sUC: 11.6 h rUC: 18.0 h Controls: 14.2 h		Overnight fast, standardized breakfast, capsule swallowed afterwards	UC: 20 (relapse n = 20, remission n = 10) Controls: 20 (Previous study)	3D-Transit telemetric capsule system (diameter 8 mm, length 21 mm, density 1.6 g/cm^3)	Haase et al [23]
						UC: 2.04 h (0.86) Controls: 1.51 h (0.51)		UC: 95 Controls: 115	Lactulose breath test	Rana et al [29]
	UC: 10.59 h (7.10) Controls: 5.19 h (2.13)	UC: 8.03 h (1.38) Controls: 7.38 h (2.04)		UC: 12.66 h (5.37) Controls: 30.68 h (21.47)			Overnight fast, breakfast, SP swallowed	UC: 5 (mild to moderate) Controls: 5	SmartPill system	Bosworth et al [27]
		UC: 4.4 h Non-IBD patients: 3.6 h					Overnight fast, light breakfast 4 h after swallowing the capsule	UC:23 aUC:20 rUC:3 Non-IBD patients: 125	Small capsule endoscopy studies	Fischer et al [30]
UC: 24 h Controls: 26 h							Overnight fast, capsule swallowed	UC: 5 (4 severe, 1 moderate) Controls: 15	Radiotelemetry capsule	Ewe et al [25]
		aUC: 7 h (2.3) Controls: 6 h (2.6)		aUC: 7 h (5.5) Controls: 8 h (9.2)	aUC: 12 h (6.9) Controls: 7 h (1.4)		Standardised ambulatory and dietary protocol	aUC: 4 Controls: 8	Radiotelemetry capsule	Nugent et al [28]

Table 1.1: (continued)

Total gastrointestinal transit	Gastric emptying time	Small intestinal transit time	Colorectal transit time	Proximal colon	Middle and distal colon	Orocecal transit time	Meal	Number of study subjects	Method	Reference
	UC: 1.6 h	UC: 3.4 h Controls: 3.2 h (0.94)					Overnight fast, standardised breakfast, tablet swallowed afterwards	UC:6 (2 active, 4 quiescent)	Gamma scintigraphy of a radiolabelled tablet with cellulose acetate coating	Hardy et al [31], Davis et al [32]
	UC:2.7 h (0.6)	UC:4.0 h (1.5)					Light breakfast, tablet swallowed afterwards	UC:5	Gamma scintigraphy of a tablet containing ^{111}indium-labelled granules and coated with Eudragit L® (Evonik Industries AG, Darmstadt, Germany)	Hardy et al [33]
UC: 8h - >122.5h	UC: 1.05 h (1.05)	UC: 8.93 h (5.90)		UC: 2 h - >97.7 h			Overnight fast, swallowed capsule, fasting until capsule had passed the stomach	UC:6 (severe)	Fluoroscopic localisation of capsule	Fallingborg et al [26]
aUC: 54.6 h (21.8) rUC: 53.0 h (32.6) daUC: 55.0 h (22.0) drUC: 60.5 h (42.0) Controls: 48.8 h (22.3)	aUC: 0.81 h (0.32) rUC: 0.88 h (0.52) daUC: 0.96 h (0.44) drUC: 1.13 h (0.45) Controls: 0.85 h (0.37)					aUC: 4.93 h (0.95) rUC: 5.28 h (1.33) daUC: 5.45 h (1.28) drUC: 5.23 h (1.47) Controls: 3.82 h (1.08)	Radiolabelled meal	aUC: 15 rUC: 23 daUC: 23 drUC: 23 Controls: 15	Hydrogen breath testing, radiolabelled meal and stool output	Rao and Read [24]

aUC= active UC, dUC=distal UC, daUC=distal active UC, Mean/Median (SD), drUC= distal UC in remission, rUC= patients with UC in remission, sUC=severe UC. Mean/median (SD).

GI motility in the jejunum and ileum as quantified by magnetic resonance imaging (MRI) was not altered in patients with UC compared to controls.[34] After the intake of a meal, the colonic motility in patients with UC in remission was similar to controls.[35] Whereas the low-amplitude propagating contractions in the colon responsible for the transport of liquid contents and gases were found more often in UC patients in remission than in controls, the amount of high-amplitude propagating contractions, which mainly transport solid contents, was similar to controls.[35]

The pH profile in patients with UC was investigated in several studies (Figure 1.1).[25-28, 36-38] In the stomach, pH was slightly higher and no major pH changes in the small intestine were observed in patients with UC compared to healthy subjects. Only the time to reach a pH of 7 in the small bowel was prolonged in patients with UC compared to controls.[27]

For colonic pH values, conflicting results have been published (Table 1.2). A decrease in colonic pH was mainly observed apart from two studies in which similar or even higher pH values were detected possibly due to the individual form of the disease, the status of the inflammation process and the current treatment of the patients.

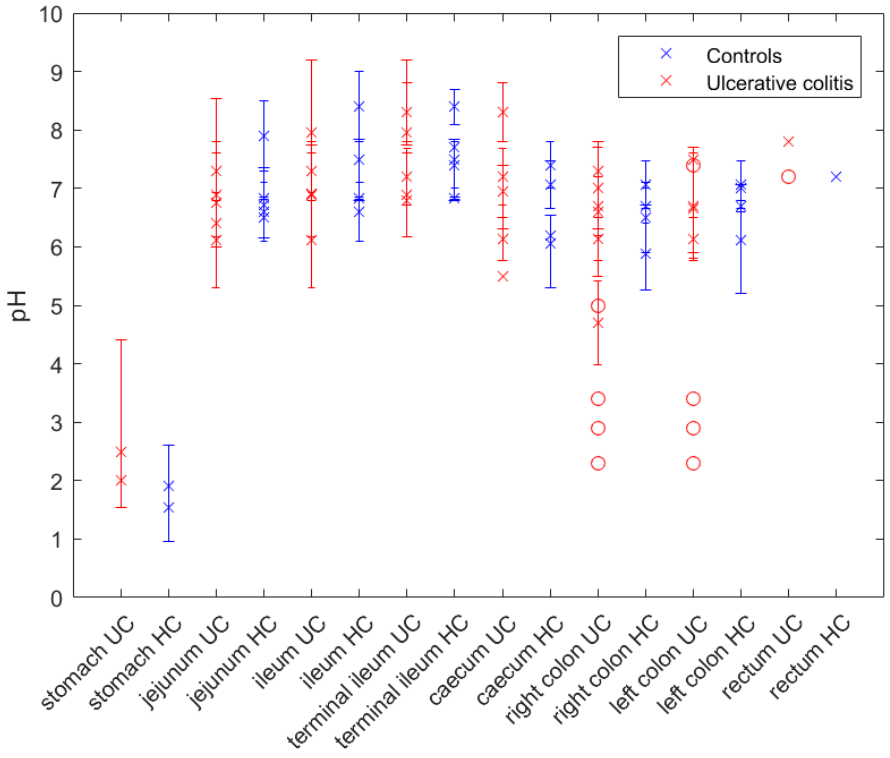

Figure 1.1: Gastrointestinal pH profile in patients with Ulcerative Colitis (x: mean/median values, open circles: single values, HC: healthy controls).

Table 1.2: Colonic pH values in patients with Ulcerative Colitis.

pH in controls	pH in patients with Ulcerative Colitis in remission	pH in patients with active Ulcerative Colitis	Special observations	Method	Reference
6.7(0.3) (n = 7)	4.90 (1.3)[a] 5.52 (1.13)[b] 5.51 (0.37)[c] (n = 6)	4.7 (0.72) (n = 7)		Radiotelemetry capsule	Raimundo et al [38]
Caecum: 5.7 Rectum: 6.6 (n = 39, previous study)		4.63 (1.93) (n = 6, very active)	Very active disease: 2 patients transferred for surgery during the study, 1 patient died	Radiotelemetry capsule, fast of at least 8 h until capsule passed the stomach	Fallingborg et al [26]
Right: 5.88 Left: 6.12 (n = 12)	Right: 7.19 Left: 6.45 (n = 4)	Right: 7 Left: 6.8 (n = 7)		Radiotelemetry capsule, overnight fast until capsule passed the stomach	Press et al [36]
Right: 6.5 Left: 7 (n = 15)		Right: 7.4 Left: 7.6 (n = 5)	Lowest individual pH values were reached in the caecum (involved in two of five cases), pH did not fall under 5.5	Radiotelemetery capsule	Ewe et al [25]
Right: 6.5 (0.6) Left: 6.7 (0.1) (n = 4)		Right: 6.7 (0.5) Left: 6.7 (0.9) (n = 8)	In 2 patients with active distal UC a low pH < 5.5 was measured	Radiotelemetry capsule, standardised ambulatory and dietary protocol	Nugent et al [28]
Colon: 7.06 (0.41) (n = 5)		Colon: 6.14 (0.37) (n = 5, mild to moderate UC)		Smart Pill following a standardised egg sandwich meal and water	Bosworth et al [27]
Right: 7.8 (n = 12)	Right: 6.5 (6.1–7.3) (n = 12)	Right: 6.6 (5.5–7.7) (n = 12)		Collection of the ascending colon fluid, measurement of pH	Vertzoni et al [37] Diakidou et al [39]

n, Number of subjects. Mean/median (SD/range), treatment with [a]sulphasalazine, [b]mesalazine, [c]olsalazine

1.2.1.2.2. Crohn's disease

An overview over the studies investigating GI transit time in CD is given in Table 1.3. Gastric emptying times in patients with CD in the fed state were prolonged as measured by scintigraphy of a capsule containing ^{111}In-labelled pellets.[40] In the fasted state, gastric emptying times in CD patients were similar to patients with different diagnosis using small capsule endoscopy studies.[40, 41] Small intestinal transit times were prolonged when measured with small capsule endoscopy studies but similar when measured by scintigraphy of labelled pellets, and thus, the GI passage could be altered according to the pharmaceutical dosage form.[30, 40, 41] This finding could also be attributed to the disease state as a recent study showed that CD patients with active disease have an increased small intestinal transit time while patients with inactive disease showed similar small intestinal transit times compared to non-IBD patients.[30] Orocecal transit times were prolonged in patients with CD.[29, 42] The passage through the ascending colon was not significantly different, but high disease activity was linked to a shorter transit time.[40]

Jejunal and ileac motility in patients with CD were similar to controls, whereas terminal ileum motility was decreased.[34] Differences in bowel hydrodynamics could occur due to the thickened bowel wall in CD and as a result of strictures which hinder the passage of GI fluids.[17]

The pH profile in patients with CD was investigated in several studies (Figure 1.2).[25, 36, 43, 44] Patients with CD showed a tendency to higher pH in the stomach compared to controls which correlated with decreased gastric acid secretion especially when patients were malnourished (mean basal acid output: 0.64 mEq/h (0.33) (malnourished), 2.12 mEq/h (0.88) (nutritional support) vs. 3.85 mEq/h (0.93) in controls, maximal acid output: 7.36 mEq/h (1.38) (malnourished), 12.76 mEq/h (2.50) (nutritional support) vs. 25.53 mEq/h (4.58) in controls).[25, 36, 45] Mean or median pH values in the small intestine of patients with CD were similar compared to controls whereas the observed pH range was higher in patients with CD. Similar results with more fluctuations were found for colonic pH values in CD patients with the exemption of one study with an overall mean decreased colonic pH (5.3 vs 6.8).[25, 36, 43]

Table 1.2: Gastrointestinal transit time in Crohn's disease.

Gastric emptying time	Small intestinal transit time	Proximal colonic transit time	Orocecal transit time	Meal	Number of subjects	Method	Reference
CD: 0.61 h (0.75) controls[a]: 0.58 h (0.29)	CD: 5.62 h (0.78) controls[a]: 4.06 h (1.39)			Overnight fast	CD:19 Patients with other diagnosis:178	Small capsule endoscopy studies	Niv et al [41]
	Active CD: 4.2 h Inactive CD: 3.1 h controls[a]: 3.6 h			Overnight fast, light breakfast 4h after swallowing the capsule	Active CD: 33 Inactive CD: 22 Patients with other diagnosis: 125	Small capsule endoscopy studies	Fischer et al [30]
			CD: 2.32 h (0.83) Controls: 1.51 h (0.51)		CD:42 Controls:115	Lactulose breath test	Rana et al [29]
			CD: 2.00 h controls: 1.47 h		CD:45 Controls:20	Lactulose breath test	Tursi et al [42]
CD: 4.0 h controls: 3.0 h	CD: 2.4 h controls: 3.0 h	CD: 8.1 h controls: 15.5 h		Fed state	CD:6 Controls:8	Scintigraphy using a capsule containing ^{111}In-labelled pellets	Edsbacker et al [40]
CD: 3.2 h (0.13) controls: 2.78 h (0.11)				Fed state	CD (inactive): 26 Controls: 19	^{13}C octanoic acid breath test	Nobrega et al [46]
CD: 6.7 h (4.2)	CD: 3.3 h (1.7) (n=3)			Fed state	CD:5	Gamma scintigraphy of a tablet containing compressed ^{111}In-labelled granules and coated with Eudragit L® (Evonik Industries AG, Darmstadt, Germany)	Hardy et al [33]

Mean/median (SD). [a]Controls in this study were patients with other diagnosis

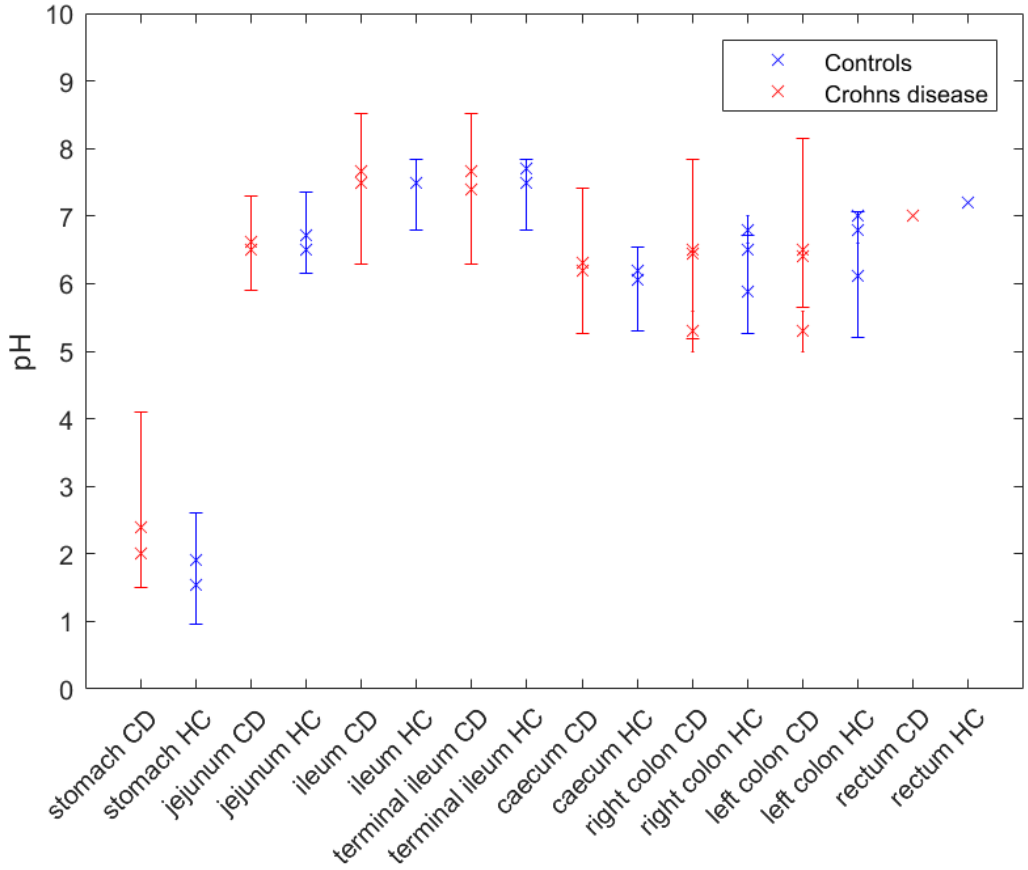

Figure 1.2: Gastrointestinal pH profile in Crohn's disease (x: mean/median values).

1.2.1.3. Composition of luminal contents

1.2.1.3.1. Ulcerative Colitis

The composition of the ascending colon fluid in the fasted state in UC patients in relapse and remission differed from healthy adults with elevated concentrations of soluble proteins (relapse: 18.9 mg/ml (8.1), remission: 19.0 mg/ml (10.8), healthy: 8.1 mg/ml (8.6)), in contrast, no difference in soluble carbohydrates (relapse: 5.4 mg/ml (2.7), remission: 6.4 mg/ml (4.1), healthy: 9.7 mg/ml (4.6)) was observed.[37] Phosphatidylcholine, an essential constituent for the normal mucus barrier function, was strongly decreased in the colonic mucus barrier of patients with UC (-70%) [as measured by mass spectrometric analysis of lipid extracts of specimens of rectal mucus]. Beneficial effects were shown when phosphatidylcholine was used as a treatment option for UC.[47-49] Due to the low number of subjects, only a trend to lower concentrations of phosphatidylcholine could be observed in

the ascending colon fluids of UC patients in relapse (0.31 mM) or remission (0.30 mM) in the fasted state compared to controls (0.36 mM).[37, 39] The faecal fluids of patients with UC were found to have a lower concentration of potassium (33.0 mmol/l vs. 84 mmol/l) and a higher concentration of sodium (67.8 mmol/l vs. 34 mmol/l) and chloride (53.1 mmol/l vs. 18.5 mmol/l) compared to healthy subjects.[50]

Regarding the properties of the ascending colon fluid of patients with UC, both the volume and surface tension were similar compared to controls (relapse: 26.8 ml (13.5), remission: 21.2 ml (8.8), controls: 22.3 ml (7.7) and relapse: 41.6 mN/m (3.1), remission: 40.6 mN/m (3.4), controls: 39.2 mN/m).[37] The buffer capacity of the ascending colon fluid in remission and relapse were similar but higher than in controls (with hydrochloric acid relapse: 32.0 mmol/l/ΔpH (18.1), remission: 37.7 mmol/l/ΔpH (15.4), controls: 21.4 mmol/l/ΔpH (7.9); with sodium hydroxide solution: relapse: 18.3 mmol/l/ΔpH (10.4), remission: 16.7 mmol/l/ΔpH (5.8), controls: 10.3 mmol/l/ΔpH).[37] Osmolality values were higher in patients with UC in relapse (199.6±127.4 mOsmol/kg) and remission (290.1±165.6 mOsmol/kg) compared to controls (80.6±102.5 mOsmol/kg).[37] Faecal fluid osmolality was similar to controls (341.1 mOsm/kg vs. 348.5 mOsm/kg).[50]

1.2.1.3.2. Crohn's disease

The composition of GI fluids in patients with Crohn's disease has not been described. The bile acid pool size (weight of total bile acids) was decreased to only 38-58% in patients with CD compared to controls as measured by induced gall bladder evacuation, subsequent aspiration of the duodenal fluid and analysis of labelled bile acid (previously administered) vs total bile acid concentrations.[51-53] It has been reported that >90% of patients with resected CD and 11-52% of patients with unresected CD suffer from bile acid malabsorption.[54] As a consequence, postprandial duodenal bile acid concentrations were decreased in 9 of 19 CD patients with a mean value of 6.04 mM (3.92).[55] The failure in the reabsorption of bile acids is a result of the disease localisation in the ileum, as the ileac sodium/bile acid cotransporter is responsible for the active reabsorption of the conjugated bile acids. As a consequence, bile acid malabsorption is particularly severe in CD patients after resection of the distal ileum.[56]

With regard to the properties of the GI fluids, faecal fluid osmolality in CD patients was increased (132-152%) as observed in two studies.[50, 57]

Changes in the exocrine pancreatic function have also been reported in CD. A significant decrease of amylase (33-85%), trypsin (29%) and lipase (28-80%) activity in the fed state in the duodenum of CD patients compared to controls was observed which was particularily strong in malnourished patients.[45, 58, 59]

1.2.1.4. Permeation and transport systems

Transporters in the GI tract can increase drug bioavailability by transferring drugs from the luminal to the basolateral site (uptake transporters) or decrease drug absorption by transport in opposite direction (efflux transporters).

For uptake transporters, differences in the transporter expression have been reported in IBD. The expression of OCTN1 and OCTN2, transporters for cationic drugs, is downregulated in UC patients, and patients with IBD were found to have mutations in the genes encoding their expression.[60, 61] The expression of PepT1, an important influx transporter for peptidomimetics, is upregulated in the colon in chronic inflammation associated with IBD, with no information being available for its expression in the small intestine of these patients.[61] In healthy adults, PepT1 is majorly expressed in the small intestine and only very low amounts of PepT1 are expressed in the colon.[61] Therefore, alterations in the colonic expression pattern of PepT1 may have only limited influence on drug absorption of peptidomimetics such as β-lactam antibiotics and angiotensin-converting enzyme inhibitors.

1.2.1.4.1. Ulcerative Colitis

The composition of the GI membranes can be altered by GI diseases, and thus, influence drug permeation. The thickness of the colonic and rectal mucus layer was reduced in patients with UC compared to controls which was more pronounced in distal regions (right colon: 90(79) vs 107(48) µm, left colon: 43 µm (45) vs 134 µm (68), rectum: 60 µm (86) vs 155 µm (54)).[62]

The efflux transporters, P-glycoprotein(P-gp), BCRP and MRP2 are the most important efflux transporters in the luminal membrane of the small intestine and they act by limiting cellular uptake into the enterocyte and enhancing the excretion of xenobiotics.[63] The expression levels of BCRP, MRP2 and P-gp in the colonic and rectal mucosa of patients with UC are strongly decreased during active inflammation.[64] In contrast, elevated levels of P-gp in the colon of patients with UC were found in another study possibly due to a milder disease state in the study subjects.[65] The bioavailability of sulfasalazine, a substrate of MRP2

and BCRP and prescribed for IBD, could thus be increased in UC and produce more side effects.[61]

1.2.1.4.2. Crohn's disease

The thickness of the colonic and rectal mucus layer was increased in patients with CD compared to controls (right colon: 190(83) vs. 107(48) µm, left colon: 232(40) vs. 134(68) µm, rectum: 294(45) vs. 155(54) µm).[62]

Baseline permeability in surgical specimens from the distal ileum of CD patients was similar compared to patients with colon cancer as measured by permeability to ^{51}Cr-EDTA and electrical resistance in Ussing chambers.[66] However, after exposure to sodium caprate, a stimulus to the luminal epithelium, the increase in paracellular permeability in CD was more pronounced.[66] This hyper responsiveness might be of particular interest because certain drugs may act as luminal stimulus.

Paracellular permeability for various compounds like ^{51}Cr-EDTA, [^{99}mTc]DTPA, sucrose and lactulose was increased in patients with CD compared to controls probably caused by the opening of tight junctions.[67-70]

Transcellular permeability, as indicated by mannitol's permeability in *in vivo* lactulose/mannitol intestinal permeability studies, was not altered in patients with CD compared to controls.[71, 72] Mannitol is absorbed via the paracellular pathway in *in vitro* permeability studies (e.g. Ussing chambers), whereas in *in vivo* intestinal permeability studies, it is used as marker for the transcellular route due to a solvent drag effect caused by the hyperosmolality of villus tips.[73]

Active transport systems can also be altered in CD. The expression of P-gp was increased to over 200% in the duodenal biopsy specimens and in the colon of patients with CD.[65, 74] This increased P-gp expression could be responsible for the decreased absorption of tacrolimus and justify the higher doses of tacrolimus required in a patient with CD.[74]

1.2.1.5. Metabolism

1.2.1.5.1. Ulcerative Colitis

The expression of metabolizing enzymes in the large intestine of patients with UC is altered compared to controls. In colorectal tissue, the expression of the most abundant metabolising enzyme, CYP3A4, was slightly elevated (125%) but the expression of CYP2C9, CYP1A1

and UDP-glucuronic acid transferase was decreased in enterocytes (74%, 81%, 72%).[65] In biopsy samples of the terminal ileum and various regions of the colon, the expression of CYP3A and CYP2D6 was not altered but the expression of CYP1A1 was increased.[75] Whereas, in the terminal ileum and colon, no difference in CYP2E1 expression compared to controls was observed, one study found increased expression (137%) in colorectal tissue probably due to the inflammation processes in active disease.[65, 75]

Considering conjugation reactions, sulphation by sulfotransferases in the colonic mucosa of patients with UC was reduced to <15% compared to controls.[76] The systemic sulphation pathway is not reduced as shown by no alteration in paracetamol metabolism in patients with UC.[77]

1.2.1.5.2. Crohn's disease

Patients with CD displayed different expression patterns for metabolizing enzymes. The expression of CYP3A4 was more than doubled in the colon of CD patients compared to controls and also increased, together with CYP3A5 expression, in duodenal biopsies of children with CD.[65, 78] This may alter the bioavailability of substrates for both enzymes such as corticosteroids. In a recent study, lower CYP3A4 activity was shown in patients with CD as assessed after intravenous and oral administration of midazolam (CYP3A4 substrate).[79] This finding was mainly attributed to a lower hepatic CYP3A4 activity (hepatic extraction ratio in CD patients 0.11 vs. 0.36-0.62 in healthy subjects; intestinal extraction ratio in CD patients 0.64 vs. 0.30-0.61 in healthy subjects). Furthermore, in the same study, 25% of the variability in budesonide pharmacokinetics (CYP3A4 substrate) was attributed to the reduced CYP3A4 activity.

Elevated expression of other metabolizing enzymes such as CYP2C9 (130%), CYP1A1 (134%) and UDP-glucuronic acid transferase (135%) was also observed.[65, 75] CYP2B6 levels were augmented to 178% in patients with CD and the expression of glutathione-S-transferase was strongly raised (159-167%).[65] A tendency to increased levels of CYP2E1 (122%) was reported.[65, 75] CYP3A and CYP2D6 expression was similar to controls.[75]

1.2.1.6. Microbiota

In recent years, the importance of the GI microbiota in IBD patients is increasingly recognised. At the early stages of IBD differences in the microbiota (dysbiosis) are already present and the role in disease etiology and disease progression is currently being

investigated.[80] The emergence of several new methodologies (metagenomic sequencing, transcriptomics and metabolomics) in the last years has provided information on bacterial functions over and above the broad taxonomic profiles.[80] The microbiota of patients with IBD was decreased in diversity, as the gene catalogue of the human gut microbiome in IBD patients showed 25% less bacterial genes compared to controls, with a shift to more potentially inflammatory and less potentially protective bacterial species.[80, 81] Reduced amounts of Faecalibacteria, Leuconostocaceae, *Odoribacter splanchnius*, *Phascolarctobacterium* and *Roseburia* in patients with IBD led to decreased levels of short chain fatty acids (SCFA) which are involved in immune regulatory functions and stimulate bile acid production and mucosal protection.[80, 82-84] Several drugs are processed by bacterial enzymatic action which is possibly affected by the altered composition of the microbiota observed in IBD (Table 1.4).

Table 1.4: Effect of IBD on drug interactions with gut bacterial enzymes.[11, 85-88]

Reaction	Enzyme	Substrates	Bacteria with high enzymatic expression	Changes in IBD
Azoreduction	Azoreductase	Sulfasalazine, prontosil, neoprontosil, balsalazine, olsalazine	*Clostridium sp.*	Azoreductase activity reduced in CD, *Clostridium* clusters IV and XIVa reduced in UC
Reduction	Nitroreductase	Nitrazepam	*Bacteroides fragilis/thetaiotamicron/vulgatus, Clostridium perfringens, Eubacterium limosum, Escherichia coli, Fusobacterium pseudonecrophorum, Peptostreptococcus asaccharolyticus*	*Bacteroides sp.* and *Eubacterium sp.* decreased
Deglucuronidation	β-glucuronidase	SN-38G (active metabolite of irinotecan)	*Bacteroides fragilis/thetaiotamicron/vulgatus, Clostridium barati/paraputrificum/perfringens, Eubactericum nitrogenes/aerofaciens, Peptostreptococcus asaccharolyticus*	*Bacteroides sp.* and *Eubacterium sp.* decreased
Thiazole ring-opening		Levamisole	*Bacteroides* and *Clostridium sp.* (Strongest metabolisers)	*Bacteroides sp.* and *Eubacterium sp.* decreased, *Clostridium* clusters IV and XIVa reduced in UC

1.2.1.6.1. Ulcerative Colitis

The microbiota of patients with UC was richer in Proteobacteria, Bacteroides, Fusobacteria and Enterobacteriaceae compared to controls.[89] Decreased levels of *Faecalibacterium prausnitzii*, *Bacteroides fragillis*, *Ruminococcus albus*, *Roseburia intestinalis*, *Clostridium coccoides*, *Eubacterium rectale*, enterohepatic *Helicobacter* species and the *Clostridium leptum* group were observed.[89]

Small intestinal bacterial overgrowth (SIBO) was slightly more prevalent in patients with UC compared to controls (17.8 % vs 0.86%).[29] In terms of enyzmatic bacterial function, differences in the colonic mucus of patients with UC were observed. Proteinase activity (657.6 units h^{-1}mg dry $wt.^{-1}$ (150.6) vs. 77.2 units h^{-1}mg dry $wt.^{-1}$ (25.9)) and non-specific esterase activity (39.8 µmol h^{-1} mg dry $wt.^{-1}$ (3.3) vs. 33.9 µmol h^{-1} mg dry $wt.^{-1}$ (3.7)) were increased compared to controls.[90]

1.2.1.6.2. Crohn's disease

Changes in bacteria species colonising the intestine of CD patients were observed with higher amounts of Bacteroidetes and Enterobacteriaceae, specifically *Eschericia coli*, and lower amounts of Firmicutes and *F. prausnitzii* compared to healthy subjects.[91]

45.2% of patients with CD suffered from SIBO compared to only 0.86% of controls.[29] With regard to bacterial enzyme activity, decreased faecal azoreductase activity (11.39 mU/g vs. 51.13 mU/g), extremely high proteinase activity (585.8 units h^{-1}mg dry $wt.^{-1}$ (202.1) vs. 77.2 units h^{-1}mg dry $wt.^{-1}$ (25.9)) and elevated non-specific esterase activity (51.7µmol h^{-1} mg dry $wt.^{-1}$ (19.7) vs. 33.9µmol h^{-1} mg dry $wt.^{-1}$ (3.7)) were observed in CD.[85, 90]

1.2.2. Coeliac disease

1.2.2.1. General information

Coeliac disease, affecting 1% of the population, is a genetic autoimmune enteropathy with a hypersensitivity of the patient to gluten.[92, 93] A small intestinal biopsy which shows villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis serves as an additional diagnostic criteria.[93] Normally, the villous atrophy, occurs in patches and is localized at the duodenal bulb and in the descending duodenum but more distal GI segments can also be affected. The villous atrophy results in decreased availability of absorptive surface area leading to impaired drug and nutrient absorption.[94]

1.2.2.2. Gastrointestinal transit time/motility and pH

The mouth-to-caecum transit time in untreated patients with coeliac disease was prolonged compared to controls using the lactulose breath test but significantly decreased after treatment with a gluten-free diet (Table 1.5).[95-97] Gastric emptying time measured with ^{13}C-octanoic acid breath test and ultrasonographic emptying studies in untreated patients with coeliac disease was increased but normalized after treatment with a gluten-free diet.[92, 98, 99] However, with another methodology (small bowel PillCam® [Given Imaging Ltd, Yoqneam, Israel]) gastric emptying was found to be similar to controls.[100] No alteration of small intestinal transit time was found in patients with coeliac disease. The faster mean colonic transit time, as measured in one study (n=40) only, was attributed to a subpopulation of patients with very fast colonic transit.[97]

Motility changes in patients with coeliac disease compared to controls were observed with increased oesophageal motility disturbances.[101]

With regard to the pH profile in patients with coeliac disease, a higher jejunal surface pH value with a pH of 6.42 (0.06) or 6.56 (0.14) in untreated patients, 6.32 (0.07) or 6.19 (0.09) in treated patients compared to 5.96 (0.05) or 5.93 (0.05) in controls was observed which might favour the absorption of weakly basic drugs.[102, 103] Intraluminal pH measurements confirmed a higher pH in the proximal small bowel and showed similar pH values in the stomach.[104]

Table 1.5: Gastrointestinal transit time in Coeliac disease [Mean/Median (SD)].

Gastric emptying time	Small intestinal transit time	Orocecal transit time	Meal	Number of study subjects	Method	Reference
Coeliac disease (children):3.75 h (1.12) (untreated), 1.46 h (0.43) (treated) Controls: 2.02 h (0.7)			Overnight fast, standard meal enriched with ^{13}C	Coeliac disease: 9 Controls: 9	^{13}C-octanoic acid breath test	Perri et al [92]
Coeliac disease: 5.43 h Controls: 3.55 h			Overnight fast, test meal	Coeliac disease: 16 Controls: 24	Ultrasonographic emptying studies	Benini et al [98]
Coeliac disease: 3.38 h (0.53) Controls: 2.22 h (0.25)			Overnight fast, test meal	Coeliac disease: 9 Controls: 9	Ultrasonographic emptying studies	Bardella et al [99]
		Coeliac disease (untreated): 4.05 h (0.17) Controls: 1.95 h (0.1)	Fasting period of at least 12 h	Coeliac disease: 16 Controls: 20	Hydrogen breath test	Battaglia et al [95]
		Coeliac disease: 2.13 h Controls: 1.01 h	Overnight fast, test meal	Coeliac disease: 25 Controls: 7	Hydrogen breath test	Spiller et al [96]
Coeliac disease: 0.51 h (0.37) Controls: 0.73 h (0.81)	Coeliac disease: 4.20 h (1.12) Controls: 4.08 h (1.47)		Bowel cleansing day before, fasting since midnight, drinking 2 h/ eating 4 h after capsule ingestions	Coeliac disease: 30 Controls: 30	Small bowel PillCam®	Urgesi et al [100]

1.2.2.3. Composition of luminal contents

The composition of GI fluids in patients with coeliac disease has not been described. About 20% of patients with untreated coeliac disease showed a decreased secretion of at least one pancreatic enzyme.[105] Reduced cholecystokinin secretion as response to a meal, which was observed in patients with coeliac disease, could lead to decreased gall-bladder motility and small intestinal transit time.[106] This could further provoke an increase and stasis of the bile acid pool.[106, 107] Additionally, increased biliary outputs of phospholipids (0.26 mg/kg*h (0.05) vs 0.08 mg/kg*h (0.02)), cholesterol (0.82 mg/kg*h (0.10) vs 0.43 mg/kg*h (0.06)) and bile acids (9.28 mg/kg*h (1.65) vs 4.64 mg/kg*h (0.45)) were all observed in patients with coeliac disease.[108]

Protein concentrations in jejunal perfusion fluids were altered in patients with coeliac disease compared to controls. The concentration of glycosaminoglycan hyaluronan, a connective membrane component, was increased twofold in the basal state of coeliac disease compared to controls.[109] After provoking an immune response by challenging the jejunal segment with gliadin (protein present in wheat), concentrations of albumin and glycosaminoglycan hyaluronan increased up to twofold indicating increased protein leakage through the GI membrane.[109]

1.2.2.4. Permeation and transport systems

Differences in paracellular passive diffusion were observed in patients with coeliac disease compared to controls with a higher GI permeability of lactulose and ^{51}Cr-EDTA, possibly due to opening of the tight junctions.[71, 110-113]

For the transcellular pathway, a lower permeability for mannitol and polyethylene glycol 400 was observed in *in vivo* intestinal permeability studies, possibly due to the decrease in the absorptive surface area.[110-113]

In the case of efflux transporters, the expression of P-gp in untreated and treated children with coeliac disease was elevated compared to controls whereupon gluten withdrawal resulted in a further increase.[114]

1.2.2.5. Metabolism

Jejunal morphological changes like flattened villi in coeliac disease were accompanied by different activity of metabolic enzymes. The CYP3A activity was decreased in patients with

coeliac disease, but treatment with a gluten-free diet subsequently resulted in increased activity.[115] Accordingly, the expression and activity of CYP3A4 in children with coeliac disease were reduced.[116]

1.2.2.6. Microbiota

The microbiota of patients with coeliac disease was found to be rich in potentially pathogenic Gram-negative bacteria and poor in species such as *Lactobacilli* and *Bifidobacteria* compared to controls.[117] After treatment with a gluten-free diet, the microbiota shifted to more beneficial species.[117] The prevalence of SIBO in patients with coeliac disease is not evident due to the heterogeneity of studies (differences in inclusion criteria, no homogeneous control groups, low study quality), whereas SIBO prevalence appears to be higher in patients with coeliac disease with persisting symptoms following withdrawal of gluten.[118-121]

1.2.3. Irritable bowel syndrome

1.2.3.1. General information

Irritable bowel syndrome (IBS) is a chronic GI disorder, prevalent in 5-11% of the population in most countries, with symptoms such as recurring abdominal pain, bloating and changes in the pattern of bowel movements.[122] The disease can either be predominated by diarrhoea (IBS-D) or constipation (IBS-C) or it can be a combination of both (IBS-M). The recrudescence of the symptoms is often linked with psychological stress.

1.2.3.2. Gastrointestinal transit time/motility and pH

Gastric emptying time and small intestinal transit time were not significantly different in patients with IBS compared to controls measured with a SmartPill GI monitoring system (51.23 min (59.1) vs 76.81 min (73.2) and 218.56 min (59.60) vs 199.20 min (82.31)).[123] Differentiation between IBS subtypes revealed that small bowel transit time and total GI transit time were shorter in patients with IBS-D (3.3 h (0.3) vs. 4.2 h (0.2) and 35 h (5) vs 53 h (4)) and prolonged in patients with IBS-C (5.4 h (0.3) vs. 4.2 h (0.2) and 87 h (13) vs 53 h (4)).[124]

The pH profile in patients with IBS in the fasted state was similar to controls throughout the four quartiles of the small intestine indicating no alteration in the ionisation of administered drugs compared to controls.[123]

1.2.3.3. Composition of luminal contents

The composition of GI fluids in patients with IBS has not been described. Around 32% of patients with IBS suffer from moderate bile acid malabsorption with a 10% prevalence of severe bile acid malabsorption.[125] Patients with IBS-D, showing a decreased bile acid deconjugation activity in the faeces, have increased levels of faecal primary bile acids, chenodeoxycholic acid, sulphated bile acids and ursodeoxycholic acid and decreased levels of faecal secondary bile acids.[126] Bile acid deconjugation activity was also decreased in the faeces of patients with IBS-C.[126]

1.2.3.4. Permeation

Not all patients with IBS showed an increase in intestinal permeability, but for the subgroup of patients with IBS-D a higher intestinal permeability was observed more frequently.[127] Rectal permeability tests in patients with IBS-D observed that the passage of macromolecular compounds through rectal biopsies was increased.[128]

1.2.3.5. Microbiota

The GI microbiota of patients with IBS has been analysed in several studies, but inconsistent results have been published due to the lack of differentiation between disease subtypes, the pathophysiology of the disease and the methods used. Patients with IBS had a higher amount of mucosa-associated bacteria at the rectal epithelium than healthy controls.[129] The faecal microbiota was reduced in the *C. coccoides* subgroup and the *Bifidobacterium catenulatum* group and a high ratio of Firmicutes to Bacteroidetes was found in a subgroup of patients with IBS.[130-132] The IBS-D subtype could be distinguished by decreased levels of *Lactobacillus* spp., Bifidobacteria and increased levels of *E. coli*.[126, 129, 132] The microbiota of patients with IBS-C was richer in *Bacteroides*, *Veillonella* spp. and *Bifidobacterium*.[126, 132]

1.2.4. Short Bowel Syndrome

1.2.4.1. General information

Short bowel syndrome (SBS) is a malabsorption disorder as a result of the loss of a large part of the bowel due to surgical resection, congenital defects or disease resulting in a remaining intestinal length of less than 200 cm.[133, 134] The diminished intestinal surface area

impedes absorption and, thus, causes the dehydration and malnutrition with micronutrients and macronutrients of patients with SBS which cannot always be overcome with enteral supplements.[135, 136] Drug absorption can equally be impaired in patients with SBS and for poorly absorbed drugs alternative routes of administration should be considered.[137]

1.2.4.2. Gastrointestinal transit time/motility and pH

GI transit time in patients with severe SBS was largely decreased impeding nutrient absorption as well as drug absorption.[138] Different GI transit times according to the method used were observed in patients with SBS: 52.5 min (lactulose hydrogen breath testing), 967 min (radiopaque markers) and 96.3 min (blue food colour to appear in ostomy effluent or stool). Limitations of the methods include that lactulose hydrogen breath testing can only be used in patients with intact ileocecal valve and the much longer transit time with a radiopaque marker indicates that anatomical changes prevent the passage of the marker.[138] Therefore, stagnation of solid oral dosage forms in the GI tract of SBS patients might also occur and result in a different exposure to the absorptive surfaces and increased variability of drug absorption.

The pH profile in the stomach of patients with SBS was similar compared to controls but higher pH values in the small intestine (6.03 vs. 5.39) and right colon (6.7 vs. 5.8) were observed (Figure 1.3).[44, 139-141]

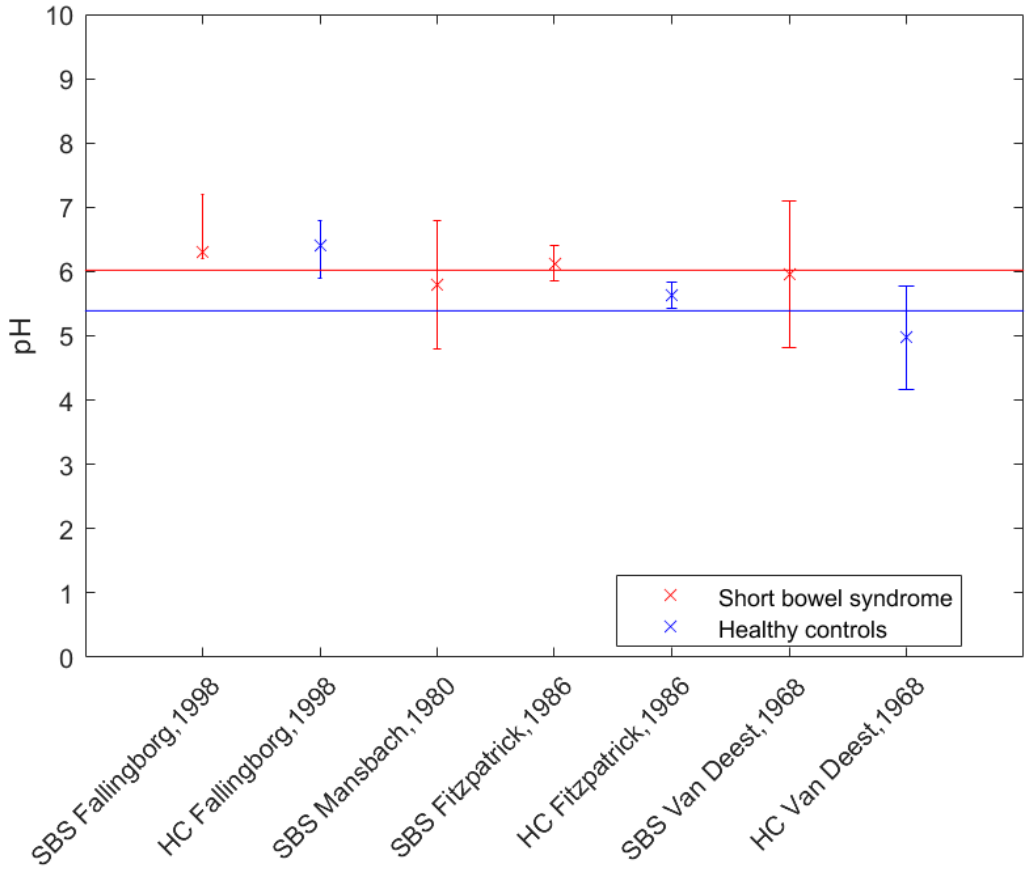

Figure 1.3: pH values in the small intestine of SBS patients (x: mean value, HC: healthy controls, blue line: mean value of controls, red line: mean value of patients with short bowel syndrome).

1.2.4.3. Composition of luminal contents

Gastric acid hypersecretion, which can be fivefold greater than basal levels in healthy subjects, is often experienced during the acute stage after surgical resection by patients with SBS.[142] This can result in a pH reduction causing the inactivation of GI fluid components such as pancreatic enzymes. Due to adaptation processes the hypersecretion is normalised during the first weeks or month after resection.[143]

Bile acid malabsorption as a result of the removal of parts of the ileum, their main reabsorption area, results in decreased recirculation of bile salts and a spill over of bile salts to the colon.[142] To compensate for the bile acid loss bile salt production is increased in SBS patients, reaching 10 to 20 fold the production of healthy individuals.[144] If the increased production cannot fully compensate the loss, lower amounts of bile acids in the intestine can

prevent the solubilisation and absorption of fatty acids as well as of lipophilic drugs.[145] Choleretic diarrhoea, caused by increased levels of bile salts in the colon and the subsequent loss of chloride and water, could also affect colonic transit time.[142]

1.2.4.4. Permeation

After removal of a large part of the intestine, the remnant parts of the bowel undergo a natural adaption process including changes in the expression of membrane transporters in order to improve the absorption of nutrients.[146] Patients with SBS had an increased amount of PepT1 mRNA in the colon 1.5–2.5 years after resection with normalization over time (9.8 ± 5.7 years after resection).[147, 148]

1.2.4.5. Microbiota

The faecal and mucosa-associated microbiota of patients with SBS was deeply altered compared to controls. It was rich in *Lactobacillus,* resulting in a greater absorption of carbohydrates in patients with SBS, and the specific species *Lactobacillus mucosae* was prevalent in most samples of SBS patients, while it was not detected in controls.[147] Decreased amounts of *C. leptum*, *C. coccoides,* Bacteroidetes, Firmicutes, *Bifidobacterium* and *Methanobrevibacter smithii* were found in patients with SBS.[134, 148]

The higher risk of SIBO in patients with SBS is a result of the stagnation of intestinal contents, the impairment of the ileocecal valve and the reduction of the terminal ileum which favours bacterial growth in higher parts of the GI tract.[142] As a consequence, deficiencies of fat-soluble vitamins, problems in fat absorption and increased intestinal permeability can occur.[142]

In summary, an overview of the changes affecting drug absorption in patients with GI disease compared to controls is given in Figure 1.4.

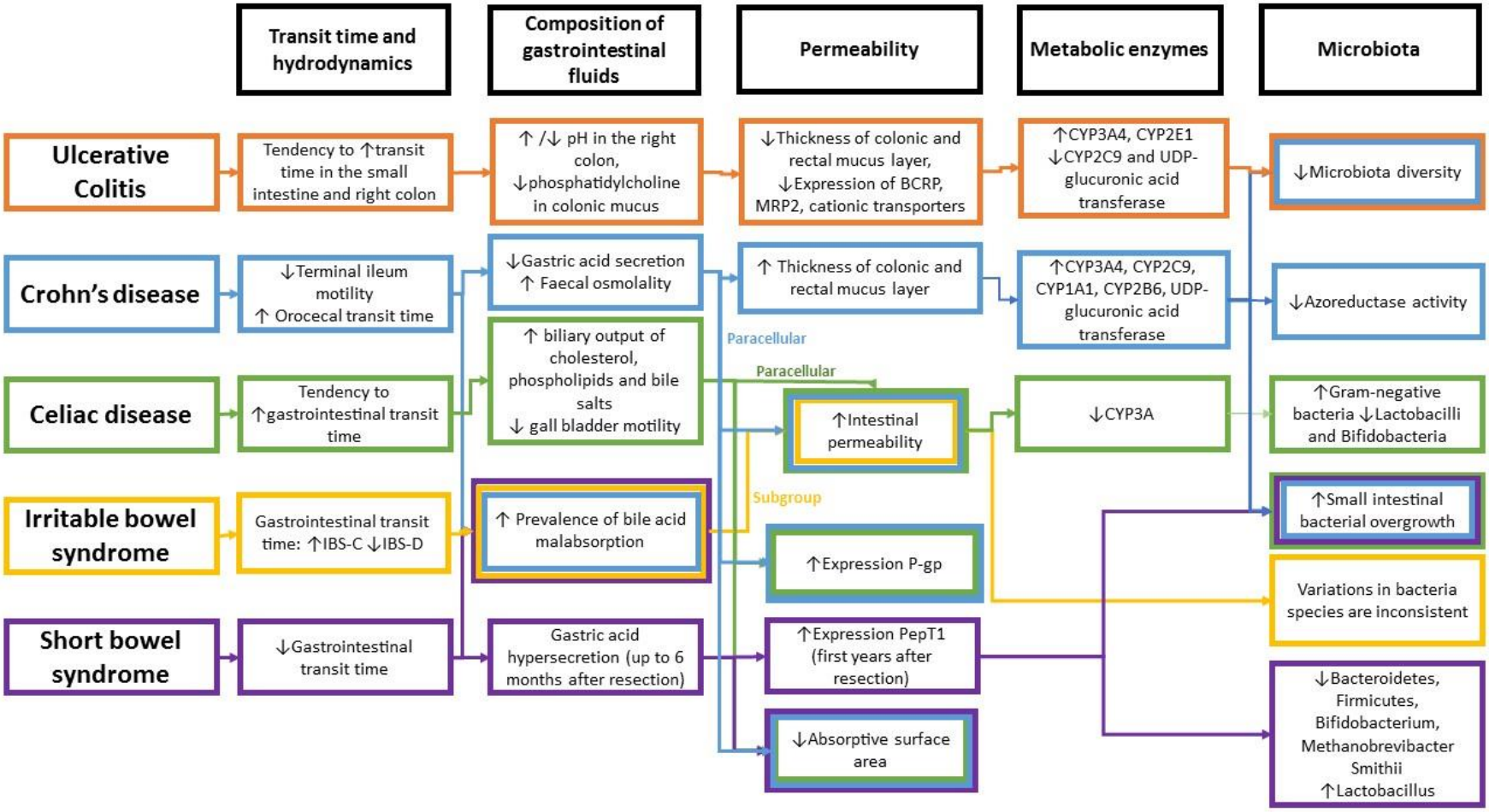

Figure 1.4: Overview of changes in GI diseases compared to healthy state.

1.3. Drug-related factors affecting absorption in gastrointestinal diseases

1.3.1. Molecular weight

The molecular weight (MW) in conjunction with other physicochemical characteristics such as the charge of the molecule, its hydrophilicity and shape determines the pathway and extent of drug permeability.[149] The rate of diffusion of a drug is inversely proportional to its molecular weight with high molecular weight compounds having low permeability.[149] Molecules with MW <200 g/mol can permeate through tight junctions between intestinal cells via paracellular passive diffusion.[150]

In CD and coeliac disease, ruptures of the tight junctions can increase the permeability of larger drugs (MW >200 g/mol) via the paracellular route by impairing the sieve effect of the tight junctions (Section 1.2.1.2.3 and 1.2.2.3). In coeliac disease, the decreased absorptive surface area hinders the absorption of small drugs (MW <200 g/mol) via the transcellular pathway, probably resulting in a decreased bioavailability compared to controls as indicated by the decreased permeability of mannitol (Section 1.2.2.3).

Passive transcellular diffusion is restricted for drugs with MW>500 g/mol whereas lipophilic drugs with MW 350±150 g/mol can readily permeate through the intestinal membrane. In coeliac disease, no correlation between drug absorption of different antibiotics and their molecular weight was observed since sulphamethoxazole (MW 253 g/mol) and erythromycin stearate (MW 1018.4 g/mol) showed a similar absorption pattern.[151] A possible explanation for this may be that the drugs use different pathways to pass the epithelial membrane.

The bioavailability of methyldopa (MW 211 g/mol, BCS class III compound) was significantly increased in coeliac disease patients (n = 10, C_{max} 5.0 µg/ml (2.2) vs 3.1 µg/ml (1.1), AUC 20.5 µg $ml^{-1}h$ (9.6) vs 13.4 µg $ml^{-1}h$ (4.9)), without a change in the pharmacological response.[152, 153] It should be noted that the patients were already on treatment (gluten-free diet), and more pronounced differences could be expected in patients without treatment. As levodopa is completely absorbed via efficient transepithelial carrier transport and the recovery of methyldopa in urine and faeces was not altered in patients with coeliac disease, increased paracellular permeability might not be relevant and the finding might be attributed to other factors such as increased renal excretion.[154] In contrast, patients with CD (n=5) had lower plasma levels of methyldopa (AUC 8.7 µg $ml^{-1}h$ (4.3) vs. 13.4 µg

$ml^{-1}h$ (4.9)) and a reduction in the pharmacological response (sedation, smaller decrease in systolic blood pressure).[153]

Acetaminophen (BCS class I compound) with a low MW of 151 g/mol is partly absorbed via the paracellular pathway.[152, 155] Acetaminophen absorption in patients with coeliac disease and CD was delayed (coeliac untreated $AUC_{0\text{-}1h}$ 9.0 µg min/ml (1.6), coeliac treated $AUC_{0\text{-}1h}$ 8.2 µg min/ml (2.0), CD 9.3 µg min/ml (3.5) vs. controls $AUC_{0\text{-}1h}$ 12.4 µg min/ml (3.2)) probably due to delayed gastric emptying, but the overall acetaminophen absorption was not impaired as indicated by urinary recovery.[156] In patients with SBS, total absorption of acetaminophen was decreased as the drug is absorbed in the jejunum, and thus, rectal drug administration should be preferred.[157] It should be noted that the changes in the jejunal morphology due to coeliac disease did not impair the overall absorption of acetaminophen.[156]

Tioguanine (MW 167 g/mol, log P -0.07) showed highly variable absorption in patients with CD possibly due to altered paracellular passive diffusion, with possible implication in treatment.[158] Differences in AUC were fourfold to sevenfold, and in two patients, no tioguanine absorption was observed within 6 h after oral intake for at least one of three different formulations investigated.[159]

1.3.2. Lipophilicity

Lipophilicity has a high influence on the bioavailability of a drug by affecting its solubility, permeability and metabolism.[160] Drugs can be classified according to their log P in highly (log P > 3), moderately (log P 1-3) and low (log P < 1) lipophilic drugs.[161] For highly lipophilic drugs (log P>3), the dissolution and solubility in the aqueous GI fluids are often the rate-limiting factor for drug absorption as only the dissolved part of a drug can permeate through the GI membranes and, thus, reach the systemic circulation. Alterations in GI diseases can provoke changes in the bioavailability of lipophilic drugs due to changes in GI transit times, reduced GI volumes leading to non-sink conditions and increased surface tension hindering the wetting of the drug surface. Micellar drug solubilisation can also be affected by decreased concentrations of amphiphilic bile components, and a reduction in absorptive surface area limits the permeation of drugs via transcellular passive diffusion.

In CD, decreased amounts of bile acids in the luminal fluids, reduced absorptive surface area depending on the location of the disease and increased small intestinal transit time can affect the absorption of lipophilic drugs (Section 1.2.1). In coeliac disease, impacting factors are

the increased concentrations of bile salts and lecithin, increased orocecal transit time and the highly decreased absorptive surface area (Section 1.2.2).

In patients with CD, a highly lipophilic drug, propranolol (log P 3.48, pKa 9.42), showed a higher bioavailability and increased plasma levels possibly due to prolonged small intestinal transit time. Since propranolol is a highly soluble compound (BCS class I), decreased bile salt concentrations are expected to be only secondary.[162, 163] Further investigations with multiple dosing are needed in order to assess if the increased bioavailability is clinically relevant. It should be noted that conflicting results regarding propranolol absorption in patients with coeliac disease have been reported with in some cases higher propranolol absorption in coeliac disease compared to controls whereas in other cases similar absorption was found.[4, 102, 162, 164, 165] Higher propranolol absorption correlated in one study with a measured higher jejunal surface pH resulting in a higher unionised fraction of propranolol but could also be the result of higher bile salt and phospholipid concentrations or the atropic mucosa favouring the transport of lipophilic drugs. However, jejunal perfusion showed lower propranolol absorption in the jejunum which was apparently compensated in lower intestinal parts.[165]

For levothyroxine, another highly lipophilic drug (log P 3.51) with a narrow therapeutic index, patients with coeliac disease needed higher initial doses to maintain an euthyroid state (154 µg (65) vs 106 µg (46)), which decreased (111 µg) after gluten withdrawal.[166, 167] This could be attributed to the reduced absorptive surface area in the small intestine in patients with coeliac disease (Section 1.2.2).

In CD and UC, the absorption of prednisolone (log P 1.62, BCS class I), a moderately lipophilic drug, was delayed possibly attributed to the increased gastric emptying time.[152, 158, 168]

In one study, overall prednisolone absorption in patients with CD was only impaired in patients with extensive disease manifestation in the small bowel, whereas in another study, a decreased bioavailability of 0.6 (0.2) compared to 0.86 (0.09) in controls was observed also for patients with CD with a different disease localisation.[168, 169] The authors of the first study postulated that the methodology of the latter study might have been more sensitive as it included measurements of serum, urine and stool recovery of prednisolone. Highly variable prednisolone serum levels in patients with CD with higher disease activity could be

attributed to altered CYP3A4 activity.[170] Surprisingly, prednisolone absorption was not altered in patients with coeliac disease where absorptive surface area is reduced due to the villous atrophy.[170, 171]

For drugs with low lipophilicity and high hydrophilicity following paracellular permeability, molecular weight (Section 1.3.1) and charge (Section 1.3.3) need to be considered for the evaluation of absorption of these drugs in GI diseases.

1.3.3. Degree of ionisation

The degree of ionisation influences both the solubility and the permeability of drugs and subsequently the rate of drug absorption. The degree of ionisation is dependent on the drug itself and the pH value of the enclosed GI fluids.

Weak bases are protonated and, therefore, more soluble in the more acidic compartments of the GI tract (stomach, proximal small intestine). Subsequent increase in pH, when the drug enters the duodenum, may result in a supersaturated state and enhance drug absorption.[172] The unionised form of a drug permeates more readily through the GI membrane, and therefore, drug absorption of weak bases is higher in GI compartments with higher pH. In CD, the pH of the stomach is elevated (Section 1.2.1.2), and decreased solubilisation of weak bases would be expected.

Weak acids are more soluble in GI compartments with a higher pH due to their ionisation profile, but membrane permeation for the more ionised fraction of the drug is impeded.[173] In coeliac disease and SBS, small intestinal pH was higher compared to controls which could possibly increase absorption of weak bases (Section 1.2).

The absorption of a weak acid, folic acid (pKa 4.7), was decreased in patients with coeliac disease possibly due to the lower absorptive surface area and the slightly elevated jejunal pH (Section 1.2.2) and, therefore, higher ionised amount of folic acid.[102, 174] Folate is highly absorbed in the more acidic milieu in the duodenum and proximal jejunum as the removal of these parts results in folate deficiency that is commonly observed in patients with coeliac disease.[175]

For two other weak acids, indomethacin (BCS class II) and acetylsalicylic acid (BCS class I), no effect on overall absorption was observed in patient with coeliac disease. Only a faster absorption rate (coeliac disease: t_{max} 0.80 h (0.60) and controls: t_{max} 1.09 h (0.16)) was found

for acetylsalicylic acid probably due to faster gastric emptying in the fasted state (Section 1.2.2.2) or differences in drug permeability.[152, 176] Thus, the slightly higher jejunal pH that might decrease the unionised fraction of the drug available for absorption has no effect on absorption (Section 1.2.2.3). With acetylsalicylic acid, therapeutic outcomes were achieved in patients with SBS revealing no impairment of drug absorption.[177]

1.4. Formulation-related factors affecting absorption in gastrointestinal diseases

Pharmaceutical formulations are designed to overcome the challenges of the GI tract and to deliver the active pharmaceutical ingredient into the systemic circulation. A variety of different approaches is used to optimise the bioavailability, safety and efficacy of the drug. Enteric-coated formulations protect the drug from gastric acid or the stomach from the toxicity of the drug. Modified-release formulations can ensure constant drug levels, facilitate drug therapy by minimizing the administration frequency and deliver the drug locally to specific compartments of the GI tract. Immediate-release formulations are a simple approach if no further modification of the drug bioavailability is needed. To fulfil their purpose, the different formulations are designed based on the conditions of the GI tract in healthy subjects, for example, pH, microbiota and transit time (Section 1.2). However, these parameters can be altered in patients with GI diseases impacting the drug release/dissolution from the formulation.

1.4.1. Immediate-release formulation

For immediate release formulations, the disintegration of the pharmaceutical formulation, the disaggregation of the granules and finally the dissolution of the particles will be affected by the hydrodynamics in the GI tract. Transit times in the different GI compartments, altered by GI diseases (Section 1.2), affect the time until the absorption site is reached and the time available for absorption. Delayed gastric emptying as observed in CD and untreated coeliac disease in the fed state (Section 1.2) can result in a delayed T_{max} since for most drugs, the main absorptive area is the large surface area of the small intestine. Patients with faster gastric emptying may also show a shorter T_{max}.[4] Differences in terms of bile salts as observed in coeliac disease, CD and SBS (Section 1.2) can affect the wetting of the pharmaceutical formulation and, therefore, change the disintegration time.

1.4.2. Modified-release formulation

1.4.2.1. Time-controlled release

For the treatment of IBD, pharmaceutical formulations with time-controlled release mechanism have been developed to deliver drugs to their target site in the colon. Depending on the transit times in the different compartments of the GI tract, the amount of drug available in each compartment may vary for these formulations. For UC, a high variability in colonic transit time was observed, while in CD, the passage through the colon was accelerated (Section 1.2.1.2.1 and 1.2.1.2.2). Faster colonic transit time can lead to a large amount of drug not being released, and therefore, failure of the therapeutic effect may occur.

When a micro pellet formulation of mesalazine coated with ethyl cellulose (Pentasa®, Ferring Pharmaceuticals, Copenhagen, Denmark) was administered to healthy subjects, drug product performance was not affected by laxative-induced diarrhoea.[178, 179] Thus, reduced colonic transit time as observed in CD (Section 1.2.1.2.2) is not expected to affect drug release from this formulation.

Administration of an enteric coated multimatrix formulation of mesalazine (Mezavant®, Shire Pharmaceutical Contracts Ltd, London, UK; Lialda®, Shire US Inc., Massachusetts, USA) in patients with UC could be affected by longer small intestinal and colonic transit times, as following the dissolution of the gastro-resistant coating drug release occurs after diffusion from the lipophilic and hydrophilic matrix (Section 1.2.1). Drug release might occur in more proximal GI compartments differing from controls in which disintegration of the formulation was observed between 4.8 and 17.4 h after administration.[178]

Administration of a controlled release pellet formulation of budesonide (Entocort®, AstraZeneca UK Ltd, London, UK) showed increased systemic bioavailability in patients with CD compared to controls (20.5% (15.1, 27.8) vs. 11.5% (8.8, 15.0), $AUC_{0-\infty}$ 114.0 nmol*h ⁄ L (81.4, 159.5) vs. 60.4 nmol*h ⁄ L (45.1, 80.8)).[40] This effect could be attributed to the delayed gastric emptying observed and other factors such as the composition of GI fluids, differences in permeability and the colonic bacterial and intestinal metabolism. Differences in the pharmacokinetics of budesonide in patients with CD could possibly result in treatment failure or increased side effects.

1.4.2.2. pH-controlled release

The alteration of the typical pH profile in GI compartments changes the release profile of pharmaceutical formulations with pH sensitive coatings. For enteric coated formulations, the reduction in acid in the stomach in CD can lead to premature drug release in the stomach (Section 1.2.1.2.2). Increased gastric residence time as observed in coeliac disease, UC and CD could delay drug absorption of enteric coated formulations (Section 1.2).

Different mesalazine formulations with pH-controlled release behaviour are available for the therapy of IBD. Formulations with a coating of Eudragit L® (e.g. Salofalk®, Dr Falk GmbH, Freiburg, Germany), dissolving at pH ≥6, target the mid-ileum and colon, whereas a tablet coated with Eudragit S® (e.g. Asacol®,Tillotts Pharma AG, Ziefen, Switzerland), dissolving at pH ≥ 7, targets the terminal ileum and colon.[178] Based on the lower colonic pH values in UC (Section 1.2.1.2.1), impairment of drug release from these formulations may take place where failure to reach the pH needed for dissolution of the polymer coating occurs.

1.4.3. Azo-bonded prodrug formulations

Colonic drug delivery, often used in IBD, can be achieved by administering prodrugs or polymer coatings, which are cleaved by colonic bacterial enzymes such as azoreductase leading subsequently to the release of the active metabolite/drug.

In GI diseases, three different aspects can affect drug release of azo-bonded prodrugs such as sulfasalazine and olsalazine. Firstly, a decreased intestinal transit time has been associated with less exposure of the prodrugs to bacterial action and enhanced faecal loss of the prodrugs.[179] The therapeutic efficacy could be affected in some IBD patients as colonic transit time was highly variable (Section 1.2.1.2). Secondly, reduced activity of bacterial azoreductase as observed in CD (Section 1.2.1.6.2) could lead to reduced prodrug activation. Thirdly, small intestinal bacterial overgrowth as observed in CD and UC (Section 1.2.1.6) could provoke prodrug activation in upper parts of the GI tract.

1.5. Methods to predict drug product performance

Throughout the different stages in pharmaceutical drug development, *in vitro* biorelevant release/dissolution models linked with physiologically based pharmacokinetic (PBPK) models are used to predict drug product performance.[12, 180] Media, that simulate closely the conditions in the GI tract of healthy subjects by incorporating, for example, phospholipids,

bile salts and lipids, are termed biorelevant. By using biorelevant media and applying hydrodynamics to reflect the conditions in healthy subjects, successful predictions of the drug product performance can be established with *in vitro* dissolution/release testing.[181, 182] Nowadays, *in vitro* dissolution/release profiles are often further linked with PBPK models resulting in better *in vivo* predictions of drug bioavailability.[183-185] It should be noted that the design of *in vitro* dissolution/release and PBPK models is based on conditions in healthy subjects. A remaining challenge is the prediction of drug product performance in patients with GI diseases where absorption is expected to be impaired (Section 1.2). Therefore, the development of biorelevant *in vitro* dissolution/release tests in patients with GI diseases linked with PBPK models would be desirable. In the following sections, the need to develop both *in vitro* dissolution/release tests and PBPK models reflecting conditions found in GI disease which can be confidently used to predict drug product performance is discussed.

1.5.1. *In vitro* dissolution and release testing

In vitro dissolution testing has been established in the pharmaceutical industry for quality control purposes for stability testing and to assure batch to batch consistency. For drug development, biorelevant *in vitro* dissolution and release testing is used for the development of pharmaceutical formulations, to predict the *in vivo* performance of a drug product and to develop *in vitro/in vivo* correlations (IVIVC) with the intention to reduce time-consuming and cost-intensive animal or human studies. For the development of a suitable biorelevant *in vitro* dissolution testing method, the physicochemical characteristics of the drug and the physiological conditions in the GI tract should be considered. Current *in vitro* dissolution tests incorporate hydrodynamic conditions and media based on the physiological conditions in healthy subjects.

There is a need for biorelevant dissolution methodology to simulate the GI conditions in patients with GI diseases since pathophysiological changes (Section 1.2) are expected to have an impact on drug solubilisation and dissolution and subsequently on drug absorption. Currently, no *in vitro* dissolution and release tests reflect changes observed in patients with GI diseases.

In vitro dissolution and release tests used for drugs in GI diseases, especially IBD, have been developed reflecting mainly the GI pH profile in healthy subjects. To study the release and dissolution of different colon-targeting mesalazine and budesonide formulations several *in*

vitro dissolution methods have been developed (Figure 1.5).[186-189] In terms of media, GI fluids were simulated using simple pharmacopeia buffers (SGF, SIF and SCoF), biorelevant media (Fasted-State Simulated Intestinal Fluid) or media enriched with enzymes. Different buffer systems were used (phosphate and bicarbonate), whereas bicarbonate buffers were superior in predicting the *in vivo* performance of mesalazine formulations.[190] The passage through the different GI compartments is simulated by media changes, modifications of the pH value at various time points and the total duration of the experiment (360-1440 min). The models vary in the applied hydrodynamics due to differences in volumes of the media (200-1000 ml), in the agitation rate (50-100 rpm, 10 dips/min) and in the choice of the dissolution apparatus (USP II or III dissolution apparatus).

Bacterial enzymatic action, needed for colon-targeting drug delivery, was included in *in vitro* dissolution tests with USP dissolution apparatus in several ways spanning the simple addition of enzymes to the addition of rat caecal contents and human faecal slurries.[191] Drug metabolism by intestinal microbiota can further be tested in more complex *in vitro* GI simulators such as semi-continuous culture systems and continuous culture systems (e.g. TNO TIM-2 *in vitro* model of the colon) with anaerobic conditions in which pH, temperature and redox potential can be controlled.[11, 192, 193]

For the development of biorelevant *in vitro* dissolution and release tests for patients with GI diseases, pathophysiological changes in terms of media, hydrodynamics and microbiota must be reflected in the experimental design.

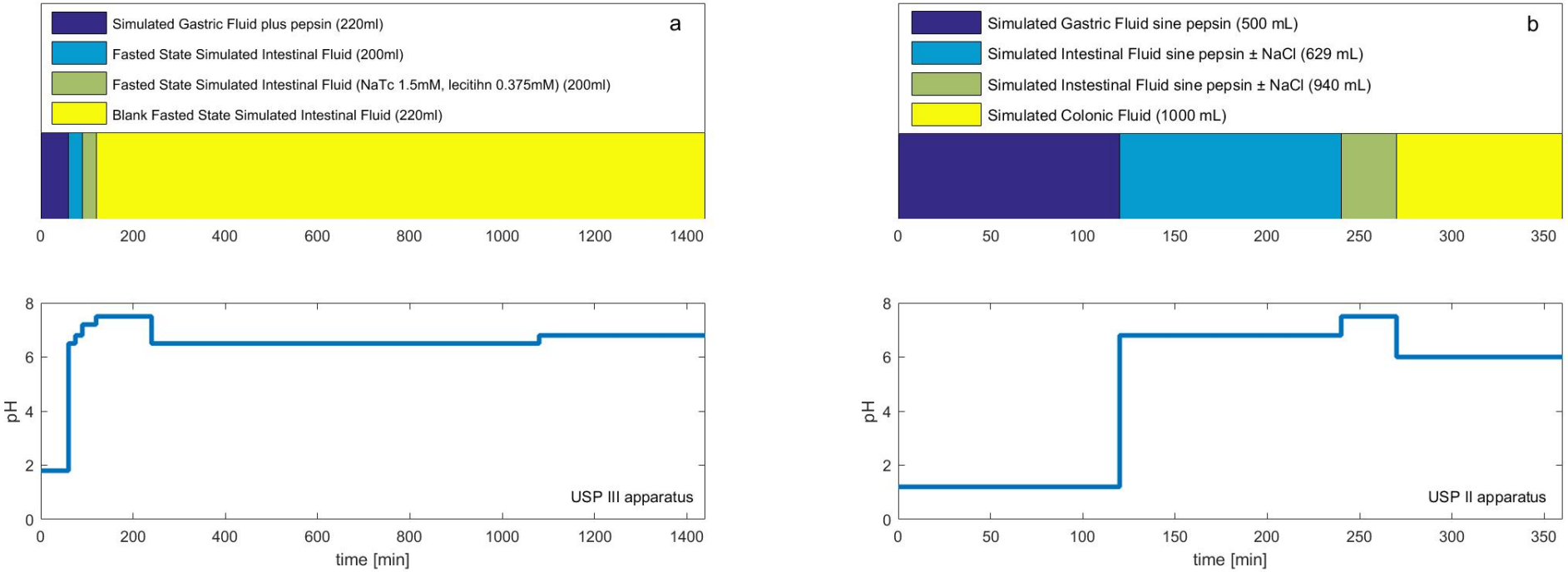

Figure 1.5: *In vitro* dissolution/release models for modified release dosage forms; a: Klein *et al*[190], b: Schellekens *et al*[187], c: Ahmed and Ayres[189], d: Goyanes *et al*[188].

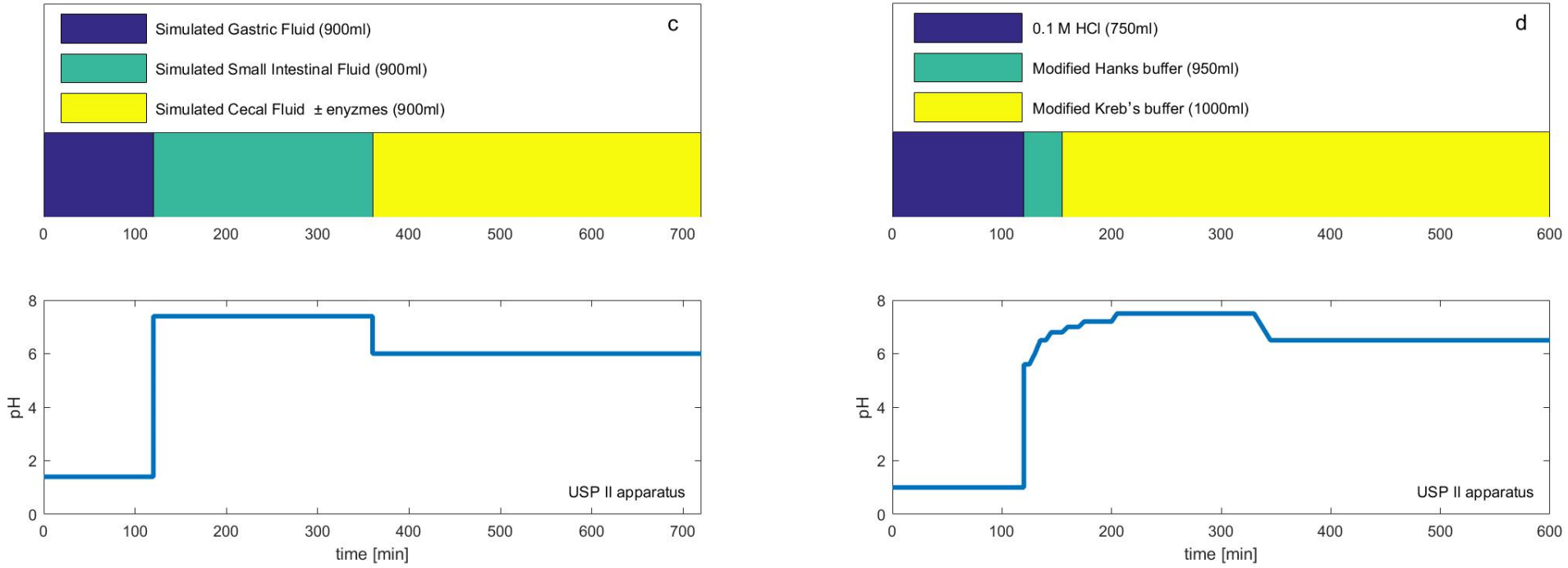

Figure 1.5: (followed).

1.5.2. PBPK models

PBPK models use preclinical *in vitro* data, physicochemical drug properties and physiological parameters to predict *in vivo* plasma concentration-time profiles.[12] PBPK modelling was first introduced to assess the toxicology of drugs and was in recent years established as useful biopharmaceutical tool to predict drug bioavailability. The mathematical modelling framework used incorporates the different compartments of the GI tract and evaluates absorption, distribution, metabolism and elimination of the studied compound.

For patients with GI diseases, PBPK models present a special opportunity to improve their drug therapy. Pathophysiological changes can affect drug absorption (Section 1.2), but only a minor part of drugs and pharmaceutical formulations is tested in a GI disease population. Especially for the medication of concomitant conditions, for example oncological or cardiovascular drugs, the impact of the GI disease on drug product performance is unknown. As human studies are very cost-intensive, this might not change in the coming years considering the heterogeneous and therefore small patient population in the different types of GI disease. Establishing predictive *in silico* models for the different GI disease states can help to implement appropriate dosing regimen and improve drug therapy management.

For GI diseases, PBPK models should include all the pathophysiological changes relevant for drug absorption in patients with GI diseases compared to healthy subjects (Section 1.2). However, due to only a limited number of studies with small patient populations and a high inter- and intra-study variability, the characterisation of the pathophysiological changes is challenging. Up to now, no PBPK models for patients with GI diseases have been developed, but recently, a PBPK model for patients after bariatric surgery (post sleeve gastrectomy, post Roux-en-Y gastric bypass, post biliopancreatic diversion with duodenal switch, post jejunoileal bypass) was developed.[194] The virtual model showed that the bioavailability of 5 drugs (omeprazole, diclofenac, fluconazole, ciprofloxacin, simvastatin) in patients after bariatric surgery was highly dependent on drug-specific parameters. The model, based on the template for morbidly obese in the Simcyp Simulator v10 (Simcyp Limited, Sheffield, UK), integrated changes in gastric volume and emptying rate, GI pH, differences in small intestinal dimensions and motility, transit time, bile properties, renal function and serum protein levels as observed in literature. Predictions of oral bioavailability of atorvastatin and cyclosporine in patients post Roux-en-Y gastric bypass were confirmed by clinical data;

however, the absorption of atorvastatin was not captured in the model for patients with post biliopancreatic diversion with duodenal switch.[195]

1.6. Conclusion and outlook

Further elucidation of drug absorption profiles in patients with GI diseases could be highly beneficial. The significance of current studies is often limited by small patient populations, conflicting data and the difficulty to assess changes in different disease states. More *in vivo* data is needed to further assess the GI physiological conditions in patients with GI diseases. Oral absorption already shows a high interindividual variability in healthy adults. Different disease states and disease localisation make it even more difficult to assess absorption profiles in this heterogeneous group. In order to improve drug therapy for patients with GI diseases, their medication should be tested under conditions specific to the particular pathophysiology. The ability to predict the *in vivo* performance of drug products in patients with GI diseases will be contingent on the development of appropriate biorelevant dissolution testing linked with PBPK models simulating pathophysiological conditions. Medication for concomitant diseases is seldom tested in GI disease patients. For these drugs, the development of more cost-effective and less time-consuming alternatives to expensive clinical trials would represent an opportunity to improve drug therapy. Predicting the probability that a drug will be affected by certain GI diseases depending on its physicochemical properties, would further limit the amount of experimental and computational work required.

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Chapter 2

Gastrointestinal diseases and their impact on drug solubility. Part I. Crohn's disease

Abstract

Objectives

Biorelevant media representative of Crohn's disease (CD) patients were developed using a Design of Experiment (DoE) approach to investigate differences in drug solubility and dissolution in luminal fluids of CD patients compared to healthy subjects. 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.

Key findings

Identified differences in CD patients compared to healthy subjects were a reduced concentration of bile salts, a higher gastric pH and an increased colonic osmolality. Differences in the properties of CD compared to healthy biorelevant media were mainly observed for surface tension and osmolality, suggesting differences in the wetting and swelling behaviour of drugs and formulations. 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.

Conclusions

Simulating the conditions in CD patients *in vitro* offers the possibility to identify relevant differences in drug solubilisation without conducting expensive clinical trials. To increase the confidence in the risk assessment tools, further studies investigating the composition of GI fluids in CD patients and the solubility of additional drugs in CD media are needed.

2.1. Introduction

Inflammatory bowel disease (IBD) is an incurable autoinflammatory disorder that affects about 3.7 million people in Europe.[1] While the aetiology of IBD is still unknown, a combination of factors (environment, genetics, microbiota) is expected to contribute to the disease.[2] 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.[3] Within the first 20 years after CD diagnosis, 50% of patients present complications such as strictures, fistulas, abscesses or obstructions.[3] These complications often necessitate surgeries and bowel resections.[4] Apart from the affected GI 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.[5] Therefore, CD necessitates a long-term drug therapy adapted to the disease localisation and disease state (relapse or remission).

Despite the location of the disease in the GI tract, drug therapy of CD patients relies highly on the oral route of drug administration. 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).[6] 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. 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.[7]

Oral drug delivery depends highly on drug absorption. To reach the systemic circulation, the drug 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 has been observed for several drugs in GI disease patients.[8] 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.[9] 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.[10, 11] 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.[12-16] This approach has previously been extended to special populations with the development of biorelevant media for paediatrics or hypochlorhydric and achlorhydric people.[17, 18] 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 biorelevant media representative of the stomach, intestine and colon of CD patients based on literature data and healthy biorelevant media, as a cost- and labour-effective tool to assess the risk of altered luminal drug solubility in patients with GI diseases *in vitro*. To take into account the interindividual variability in patients with CD, a Design of Experiment (DoE) approach was followed. The developed simulating 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 solubility results were analysed with partial least squares (PLS) regression to identify the impact of media-dependent factors on the solubility of the investigated drugs according to their physicochemical characteristics.

2.2. 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.

2.3. Methods

2.3.1. Media development

For the development of biorelevant media for CD patients, a DoE approach (Section 2.3.3) 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. Healthy biorelevant media were used as reference for all media properties and components that were not used as factors in the DoE.

2.3.2. 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. 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

$$x_{CD-BM} = \frac{y_{CD}}{y_H} * x_{H-BM} \quad (2.1)$$

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 healthy biorelevant media. In the case of a decrease of the factor in CD patients compared to healthy subjects, Equation 2.1 was used to set the low level and the high level was set to the level in healthy biorelevant media. The opposite was the case for an increase of the factor in CD. 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.

2.3.2.1. 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.[19] 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.[19] The bile acid pool is the total amount of bile acids circulating in the enterohepatic circulation. CD can affect any part of the GI tract but most frequently the inflammation is localised 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 (Table 2.1).[20-22] An increased loss of bile salts can be compensated by higher production. However, constant bile salt loss during the day, when bile salts are released as response to meals, is expected to lower the bile salt concentrations in GI fluids. Bile salts are present in the luminal fluids of all GI compartments and thus, lower bile salt concentrations were integrated in the DoE of all CD media.

Table 2.1: Bile acid pool in CD patients and controls.

	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	[20]
	3.09 (0.27)	1.48 (0.16)	10/14	[21]
	3.10 (0.27)	1.18 (0.2)	13/10	[22]
Median	**3.09**	**1.32**		

2.3.2.2. pH in the stomach

The pH profile in the stomach of CD patients was in the range of 1.5 to 4.1 as investigated in two studies.[23, 24] 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.[25]

2.3.2.3. Osmolality in the colon

The faecal osmolality in CD patients was increased by 32% to 52%, as observed in two studies (Table 2.2).[26, 27] This observation was accompanied with a large osmotic gap indicating osmotic diarrhoea in CD patients from osmotic active agents other than electrolytes, for example 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. Therefore, an increased osmolality in colonic luminal fluids was reflected by integrating the osmolality as factor in the DoE of colonic CD media.

Table 2.2: Osmolality of the faecal fluids of CD patients and controls.

	Osmolality in CD [mOsm/kg]	**Osmolality in controls [mOsm/kg]**	**Number of subjects (CD/Controls)**	**Reference**
	487 (87)	321 (254-464)	13/11	[26]
	463 (21)	350 (20)	20/16	[27]
Median	**475**	**336**		

2.3.3. 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 2.3) were used as reference and modifications were made to reflect the changes in the composition of luminal contents in patients with CD (Section 2.3.2). For the gastric medium in the fasted state, pH (*p*) and bile salt (*b*) concentration were included as factors in the DoE. 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 2.1):

- 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 2.3.2.2). For the bile salt concentrations in all CD media, the low level was set based on the percental approach described in Section 2.3.2.1 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 2.3), 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 2.3.2.3) 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* was followed for the preparation of gastric and intestinal biorelevant media.[14] Colonic biorelevant media were prepared according to Vertzoni *et al*.[15]

Table 2.3: 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
1 M 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		
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
Reference	[13]	[14]	[15, 16]	[14]	[15, 16]

	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
Bile salts/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 in healthy biorelevant media

Figure 2.1: Design of Experiment for the development of biorelevant media for CD patients.

2.3.4. 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.

2.3.4.1. Surface tension

A Du Noüy ring tensiometer (Sigma 700 Force tensiometer, Attension, UK) was used to measure the surface tension of biorelevant media. Therefore, a platinum ring is lifted from the surface of the medium and the required force for the raising of the ring is measured. The surface tension of the medium can be related to the measured force according to

$$F = w_{ring} + 2\pi * (r_i + r_a) * \gamma \quad (2.2)$$

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.[28]

2.3.4.2. 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).

2.3.4.3. 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.

2.3.4.4. 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 (2.3)

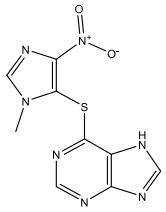
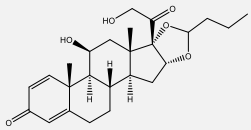
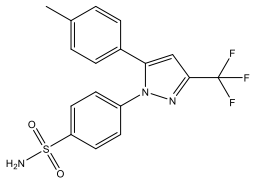
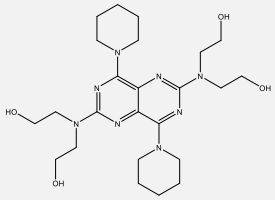
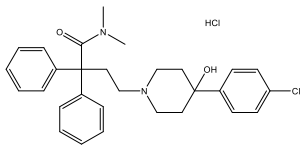
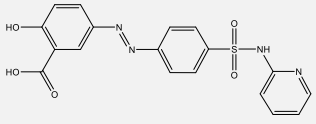
$$\beta = \left(\frac{M_{acid} * V_{acid}[ml]}{\Delta pH}\right) * \frac{1000}{V_{sample}[ml]} \quad (2.3)$$

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.[29]

2.3.5. 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. While drugs with an indication for GI diseases were preferred, the main selection criterion was to cover a range of different physicochemical properties. Therefore, the six drugs vary in their ionisation properties (pKa) and lipophilicity (log P) as shown in Table 2.4.

Table 2.4: Physicochemical characteristics and indication of selected compounds for solubility studies.

Drug	Molecular weight [g/mol]	pKa (acid/base)	log P	BCS class	Indication
Azathioprine 	277.3	7.9 (acid)[30]	0.1[31]	IV[32]	Immunosuppressive
Budesonide 	430.5	12.0 (acid)[33]	2.6[34]	II[35]	Locally acting corticosteroid in IBD
Celecoxib 	381.4	11.1 (acid)[36]	3.5[36]	II[37]	Nonsteroidal anti-inflammatory drug
Dipyridamole 	504.6	6.4 (base)[38]	2.2[39]	II[40]	Platelet aggregation inhibitor
Loperamide 	477.0	8.6 (base)[41]	5.5[42]	II[40]	Anti-diarrheal agent
Sulfasalazine 	398.4	2.3, 7.9 (acid) [43]	2.9[44]	II/IV[32]	Anti-inflammatory agent in IBD

2.3.6. Solubility studies

The solubility studies of the investigated drugs were performed using the shake-flask method.[45] 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 supernatant was filtered with GF/D membrane filters with a pore size of 2.7 μm (Whatman® Puradisc, diameter 13 mm) and analysed by HPLC-Ultraviolet (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 % 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, US): 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 2.5.

Table 2.5: HPLC-UV analytical methods used for the quantification of investigated drugs.

Drug	Column	Mobile phase	Flow rate [mL/min]	Temperature [°C]	Injection Volume [µL]	UV detection [nm]
Budesonide[46]	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[47]	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[48]	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[49]	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[50]	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

2.3.7. 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 healthy biorelevant media 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 their physicochemical properties. Hence, 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 n-octanol:water partition coefficient, log P, 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.[51] Interactions between media- 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.[52] 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.[53] 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. 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.[52]

2.4. Results and discussion

2.4.1. Media characterisation

Surface tension of CD and healthy biorelevant media is presented in Figure 2.2. 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). 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.[54] Considering the surface tension of fasted state colonic media, only for CD-FaSSCoF l*b*-h*o* the surface tension was with 8% significantly decreased 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 concentrations has previously been observed for fed state simulated intestinal fluids in a range of 1-7 mM, and could be related to the reduced concentration of surfactants being above the CMC.[54] 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 2.2. 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 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.[14, 55]

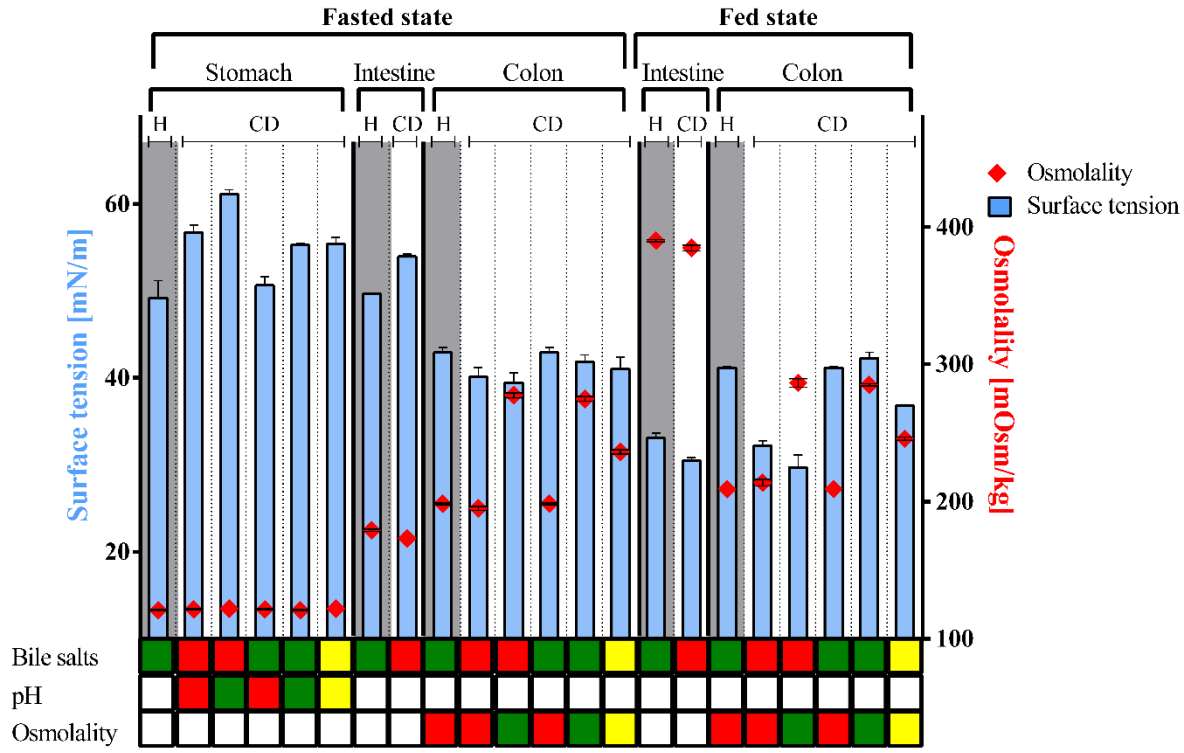


Figure 2.2: Surface tension (blue, left y-axis) and osmolality (red, right y-axis) of CD biorelevant media according to the Design of Experiments (green: high level, yellow: medium level, red: low level, white: healthy level) and healthy media (H).

The dynamic viscosity of CD biorelevant media at three different shear stresses is presented in Figure 2.3. 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 ($p<0.05$).

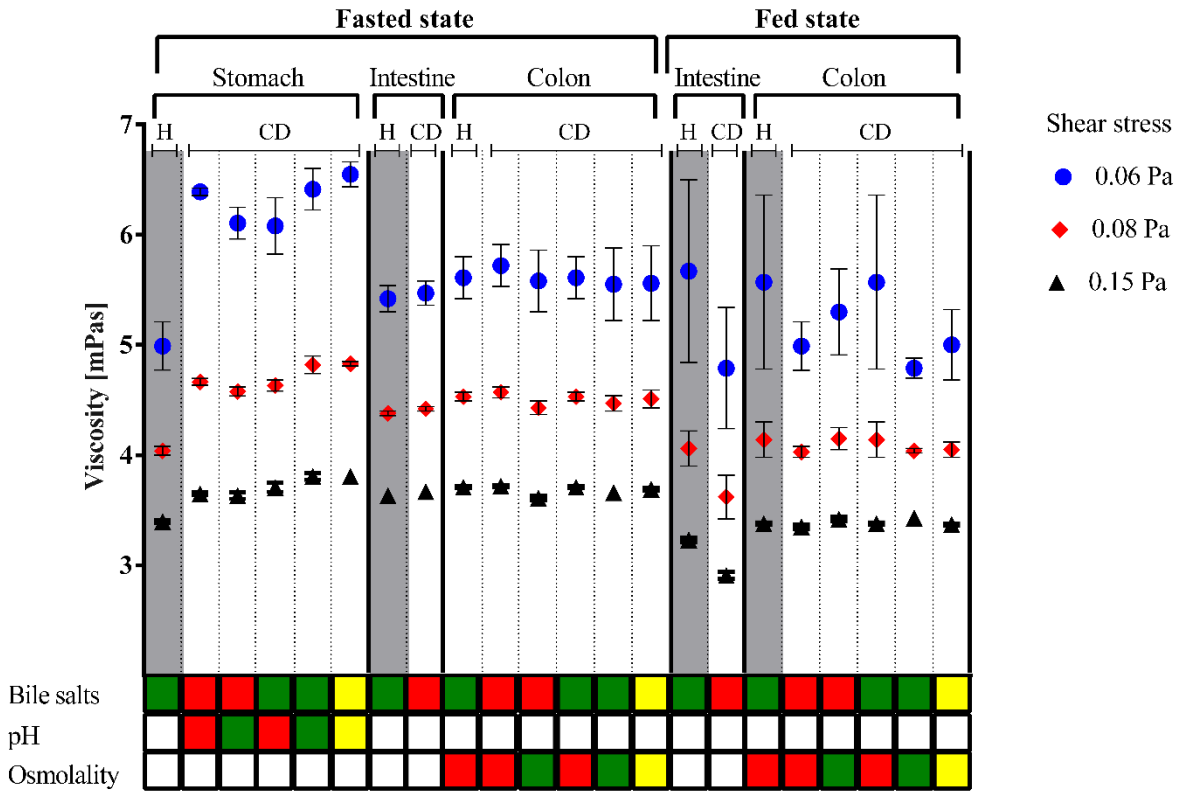


Figure 2.3: Dynamic viscosity of CD biorelevant media according to the Design of Experiments (green: high level, yellow: medium level, red: low level, white: healthy level) and the corresponding healthy biorelevant media (H) at different shear stress (0.06 Pa: blue, 0.08 Pa: red, 0.15 Pa: black).

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

2.4.2. Solubility of drugs in CD biorelevant media

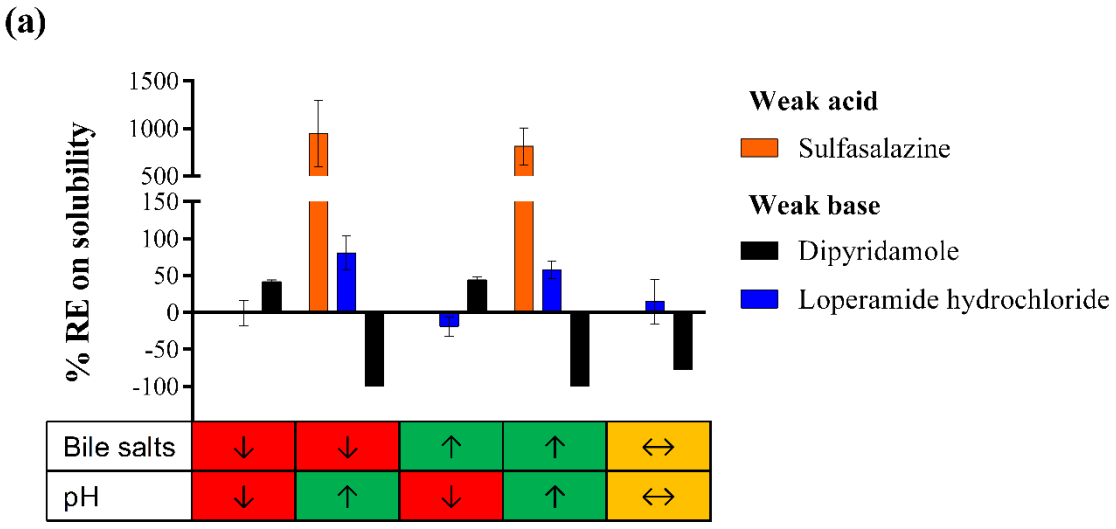
The solubility of six different drugs was investigated in CD and healthy biorelevant media simulating stomach, small intestine and colon in the fasted state and small intestine and colon in the fed state. Drug solubility of all investigated drugs in healthy biorelevant media is presented in Table 2.6.

Table 2.6: Drug solubility of investigated drugs in healthy biorelevant media.

	Solubility in healthy biorelevant media [µg/mL]				
Drug	**FaSSGF**	**FaSSIF-V2**	**FaSSCoF**	**FeSSIF-V2**	**FeSSCoF**
Azathioprine	242.9 (7.97)	242.53 (6.82)	316.27 (11.09)	254.33 (1.14)	252.82 (8.41)
Budesonide	17.83 (0.19)	22.72 (0.64)	18.43 (0.15)	43.75 (4.68)	17.48 (0.40)
Celecoxib	2.94 (0.05)	14.77 (0.44)	12.34 (0.95)	97.98 (0.81)	22.50 (0.88)
Dipyridamole	13.1 (4.4) x 10^3	11.91 (0.46)	7.10 (0.33)	80.02 (5.72)	18.91 (0.58)
Loperamide-HCl	266.74 (0.84)	204.69 (13.76)	29.31 (2.87)	241.13 (7.43)	231.19 (30.06)
Sulfasalazine	[a]	1.28 (0.03) x 10^3	7.34 (0.11) x 10^3	1.07 (0.02) x 10^3	561.71 (2.75)

[a]Measurement value of 1.17 ug/mL (>Limit of Detection, <Limit of Quantification) was only used as reference value for comparative purposes

In fasted state gastric media, differences in drug solubility between CD biorelevant media and healthy biorelevant media were observed (Figure 2.4). 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 an increasing fraction of the drug being ionised. 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 ($p<0.05$).



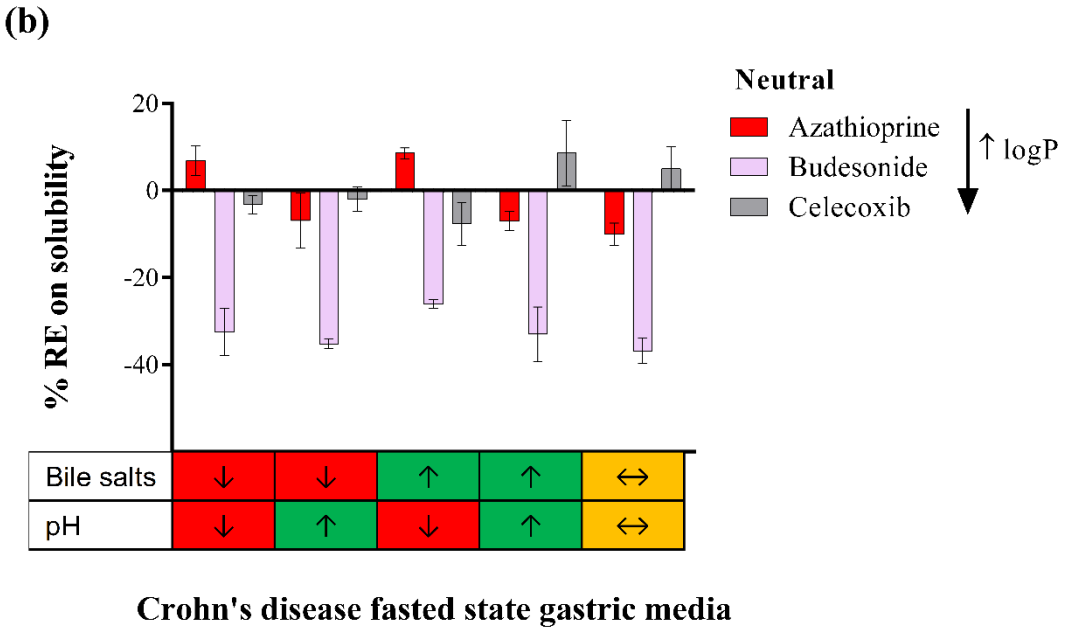


Figure 2.4: % Relative Effect (RE) on solubility of investigated drugs in CD gastric biorelevant media according to the Design of Experiments (green: high level, yellow: medium level, red: low level) in the fasted state compared to the corresponding healthy medium for ionisable drugs (a), and neutral drugs (b).

The % Relative Effect of CD on drug solubility in fasted and fed state intestinal media is shown in Figure 2.5. 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.[11] 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.[56]

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.

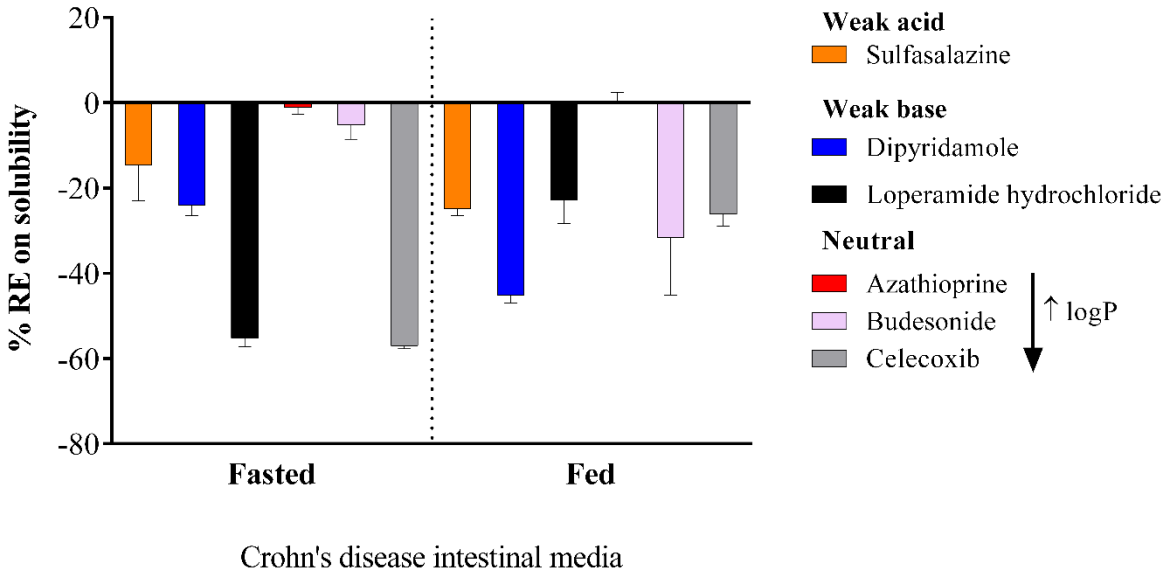


Figure 2.5: % Relative Effect (RE) on solubility of investigated drugs in CD intestinal biorelevant media in the fasted state and fed state compared to the corresponding healthy media.

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 2.6. In colonic media, the CD biorelevant medium with high bile salt concentration and low osmolality corresponds to FaSSCoF in the fasted state and FeSSCoF in the fed state, respectively. 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. Since the higher osmolality in the faecal fluid of CD patients was related to a higher amount of insoluble carbohydrates instead of a higher chloride concentration, it is questionable if the solubility of loperamide hydrochloride would also be decreased in the colonic luminal fluid of CD patients.

In fed state colonic media, the solubility of sulfasalazine was decreased in all CD media ($p<0.05$) suggesting a negative impact of decreased bile salt and lecithin concentrations 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 ($p<0.05$). For dipyridamole, the solubility was decreased in CD-FeSSCoF with low bile salt concentration and low osmolality ($p<0.05$). 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.

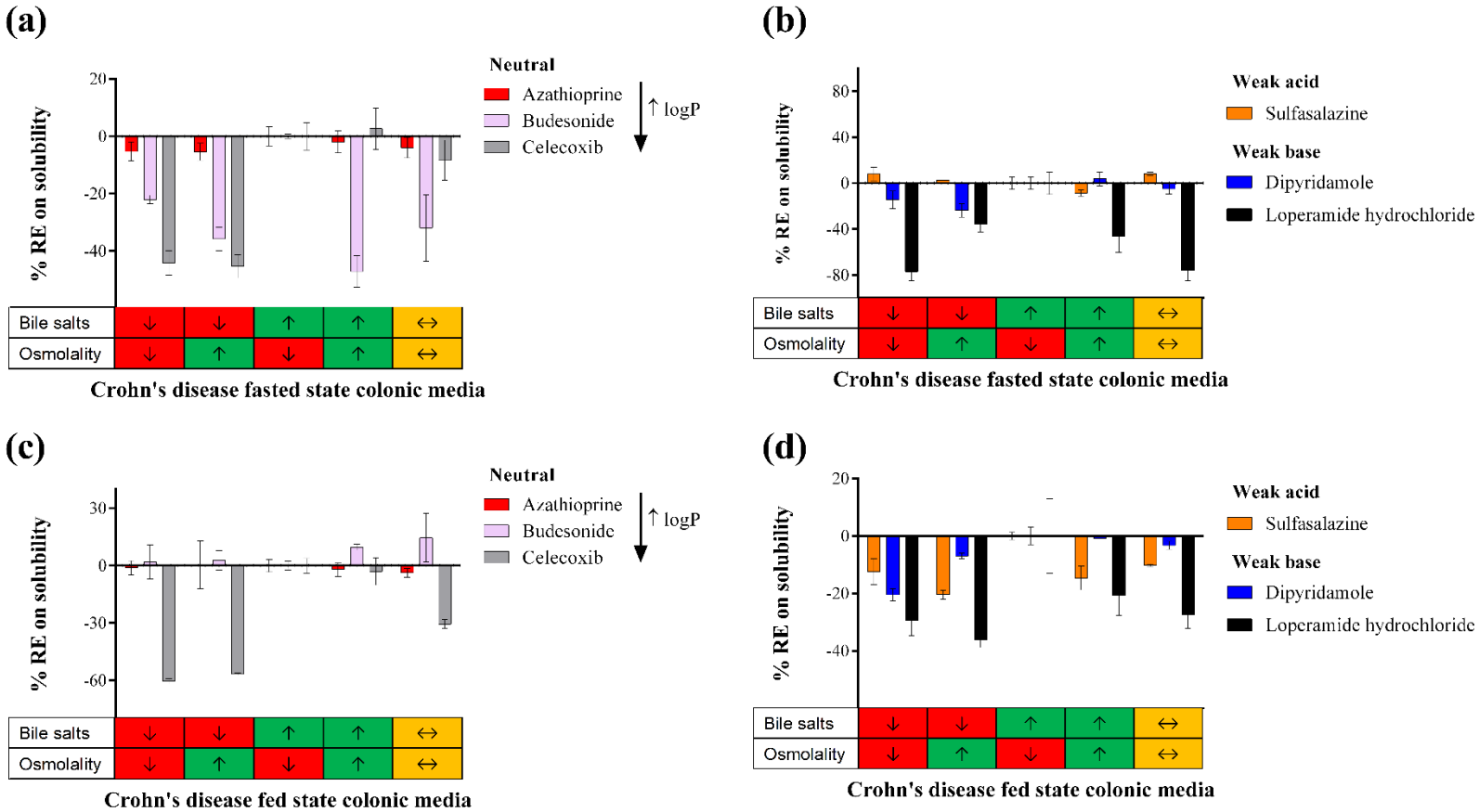


Figure 2.6: % Relative Effect (RE) on solubility of investigated drugs in CD colonic biorelevant media in the fasted state (top) and fed state (bottom) according to the Design of Experiments (green: high level, yellow: medium level, red: low level) compared to the corresponding healthy media for neutral drugs (a, c), and ionisable drugs (c, d).

2.4.3. Multivariate statistical analysis

The PLS models for the different GI compartments and prandial states are shown in Figure 2.7 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 patients with CD 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.

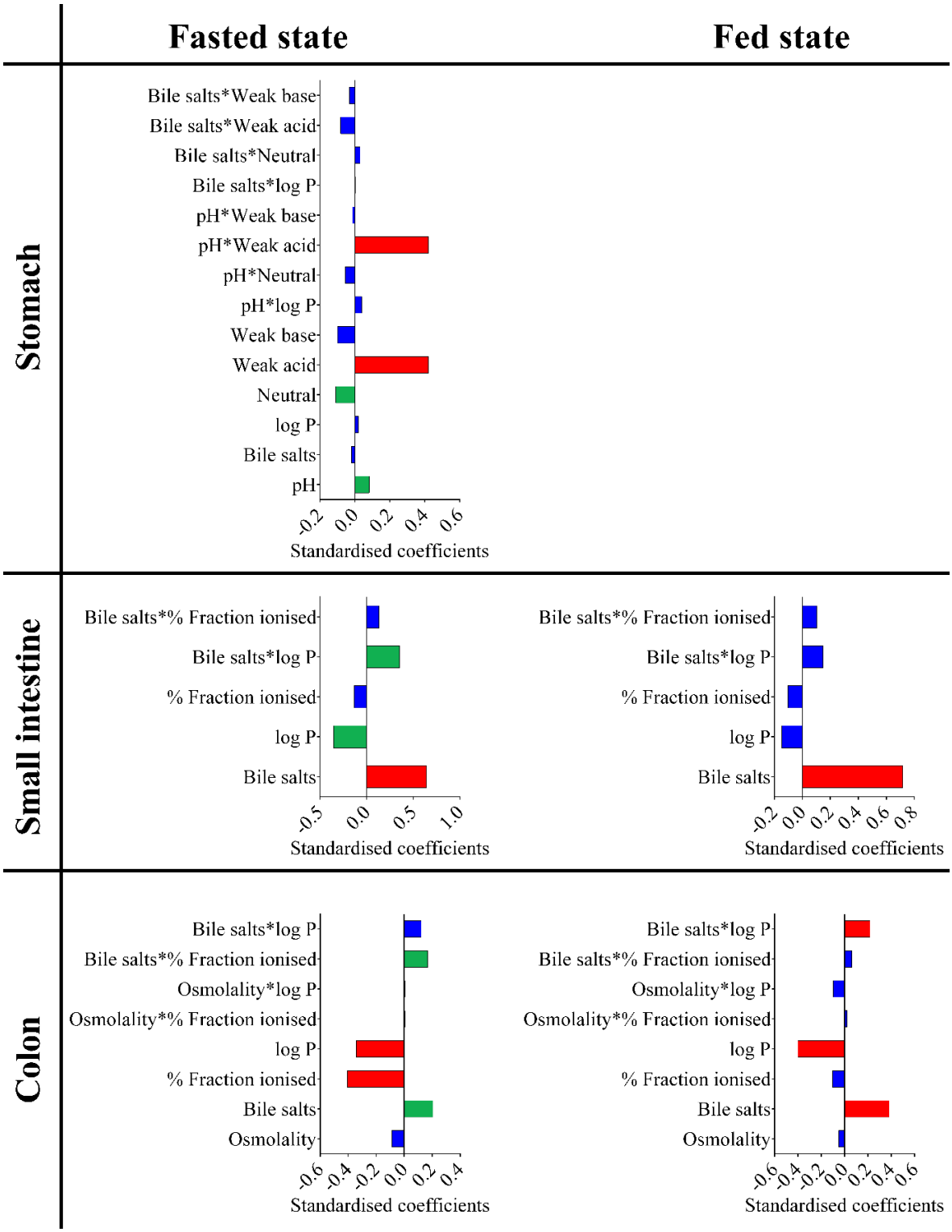


Figure 2.7: Standardised coefficients of factors and interactions in the PLS regression of drug solubility in CD simulated gastrointestinal fluids in the fasted state (left) and fed state (right) and different compartments of the GI tract (top: stomach, middle: small intestine, bottom: colon). Red colour denotes coefficients of VIP values > 1, green > 0.7 and blue < 0.7.

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

In simulated gastric fluids of CD patients compared to healthy biorelevant media, 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 CD and healthy intestinal biorelevant media.

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 2.4.3), 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. Further studies with a higher number of compounds would additionally increase the confidence in the risk assessment tools.

2.5. Conclusion

Based on current literature about pathophysiological changes in CD patients, simulated GI fluids of CD patients were developed for different GI compartments and prandial states. Differences in the properties of CD biorelevant media compared to healthy biorelevant media were mainly observed for surface tension and osmolality. Consequently, for example the common ion effect or differences in wetting behaviour could affect drug dissolution and drug release from pharmaceutical formulations in CD patients. Drug product performance, especially of drugs with solubility- or dissolution rate-limited absorption, may therefore be altered as a result of CD.

Differences of drug solubility in simulated gastric fluids of CD patients compared to healthy biorelevant media were related to differences in media pH and drug ionisation as observed for a weak acid and a weak base in gastric CD media with high pH. At high risk of altered

drug solubilisation in CD are drugs with moderate to high lipophilicity, since the log P showed a significant negative effect on drug solubility for colonic and fasted state intestinal CD media. A lower drug solubility for lipophilic drugs is further expected in CD GI fluids with low bile salt and lecithin concentrations as a significant positive effect of bile salts/lecithin on drug solubility was observed in CD intestinal and colonic media. Further investigation of the luminal fluid composition of CD patients would increase the confidence in simulated biorelevant media of this patient population.

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, the high cost associated with clinical trials limits their conduction in CD patients. Apart from drug dissolution, drug product performance can also be affected by differences in permeability, distribution, gut wall/hepatic metabolism and elimination. Therefore, pathophysiological differences considering all ADME processes need to be considered to identify all drugs at risk of altered drug product performance in patients with CD. Results from solubility and dissolution experiments with CD media can be integrated in PBPK models offering the opportunity to integrate ADME processes mechanistically and to consider the special physiology of patient populations in order to predict a drug's plasma concentration profile *in vivo*.

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Chapter 3

Gastrointestinal diseases and their impact on drug solubility. Part II. Ulcerative Colitis

Abstract

Objectives

For poorly soluble compounds, drug product performance in patients with Ulcerative Colitis (UC) compared to healthy subjects can be affected due to differences in drug solubility in GI fluids. To assess this risk *in vitro*, biorelevant media for different prandial states and GI compartments were developed representative of UC patients based on pathophysiological changes in UC and with a Design of Experiment approach. The UC media were characterised and drug properties, posing a high risk of altered drug solubility in UC, were identified by assessing the solubility of six drugs in UC media and relating the results to their properties.

Key findings

The characterisation of UC biorelevant media showed differences in terms of surface tension, buffer capacity and osmolality compared to healthy biorelevant media. A lower drug solubility in UC intestinal media was observed for compounds with a high lipophilicity. For weak bases and weak acids, drug solubility was altered in UC colonic fasted state simulated fluids compared to healthy media. Additionally, a higher solubility of neutral lipophilic drugs was observed in UC media with increased concentrations of soluble proteins. In UC colonic fed state simulated fluids, differences in drug solubility of ionisable compounds were observed compared to the healthy medium and a lower solubility of neutral lipophilic drugs was observed in UC media with low lecithin concentration.

Conclusions

The developed UC biorelevant media offer the possibility to identify the risk of altered drug solubilisation in UC patients without conducting expensive clinical trials. A high risk was related to drug ionisation properties and lipophilicity in the current study with all investigated drugs showing differences in solubility between healthy and UC media. Therefore, drug product performance of drugs with dissolution rate- or solubility-limited absorption may be altered in patients with UC.

3.1. Introduction

Ulcerative Colitis (UC), a main type of inflammatory bowel disease (IBD), is an autoinflammatory disorder that affects approximately 2.1 million people in Europe.[1] The inflammation manifests itself in ulcerations of the lining of the large intestine which are confined to the mucosa and submucosa. Typically, the first appearance of the disease is limited to the rectum and further disease progression leads to a proximal extension to the colon. According to the disease location, the Montreal classification system groups UC in Ulcerative proctitis (rectum is affected), left-sided UC (a proportion of the colorectum distal to the splenic flexure is affected) or extensive colitis (entire large intestine is affected).[2] UC can also be grouped in four different disease states according to symptom severity: mild, moderate, severe or a state of clinical remission.[2]

The different states and locations of UC necessitate different treatment options and drug formulation approaches. The classic step-up approach includes aminosalicylates as first treatment option in mild to moderate UC.[3] For this treatment, different drug formulations can be used based on disease location with suppositories and enemas for distal UC and/or controlled-release or prodrug formulations of mesalamine, when more proximal parts of the colon are affected. Corticosteroids are used to induce remission in moderate to severe disease states. Drug formulations include immediate-release formulations of systemic corticosteroids or controlled-release formulations of the topical steroid budesonide (e.g., Uceris® [Santarus, San Diego, CA, USA]). For active UC, the next therapeutic option is a co-treatment with thiopurines such as azathioprine due to their slow onset of therapeutic action. Further treatment options are calcineurin inhibitors for severely active UC or monoclonal antibodies as last therapeutic option.

Consequently, drug delivery via the oral route is commonly used in UC for locally-acting as well as systemic drug therapy. In addition, the use of several other drug classes, which are most often administered orally (e.g., antidepressants, antibiotics and nonsteroidal anti-inflammatory analgesics), was increased in IBD patients compared to the general population.[4] Successful drug delivery via the oral route is dependent on gastrointestinal (GI) physiology and drug/formulation properties. Various processes such as drug release/dissolution, permeation through the GI membrane and gut or hepatic metabolism can be influenced by an altered GI physiology in UC.[5] Since clinical trials to assess drug product performance in UC patients are rarely performed due to high costs, a heterogenous patient population and a high time effort, possible effects on the drug therapy of UC patients are not

investigated in most cases. Therefore, alternative tools to predict drug product performance in UC patients are needed.

In vitro release and dissolution testing can be used as surrogate for the *in vivo* performance of poorly soluble compounds with solubility- or dissolution rate-limited absorption.[6] For this purpose, biorelevant media have been developed based on healthy subjects to simulate GI fluids of different GI compartments and prandial states and to evaluate drug products *in vitro*.[7-11] Since UC can alter the GI fluid composition of patients, drug product performance could be affected for these drugs. The development of biorelevant media for UC patients allows to identify if differences in drug solubility or dissolution exist compared to the healthy biorelevant medium which would indicate a high risk of altered drug product performance in UC patients.

This study aims to develop a risk assessment tool to identify compounds with a high risk of altered solubility in the GI fluids of UC patients. Therefore, pathophysiological changes impacting on the composition of GI fluids in UC patients are considered and UC biorelevant media representative of the stomach, intestine and colon were developed based on healthy biorelevant media and published data using a Design of Experiment (DoE) approach. Subsequently, the developed UC biorelevant media were characterised according to their surface tension, osmolality, buffer capacity and dynamic viscosity and the solubility of six poorly soluble compounds with different physicochemical properties was determined in UC biorelevant media. To identify if certain drug characteristics contribute to a higher risk of altered drug solubility in GI fluids of UC patients, Partial least Squares (PLS) regression was used to correlate drug properties and media-dependent factors with the Relative Effect on drug solubility.

3.2. Materials

Acetic acid High Performance Liquid Chromatography (HPLC) grade, 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, cholic acid sodium salt and acetonitrile HPLC grade were purchased from VWR International Ltd, Lutterworth, UK. Tris(hydroxymethyl)aminomethane, hydrochloric acid 36.5–38%, sodium chloride, trifluoroacetic acid (TFA), potassium dihydrogen orthophosphate, bovine serum albumin (BSA) protease free powder fraction V, dimethyl sulfoxide 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.

3.3. Methods

3.3.1. Media development

3.3.1.1. GI pathophysiological changes in UC patients integrated in the experimental design

Information from literature was collected to identify differences in the composition of GI fluids of UC patients compared to healthy subjects. For studies with graphically displayed data, the relevant information was extracted with WebPlotDigitizer (Ankit Rohatgi, USA).[12] Apart from components and properties directly measured in the GI fluids of UC patients, an additional factor, namely the lecithin levels measured in the GI mucosa, was considered as indirect factor due to the limited number of studies performed in UC patients. All factors were integrated with two levels in the experimental design. The low and the high level were selected based on the available information on the respective parameter as described in Sections 3.3.1.1.1 and 3.3.1.1.2.

3.3.1.1.1. Indirect factor

The lecithin concentration was included as indirect factor in the experimental design of gastric, intestinal and fed state colonic UC media.

Lecithin is a constituent of the GI mucosa and essential to maintain the normal mucus barrier function. It has been shown that the lecithin concentration in the intestinal mucus barrier of patients with UC was decreased by over 70% compared to healthy subjects.[13, 14] The lecithin in the colonic mucus barrier is likely to origin from secretions by jejunal and ileal enterocytes as investigated in rat intestinal perfusion studies.[15] Therefore, decreased lecithin concentrations are likely to be present also in more proximal parts of the GI tract than the colon. The treatment of UC patients with a delayed-release oral formulation of lecithin has shown to increase the amount of lecithin in rectal mucus and reduce inflammatory activity.[16]

Lecithin is also an essential constituent of bile and can emulsify hydrophobic molecules due to its amphiphilic structure. Hepatobiliary manifestations are common in UC patients and include primary sclerosing cholangitis (PSC), small duct PSC, chronic hepatitis, cryptogenic cirrhosis, cholangiocarcinoma and cholelithiasis.[17] The most common of these conditions is

PSC with an incidence of 2.5 to 7.5% in patients with UC.[17] PSC leads to the formation of bile duct strictures impeding the flow of bile to the intestine. Consequently, reduced bile salt and lecithin concentrations are likely to be present in the GI fluids of the affected patients with UC. Reduced concentrations of bile acids and lecithin were already observed in intrahepatic bile specimens of patients with PSC.[18] Additionally, decreased lecithin concentrations in UC patients compared to healthy subjects were also observed in gallbladder bile in the fasted state obtained by cholecystokinin-stimulated, duodenal biliary drainage.[19]

Apart from the ascending colon fluid, no studies investigated the concentration of lecithin in the remaining luminal fluids of UC patients. Therefore, the lecithin levels for the DoE were based on an indirect percental approach in all media except UC-Fasted-State Simulated Colonic Fluid (FaSSCoF) according to

$$x_{CD-BM} = 0.30 * x_{H-BM} \quad (3.1)$$

where x_{CD-BM} is the low level of the lecithin concentration in CD media, x_{H-BM} is the lecithin concentration in healthy biorelevant media and the factor 0.30 represents the ratio of lecithin previously observed in the colonic mucus layer of CD patients compared to healthy subjects.[13, 14] Hence, the low lecithin level in UC biorelevant media is set to 30% of the concentration in corresponding healthy biorelevant media and the high lecithin level corresponds to the concentration in healthy biorelevant media.

3.3.1.1.2. Direct factors

3.3.1.1.2.1. Fasted state ascending colon fluid

The fasted state ascending colon fluid of UC patients in states of relapse and remission as defined based on the Clinical Rachmilewitz Index (CRI) has previously been characterised.[20]

A higher osmolality was observed in patients with UC in remission (290.1±165.6 mOsmol/kg) compared to patients in relapse (199.6±127.4 mOsmol/kg) and healthy subjects (80.6±102.5 mOsmol/kg).[20, 21] For the experimental design, the osmolality was integrated with a low level of 196.0 mOsmol/kg, corresponding to the osmolality of FaSSCoF and similar to the osmolality observed in UC patients in relapse, and a high level of 290.0 mOsmol/kg representative of UC patients in remission.

The mean total bile acid concentration was lower in UC patients in relapse (75.83±42.96 µM) compared to patients in remission (115.15±100.20 µM) and healthy

subjects (115.20±119.30 µM), but the difference reached no statistical significance as the power of the test was very low.[20, 21] For the experimental design, the bile salt concentration was integrated with a low level of 75.00 µM representative of UC patients in relapse and a high level of 150.00 µM (bile salt concentration of FaSSCoF, similar bile salt concentration in healthy and UC patients in remission).

The concentration of soluble proteins was not significantly different between patients in relapse (18.9±8.1 mg/mL) and remission (19.0±10.8 mg/mL), but significantly higher compared to healthy subjects (9.8±4.6 mg/mL).[20, 21] For the experimental design, the concentration of soluble proteins was integrated using BSA with a high level of 19.0 mg/mL representative of UC patients in relapse and remission and a low level of 0.0 mg/mL based on the concentration in FaSSCoF.

The lecithin concentrations in the fasted state ascending colon fluid in UC patients in remission and relapse were in the range of 0.13 to 0.62 mM (graphically extracted).[20] While the mean concentration of lecithin in the fasted state ascending colon fluid of UC patients was lower compared to healthy subjects, the difference did not reach statistical significance due to high data variability. For the experimental design, the lecithin concentration was included as factor with the observed range as low and high level.

The pH in the fasted state colonic fluid of UC patients in remission and relapse was in the range of 6.1 to 7.3 with a median of 6.5 and in the range of 5.5 to 7.7 with a median of 6.6, respectively.[20] For the experimental design, the pH was included as factor with a low level of 5.5 and a high level of 7.7 representative of the pH range observed in UC patients.

The buffer capacity of the fasted state ascending colon fluid was higher in UC patients in relapse (32.0±18.1 mmol/L/ΔpH) and remission (37.7±15.4 mmol/L/ΔpH) compared to healthy subjects (21.4±7.9 mmol/L/ΔpH) as measured with hydrochloric acid. Due to the high number of factors integrated in the experimental design for the fasted state colonic UC media, the buffer capacity was not included.

3.3.1.1.2.2. Fed state colon fluid

Several studies investigated the pH in the colon of UC patients in the fed state.[22-26] Very low pH values (pH 2.3-3.4), observed in one study were excluded due to analytical uncertainties (e.g., no confirmatory pH measurements, possibly artificial low pH values when a certain distance to antenna was exceeded).[24, 27] The highest colonic pH value observed in UC patients in the fed state was 7.8 and the lowest was 4.7.[24, 26] Therefore, the pH in the fed state

colonic medium was included as factor in the experimental design with a low level of 4.7 and a high level of 7.8 representative of the pH range observed in UC patients.

3.3.1.2. Development of UC media with DoE

A DoE approach was followed to develop the UC biorelevant media with the aim to assess the impact of each of the factors and to reflect the interindividual variability in UC patients. The development of UC biorelevant media was based on observed differences in UC patients compared to healthy subjects identified in literature (Section 3.3.1.1) and previously developed biorelevant media for healthy subjects including Fasted-State Simulated Gastric Fluid (FaSSGF), Fasted-State Simulated Intestinal Fluid-Version 2 (FaSSIF-V2), FaSSCoF, Fed-State Simulated Intestinal Fluid-Version 2 (FeSSIF-V2) and Fed-State Simulated Colonic Fluid (FeSSCoF).[8-11]

The DoE was performed using XLSTAT (Addinsoft, France) with a full factorial design in UC patients for stomach and intestine in the fasted state and intestine and colon in the fed state. For fasted state gastric and fasted and fed state intestinal media, the lecithin concentration was included as factor in the experimental design. Additionally, the ratio of bile salts to lecithin was integrated as factor and set to the ratio in the corresponding healthy biorelevant media for the low level. This approach was used to keep a similar composition of the mixed micelles to healthy biorelevant media in some UC media. For fed state colonic UC media, the pH and the concentration of lecithin and bile salts were the investigated factors.

For fasted state colonic UC media, the factors investigated were bile salts, lecithin, pH, osmolality and soluble proteins. Due to the high number of factors, a fractional factorial design (2^(5-2)) was used for the UC fasted state colonic media using Dataplot (NIST, US).[28] Therefore, the main effects are confounded with the two-factor interaction in the case of UC-FaSSCoF media, while permitting the exploration of the effects of many factors with a minimum number of media. The factor soluble proteins was represented in UC-FaSSCoF media by BSA.

Each factor changed in UC compared to healthy subjects was integrated in the DoE with two levels (low and high). Additionally, centre points with medium levels of each parameter were included for gastric and intestinal media. An overview of the factors and levels of the DoE is given in Figure 3.1. For UC-FaSSCoF media with osmolality as factor in the DoE, sodium chloride was added to adjust the osmolality. For all other media, the osmolality was

set to the value in corresponding healthy biorelevant media by adjusting the concentration of sodium chloride.

Biorelevant media were prepared according to the method described in Jantratid *et al* for gastric and intestinal media and Vertzoni *et al* for colonic media.[9, 10]

	Ulcerative colitis											
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]	0.024	0.08	0.90	3.0	0.075	0.15			3.0	10	0.18	0.6
Lecithin [mM]	0.006	0.02	0.06	0.2	0.130	0.62			0.6	2	0.15	0.5
Bile salts/Lecithin	4:1	40:3	15:1	50:1	15:62	15:13			5:1	50:3	6:5	4:1
pH					5.5	7.7					4.7	7.8
Osmolality [mOsm/kg]					196	290						
Soluble proteins [mg/ml]					0	19						

	no changes
	decrease
	increase
	Value in healthy biorelevant media

Figure 3.1: Design of Experiments for the development of Ulcerative Colitis media.

3.3.2. Media characterisation

Surface tension, osmolality, dynamic viscosity and buffer capacity of biorelevant media previously developed based on healthy subjects and newly developed for UC patients were measured. All measurements were performed in triplicate. The results were reported as mean with standard deviation.

3.3.2.1. Surface tension

Surface tension measurements were performed with a ring tensiometer (Sigma 700 Force tensiometer, Attension, UK) and a glass vessel (diameter of 46 mm) filled with 10 mL of each medium. The force to pull a Du Noüy ring from the surface of the medium was measured and related to the medium's surface tension.[29]

3.3.2.2. Osmolality

Osmolality 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.

3.3.2.3. Dynamic viscosity

A Bohlin Rheometer C-VOR (Malvern instruments, UK) with a cone-plate system (4°, 40 mm) was used to determine the dynamic viscosity of the media at a temperature of 37ºC. A small amount of sample was placed between the plate and the cone, sheared with different shear stresses (20 points, logarithmically distributed between 0.05 and 0.15 Pa) and the shear rate was measured. Dynamic viscosity corresponds to the ratio of shear stress to shear rate.

3.3.2.4. Buffer capacity

To determine the buffer capacity of the media, small volumes of 0.5 M hydrochloric acid were added to 10 mL of medium until a change of one pH unit was measured with a Mettler Toledo SevenCompact S220 pH meter (Schwerzenbach, Switzerland). Subsequently, equation (3.2) was used to calculate the buffer capacity according to

$$\beta = \left(\frac{M_{acid} * V_{acid}[ml]}{\Delta pH}\right) * \frac{1000}{V_{sample}[ml]} \quad (3.2)$$

where β is the buffer capacity, M_{acid} is the molarity, ΔpH is the change in pH, V_{acid} and V_{sample} are the volume of the acid added and the volume of the sample, respectively.[30]

3.3.3. Compound selection

For the solubility studies, poorly soluble compounds belonging to class II (low solubility, high permeability) or IV (low solubility, low permeability) of the Biopharmaceutics Classification System (BCS) were selected as presented in Figure 3.2.[31-45] Drug selection was based on different physicochemical characteristics of the drugs such as ionisation properties (pKa) and lipophilicity (log P) and drugs with an indication for GI diseases were preferred.

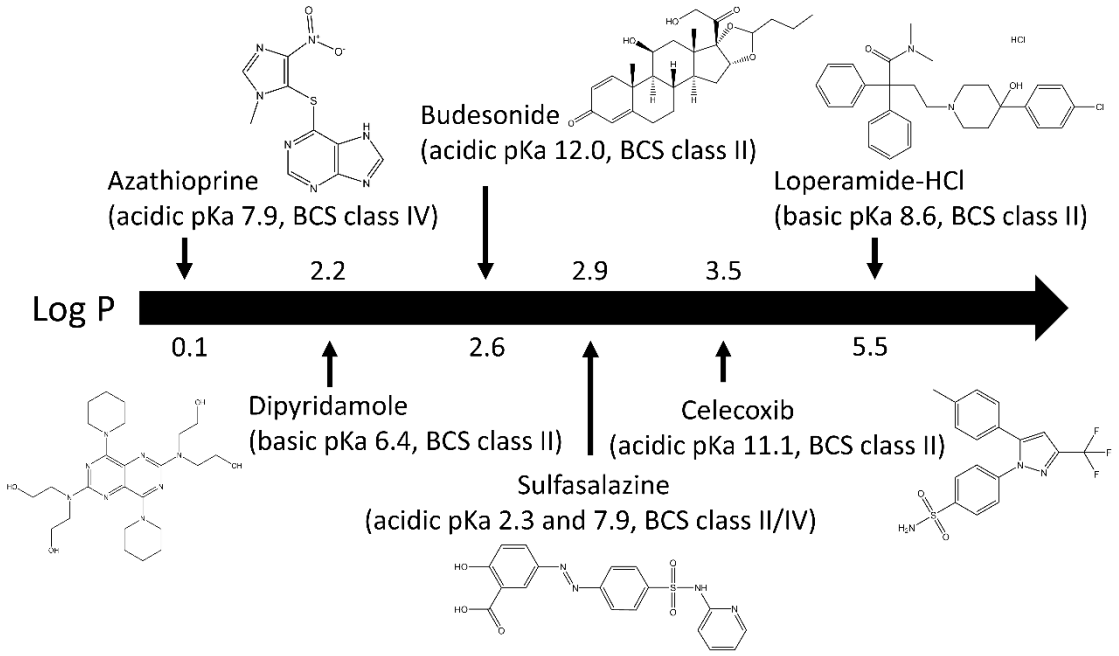

Figure 3.2: Physicochemical characteristics and indication of investigated drugs.[31-45]

3.3.4. Solubility studies

The shake-flask method was used to determine the solubility of the six selected drugs.[46] Therefore, 5 mL of medium were added to an excess amount of drug in a glass tube and placed in a shaking water bath (Grant instruments, Royston, UK) at 37°C with 200 strokes/min. After 24 h, the supernatant was filtered with GF/D membrane filters with a pore size of 2.7 µm (Whatman® Puradisc, diameter 13 mm) and analysed by HPLC-Ultraviolet (UV). HPLC analysis was performed with an Agilent Technologies 1200 series HPLC system (Santa Clara, CA, US) with a binary pump, autosampler, thermostatted column compartment and diode array detector. The details of the HPLC-UV methods used

for the quantitative analysis of the six compounds are presented in Gastrointestinal diseases and their impact on drug solubility. Part I. Crohn's disease (Chapter 2).

For biorelevant media including BSA, an additional treatment step for protein precipitation was added after sample filtration. 1 mL of protein precipitation reagent was added to 500 µL of sample, the mixture was vortexed for 30 s and centrifuged for 10 min at 12000 rpm and 4°C (Eppendorf Heraeus Fresco 17 centrifuge, ThermoElectron LED GmbH, Germany). The protein precipitation reagent was methanol for all drugs except sulfasalazine, for which dimethyl sulfoxide was used due to the poor solubility of sulfasalazine in methanol. For the sulfasalazine samples with dimethyl sulfoxide, the ratio of the mobile phase used for the HPLC-UV analysis was modified to 60:40 MeOH: Acetic acid 3.3% in H_2O. Solubility studies were performed in triplicate in UC media and healthy media. Average solubility differences between UC media and healthy media were expressed as a % Relative Effect on solubility [$((S_{UC}-S_{Healthy})/ S_{Healthy}) \times 100$]. Positive values indicate that drug solubility in UC media exceeds the solubility in healthy media, whereas negative values indicate the opposite.

3.3.5. Statistical analysis

All statistical analysis was performed using XLSTAT (Addinsoft, France).

To identify statistically significant differences of media properties and drug solubility between UC biorelevant media and the corresponding healthy media, one-way analysis of variance (ANOVA) with a post-hoc Tukey's test was applied with a significance level of $p \leq 0.05$.

Multivariate statistical analysis was used to identify drug properties that present a high risk of altered drug solubility in UC. Therefore, the % Relative Effect on drug solubility was correlated with media-dependent factors of the DoE and drug physicochemical properties by Partial Least Squares (PLS) regression. Media-dependent factors were for gastric and intestinal UC media the bile salt and lecithin concentration. For fasted state colonic UC media, the media-dependent factors were osmolality, pH and the concentrations of bile salts, lecithin and soluble proteins. For fed state colonic UC media, media-dependent factors were pH and the concentration of bile salts and lecithin. In terms of drug-dependent parameters, the partition coefficient, log P, was included for all UC media. For media with pH as media-dependent factor (colonic UC media), a categorical variable discriminating between weak acids, weak bases and neutral compounds was introduced. For the gastric and intestinal UC media, 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 used as additional drug-dependent factor.[47] Interactions between media-dependent and drug-dependent factors were included in the model as shown in Table 3.1.

The quality assessment of the PLS models was based on the square of coefficient of determination (r^2) and goodness of prediction (q^2), both indicating a good fit of the data and a good predictive ability of the model, respectively, when close to 1. A difference higher than 0.3 between r^2 and q^2 indicates model over-fitting and consequently an inappropriate model.[48] Models were selected for optimum model predictive ability based on the lowest predicted residual error sum of squares (PRESS) and the highest q^2. Usually good model predictability is given when q^2 is higher than 0.5, in certain cases, however, lower limits can be accepted since q^2 is dependent on the properties of the data set (e.g., number of observations).[49] In our models, a high influence on the % Relative Effect on solubility is indicated for the media- and drug-dependent factors with high absolute value of the standardised coefficients. If the standardised coefficient is positive, this indicates a positive impact on the % Relative Effect on solubility, while a negative standardised coefficient indicates the opposite. The Variable Importance in Projection (VIP) of a factor summarizes the influence of each individual independent factor on the PLS model. In the current study, we considered factors with VIP ≥ 0.7 as influential to the model and factors with VIP >1 as most influential.[48, 50]

Table 3.1: Overview of predictive factors in the PLS model of the different UC biorelevant media.

Medium	Media-dependent factors	Drug-dependent factors	Interactions
UC-FaSSGF **UC-FaSSIF** **UC-FeSSIF**	Bile salts Lecithin	Log P % Fraction ionised	Bile salts*log P Bile salts*% Fraction ionised Lecithin*log P Lecithin*%Fraction ionised
UC-FaSSCoF	Bile salts Lecithin Osmolality pH Soluble proteins	Categorical variable (weak acid, weak base, neutral) log P	Bile salts*weak acid/weak base/neutral Bile salts*log P Lecithin*weak acid/weak base/neutral Lecithin*log P Osmolality*weak acid/weak base/neutral Osmolality*log P pH*weak acid/weak base/neutral pH*Log P Soluble proteins*weak acid/weak base/neutral Soluble proteins*log P
UC-FeSSCoF	Bile salts Lecithin pH	Categorical variable (weak acid, weak base, neutral) Log P	Bile salts*weak acid/weak base/neutral Bile salts*log P Lecithin*weak acid/weak base/neutral Lecithin*log P pH*weak acid/weak base/neutral pH*log P

3.4. Results and discussion

3.4.1. Media characterisation

An overview of the surface tension and osmolality of healthy and UC biorelevant media is given in Figure 3.3.

Surface tension in fasted state gastric media was significantly higher (+24%, $p<0.05$) in the UC medium with low lecithin and low bile salt concentrations compared to the healthy medium, possibly due to the low surfactant concentration being below the critical micellar concentration. In fasted state intestinal media, a significantly higher surface tension compared to the healthy medium was observed for both UC media with low lecithin concentrations (+4%, +15%, $p<0.05$). In fasted state simulated colonic media, the surface tension in three UC media with low pH (low lecithin/low bile salt/low osmolality/high soluble proteins -11%, low lecithin/high bile salt/high osmolality/low soluble proteins -26% and high lecithin/low bile salt/high osmolality/low soluble proteins -31%, $p<0.05$) was significantly lower compared to the healthy medium and the surface tension of one UC medium (low lecithin/low bile salt/high pH/high osmolality/high soluble proteins) was increased by 7% ($p<0.05$). The surface tension of UC-FaSSCoF media was in the range of 29.3 mN/m to 46.0 mN/m, which is in accordance with the surface tension observed in the ascending colon fluid of UC patients in relapse (41.6±3.1 mN/m) and in remission (40.6±3.4 mN/m).[20] In the fed state, the surface tension of intestinal UC media was significantly decreased compared to the healthy medium (-7 to -12%, $p<0.05$). The surface tension of FeSSCoF was significantly higher compared to six of the UC media including the media with low pH and media with high pH/low lecithin concentrations ($p<0.05$).

Osmolality in UC biorelevant media was only different according to the specified levels for fasted state colonic media when osmolality was included as factor in the experimental design (Figure 3.3).

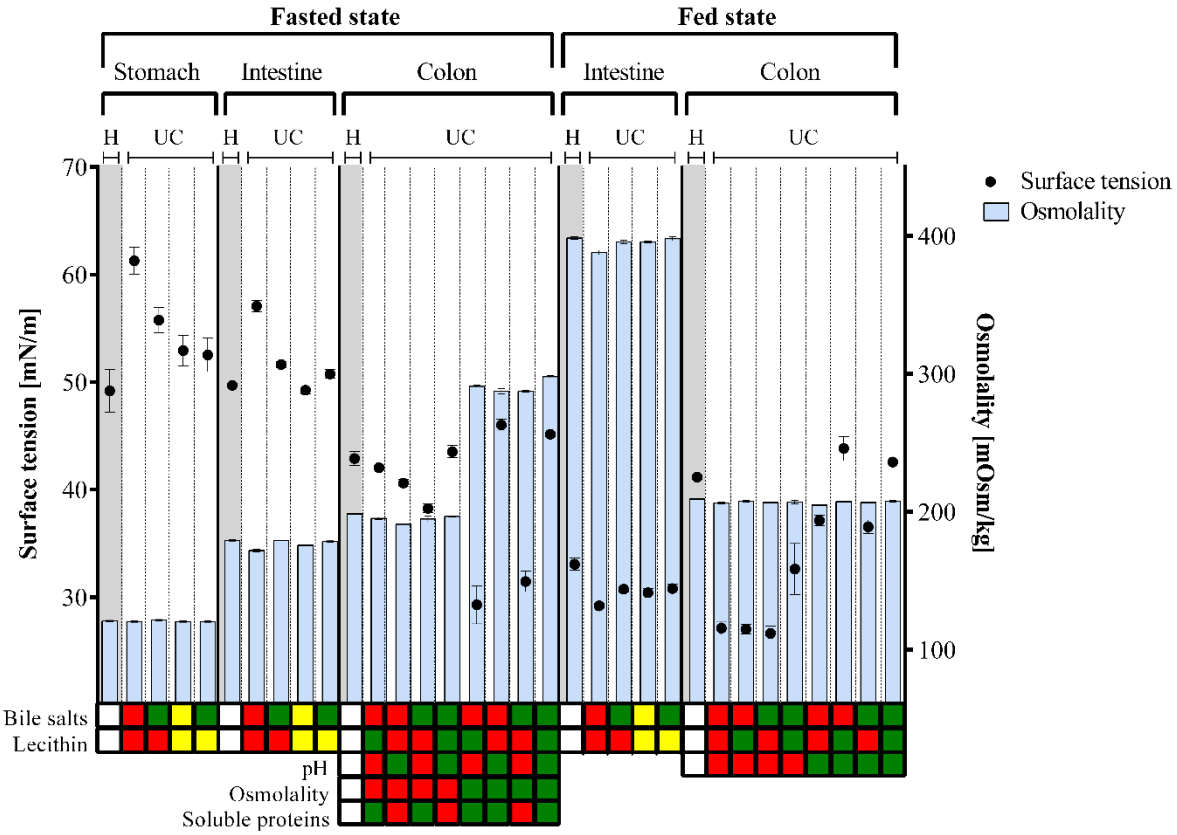

Figure 3.3: Surface tension (blue, left y-axis) and osmolality (black, right y-axis) of UC biorelevant media according to the Design of Experiments (green: high level, yellow: medium level, red: low level, white: healthy level) and healthy media.

The dynamic viscosity of the investigated biorelevant media is presented at three different shear stresses in Figure 3.4. All healthy and UC media showed pseudoplastic behaviour. The viscosity at an applied shear stress of 0.15 Pa was in the range of 3.23 to 3.50 mPas, at 0.08 Pa in the range of 3.74 to 4.28 mPas and at 0.06 Pa in the range of 4.59 to 5.99 mPas, respectively. Significant differences between UC and healthy biorelevant media for all three different shear stresses were not observed ($p<0.05$).

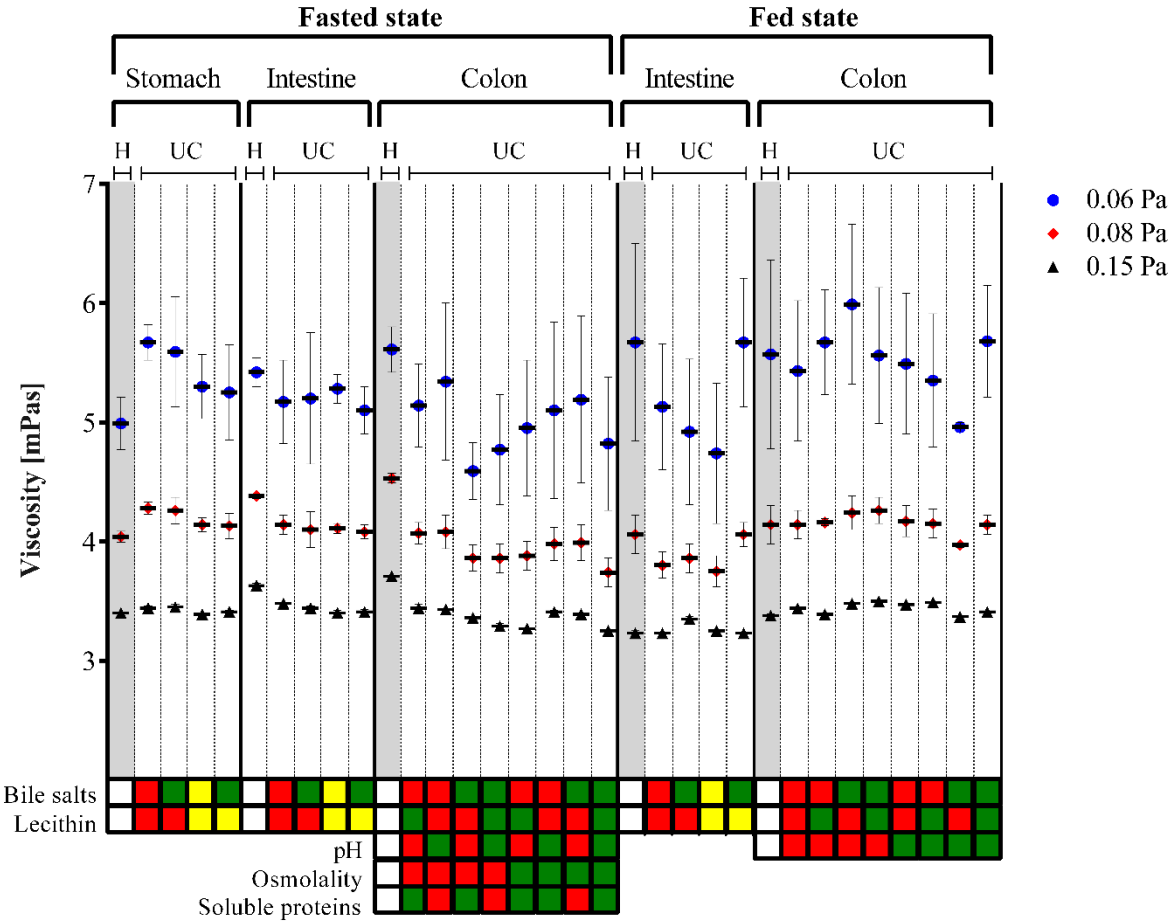

Figure 3.4: Dynamic viscosity of UC biorelevant media and the corresponding healthy biorelevant media at different shear stress (0.06 Pa: blue, 0.08 Pa: red, 0.15 Pa: black) according to the Design of Experiments (green: high level, yellow: medium level, red: low level, white: healthy level).

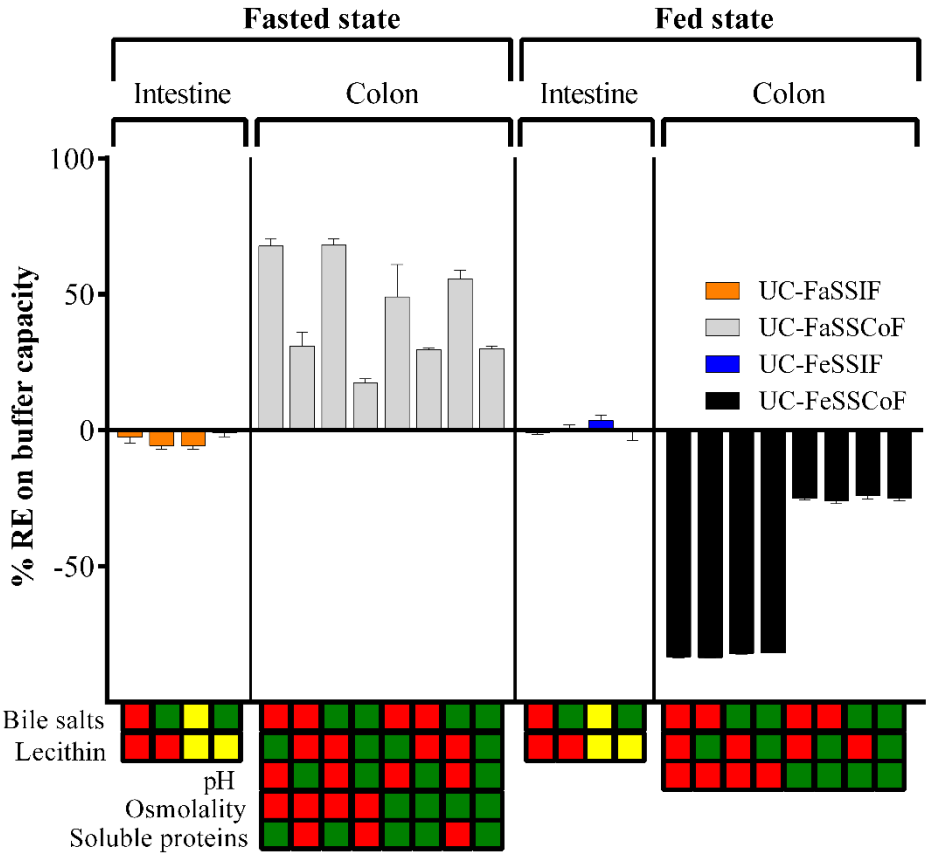

Figure 3.5: % Relative Effect on buffer capacity in UC biorelevant media compared to the corresponding healthy biorelevant media according to the Design of Experiments (green: high level, yellow: medium level, red: low level).

The buffer capacity was not significantly different in healthy fasted and fed state intestinal media compared to UC media (Figure 3.5). In fasted state colonic media, the healthy medium had a significantly lower buffer capacity compared to all UC media ($p<0.05$) and the increase was more pronounced for UC-FaSSCoF media with low pH compared to UC-FaSSCoF media with high pH. In contrast, in the fed state colonic media the buffer capacity was significantly lower in the UC media ($p<0.05$), whereby the decrease was more pronounced for UC-FeSSCoF media with low pH compared to UC-FeSSCoF media with high pH.

3.4.2. Solubility of drugs in UC biorelevant media

The % Relative Effect of UC on the solubility of six different drugs, as investigated with UC biorelevant media and healthy biorelevant media simulating stomach, small intestine and colon in the fasted state and small intestine and colon in the fed state, is shown in Figure 3.6.

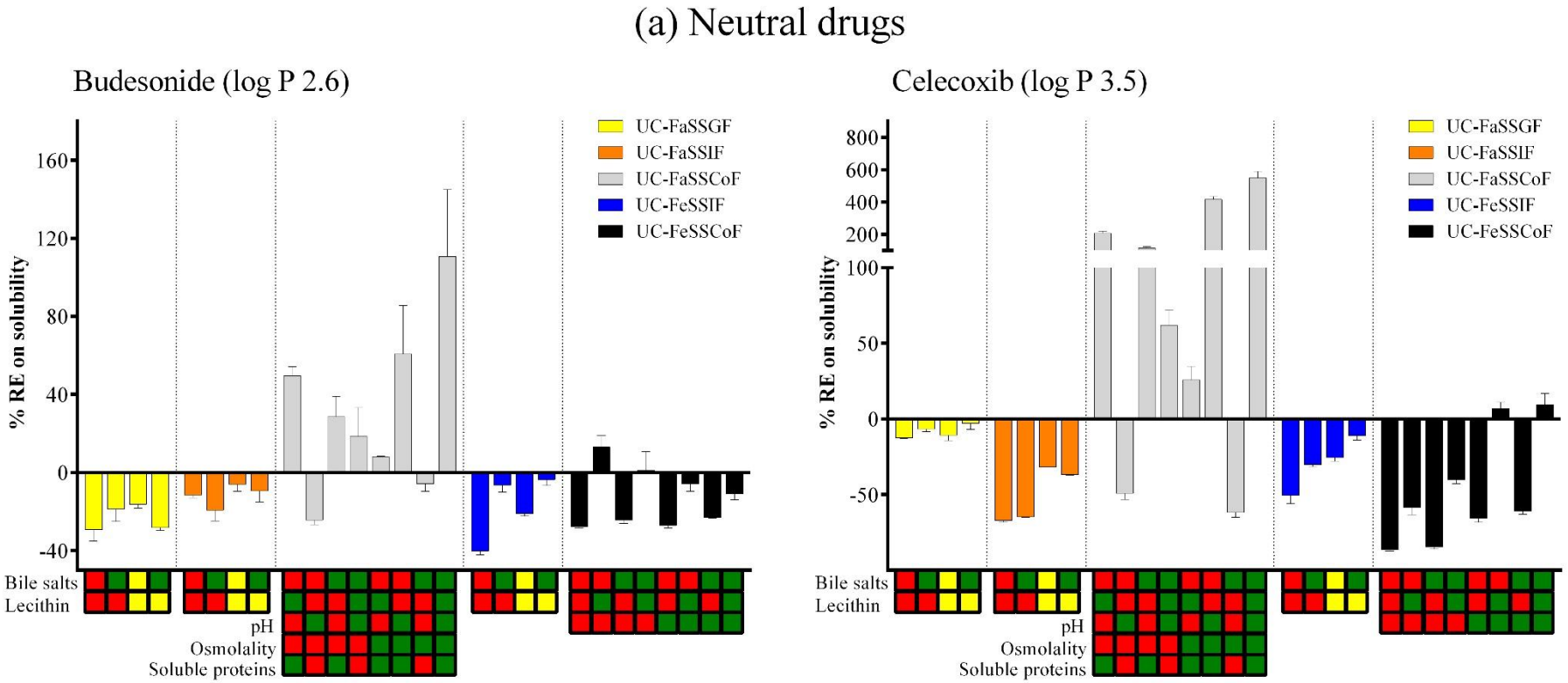

Figure 3.6: % Relative Effect on solubility of investigated drugs in UC biorelevant media compared to the corresponding healthy media according to Design of Experiments for (a) neutral drugs, (b) weak bases and (c) weak acids. [green: high level, yellow: medium level, red: low level].

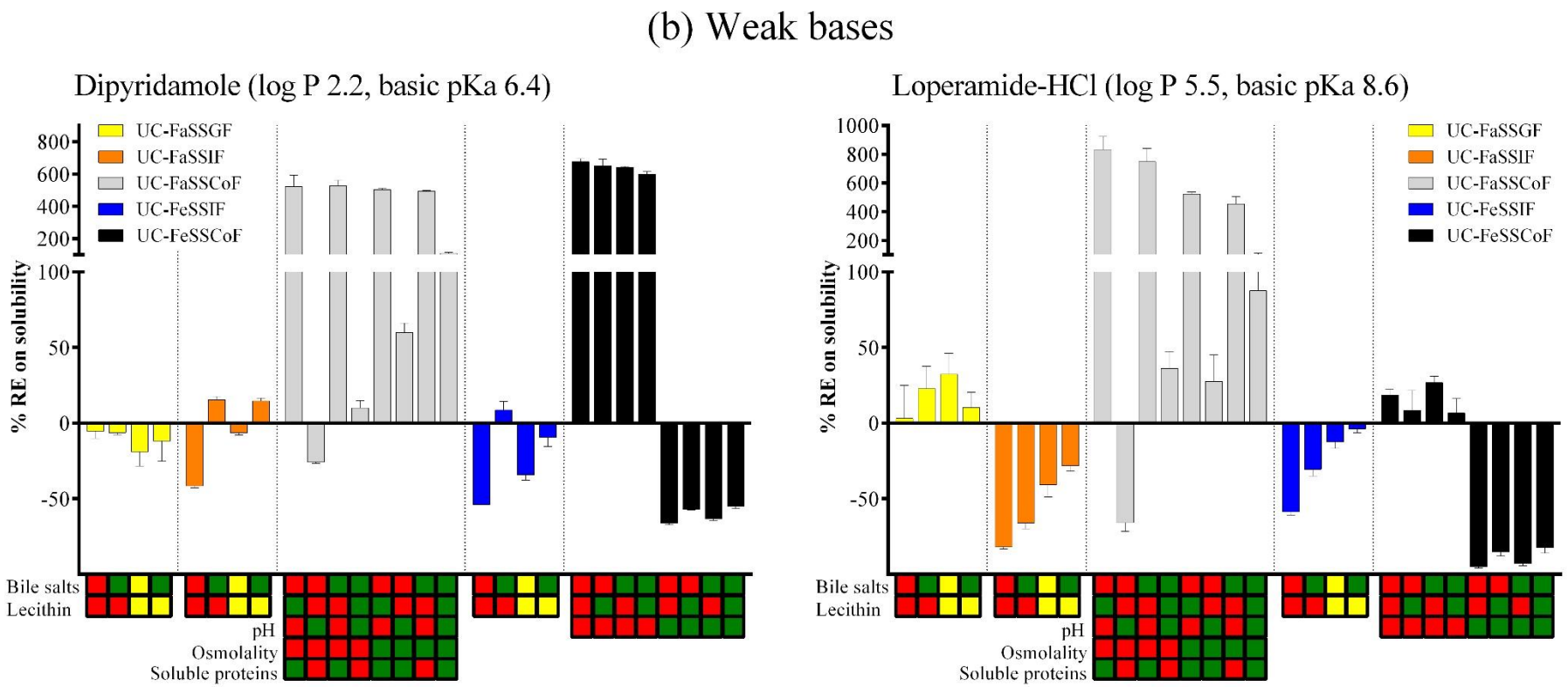

Figure 3.6: (followed).

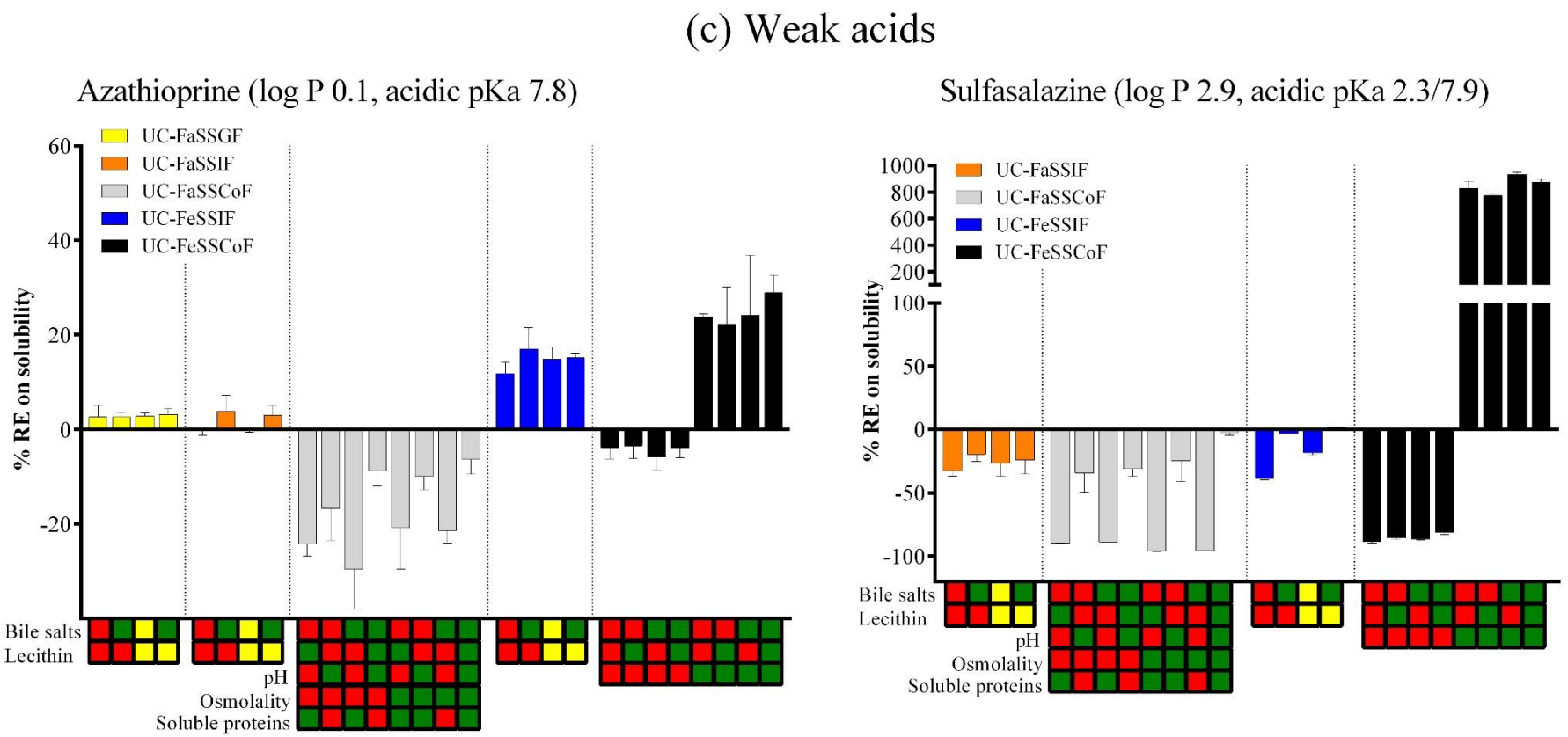

Figure 3.6: (followed).

3.4.2.1. Neutral drugs

For the investigated neutral drugs, differences in drug solubility in gastric and intestinal media were observed due to decreased lecithin or bile salt concentrations. For budesonide, the decrease was significant in all gastric fasted state UC media, in the fasted state intestinal UC medium with low lecithin and high bile salt concentrations and in the fed state intestinal medium with low lecithin and low bile salt concentrations ($p<0.05$). For celecoxib, a significantly reduced solubility was observed in the fasted state gastric UC medium with low lecithin and low bile salt concentrations and in all fasted and fed state intestinal UC media ($p<0.05$). These findings are consistent with lower concentrations of bile salts and lecithin resulting in a decreased concentration of mixed micelles available for drug solubilisation of lipophilic compounds.[51]

In fasted state colonic UC media, budesonide solubility was significantly higher in media with high pH, high osmolality and high soluble proteins ($p<0.05$). For celecoxib, the solubility was increased in all UC media with high concentrations of soluble proteins and one other UC medium (high bile salt and lecithin concentrations, high pH, low concentration of soluble proteins and a low osmolality), while the solubility was decreased in media with low concentrations of lecithin and soluble proteins ($p<0.05$). The positive effect of soluble proteins, represented by BSA, on the solubility of non-ionised compounds has previously been reported for danazol, felodipine and prednisolone.[10, 52] Additionally, it has been shown that the octanol:water partition coefficient is positively correlated to the BSA:water partition coefficient for neutral compounds.[53] In fed state colonic media, the solubility of budesonide and celecoxib was significantly decreased in UC media with low lecithin concentrations ($p<0.05$), indicating lower solubilisation due to decreased surfactant concentration. For celecoxib, this was also the case for UC media with low pH. This could be due to the low pH (4.7) resulting in more sodium cholate (pKa 5.13) being present in its unionised form and hindering the formation of micelles.[54]

3.4.2.2. Weak bases

For the investigated weak bases, no significant differences in drug solubilisation were observed in fasted state gastric UC media. The solubility of loperamide hydrochloride was decreased in all fasted state intestinal UC media ($p<0.05$), indicating a lower solubility with a lower concentration of surfactants. For dipyridamole, a lower solubility was observed in UC-FaSSIF with low lecithin and low bile salt concentrations, while the solubility was increased in UC-FaSSIF with high bile salt concentrations and either low or medium lecithin

concentration ($p<0.05$). For dipyridamole, lower lecithin concentrations seem to promote drug solubilisation, probably due to electrostatic interactions between dipyridamole and bile salts. In fed state intestinal media, the solubility of loperamide hydrochloride was decreased in UC-FeSSIF with low lecithin concentrations and medium lecithin and medium bile salt concentrations. For dipyridamole, the solubility was decreased in UC-FeSSIF with low and medium bile salt concentrations indicating again the importance of bile salts for the solubilisation of weak bases. In fasted state colonic media, the solubility of loperamide hydrochloride and dipyridamole was increased in media with low pH due to a higher fraction of the drug being ionised. Additionally, the solubility of dipyridamole was also increased in the UC-FaSSCoF media with high level of all factors. In fed state colonic media, loperamide hydrochloride had a lower solubility in all UC media with high pH due to a smaller protonated fraction of the drug. Similarly, the solubility of dipyridamole was increased in UC media with low pH due to a higher fraction of the drug being ionised. Additionally, loperamide hydrochloride had a higher solubility in UC-FeSSCoF with high bile salt and low lecithin concentrations and low pH. The solubility of dipyridamole was decreased in the UC-FeSSCoF media with high pH and low lecithin concentrations.

3.4.2.3. Weak acids

For the investigated weak acids, most differences were observed due to pH changes. For azathioprine, a hydrophilic compound with a log P of 0.1, the solubility was significantly decreased in UC-FaSSCoF with low pH, while the solubility was increased in UC-FeSSCoF with high pH. For sulfasalazine, the solubility in the fasted state gastric media was below the limit of quantification. In intestinal fasted and fed state media, the solubility of sulfasalazine was significantly decreased in UC media with low lecithin and low bile salt concentration and medium lecithin and medium bile salt concentration. In fasted state colonic media, sulfasalazine solubility was decreased in UC media with low pH and other media with high pH, low osmolality and low concentration of soluble proteins. In fed state colonic media, the solubility of sulfasalazine was increased in UC media with high pH and decreased in UC media with low pH.

3.4.3. Multivariate statistical analysis

Successful PLS models were developed for small intestinal and colonic UC media in the fasted and fed state. The plots of the standardised coefficients of the respective drug- and media-dependent factors are shown in Figure 3.7. For the fasted state gastric media, it was not possible to develop a predictive PLS model (q^2 -0.04, r^2 0.09).

For fasted state intestinal media, the developed PLS model for the % Relative Effect of UC on drug solubility showed a good fit of the experimental data (r^2 0.76) and a high predictive power (q^2 0.70). The model depicted a positive effect of bile salts, lecithin and the interaction between lecithin and log P, while log P had a negative impact. Consequently, in the luminal fluids of UC patients with low bile salt and lecithin concentrations a high risk of reduced drug solubility is expected for compounds with a high lipophilicity. This is in accordance with another study, where a positive effect of bile salt and lecithin concentration on drug solubility in fasted state simulated fluids has previously been shown for seven out of twelve compounds with a clog P in the range of 1.43 to 6.15 (ACD/Labs) including three neutral compounds (felodipine clog P 4.83, griseofulvin clog P 3.53, fenofibrate clog P 4.80), three weak bases (tadalafil clog P 1.43, zafirlukast clog P 6.15, aprepitant clog P 4.80) and one weak acid (phenytoin clog P 2.52).[47, 55] It should be noted that five drugs with a clog P of 1.71-10.27 (ACD/Labs) (probucol clog P 10.27, carvedilol clog P 4.11, piroxicam clog P 1.71, indomethacin clog P 3.1, naproxen clog P 3.0) did not follow this pattern in the respective study indicating drug-specific effects in certain cases.[47, 55] Therefore, a difference in luminal drug solubility in UC patients may not be fully predicted for certain drugs by the sole use of drug properties employed in the current study.

For fed state intestinal media, the model quality of the developed PLS model was accurate with a high predictability (r^2 0.73, q^2 0.66). As for the PLS model of the fasted state, bile salts and lecithin had a positive effect on the % Relative Effect on drug solubility with a higher impact of the bile salt concentration. The interaction between lecithin and log P had also a positive influence (VIP>0.7). In contrast, log P had a negative impact. In another study, a positive impact of higher bile salt concentration on drug solubility in fed state simulated intestinal media was observed for nine of thirteen compounds (itraconazole, probucol, felodipine, tadalafil, aprepitant, carvedilol, zafirlukast, indomethacin, phenytoin) with a clog P in the range of 1.43 to 10.27 (ACD/Labs).[47, 56] In the same study, a positive effect of lecithin on the solubility of eight out of thirteen compounds was also observed (itraconazole, probucol, felodipine, fenofibrate, carvedilol, zafirlukast, indomethacin, phenytoin).[56] However, bile salts or lecithin had a negative impact on drug solubility for

certain lipophilic drugs in the respective study indicating again drug-specific effects in some cases.[56]

For fasted state colonic media, the good quality PLS model (r^2 0.90, q^2 0.82) revealed a positive effect of log P, weak base and the interplay between soluble proteins and neutral drugs, and a lower positive influence (VIP>0.7) of soluble proteins and the interplay between log P and neutral drugs. In contrast, the model showed a negative influence of pH, weak acids, the interplay between pH and log P and the interplay between pH and weak base. This indicates that differences in drug ionisation determine the drug solubility in the fasted state colonic fluid of UC patients. Additionally, a higher drug solubility of neutral lipophilic compounds is expected in the fasted state colonic fluids of UC patients.

For fed state colonic media, the predictive power of the developed PLS model was acceptable (q^2 0.49, r^2 0.71). Most influential variables of the model with positive impact were the categorical variable weak acid and the interplay between pH/log P and pH/weak acid. Additionally, a positive effect of log P was influential to the model (VIP>0.7). A negative impact on the % Relative Effect on drug solubility was observed for the categorical variable neutral and the interplay between pH and weak base. Differences in ionisation are therefore, expected to be the major influence on drug solubility in the fed state colonic fluid of UC patients.

Given the high number of UC media and the solubility studies of six compounds, the statistical models were acceptable. Further studies with more compounds would additionally increase the confidence in the models.

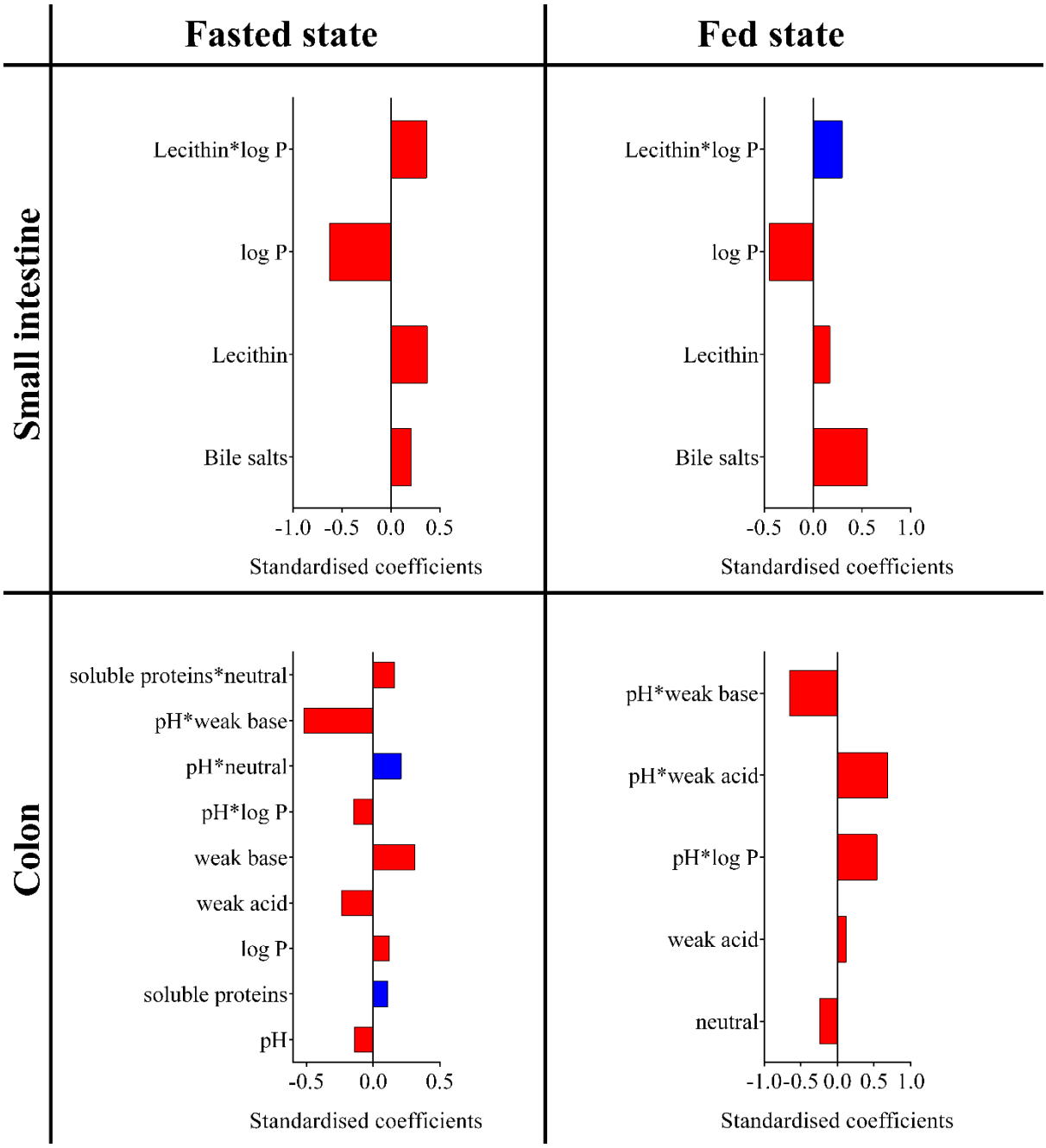

Figure 3.7: Standardised coefficients of the PLS regression of drug solubility in UC simulated gastrointestinal fluids in the fasted state (left) and fed state (right) and different compartments of the GI tract (top: small intestine, bottom: colon). Red colour denotes coefficients of VIP values > 1 and blue > 0.7.

3.4.4. Drugs at risk of altered solubility in luminal fluids of UC patients

Considering intestinal fluids in the fasted and fed state, compounds with a higher lipophilicity are expected to show a lower drug solubility in UC patients compared to healthy

subjects. This is especially expected for UC patients with low concentrations of bile salts and lecithin in their intestinal fluids.

In terms of fasted state colonic fluids of UC patients, a high risk of altered drug solubility is indicated for weak bases and weak acids. For weak acids, a lower drug solubility is expected in fasted state colonic fluids of UC patients compared to healthy subjects. For weak bases, a higher drug solubility is expected in UC patients with a low pH in their fasted state colonic fluids. Additionally, neutral moderately lipophilic drugs are expected to have a higher solubility in UC patients with increased concentrations of soluble proteins (relapse and remission) in their fasted state colonic fluids.

Regarding the fed state colonic fluid of UC patients, the altered colonic pH in UC patients poses a risk for ionisable drugs. For weak acids, a higher drug solubility is expected in UC patients with increased pH in their fed state colonic fluids, whereas for weak bases a lower drug solubility is expected. In addition, a lower solubility of neutral moderately lipophilic drugs is expected in the fed state colonic fluids of UC patients with low lecithin concentration.

3.5. Conclusion

Biorelevant media were developed as *in vitro* tool to assess drug solubility and dissolution in UC patients for different GI compartments and prandial states based on literature data investigating pathophysiological changes in UC. The characterisation of UC biorelevant media showed differences in terms of surface tension, buffer capacity and osmolality compared to healthy biorelevant media. These findings suggest that drug product performance could be affected for certain drugs and formulations due to e.g., changes in the wetting behaviour or drug solubility due to the common ion effect or pH.

A lower drug solubility in UC intestinal luminal fluids is expected for compounds with high lipophilicity, especially in patients with low concentrations of bile salts and lecithin. In the fasted state colonic fluid of UC patients, weak bases and weak acids are at high risk of altered drug solubility compared to healthy subjects. Additionally, neutral drugs are likely to have a higher solubility due to increased concentrations of soluble proteins. In the fed state colonic fluids of UC patients, ionisable drugs have a high risk of altered drug solubility. Furthermore, a lower solubility of neutral drugs is expected in the fed state colonic fluids in UC patients with low lecithin concentration.

To increase the confidence in the predictions of drug solubility in UC patients, further studies assessing the luminal fluid composition in UC patients are needed. Furthermore, the confidence in the presented statistical models can be increased by investigating additional compounds.

Differences in drug solubility in luminal fluids of UC patients compared to healthy subjects can be identified using biorelevant UC media, without the need to conduct expensive clinical trials. Apart from differences in drug solubility and dissolution, UC can affect a drug's permeability, distribution, gut wall/hepatic metabolism and elimination. Therefore, *in vitro* solubility or dissolution tests can be used as input for physiologically-based pharmacokinetic models that offers the possibility to predict drug product performance considering all ADME (absorption, distribution, metabolism, excretion) processes.

3.6. References

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Chapter 4

Gastrointestinal diseases and their impact on drug solubility.

Part III. Coeliac disease

Abstract

Objectives

The aim of this study was to develop biorelevant media for patients with Coeliac disease (CED) using a Design of Experiment (DoE) approach as *in vitro* tool for predicting drug solubility and dissolution in their intestinal fluids. The CED biorelevant media were characterised according to their surface tension, osmolality, dynamic viscosity and buffer capacity. By performing solubility studies of six drugs with different physicochemical properties in CED media, we aimed to identify drugs at high risk of altered luminal solubility in CED patients.

Key findings

Identified published differences in CED patients compared to healthy subjects were related to a higher concentration of bile salts, lecithin and cholesterol and included as factors in the DoE resulting in 8 CED biorelevant media. Differences in media properties were observed for the surface tension between CED and healthy biorelevant media. In terms of solubility, only a minimal effect of CED on the solubility of the hydrophilic neutral compound azathioprine was observed. For neutral moderately lipophilic compounds (budesonide, celecoxib), a higher surfactant concentration resulted in most cases in a higher drug solubility, while it was specific to each drug whether this was driven majorly by bile salts or lecithin. In comparison, drug solubilisation of ionisable compounds with moderate to high lipophilicity was less impacted by CED differences.

Conclusions

The developed biorelevant CED media serve as *in vitro* tool to identify the main media factors impacting on drug solubility in CED. Further studies assessing the luminal fluid composition in patients with CED are needed to increase the confidence in the developed *in vitro* tool.

4.1. Introduction

Coeliac disease (CED) is a chronic auto-inflammatory disease induced by an intolerance to dietary gluten, a storage protein of wheat, rye, barley and oats. Approximately 1% of the population is affected by CED and its aetiology is a combination of genetic predisposition and environmental factors (e.g., breastfeeding, time of gluten introduction and the microbiota).[1] CED mainly affects the small intestine resulting in gastrointestinal (GI) symptoms such as bloating, diarrhoea, malabsorptive symptoms and weight loss. Additionally, CED patients can present extra-intestinal symptoms such as dermatitis herpetiformis, anaemia or osteoporosis.[2] The diagnosis involves serological testing for autoantibodies (anti-tTG, anti-EMA) and an endoscopic biopsy.[3] Depending on the damage of the small intestine, the disease can be classified in different disease grades based on histological findings such as crypt hyperplasia, the constitution of the villi and the intra-epithelial lymphocytes in the jejunum and duodenum.[4] For the treatment of CED, patients need to adhere to a gluten-free diet, the only known effective treatment to date, since the reintroduction of dietary gluten results in a relapse of the disease.[5] More treatment options are expected to emerge in the near future, since several new active pharmaceutical ingredients have reached clinical phases of drug development in recent years.[5]

Oral administration is due to patient convenience the preferred route of drug administration for most drugs. Consequently, patients with CED are likely to be treated with orally administered drug products for concomitant conditions or extra-intestinal manifestations of CED. Since oral drug administration is, apart from drug and formulation properties, dependent on GI physiology, pathophysiological changes in CED could affect drug safety and efficacy. GI diseases can affect various processes involved in oral drug delivery e.g., drug release from the formulation, drug dissolution, permeation through the GI membrane and gut or hepatic metabolism.[6] Altered drug absorption in CED patients compared to healthy subjects has previously been attributed to a reduced small intestinal surface area, a different intestinal CYP enzyme abundance, a higher jejunal permeability and differences in gastric emptying.[7]

So far, there is only a small number of drugs for which drug product performance has been investigated in CED patients and these studies included only a small number of patients.[7] Due to the high costs of clinical trials, it is expected that in the future investigations in CED patients will remain rare.

For poorly soluble drugs, drug absorption can be limited by the dissolution rate or the solubility of the drug in GI fluids.[8] If this is the case, *in vitro* release and dissolution testing can be used as surrogate for a drug's *in vivo* performance.[8] *In vitro* setups with a close representation of *in vivo* conditions are expected to result in better predictions. To simulate closely the conditions present in the GI tract, biorelevant media have been developed mimicking the composition of the GI fluids of healthy subjects.[9-13] The composition of the GI fluids can be altered in patients with GI disease and therefore, *in vitro* dissolution and solubility studies with biorelevant media adapted to pathophysiological conditions could result in better predictions of drug product performance in patient populations. [6]

This study aims to identify drugs at risk of altered solubility in GI fluids of patients with CED by developing biorelevant media for patients with CED representative of the small intestinal fluid in the fasted and fed state. Therefore, information from literature was collected to identify differences in the composition of luminal contents of patients with CED compared to healthy subjects. Biorelevant media for patients with CED were developed based on biorelevant media for healthy subjects and a Design of Experiment (DoE) approach by integrating the identified differences as factors with two levels. Subsequently, the CED biorelevant media were characterised in terms of surface tension, osmolality, buffer capacity and dynamic viscosity. Additionally, the solubility of six compounds with different physicochemical properties in the developed CED and healthy biorelevant media was determined.

4.2. Materials

Acetic acid High Performance Liquid Chromatography (HPLC) grade, chloroform, sodium oleate, budesonide, phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich Company Ltd., Dorset, England. Sulfasalazine, loperamide hydrochloride, dipyridamole, celecoxib, azathioprine, methanol HPLC grade and acetonitrile HPLC grade were purchased from VWR International Ltd, Lutterworth, UK. Sodium chloride, trifluoroacetic acid (TFA), potassium dihydrogen orthophosphate, dimethyl sulfoxide 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), glyceryl monooleate–Rylo Mg 19 (Danisco, Brabrand, Denmark) and cholesterol (95%, Acros Organics, Geel, Belgium). Water was ultra-pure (Milli-Q) laboratory grade.

4.3. Methods

4.3.1. Media development

4.3.1.1. GI physiological differences in CED compared to healthy subjects

To identify differences in the composition of GI fluids of CED patients compared to healthy subjects, a literature search was performed. Since to date the GI fluids of CED patients have not been directly characterised, studies investigating parameters that most likely impact on GI fluids were considered.

The bile flow and biliary lipid output was previously investigated in CED patients and healthy subjects using a duodenal intubation technique with a constant infusion of a liquid formula diet.[14] Biliary lipid outputs such as cholesterol, bile acids and phospholipids could then be estimated by the dilution of a marker, polyethylene glycol 4000. The bile flow was with 232±29 mL/h (mean ± SD) significantly higher in CED patients compared to 132±24 mL/h in healthy subjects (Student's t-test, $p<0.05$). The biliary cholesterol output normalised to the body weight was significantly increased in CED patients (0.82±0.10 vs 0.43±0.06 mg/kg*h, $p<0.02$). Similarly, the biliary output of phospholipids was also highly increased in CED patients compared to healthy subjects (0.26±0.05 vs 0.08±0.02 mg/kg*h, $p<0.02$). Additionally, a higher bile acid output was observed in CED patients (9.28±1.65 vs 4.64±0.45 mg/kg*h). In accordance, it was observed that the bile salt pool is three times higher in CED patients compared to healthy subjects which could be related to a very effective ileal reabsorption of bile acids or a sluggish contraction of the gall bladder.[15]

4.3.1.2. Development of CED media with Design of Experiment

The development of biorelevant media for CED patients followed a DoE approach and CED biorelevant media representative of the small intestinal fluid in the fasted and fed state were developed. Biorelevant media previously developed based on healthy subjects were used as the basis for CED biorelevant media and included Fasted-State Simulated Intestinal Fluid-Version 2 (FaSSIF-V2) and Fed-State Simulated Intestinal Fluid-Version 2 (FeSSIF-V2).[11] According to the identified differences described in Section 4.3.1.1, healthy biorelevant media were modified by including the respective differences as factors in the experimental design. For both prandial states, the integrated factors were the concentration of bile salts, lecithin and cholesterol. Since the biliary secretion is the main source of bile salts, lecithin and cholesterol present in the intestinal fluids, a direct correlation between biliary output and intestinal concentration was assumed. Due to the three parameters not being directly

measured in the GI fluids, an indirect percental approach was followed to determine the level of the corresponding factor according to

$$x_{CD-BM} = \frac{y_{CD}}{y_H} * x_{H-BM} \quad (4.1)$$

where x_{CD-BM} is the high level of the factor in CD media, y_{CD} and y_H are the median of the corresponding biliary output observed in CD patients and healthy subjects, respectively and x_{H-BM} is the level of the factor in healthy biorelevant media.

The three factors were integrated with two levels in the experimental design, a low and a high level. The low level was based on the concentration in healthy biorelevant media (Table 2.3) and the high level corresponded to the median percentage of the respective concentration in the healthy medium. For cholesterol, the low level concentration was based on the median concentration of cholesterol observed in human intestinal fluid as observed previously (fasted state: 0.08 mM, fed state: 0.57 mM), since cholesterol is not a component of FaSSIF-V2 and FeSSIF-V2.[16]

The DoE was performed with Statgraphics Centurion 18 (Statpoint Technologies Inc., VA, US) with a full factorial design for CED intestinal biorelevant media for the fasted and fed state. An overview of the DoE is given in Figure 4.1. Biorelevant media were prepared as previously described with an additional step of adding cholesterol.[11] The cholesterol solution (50 mg/mL in chloroform) was mixed with a lecithin solution (100 mg/mL in dichloromethane) using a magnetic stirrer, before being added to the bile salt/buffer mixture and driven off using a rotary evaporator Büchi Rotovapor R-114 (Büchi Labotechnik, Flawil, Switzerland) according to the published protocol. The osmolality of CED media was set to the value in the corresponding healthy biorelevant medium by adjusting the concentration of sodium chloride.

	Celiac disease			
Prandial state	**Fasted state**		**Fed state**	
Compartment	**intestine**		**intestine**	
Level	**low**	**high**	**low**	**high**
Bile salts [mM]	3.0	5.1	10.0	17.0
Lecithin [mM]	0.2	0.6	2.0	6.0
Cholesterol [mM]	0.08*	0.16	0.57*	1.14

	increase
	value in healthy biorelevant media

Figure 4.1: Design of Experiments for the development of Coeliac disease intestinal biorelevant media (*value observed in human intestinal fluids[16]).

4.3.1.3. Media characterisation

Surface tension, osmolality, dynamic viscosity and buffer capacity of biorelevant media previously developed based on healthy subjects and newly developed for CED patients were measured in triplicate. The results are reported as mean with standard deviation.

4.3.1.3.1. Surface tension

Surface tension measurements were performed with a ring tensiometer (Sigma 700 Force tensiometer, Attension, UK) using approximately 10 mL of each medium, placed in a glass vessel with a diameter of 46 mm. A platinum Du Noüy ring was lowered below the meniscus of the medium. Subsequently, by pushing and pulling the ring through the surface of the medium, the force exerted by the meniscus was measured and related to the surface tension of the medium.[17]

4.3.1.3.2. Osmolality

Osmolality was determined with an Advanced Instruments Inc. micro-osmometer Model 3300 (Norwood, MA, US). Therefore, the freezing-point depression of a 20 µl sample was measured with a high-precision thermistor following the supercooling and induced crystallisation of the sample.

4.3.1.3.3. Dynamic viscosity

The dynamic viscosity at 37ºC was measured with a Bohlin Rheometer C-VOR (Malvern instruments, UK). Therefore, a cone-plate measuring system, including a rotating upper cone (4°, 40 mm) and a fixed lower plate with the medium contained between them, was used. The shear rate was measured while twenty different shear stresses, logarithmically distributed in the range of 0.05 to 0.15 Pa, were exerted on the sample of the medium. The ratio of shear stress to shear rate corresponds to the dynamic viscosity.

4.3.1.3.4. Buffer capacity

Buffer capacity was determined using a potentiometric titration method. Therefore, small volumes of 0.5 M hydrochloric acid were added to 10 mL of sample until a change of one pH unit was recorded by a Mettler Toledo SevenCompact S220 pH meter (Schwerzenbach, Switzerland). Equation (4.2) was used to calculate the buffer capacity (β) according to

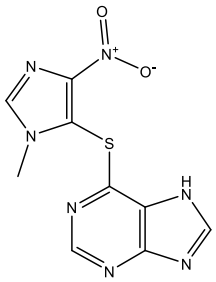
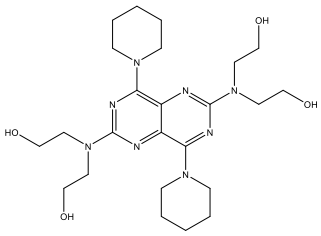
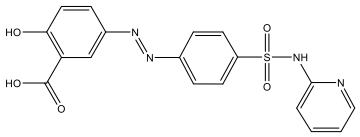
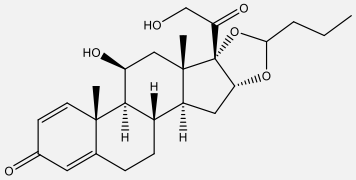
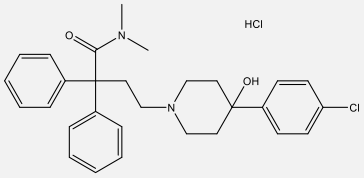
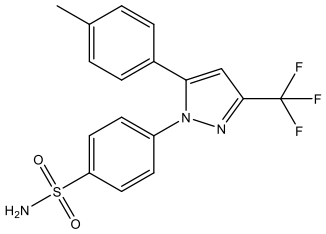
$$\beta = \left(\frac{0.5M * V_{acid}[ml]}{\Delta pH}\right) * \frac{1000}{10mL} \quad (4.2)$$

where V_{acid} is the volume of the acid added and ΔpH corresponds to the change in pH.[18]

4.3.1.4. Compound selection

For the solubility studies, poorly soluble compounds belonging to Biopharmaceutics Classification System (BCS) class II (low solubility, high permeability) or IV (low solubility, low permeability) were selected as shown in Table 4.1. Additionally, the selected drugs varied in their ionisation properties (pKa) and lipophilicity (log P). Drugs with an indication for GI diseases were preferred.

Table 4.1: Overview over physicochemical characteristics and indication of selected compounds for solubility studies.

Neutral drugs	**Weak bases**	**Weak acid**
Azathioprine: • pKa 7.9 (acid)[19] • log P 0.1[20] • BCS class IV[21] • Immunosuppressive 	Dipyridamole • pKa 6.4 (base)[22] • log P 2.2[23] • BCS class II[24] • Platelet aggregation inhibitor 	Sulfasalazine • pKa 2.3 (acid), 7.9[25] • log P 2.9[26] • BCS class II or IV[21] • Anti-inflammatory agent in IBD 
Budesonide • pKa 12.0 (acid)[27] • log P 2.6[28] • BCS class II[29] • Locally acting corticosteroid in IBD 	Loperamide-HCl • pKa 8.6 (base)[30] • log P 5.5[31] • BCS class II[24] • Anti-diarrheal agent 	
Celecoxib • pKa 11.1 (acid)[32] • log P 3.5[32] • BCS class II[33] • Nonsteroidal anti-inflammatory drug 		

4.3.1.5. Solubility studies

The shake-flask method was used to determine the solubility of the investigated compounds.[34] Therefore, an excess amount of drug was added to 5 mL of the respective medium in a glass tube, which was placed in a shaking water bath (Grant instruments, Royston, UK) and maintained at 37 °C and 200 strokes/min for 24 h. Subsequently, GF/D membrane filters with a pore size of 2.7 μm (Whatman® Puradisc, diameter 13 mm) were used to filter, followed by the quantitative analysis of the supernatant with HPLC-Ultraviolet (UV). The solubility studies were performed in triplicate in CED disease media and healthy media and average solubility differences between CED media and healthy media were expressed as a % Relative Effect on solubility [$((S_{CED}-S_{Healthy})/ S_{Healthy}) \times 100$]. A higher drug solubility in CED media compared to healthy media is indicated by a positive value, whereas the opposite is indicated for negative values. HPLC analysis was performed with an Agilent Technologies 1200 series HPLC system (Santa Clara, CA, US) including a binary pump (G1212A), an autosampler (G1329A), a thermostatted column compartment (G1316A) and a diode array detector (G1315D). The methods used for the HPLC-UV analysis of the six drugs were modifications of previously published methods (presented in Gastrointestinal diseases and their impact on drug solubility. Part I. Crohn's disease [Chapter 2]).

4.3.1.6. Statistical analysis

Differences between media properties and drug solubility in CED disease biorelevant media compared to healthy biorelevant media were identified with the software XLSTAT (Addinsoft, France) using one-way analysis of variance (ANOVA) with a post-hoc Tukey's test and a significance level of $p \leq 0.05$.

A multifactorial ANOVA performed in Statgraphics Centurion 18 (Statpoint Technologies Inc., VA, US) was used to estimate the effects of the three categorical variables (bile salts, lecithin, cholesterol) and two-factor interactions in the DoE on the solubility of each of the six investigated compounds. Factors were considered statistically significant if the p-value was less than 0.05, indicating an effect on drug solubility at the 95.00% confidence level.

4.4. Results and discussion

4.4.1. Media characterisation

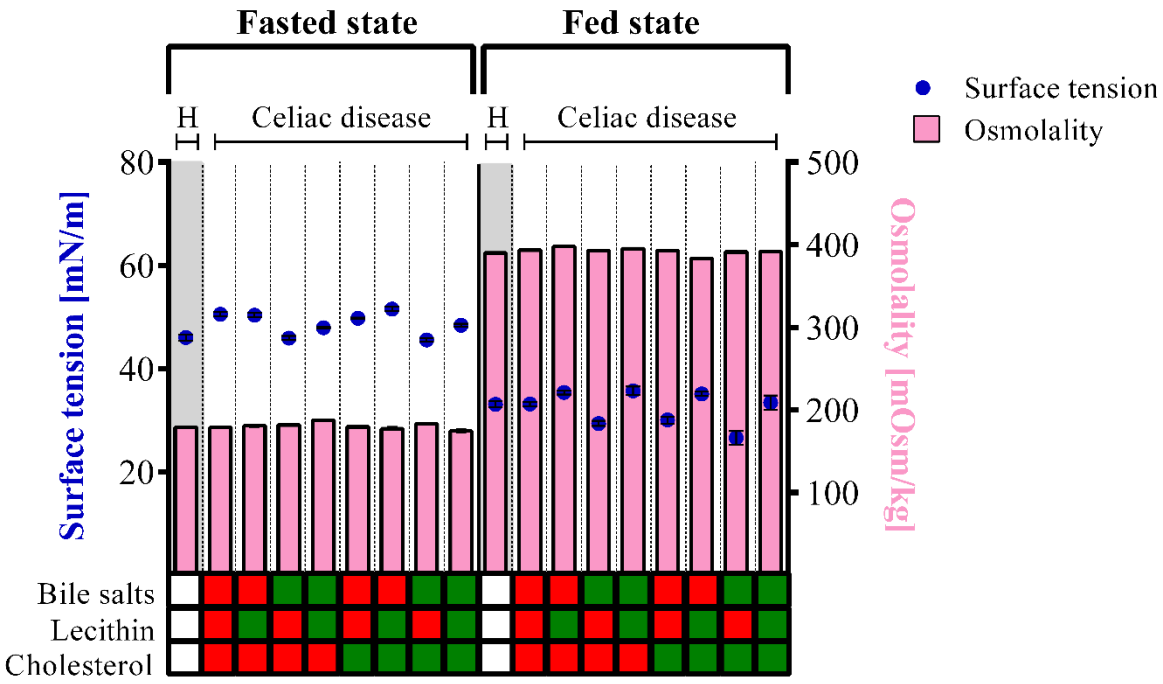


Figure 4.2: Surface tension (blue, left y-axis) and osmolality (rose, right y-axis) of Coeliac disease biorelevant media according to the Design of Experiment (green: high level, red: low level, white: healthy level) and healthy media (H).

The surface tension of fasted and fed state intestinal CED biorelevant media is shown in Figure 4.2. In the fasted state, the surface tension of all media with low bile salt concentration was increased compared to the healthy medium ($p<0.05$). This finding is consistent with another study, where a higher surface tension was observed for reduced bile salt concentrations in fasted state simulating fluids without cholesterol.[35] Additionally, media with at the same time high bile salt and lecithin concentrations possessed a significantly higher surface tension compared to the healthy medium but a lower surface tension compared to all CED media with low bile salt concentrations ($p<0.05$). In the fed state, the surface tension of all CED media with low lecithin concentrations, except for the medium with at the same time low bile salt and cholesterol concentrations, was significantly decreased ($p<0.05$).

The osmolality of CED and healthy biorelevant media was not significantly different.

The measured dynamic viscosities of CED biorelevant media at a shear stress of 0.06 Pa, 0.08 Pa and 0.15 Pa are presented in Figure 4.3. All healthy and CED media showed shear

thinning behaviour. The viscosity of CED biorelevant media at an applied shear stress of 0.15 Pa was in the range of 3.26 to 3.56 mPas, at 0.08 Pa in the range of 3.70 to 4.56 mPas and at 0.06 Pa in the range of 4.28 to 6.42 mPas. No significant differences between CED and healthy biorelevant media were observed considering all three different shear stresses ($p<0.05$).

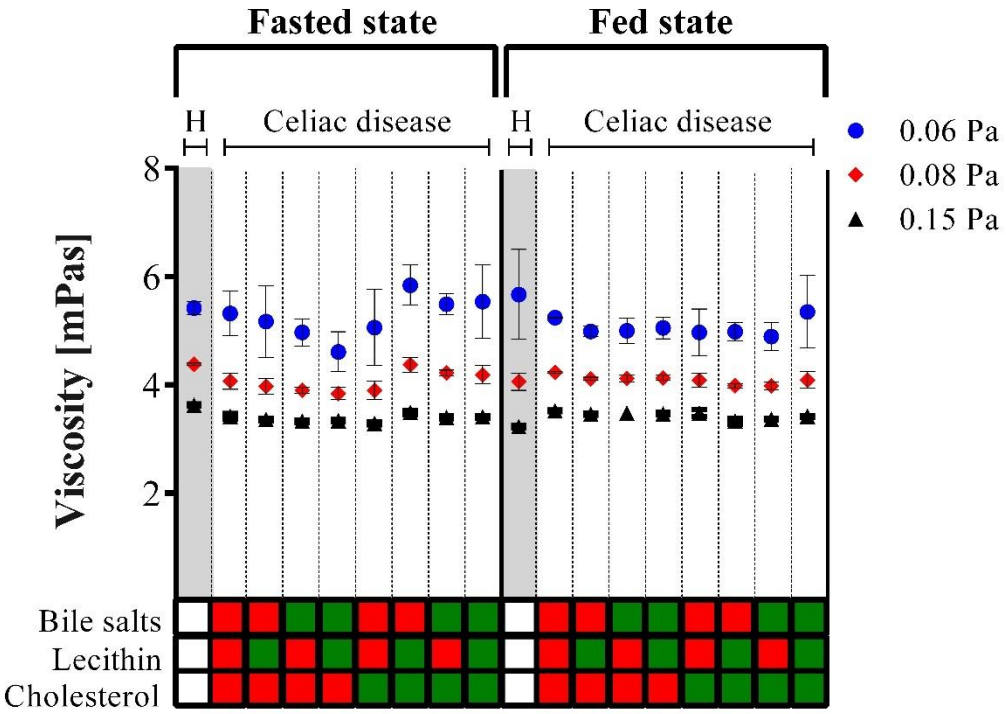


Figure 4.3: Dynamic viscosity of Coeliac disease biorelevant media and the corresponding healthy biorelevant media (H) at different shear stress (0.06 Pa: blue, 0.08 Pa: red, 0.15 Pa: black) according to the Design of Experiments (green: high level, red: low level, white: healthy level).

The buffer capacity was not significantly different in healthy fasted and fed state intestinal media compared to CED media, since the same buffer composition was used and no changes to the media pH were applied (data not shown).

4.4.2. Solubility of drugs in CED biorelevant media

4.4.2.1. Neutral drugs

The results of the solubility studies with neutral compounds in CED fasted and fed state intestinal media are illustrated in Figure 4.4.

For azathioprine, the solubility in the fasted state was not significantly different in CED media compared to healthy media. In the fed state, the solubility of azathioprine was

significantly higher in CED biorelevant media with high concentrations of bile salts but the relative increase was for all media below 15%.

For budesonide, the solubility in all fasted state CED biorelevant media was significantly higher compared to the healthy medium ($p<0.05$), whereby the solubility of budesonide was highest in CED media with high bile salt concentrations. The positive effect of bile salts is in accordance with a previous study showing that an increase of the concentration of bile salts in a fixed 4:1 ratio of bile salts to lecithin resulted in an increase in budesonide solubility.[36] Additionally, the positive effect of cholesterol on budesonide solubilisation indicates a drug-cholesterol interaction or a positive solubilisation effect of more complex vesicles (sodium taurocholate-lecithin-cholesterol) as previously reported for fenofibrate.[37]

In the fed state, the solubility of budesonide in the CED media with at the same time low concentrations of bile salts and lecithin was significantly decreased compared to the healthy medium ($p<0.05$), indicating a competition for solubilisation between cholesterol and budesonide possibly due to the similarity of their chemical structure. In contrast, a significantly higher solubility was observed in CED media with high concentrations of bile salts and lecithin and CED media with either a high concentration of bile salts or lecithin and a low concentration of cholesterol ($p<0.05$), indicating a positive effect of higher surfactant concentration and a negative effect of cholesterol on budesonide solubility.

For celecoxib, the solubility in fasted state CED media with a high concentration of lecithin and a low concentration of cholesterol was significantly higher compared to the healthy medium. In contrast, in all other CED fasted state media, the solubility of celecoxib was significantly lower ($p<0.05$). The positive effect of lecithin on celecoxib solubility is in accordance with previous results revealing a higher solubility of celecoxib in FaSSIF (higher concentration of lecithin) compared to FaSSIF-V2.[38]

In the fed state, the solubility of celecoxib was significantly higher in CED media with at the same time high concentrations of bile salts and lecithin ($p<0.05$), suggesting a positive effect of luminal surfactants on celecoxib solubility.

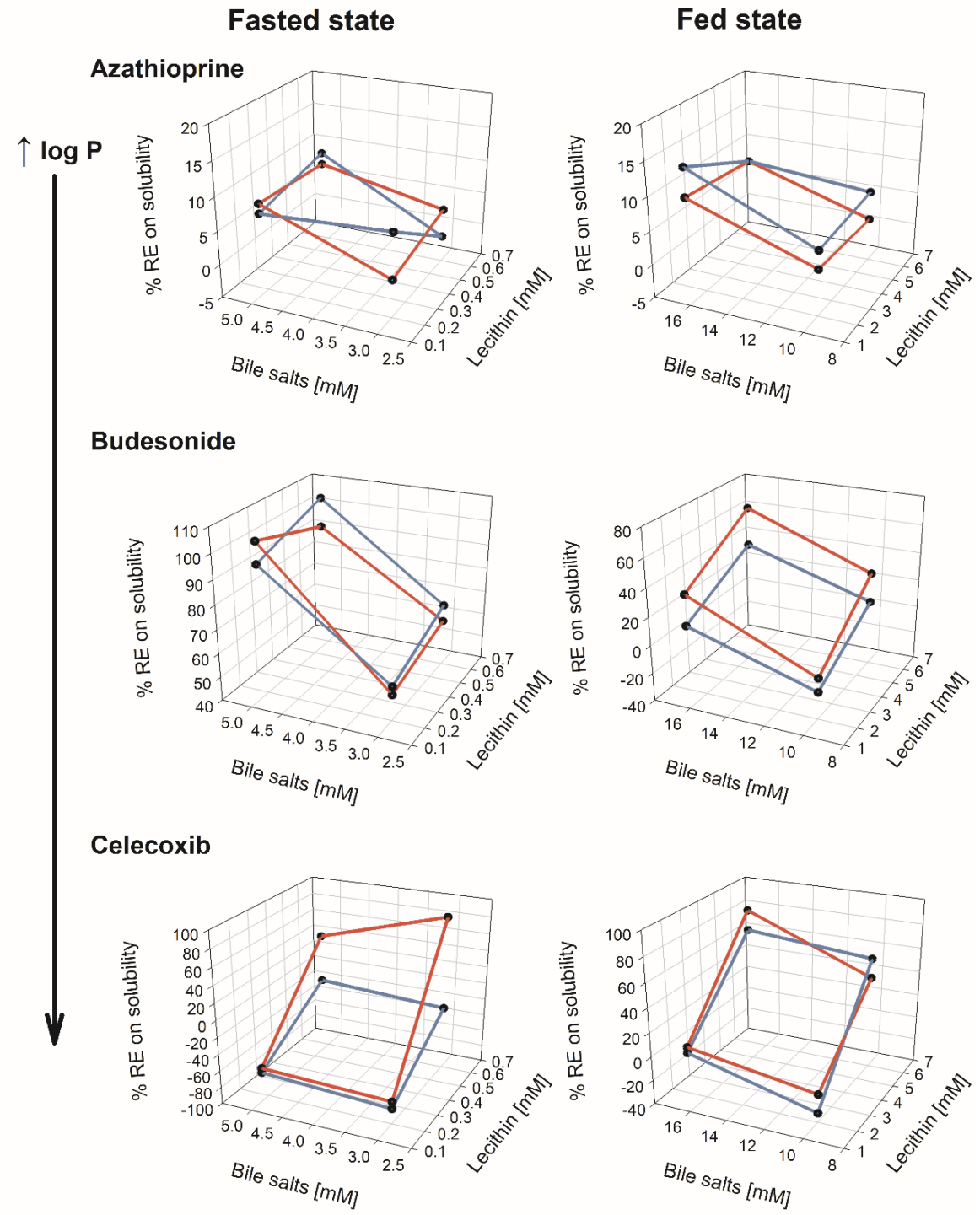


Figure 4.4: % Relative Effect (RE) on the solubility of neutral (at pH 5.8-6.5) investigated drugs in Coeliac disease intestinal biorelevant media compared to the corresponding healthy media according to Design of Experiment (red lines: low concentration of cholesterol, blue lines: high concentration of cholesterol).

4.4.2.2. Weak acid

The results of the solubility studies in CED fasted and fed state intestinal media with compounds possessing different ionisation properties are presented in Figure 4.5.

For the weak acid sulfasalazine, the solubility in fasted state CED media with at the same time high concentrations of lecithin and low concentrations of cholesterol is significantly lower compared to the healthy medium ($p<0.05$). In fed state intestinal media, the solubility of sulfasalazine was significantly higher in CED media with high bile salt concentrations and in the medium with a low concentration of bile salts and lecithin and a high concentration of cholesterol.

4.4.2.3. Weak bases

For the weak base dipyridamole, the solubility was significantly higher in fasted state CED media with high bile salt concentrations and to a lower extent also in the medium with a high concentration of lecithin and low concentrations of bile salts and cholesterol ($p<0.05$). The positive effect of bile salts on the solubility of dipyridamole is most likely the result of electrostatic interactions of the weak base with sodium taurocholate. In the fed state, the solubility of dipyridamole in the CED medium with a high concentration of lecithin and low concentrations of bile salts and cholesterol was significantly lower compared to the corresponding healthy medium ($p<0.05$).

For loperamide hydrochloride, the solubility in the fasted state CED media with high concentrations of lecithin and cholesterol and a low concentration of bile salts was significantly lower compared to the corresponding healthy medium ($p<0.05$). This is possibly due to less bile salts being available for drug solubilisation due to the need for lecithin and cholesterol solubilisation. In the fed state, the solubility of loperamide hydrochloride was not significantly different in CED media compared to the corresponding healthy medium ($p<0.05$).

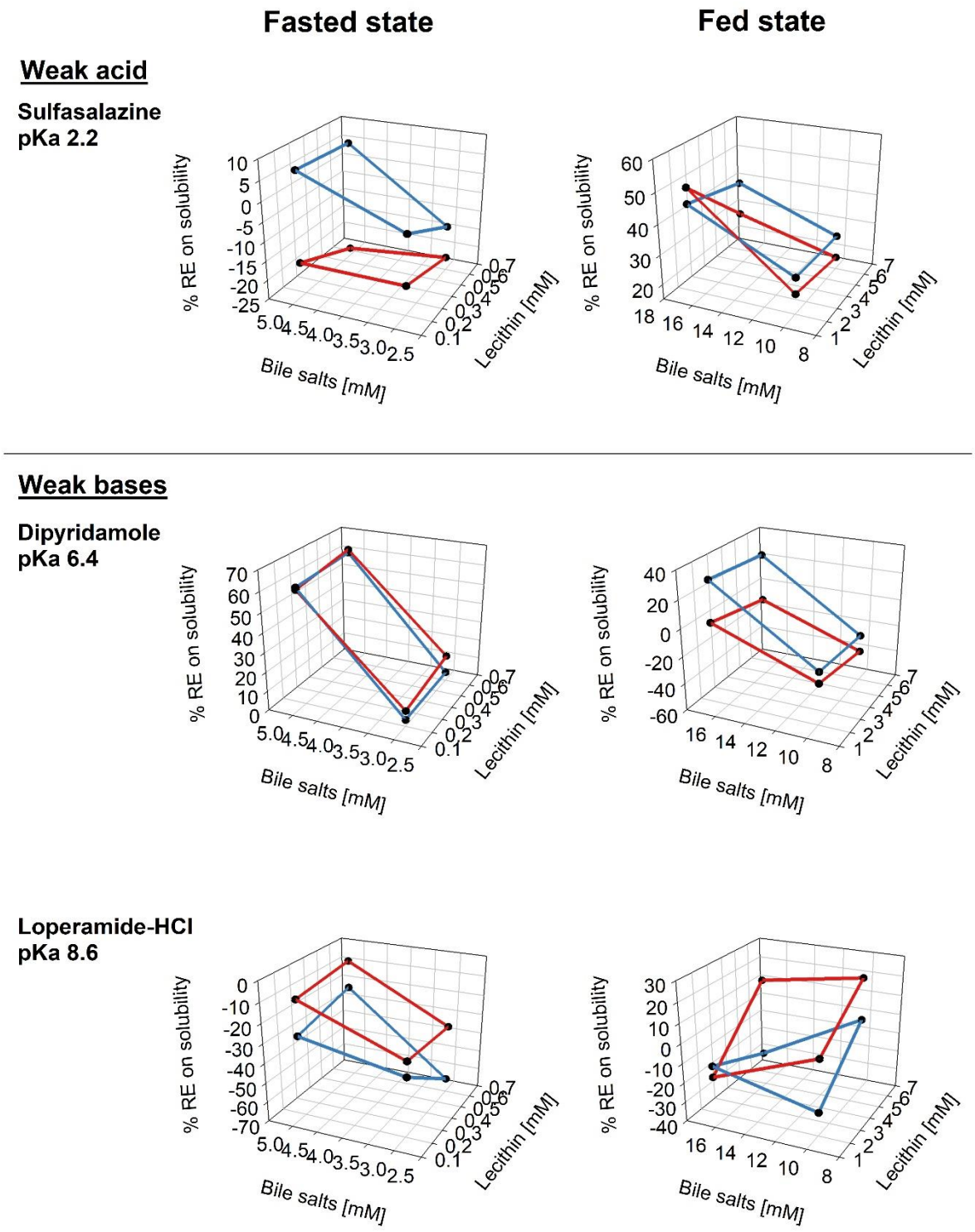


Figure 4.5: % Relative Effect (RE) on the solubility of weak acids and bases in Coeliac disease intestinal biorelevant media compared to the corresponding healthy media according to Design of Experiment (red lines: low concentration of cholesterol, blue lines: high concentration of cholesterol).

4.4.3. Multifactorial statistical analysis of solubility in CED media

For CED fasted state intestinal media, the significant effects and two-factor interactions affecting the drug solubility of the six investigated drugs are presented in Table 4.2.

For azathioprine and budesonide, only the bile salt concentration had a positive impact on their solubility. For celecoxib, the highest positive effect on solubility had the lecithin concentration, followed by a negative effect of cholesterol. Additionally, all two-factor interactions were significant for the solubility of celecoxib but less influential in comparison to both main effects. For dipyridamole, the highest positive impact on its solubility was observed for bile salts. Other significant effects for dipyridamole were a positive effect of lecithin, a negative effect of cholesterol and the interaction between bile salts and cholesterol was significant. Considering loperamide, bile salts showed a positive and cholesterol a negative impact on solubility. For sulfasalazine solubility, a positive effect of cholesterol was observed, followed by a significant interaction of bile salts and cholesterol and a positive effect of the bile salt concentration.

Table 4.2: Significant effects and two-factor interactions in CED fasted state intestinal media.

Main effects/ Interactions	**AZA**	**BUD**	**CEL**	**DIP**	**LOP**	**SSZ**
BS	+ (9.07)	+ (32.52)		+ (1165.22)	+ (10.11)	+ (4.64)
Lec			+ (908.98)	+ (4.70)		
Chol			- (199.47)	- (5.43)	- (4.92)	+ (37.77)
BS/Lec			- (15.79)			
BS/Chol			+ (19.59)	+ (4.82)		+ (6.74)
Lec/Chol			- (141.76)			

+: positive effect, -: negative effect, (F-ratio), BS: bile salts, Lec: lecithin, Chol: cholesterol, AZA: azathioprine, BUD: budesonide, CEL: celecoxib, DIP: dipyridamole, LOP: loperamide, SSZ: sulfasalazine

For CED fed state intestinal media, the significant effects and two-factor interactions with an impact on the drug solubility of all six drugs are shown in Table 4.3.

For azathioprine, the bile salt concentration had the highest positive impact on solubility, followed by a positive impact of cholesterol. Considering budesonide solubility, all three main effects were significant with the highest positive impact of bile salts, followed by a positive impact of lecithin and a negative impact of cholesterol. The two-factor interactions bile salts/cholesterol and lecithin/cholesterol were also significant but less influential compared to the main effects. For celecoxib, the lecithin concentration had the highest positive impact on its solubility, followed by a positive effect of the bile salt concentration. For dipyridamole, bile salts and cholesterol had a positive impact on solubility. Additionally, the interaction of bile salts and cholesterol was significant. Considering loperamide solubility, a negative impact of cholesterol was observed and a smaller positive effect of the lecithin concentration. For sulfasalazine, only the bile salt concentration had a positive impact on its solubility.

Table 4.3: Significant effects and two-factor interactions in CED fed state intestinal media.

Main effects/ interactions	**AZA**	**BUD**	**CEL**	**DIP**	**LOP**	**SSZ**
BS	+ (24.83)	+ (328.11)	+ (6.56)	+ (78.90)		+ (17.47)
Lec		+ (309.56)	+ (62.68)		+ (8.09)	
Chol	+ (6.44)	- (125.57)		+ (23.48)	- (12.55)	
BS/Lec						
BS/Chol		- (6.51)		+ (6.18)		
Lec/Chol		- (4.97)				

+: positive effect, -: negative effect, (F-ratio), BS: bile salts, Lec: lecithin, Chol: cholesterol, AZA: azathioprine, BUD: budesonide, CEL: celecoxib, DIP: dipyridamole, LOP: loperamide, SSZ: sulfasalazine

4.4.4. Drugs at risk of altered solubility in luminal fluids of CED patients

For hydrophilic compounds, only small differences in drug solubility are expected between intestinal fluids of CED patients and healthy subjects as shown by the low impact of CED alterations on azathioprine solubility.

A higher impact of CED on drug solubility is expected for neutral compounds with moderate to high lipophilicity. For these drugs, a higher luminal surfactant concentration (bile salts, lecithin) is expected to result in a higher solubility. It seems to be specific to each drug whether this increase in solubility is mainly driven by bile salts as in the case of budesonide or lecithin as in the case of celecoxib.

A lower risk of altered intestinal solubility in CED is expected for ionisable compounds with moderate to high lipophilicity since drug solubilisation was less impacted by CED changes integrated in the DoE compared to neutral lipophilic compounds.

4.5. Conclusion

In the current study, biorelevant media developed representative for the small intestinal fluid in fasted and fed state of patients with CED showed differences in media properties and drug solubilisation compared to biorelevant media developed based on healthy subjects. In terms of media properties, CED biorelevant media showed different surface tensions with some CED media possessing a higher surface tension in the fasted state, whereas a lower surface tension was observed in some CED media in the fed state. Most likely, this is the result of different concentrations of sodium taurocholate, lecithin and cholesterol resulting in self-assembled structures with a different composition. The different surface tension indicates that the wetting of the surface of a drug or formulation could be altered in CED patients compared to healthy subjects.

In terms of solubility, hydrophilic neutral compounds possess a minimal risk of altered drug solubility in intestinal fluids of patients with CED. Considering neutral compounds with moderate to high lipophilicity, a higher impact of CED on drug solubility is expected with a higher surfactant concentration (bile salts, lecithin) resulting in most cases in a higher drug solubility. The driving factor behind the increase in drug solubility, either the higher bile salt or lecithin concentration, seems to be specific to each drug. For ionisable compounds with moderate to high lipophilicity, drug solubilisation was less impacted by CED differences compared to neutral lipophilic compounds.

To increase the confidence in the developed *in vitro* tool, further studies assessing the luminal fluid composition in patients with CED are needed. Additionally, drug product performance in patients with CED can also be affected by other pathophysiological changes in terms of GI transit time, enzyme and transporter abundance or available absorptive surface area. To also account for these differences in patients with CED compared to healthy subjects, physiologically-based pharmacokinetic (PBPK) models can be used. PBPK models are complex mechanistic models that predict the absorption, distribution, metabolism and elimination of a drug using various physiological information, experimental data and drug characteristics. By using a trial population adapted to the physiology of patients with CED, PBPK models can be used to predict the plasma concentration profile of the investigated drugs in patients with CED. To account for differences in drug dissolution in CED, the results from *in vitro* solubility and dissolution studies using the developed CED biorelevant media can be integrated in PBPK models.

4.6. References

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Chapter 5

Predicting budesonide performance in healthy subjects and patients with Crohn's disease using biorelevant *in vitro* dissolution testing and PBPK modelling

Abstract

Objectives

Drug product performance might be affected in Crohn's disease (CD) patients compared to healthy subjects due to pathophysiological changes. Since a low number of clinical studies is performed in this patient population, physiologically-based pharmacokinetic (PBPK) models with integrated results from biorelevant *in vitro* dissolution studies could be used to assess differences in the bioavailability of drugs. Using this approach, budesonide was used as model drug and its performance in healthy subjects and patients with CD was predicted and compared against observed pharmacokinetic (PK) data.

Key findings

The *in vitro* release tests revealed a similar extent of drug release from a controlled-release budesonide formulation in the fasted state, whereas in the fed state a lower extent was observed. Differences in the physiology of patients with CD were identified in literature and their impact on budesonide performance was investigated with a PBPK model, revealing the highest impact on the simulated bioavailability for the reduced hepatic CYP3A4 enzyme abundance and lower human serum albumin concentration. For patients with CD, a higher budesonide exposure compared to healthy subjects was predicted with a PBPK population adapted to CD physiology and in agreement with observed PK data.

Conclusions

Budesonide performance in the fasted and fed state was successfully predicted in healthy subjects and patients with CD using PBPK modelling and *in vitro* release testing. Following this approach, predictions of the direction and magnitude of changes in bioavailability due to CD could be made for other drugs and guide prescribers to adjust dosage regimens for CD patients accordingly.

5.1. Introduction

Crohn's disease (CD) is, along with ulcerative colitis (UC), one of the main types of inflammatory bowel disease (IBD). It is estimated that 3.7 million persons in Europe are affected by IBD, whereof CD is estimated to affect 1.6 million people.[1] CD is to date a non-curable disease and therefore, lifelong medical treatment adapted to the disease state (relapse or remission) is required.[2] Apart from the treatment for CD, it has been shown that the use of various other medications such as antidepressants, cardiovascular medication or non-steroidal analgesics was increased in IBD patients compared to control subjects.[3]

The oral drug delivery route is important for IBD patients not only for local drug therapy, but also for the medical treatment of concomitant conditions. Since oral drug delivery relies on the physiological conditions in the gastrointestinal (GI) tract, pathophysiological changes could affect drug product performance in patients with CD. In the worst case, the drug release from a formulation can be impeded and the formulation can accumulate in the GI tract, as recently reported for mesalamine pills in the medium ileum of a patient with CD.[4] Reported changes in patients with CD with potential impact on drug product performance include differences in GI transit time, in the composition of GI fluids and in the abundance of metabolizing enzymes.[5] Apart from the GI tract, CD also presents systemic symptoms with potential to alter a drug's pharmacokinetics. Moreover, pharmacodynamic effects can differ between healthy subjects and patients with CD as suggested in the cases of alfentanil and verapamil.[6, 7] This highlights the need to test medications in patients with CD. However, expensive clinical trials are rarely performed in this patient population, especially for drugs for the treatment of concomitant diseases. Hence, the use of *in vitro* and *in silico* tools to identify drugs at risk of altered performance in patients with CD could be a less expensive and time-saving alternative.

Drug release from pharmaceutical formulations and drug dissolution in the GI fluids has been successfully simulated using *in vitro* dissolution testing with the USP IV apparatus (flow-through cell).[8-10] For poorly-soluble compounds, the use of biorelevant dissolution media, simulating closely the GI fluid composition of healthy subjects, resulted in successful predictions of drug absorption.[11-14] This approach has previously been extended by considering pathophysiological differences in patients with CD for the development of CD biorelevant media, which can be used for solubility or dissolution studies (Chapter 2).

The experimentally obtained dissolution profiles can be integrated in physiologically-based pharmacokinetic (PBPK) models. PBPK models are mathematical models that predict a drug's pharmacokinetic plasma profile by integrating various ADME (absorption,

distribution, metabolism, excretion) processes and considering the physiology of the study subject, the physicochemical properties of the drug, *in vitro* or *in silico* ADME information and the trial design. In terms of the physiology of the subjects, it can be accounted for pathophysiological differences by developing a virtual patient population in the PBPK model. This approach has resulted in improved predictions of drug product performance in patients with liver cirrhosis[15], critically septic patients[16], chronic kidney disease[17] and patients after post-bariatric surgery[18, 19] or in the perioperative setting[20].

Budesonide is a locally-acting corticosteroid due to its high ratio of topical to systemic activity and is indicated for the treatment of asthma after pulmonary administration or for the treatment of IBD after oral administration.[21] To deliver budesonide to the affected regions in the GI tract of patients with IBD, available formulations on the market include multi-particulate controlled-release formulations such as Entocort® (Tillotts Pharma UK Ltd., Wellingore, UK) with a triggering pH of 5.5 or Budenofalk (Dr. Falk Pharma, Freiburg, Germany) with a triggering pH of 6.4 and a multimatrix monolithic formulation with time-dependent release (Uceris, Santarus, San Diego, CA, USA).[22] Both multi-particulate formulations aim to deliver budesonide to the terminal ileum and ascending colon, the region most often affected in patients with CD. Budesonide can be grouped as drug with intermediary hepatic extraction ratio ($0.3 < EH < 0.7$), due to its high intestinal extraction ratio of at least 0.50 contributing to the low bioavailability in healthy subjects in the range of 9% in the fasted state and 12% in the fed state.[23, 24] In patients with CD, a higher bioavailability after oral budesonide administration has been reported in several studies.[24-26]

The aim of the present study was to develop a biorelevant *in vitro* dissolution method and a PBPK model to predict the performance of budesonide in healthy subjects and patients with CD. Therefore, the release of budesonide from the controlled-release formulation Entocort® was simulated with the USP IV dissolution apparatus in healthy and CD conditions. A PBPK model for budesonide was developed for healthy subjects considering intravenous and oral administration. The effect of pathophysiological differences in patients with CD on budesonide performance was investigated with parameter sensitivity analysis (PSA) and a population representative of patients with CD was developed and used to predict budesonide performance in this patient population.

5.2. Materials

Acetic acid High Performance Liquid Chromatography (HPLC) grade, pepsin from porcine gastric mucosa, sodium oleate, α-D-glucose, budesonide and sodium hydroxide were

purchased from Sigma-Aldrich Company Ltd., Dorset, England. Methanol HPLC grade, acetonitrile HPLC grade and cholic acid sodium salt were purchased from VWR International Ltd, Lutterworth, UK. Tris(hydroxymethyl)aminomethane, hydrochloric acid 36.5–38.0%, 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. Entocort® CR 3 mg capsules were used from Tillotts Pharma UK Ltd., Wellingore, UK.

5.3. Methods

5.3.1. *In vitro* release tests

A flow-through cell dissolution apparatus (Sotax CE7 smart, Sotax, Aesch, Switzerland), equipped with cells with a diameter of 22.6 mm and connected to a piston pump (Sotax CP7, Sotax, Aesch, Switzerland), was used in open mode and maintained at a temperature of 37ºC. A 5 mm rubi bead was placed at the bottom of the cell, followed by 6 g glass beads with a diameter of 1 mm to fill the conical part of the cell. The investigated capsules were placed on top of the glass beads and a tablet holder was reversely positioned to avoid the floating of the capsules. Glass fibre filters (GF/D with a diameter of 2.7 μm and GF/F with a diameter of 0.7 μm) were placed on top of the cell. Different setups were used to simulate the fasted and fed state and healthy and CD conditions (Sections 5.3.1.1 and 5.3.1.2). Samples were collected every 30 min and analysed by HPLC-Ultraviolet (UV). All experiments were performed in triplicate.

5.3.1.1. Healthy conditions

5.3.1.1.1. Biorelevant media

For healthy subjects, the passage through the GI tract was simulated with biorelevant media in the fasted state [Fasted-State Simulated Gastric Fluid (FaSSGF), Fasted-State Simulated Intestinal Fluid-Version 2 (FaSSIF-V2) and Fasted-State Simulated Colonic Fluid (FaSSCoF)] and fed state [Fed-State Simulated Gastric Fluid (FeSSGF), Fed-State Simulated Intestinal Fluid-Version 2 (FeSSIF-V2) and Fed-State Simulated Colonic Fluid (FeSSCoF)], mimicking closely the composition and properties of the gastric fluid, the small intestinal fluid and the ascending colon fluid, respectively.[27-30] Biorelevant media were prepared as previously described.[28,30]

5.3.1.1.2. Hydrodynamics

GI hydrodynamics are expressed in the USP IV apparatus by the flow rate and the duration of exposure to the specific dissolution medium. For healthy conditions, the hydrodynamics were defined based on a previously published method, confirmed with literature data of the GI passage of Entocort® (measured with scintigraphy) and modified according to recent literature data on gastric fluid volumes.[8, 9, 24, 31, 32] An overview of the experimental setup is given in Table 5.1.

Table 5.1: Healthy experimental conditions for *in vitro* release tests with the USP IV dissolution apparatus.

	Fasted state			**Fed state**		
GI compartment	**Medium**	**Time from start [min]**	**Flow rate [mL/min]**	**Medium**	**Time from start [min]**	**Flow rate [mL/min]**
Stomach	FaSSGF	0-60	6	FeSSGF	0-120	6
Small intestine	FaSSIF-V2	60-270	4	FeSSIF-V2	120-330	6
Colon	FaSSCoF	270-420	4	FeSSCoF	330-450	4

5.3.1.2. CD conditions

5.3.1.2.1. Biorelevant media

To reflect the differences in the composition of the GI fluids of patients with CD compared to healthy subjects, previously developed biorelevant media adapted to CD conditions were used for the *in vitro* release tests and prepared using the same method as for healthy biorelevant media (Chapter 2). Since the development of CD biorelevant media followed a Design of Experiment (DoE) approach to reflect interindividual variability, several media have been developed for one prandial state and GI compartment with different levels of the investigated factors in the DoE. For the gastric fasted state CD media, the integrated factors were the pH and bile salt/lecithin concentration. For the fasted and fed state intestinal CD media, only the bile salt/lecithin concentration was used as factor. Considering the fasted and fed state colonic CD media, the osmolality and bile salt/lecithin concentration were integrated in the DoE. Details of the CD biorelevant media with integrated factors and their two levels, low and high, are presented in Table 5.2. For the current study, two different approaches were selected with one approach including all CD media with the low level of all factors and one approach with the high level of all factors.

Table 5.2: CD biorelevant media used for *in vitro* release studies.

		Factor	Low level	High level
Fasted state	Stomach	Bile salt/lecithin concentration [mM]	0.035/0.008	0.08/0.02
		pH	1.5	4.1
	Intestine	Bile salt/lecithin concentration [mM]	1.29/0.09	3.00/0.20
	Colon	Bile salt/lecithin concentration [mM]	0.07/0.13	0.15/0.30
		Osmolality [mOsm/kg]	196	278
Fed state	Intestine	Bile salt/lecithin concentration [mM]	4.30/0.86	10.00/2.00
	Colon	Bile salt/lecithin concentration [mM]	0.26/0.22	0.60/0.50
		Osmolality [mOsm/kg]	207	294

5.3.1.2.2. Hydrodynamics

The experimental setup simulating conditions in patients with CD in the fasted and fed state is described in Table 5.3. In the fasted state, the time in the gastric compartment was reduced to 0.5 h as reported *in vivo* and the flow rate was increased to 12 mL/min, since no difference in gastric volume has been reported.[34] The small intestinal phase was increased by 0.5 h in CD conditions compared to healthy conditions as indicated for active disease state.[33] For the colonic phase, no adjustments to the healthy setup were made considering the hydrodynamics.

In the fed state, the time in the gastric compartment was prolonged by 1.0 h according to *in vivo* data and the flow rate was reduced to 4 mL/min, since no difference in gastric fluid volume is expected.[24] For the small intestinal phase, the time was reduced by 0.5 h as reported *in vivo*.[24] For the colonic phase, the same hydrodynamics as in healthy conditions were used.

Table 5.3: Crohn's disease experimental conditions for *in vitro* release tests with the USP IV apparatus.

	Fasted state			
GI compartment	**Medium**	**Time from start [min]**	**Flow rate [mL/min]**	***In vivo* transit times [h]**
Stomach	CD-FaSSGF 1) Low level 2) High level	0 - 30	12	0.6 [34]
Small intestine	CD-FaSSIF 1) Low level 2) High level	30 - 300	4	5.6[34], 4.2 (active CD)/ 3.1(inactive CD) [Healthy 3.6][33]
Colon	CD-FaSSCoF 1) Low level 2) High level	300 - 450	4	-
	Fed state			
GI compartment	**Medium**	**Time from start [min]**	**Flow rate [mL/min]**	***In vivo* transit times [h]**
Stomach	FeSSGF	0 - 180	4	4.0 [Healthy 3.0][24]
Small intestine	CD-FeSSIF 1) Low level 2) High level	180 - 360	6	2.4 [Healthy 3.0][24]
Colon	CD-FeSSCoF 1) Low level 2) High level	360 - 480	4	8.1 [Healthy 15.5][24]

5.3.1.3. HPLC-UV analysis of budesonide

The HPLC-UV analysis was performed with an Agilent Technologies 1200 series equipped with a binary pump (G1312A), a diode-array detector (G1315D), an autosampler (G1329A) and a controller (G1316A) [Agilent Technologies, Santa Clara, US]. A Waters Spherisorb ODS2 C18 column (250 mm x 4.6 mm, 5 µm) was used and set to a temperature of 25ºC. An isocratic method with a mobile phase consisting of 0.1% acetic acid in water:methanol (25:75, V/V) and a flow rate of 1 mL/min was applied. The injection volume was 100 µL and the detection wavelength was set to 245 nm. The limit of detection and quantification were 46 ng/mL and 138 ng/mL, respectively.

5.3.2. PBPK model development

A PBPK model for budesonide was developed for healthy subjects and CD patients according to the workflow described in Figure 5.1.

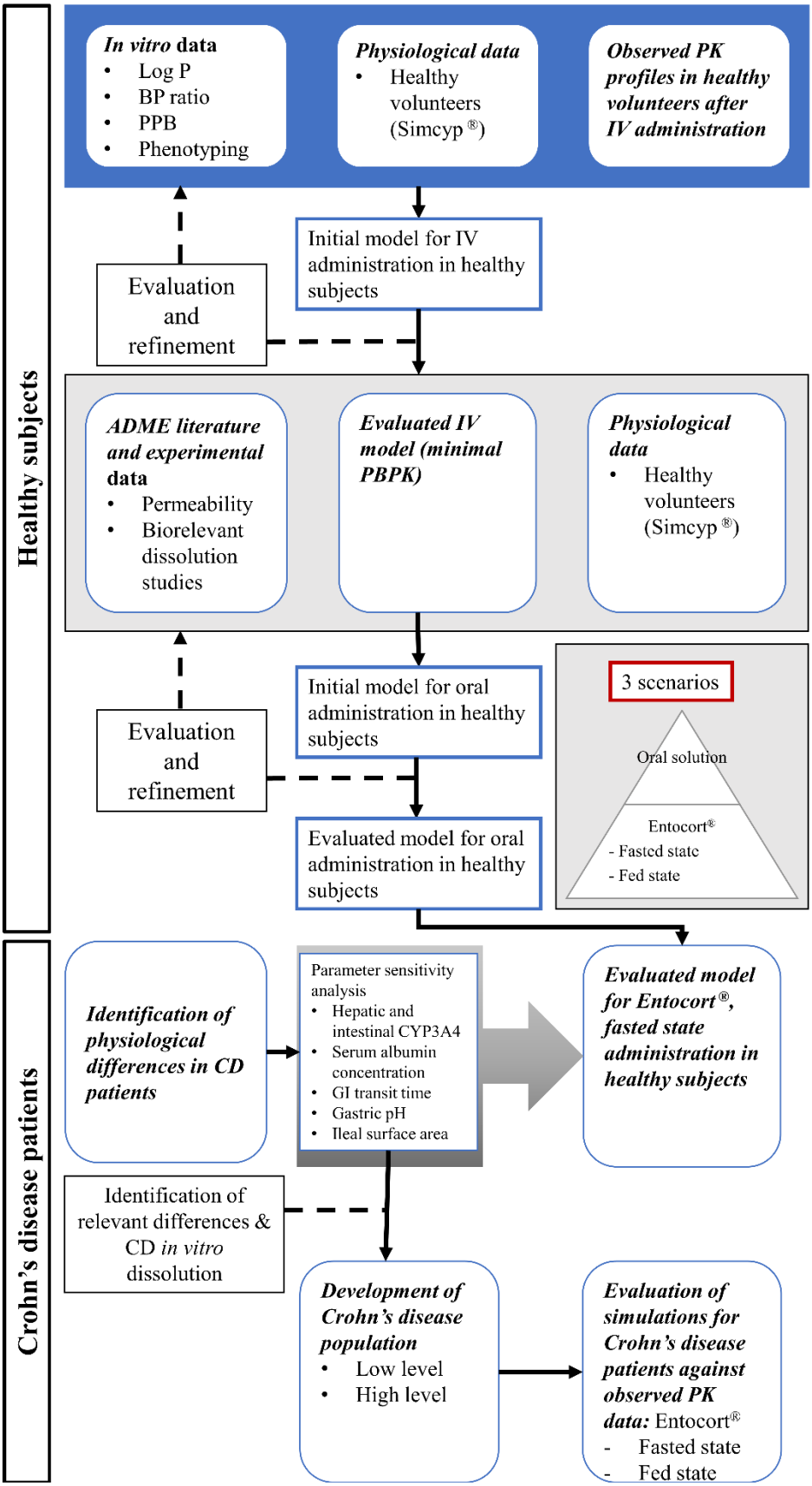

Figure 5.1: Schematic workflow for the development of a PBPK model for budesonide in healthy subjects and patients with CD (BP: blood plasma, PPB: plasma protein binding, IV: intravenous).

5.3.2.1. PBPK model development in healthy subjects

The Simcyp® Simulator Version 17 (Certara, Sheffield, UK) was used to develop a PBPK model for budesonide using drug-specific, anatomic and physiological information. Table 5.4 gives an overview of the input parameters derived from literature.

Budesonide-specific information includes drug physicochemical properties, plasma protein binding and blood plasma ratio. For the disposition model of budesonide, a minimal PBPK model with a single non-physiological adjusting compartment, representing all tissues except the liver and portal vein, was used. The disposition model for healthy subjects was developed based on fitting of parameters (intravenous clearance, volume of distribution at steady state (V_{SS}), volume of single adjusting compartment (V_{SAC}), input rate (k_{in}) and output rate (k_{out})) to previously published pharmacokinetic data after intravenous administration of budesonide.[35] The intravenous clearance was further integrated mechanistically as intrinsic enzymatic clearance using the retrograde model within the Simcyp® simulator and literature data from CYP phenotyping experiments.[36] Due to the high contribution of CYP3A4 to the budesonide clearance, the gender differences for the hepatic CYP3A4 abundance were taken into account in Simcyp®. For oral budesonide administration, the advanced dissolution, absorption and metabolism (ADAM) model was used. To account for transit time differences between different types of formulations, the segregated transit time model was selected. For the simulations for Entocort®, even though the formulation is composed of multiparticulate units, the GI transit time for a controlled-release monolithic formulations was selected due to a software issue and the similarity of these transit times to previously published transit times for Entocort®.[24] For the input of dissolution/release data, the experimental data was fitted to the following Weibull function using DDSolver

$$F=100*(1-Exp((t-tlag)^\beta/\alpha)) \quad (5.1)$$

where F is the percentage of drug released, t is time, t_{lag} is lag time, β is a shape parameter and α is a scale parameter.[37] Two different dissolution input options were evaluated: Option 1. The input of the release profile as Weibull function with substitution of the lag time with a triggering pH; Option 2. The input of the release profile as discrete *in vitro* dissolution profile (after the last experimental time point extrapolation up to 15 h was performed based on the fitted Weibull function).

Considering the permeability input, budesonide permeability was calculated in a previous study based on pharmacokinetic data after regional budesonide administration in the gut

(jejunum, ileum, colon) with concomitant administration of ketoconazole to inhibit gut wall metabolism, deconvolution of pharmacokinetic data after intravenous budesonide administration and information about intestinal surface area.[23, 38] The retrieved data was integrated in the ADAM model as regional permeability (Table 5.4).

In terms of physiology, all simulations for healthy subjects were performed with the healthy volunteer population model of the Simcyp® simulator. For the trial design, all simulations were performed with 10 trials and 10 subjects in each trial. The minimum and maximum age of the trial population and the percentage of females was adjusted according to the study population in the pharmacokinetic trials used for validation. Based on a correlated Monte-Carlo approach, realistic virtual subjects were generated within the Simcyp® simulator based on demographic information (e.g., age is linked to height, body weight and body surface area and organ volumes are correlated with body size).

Table 5.4: Input parameters for budesonide PBPK model.

Parameter	Unit	Input	Reference
Compound type		Neutral	
B/P		0.80	[21]
Fraction unbound (PPB%)		0.15 (85-90%)	[21]
log P		2.62	[39]
Mw	g/mol	430.50	
Compound type		neutral	
K_{in}	1/h	10.49[1]	[35]
K_{out}	1/h	1.65[1]	
V_{ss}	L/kg	3.20[1]	
V_{sac}	L/kg	3.01[1]	
CL IV	L/h	63.00[1]	
CLint CYP1A2	µL/min/pmol of isoform	1.18[2]	[36]
CLint CYP2C9	µL/min/pmol of isoform	0.21[2]	
CLint CYP3A4	µL/min/pmol of isoform	4.42[2]	
Additional CL, human liver microsomes	µL/min/mg protein	84.26[2]	
Degradation rate constant in colon	1/h	0.65	[40]
Difference in male/female CYP3A4 abundance		Option activated	
Permeability: Peff, man			
Duodenum	10^{-4} cm/s	1.90	[38]
Jejunum I	10^{-4} cm/s	1.90	
Jejunum II	10^{-4} cm/s	1.90	
Ileum I	10^{-4} cm/s	3.40	
Ileum II	10^{-4} cm/s	3.40	
Ileum III	10^{-4} cm/s	3.40	
Ileum IV	10^{-4} cm/s	3.40	
Colon	10^{-4} cm/s	0.59	

[1]Parameter fitted to observed PK data
[2]Calculated with retrograde model enzyme kinetics using the percentage of enzymatic contribution from published reactive CYP 450 phenotyping experiments and the clearance after intravenous administration of budesonide

5.3.2.2. Pathophysiological differences in CD patients

A literature search was carried out to quantify major physiological and anatomical changes in patients with CD compared to healthy subjects. In the following sections, the identified key differences are presented and used to develop a CD population as input for the PBPK model.

5.3.2.2.1. Hepatic and intestinal CYP3A4

Differences in hepatic and intestinal CYP3A4 activity were observed in patients with CD compared to healthy subjects by comparison of the hepatic and intestinal extraction ratio of midazolam, a CYP3A4 substrate.[25] Since no direct control group was included in the study with CD patients, the hepatic and intestinal extraction ratio (ER) of midazolam in CD patients (hepatic ER 0.11, intestinal ER 0.64) was compared to several published studies in healthy subjects (hepatic ER 0.36-0.44, intestinal ER 0.43-0.70).[25, 41-44] The relative difference of the ER in CD patients compared to healthy subjects varies depending on the chosen reference study. Therefore, a range (comparison to study with healthy subjects with lowest and highest value) was used to reflect the lowest and highest impact. Hence, the hepatic CYP3A4 activity is estimated to be decreased in patients with CD to 25 to 31% of the healthy value and the intestinal activity to be in the range of 91 to 149%. For the simulations, this ratio was used to adjust the enzyme abundance by multiplying the ratio with the healthy enzyme abundance given in the Simcyp® simulator according to $ER_{CD}/ER_{Healthy}$ x Enzyme $abundance_{Healthy}$ (hepatic CYP3A4 in females 183 pmol/mg protein, hepatic CYP3A4 in males 126 pmol/mg protein, intestinal CYP3A4 66.2 nmol/small intestine).

Limitations of this approach are firstly, that it is assumed that the ratio of intestinal or hepatic ER in CD patients compared to healthy subjects is similar to the proportion of their intestinal or hepatic CYP3A4 abundance, respectively. Secondly, the intestinal ER is determined based on intravenous and oral administration of midazolam. Therefore, it is assumed that the extrahepatic metabolism has a negligible contribution to the overall clearance after intravenous administration of midazolam and other factors contributing to differential estimates of the fraction metabolised in the gut (e.g, differences in fraction absorbed, blood:plasma ratio, hepatic blood flow) are not considered.[45]

5.3.2.2.2. Human serum albumin

The concentration of human serum albumin (HSA) in patients with CD has been reported between 28.0 g/L and 41.0 g/L as shown in Table 5.5. The normal range of HSA is 35.0-55.0 g/L and therefore, some CD patients are hypoalbuminemic.[46] In 17.6% of inflammatory bowel disease patients the HSA concentration was below the normal range and median serum levels of albumin were significantly lower in patients with active disease compared to patients in remission.[47] Reduced HSA concentrations are likely to be the result of the inflammation processes during which albumin synthesis is decreased, albumin catabolism is increased and albumin is lost from the vascular compartment due to an increased vascular

permeability.[48] Additionally, an increased amount of albumin can be lost through the damaged tissue in the GI tract in IBD patients and malnutrition can contribute to a lower HSA concentration.[49]

Table 5.5: Human serum albumin concentrations in CD patients (mean ± SD).

	Female CD patients	**Male CD patients**	**Reference**
Human serum albumin [g/L]	27.7 ± 6.0	31.7 ± 4.6	[50]
	38.1 ± 5.1		[47]
	41.0 ± 5.0 (low-grade inflammation) 33.0 ± 6.0 (severe inflammation)		[51]
	40.0 ± 8.0		[52]

5.3.2.2.3. Gastric pH

The pH range in CD patients has been reported between pH 1.5 to 4.1, as observed in two studies.[53, 54] Additionally, gastric acid secretion (mean basal acid output and maximal acid output) was lower in CD patients compared to healthy subjects, especially when patients were in a malnourished state.[55] Gastric acid-reducing agents such as proton pump inhibitors are an additional risk factor for increased gastric pH and are commonly prescribed in patients with IBD.[56]

5.3.2.2.4. GI transit time

Considering GI transit times, only a limited amount of studies has investigated the GI transit time in the fasted and fed state in patients with CD.[5] It has previously been shown that small intestinal transit time in patients with CD varied according to disease state.[33] Additionally, diarrhoea caused by inflammatory and non-inflammatory mechanisms is a frequent symptom in patients with CD.[57]

5.3.2.2.5. Absorptive surface area in the ileum

The available absorptive surface area is likely to be reduced in CD due to ulcerated and inflamed parts of the GI wall. The extent will be highly dependent on the location of the disease in the individual patient and the disease severity. In CD patients, the terminal ileum is the most commonly affected area.[58] For individual patients, the Simple Endoscopic Score

for Crohn's Disease (SES-CD) can give an indication about the affected area since the extent of ulcerated surface (none = 0; <10% =1; 10%–30% =2; >30% =3) and the extent of affected surface (none = 0; <50% =1; 50–75% =2; >75% =3) are two of four endoscopic variables considered for the classification in each bowel segment separately (ileum, right/transverse/left colon, and rectum).[59]

5.3.2.3. Parameter sensitivity analysis

Automated PSA was used to estimate the impact of the identified pathophysiological differences in patients with CD compared to healthy subjects (Section 5.3.2.2) on the predicted PK parameters C_{max} and AUC. As basis for the PSAs, the simulation after oral administration of Entocort® in the fasted state in healthy subjects using dissolution input option 1 with triggering pH and Weibull function was used. Considering hepatic CYP3A4 abundance, the investigated range for PSA included the abundance in the healthy volunteer population as highest value and the lowest value was based on the percental approach (25%) described in Section 5.3.2.2.1. The percental approach was also used to define the investigated range for the intestinal CYP3A4 abundance (91-149%). For the human serum albumin concentration, the investigated range included the lowest value observed in patients with CD up to the value in the healthy volunteer population. Additional parameters were investigated, when literature information was limited in patients with CD with the aim to identify their risk of altering the performance of budesonide. Therefore, the gastric pH in the range of 1.0 to 7.5, the gastric residence time in the range of 0.5 to 2.5 h, the small intestinal transit time in the range of 3.0 to 6.0 h and the ileal surface area in the range of 16% to 100% were investigated. PSA served to identify the most relevant pathophysiological differences in CD impacting on budesonide pharmacokinetics.

To investigate the effect of a reduced available absorptive surface area in the ileum on the simulation results, the permeability input was changed to the MechPeff model and the intrinsic transcellular permeability was adjusted to $22*10^{-6}$ cm/s in order to match the regional effective permeability of the jejunum I defined for the initial PBPK model. Subsequently, the plicae circulares fold expansion was adjusted to match the regional effective permeability values of the initial model in the remaining GI compartments (duodenum and jejunum: 1.97, ileum: 6.4, colon: 1.6). A reduction in available absorptive surface area was subsequently investigated with PSA by stepwise reducing the plicae circulares fold expansion in each of the four parts of the ileum.

5.3.2.4. Development of budesonide PBPK model for CD patients

The pathophysiological key differences in CD patients compared to healthy subjects were used to define a CD population for the PBPK model. The healthy volunteer population was used as basis and modifications were made to reflect the main differences as identified with PSA. The considered differences in the CD population were a reduced hepatic CYP3A4 abundance, an altered intestinal CYP3A4 abundance, a reduced human serum albumin concentration and a different dissolution/release input according to the CD conditions described in Section 5.3.1.2. To account for the variability observed in CD patients, two different CD populations were defined with the CD low level population reflecting the low level for each parameter and the CD high level population reflecting the high level for each parameter, respectively. An overview of the CD populations and the defined range for each parameter is presented in Table 5.6.

Table 5.6: Development of CD population in the PBPK model.

	CD Low level	CD High level	Healthy population
Hepatic CYP3A4, male [pmol/mg protein]	31.50	38.49	126.00
Hepatic CYP3A4, female [pmol/mg protein]	45.75	55.91	183.00
Intestinal CYP3A4 [nmol/SI]	60.53	98.53	66.20
HSA, male [g/L]	31.72	41.00	50.34
HSA, female [g/L]	27.70	41.00	49.38
Dissolution input	Profile in CD biorelevant media with low levels of all factors	Profile in CD biorelevant media with high levels of all factors	

HSA: Human serum albumin

5.3.2.5. Validation of budesonide PBPK model

5.3.2.5.1. Treatment of *in vivo* PK data

For the evaluation of the simulations, data from various PK studies was extracted graphically using WebPlotDigitizer Version 4.2[60] and PK parameters were derived from non-compartmental analysis (NCA) using PKsolver.[61] An overview of the PK literature data is given in Table 5.7. For studies where the budesonide plasma concentration-time profile was not presented, reported parameters were only used for discussion but excluded from the PK parameter mean values used for simulation validation. For Entocort® simulations, several published PK studies with different doses were used for model validation and therefore, PK parameters were normalised to a dose of 3 mg. In order to compare the simulations against observed PK data, the mean values of all studies with PK profiles weighed according to the number of subjects in each study were used. The mean C_{max}, $AUC_{0\text{-}inf}$ and T_{max} used for the validation of Entocort® administration in healthy subjects and CD patients in the fasted state and in the fed state are presented in Table 5.7.

5.3.2.5.1.1. External validation

Predicted plasma concentration profiles were visually assessed against observed PK profiles. In addition, the predictive performance of the simulations was assessed using the ratio of predicted to observed C_{max} and $AUC_{0\text{-}inf}$. For external validation (PK data not used in the model building process), simulations were considered successful when the ratio was within a 2-fold range.

5.3.2.5.1.2. Internal validation

For internal validation (observed PK data used for model development), a tighter criterion was set for the ratio of predicted to observed C_{max} and $AUC_{0\text{-}inf}$ corresponding to the bioequivalence range of 0.8-1.25.

Table 5.7: Pharmacokinetic data used for the evaluation of the PBPK model of budesonide.

Details	Dose [mg]	Number of subjects (M/F)	C_{max} [µg/L] (/[1])	AUC_{0-t} [µg/L*h]	AUC_{0-inf} [µg/L*h] (/[1])	T_{max} [h]	Reference
Healthy subjects, IV administration	0.1	4 (4/0)	2.23/22.3	1.54	1.69/16.9	0.08	[35]
Healthy subjects, oral solution	3.0	12 (6/6)	0.92/0.31	2.82 (12 h)	2.84/0.95	0.68	[62]
Healthy subjects, Entocort®, fasted state	18.3	8 (8/0)	2.55/0.14	28.16	32.68/1.79	2.95	[24]
	9.0	13 (6/7)	1.80/0.20	11.37 (12 h)	13.03/1.45	3.02	[63]
	4.5	6 (6/0)	0.95/0.21	8.06	8.38/1.86	2.74	[31]
	9.0	12 (6/6)	1.33/0.15	14.46	15.26/1.70	4.90	[64]
	3.0	8 (8/0)	0.50/0.17	5.27	5.57/1.86	5.00	[65]
	4.5	20 (0/20)	0.60/0.13	5.66	5.95/1.32	6.10	[66]
	3.0	8 (8/0)	0.55/0.18	5.06	5.91/1.97	5.02	[67]
	3.0	13 (5/8)	0.76/0.25	5.59	6.21/2.07	1.50	[68]
	9.0	13 (5/8)	1.61/0.18	16.64	17.34/1.93	1.50	[68]
	15.0	13 (5/8)	3.05/0.20	25.56	27.01/1.80	3.00	[68]
	Mean		**0.18**		**1.73**	**3.57**	
Healthy subjects, Entocort®, fed state	4.5	6 (6/0)	1.09/0.24	7.95 (24 h)	8.27/1.84	4.64	[31]
	17.9	8 (8/0)	3.49/0.19	29.65 (30 h)	33.85/1.89	6.04	[24]
	Mean		**0.21**		**1.87**	**5.34**	
CD, Entocort®, fasted state	various	8 (1/7)	/0.24	/1.90	/2.30	2.95	[25]
	9.0	8 (4/4)	1.86/0.21	11.74	12.86/1.43	3.95	[69]
	9.0	6 (5/1)	1.38/0.15	16.01	18.34/2.04	4.00	[70]
	Mean		**0.20**		**1.91**	**3.63**	
CD, Entocort®, fed state	9.0	8 (4/4)	1.30/0.14	9.97 (12 h)	16.66/1.85	6.96	[69]
	18.0	8 (8/0)	6.99/0.39	54.47	54.54/3.03	6.06	[24]
	Mean		**0.27**		**2.44**	**6.51**	
CD, Entocort®, -, single administration*	4.5	18 (-)	1.77/0.39	-	12.27/2.73	-	[26]
CD, Entocort®, -, repeated administration*	4.5	18 (-)	1.38/0.31	-	9.08/2.02	-	[26]

M: male, F; female

*No budesonide plasma concentration profiles, PK values are reported as in publication and data was only used for discussion

[1]Normalised to a dose of 3 mg

5.4. Results and discussion

5.4.1. *In vitro* release studies

The *in vitro* release profiles of Entocort® in healthy and CD conditions in the fasted and fed state are shown in Figure 5.2. As expected, no drug release was observed in both prandial states, when Entocort® was exposed to the gastric media due to the formulation's triggering pH of 5.5 exceeding the pH of all gastric media.

In the fasted state, the onset of drug release in healthy conditions was delayed by 0.5 h compared to CD conditions due to the prolonged time of the formulation being exposed to FaSSGF. For CD low level and high level conditions, similar *in vitro* release profiles were observed. After the onset of drug release, the dissolution rate in CD conditions was slightly lower compared to healthy conditions resulting in a similar drug release between healthy and CD conditions starting from 2 h until the end of the experiment.

In the fed state, the longer gastric residence time resulted in a delayed release of budesonide in CD conditions. After the media change to small intestinal conditions, the rate of drug release was similar between healthy and CD conditions with high level media, while a lower rate was observed for CD conditions with low level media. In contrast, when changing to the colonic media, the budesonide release was faster in CD conditions with low level media compared to both other setups. This could be due to the high percentage of budesonide already released in the other two setups (healthy conditions 88%, CD high level 75% compared to 63% CD low level) or the different media composition. At the end of the dissolution experiment, 94% of the budesonide dose were released after 7.5 h in healthy conditions, while only 76% and 82% of budesonide were released after 8.0 h in CD low level and high level conditions, respectively. By comparing fasted and fed state conditions, a longer lag time due to the prolonged time in stomach conditions was observed but the subsequent drug release rate in the intestinal compartment was higher in the fed state.

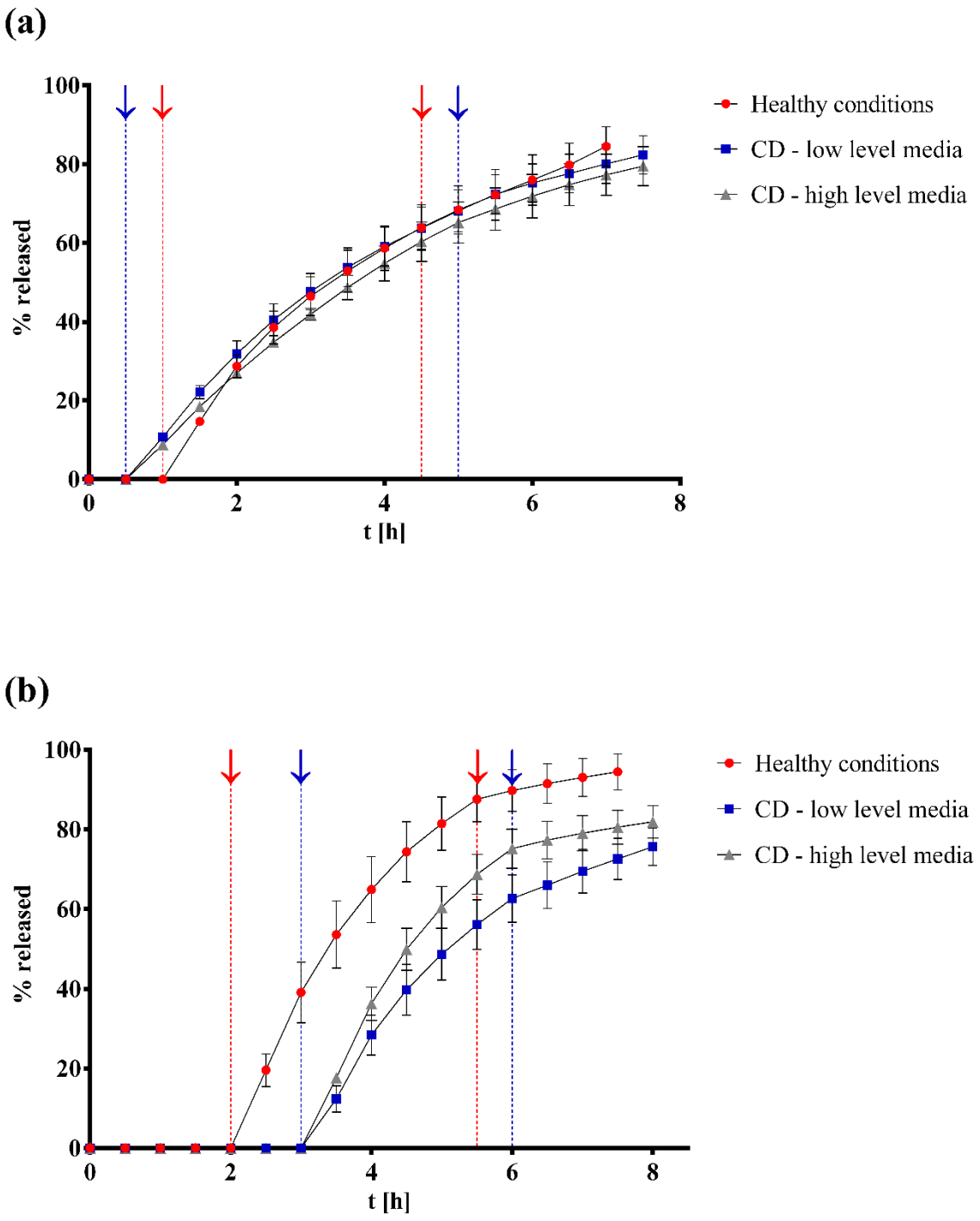

Figure 5.2: *In vitro* release of Entocort® in healthy and CD conditions in (a) the fasted state and (b) the fed state (Red arrows indicate the media change in healthy conditions and blue arrows in CD conditions, respectively).

5.4.2. PBPK predictions for healthy subjects

5.4.2.1. Intravenous administration

The performance of the PBPK model considering the disposition of budesonide was assessed by simulating the administration of 100 µg budesonide given as intravenous infusion over 5 min. The disposition of budesonide was successfully simulated as shown in Figure 5.3 and predicted PK parameters were within the predefined range set for internal model verification.

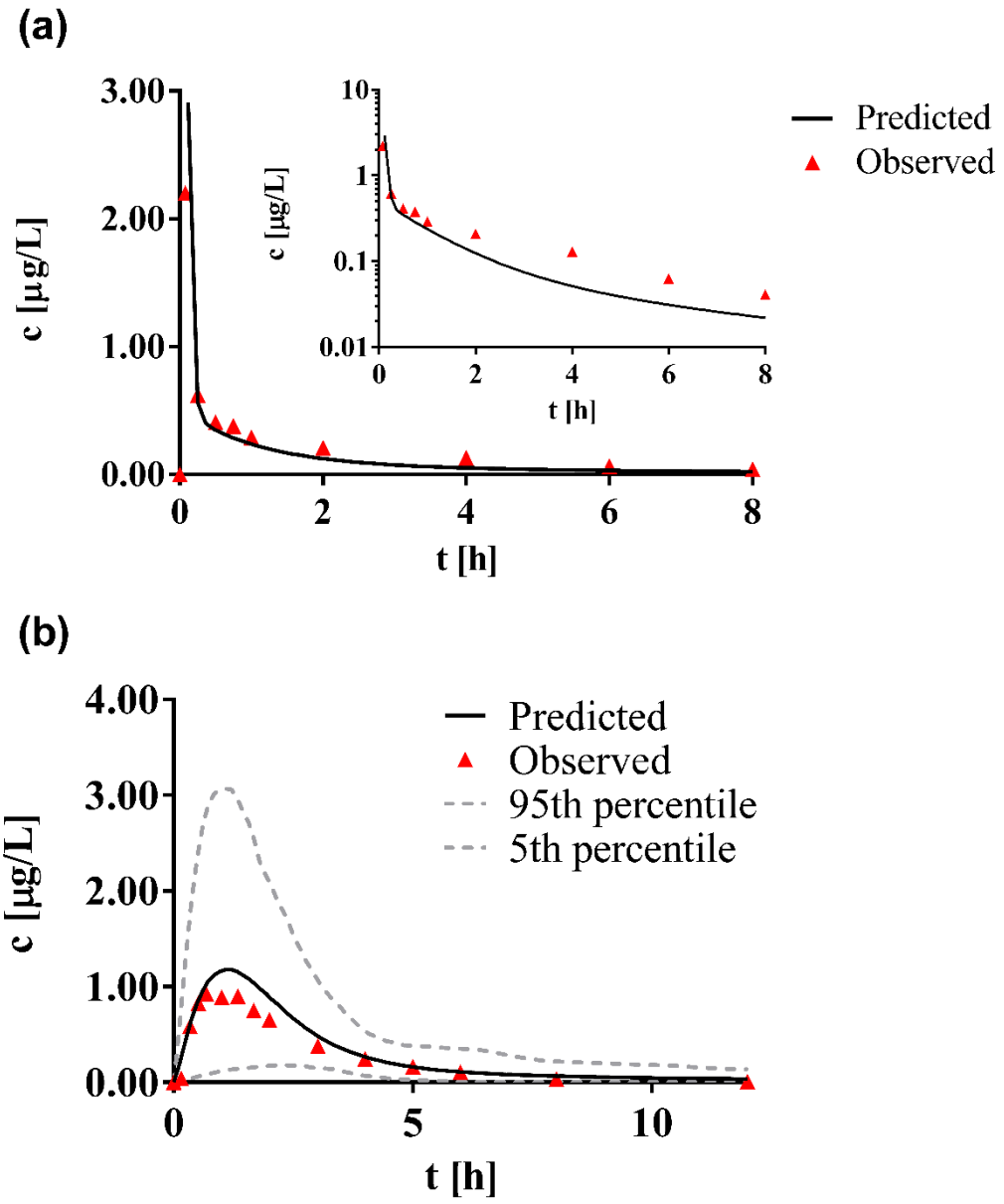

Figure 5.3: Prediction of systemic budesonide concentration in healthy subjects and observed PK profiles (a) after intravenous administration of 0.1 mg budesonide, and (b) after administration of an oral solution of 3 mg budesonide.[35, 62]

5.4.2.2. Oral administration in the fasted state

Considering oral administration, the PBPK model was first used to simulate budesonide exposure after administration of an oral solution and externally validated against observed PK data as illustrated in Figure 5.3.[62] Despite a slight overprediction of C_{max}, AUC and T_{max}, the predicted PK parameters were within the 2-fold criterion set for model verification as shown in Table 5.8. Since budesonide exposure after administration of an oral solution is independent of drug release and dissolution, intestinal permeability and gut metabolism were adequately reflected in the PBPK model. The prediction revealed an apparent fraction absorbed of 95%, whereof the main part (49%) was absorbed in the jejunum, which was consistent with complete budesonide absorption reported in literature.[71] Additionally, the predicted fraction of budesonide metabolised in the gut was 43%, whereof the metabolism in the jejunum contributed to 53%. A slightly higher gut extraction ratio of at least 0.50 has previously been indicated by the pre-systemic elimination of budesonide after local administration in the intestine with and without the local inhibition of gut wall metabolism.[23]

For simulations for the controlled-release formulation Entocort®, the results of the *in vitro* release studies were integrated in the PBPK model to predict the dissolved budesonide available for intestinal absorption. Simulations of Entocort® administration (3 mg dose) in healthy subjects using two different dissolution input options (option 1 and 2) are shown in Figure 5.4 and were compared against PK studies presented in Table 5.7. The PK parameters of both simulations met the 2-fold criterion set for external validation (Table 5.8) and both simulations were similar in their performance.

When comparing the two dissolution input options, the main difference relates to a 41% higher C_{max} for option 2 compared to option 1, while $AUC_{0\text{-}inf}$ was only 2% higher. The mean observed C_{max} was approximately in the middle of both simulated C_{max}. The limitation of the discrete dissolution input (option 2) is that it only accounts for experimental variability in the *in vitro* release experiment. In contrast, intersubject variability in terms of GI pH is considered when option 1 is used. In this case, the onset of drug release from the Entocort® formulation depends on the virtual subject's GI pH and indirectly also on its GI transit times. This is reflected in the 5^{th} and 95^{th} percentiles of the simulations with option 1 showing a higher variability for the onset of budesonide absorption.

In comparison to the simulation for the oral solution, the apparent fraction absorbed of budesonide is lower with 68-70% for Entocort® compared to 95% for the oral solution, whereof a higher percentage of 63-64% vs 39% is absorbed in the ileum. This is in agreement

with a previous study showing an increased budesonide delivery to the ileo-colonic region for Entocort® compared to an immediate-release formulation.[24]

Intersubject variability was evaluated based on the comparison of the simulation with observed single subject PK profiles (Figure 5.4).[64] Considering the 90% confidence interval of the Entocort® simulation, only one out of 12 subjects fell outside the 5th and 95th percentiles (dissolution option 1) indicating that interpatient variability is satisfactorily captured.

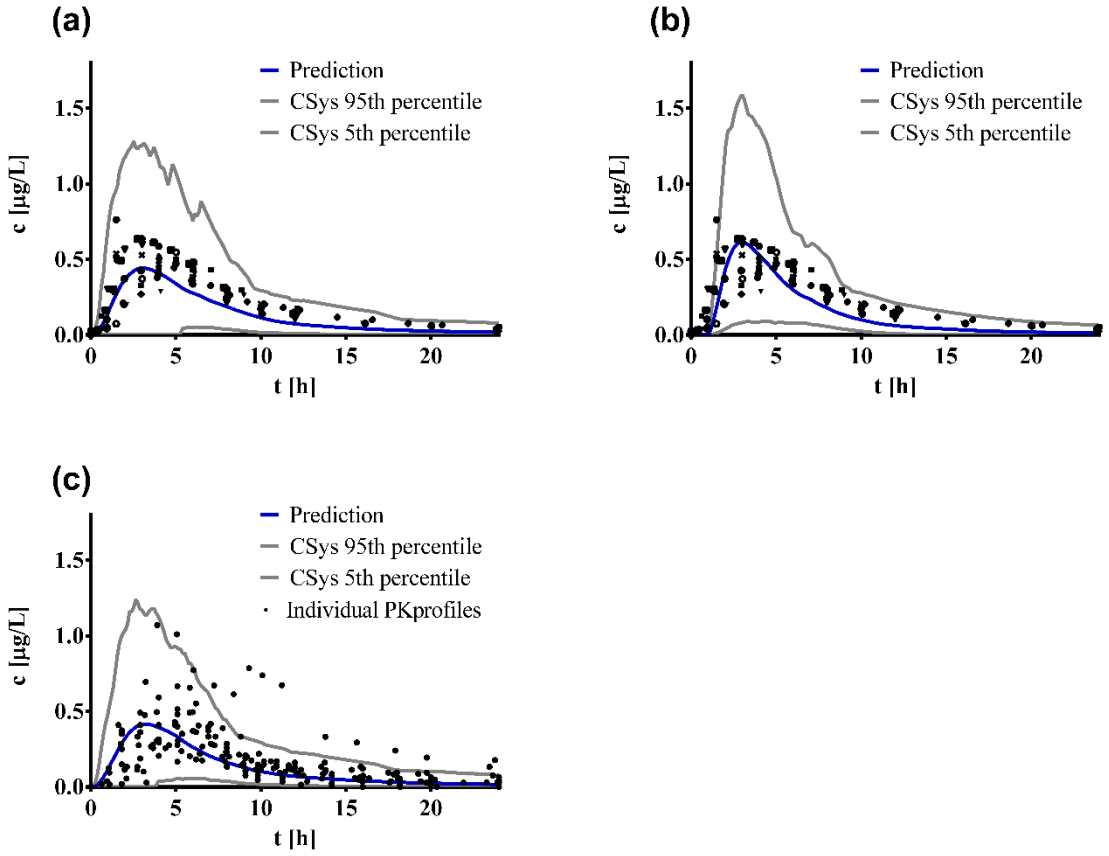

Figure 5.4: Simulation of budesonide plasma concentration for healthy subjects after administration of 3 mg Entocort® in the fasted state with dissolution input option 1 (a) compared to observed mean profiles (Table 5.7), and (c) compared to individual PK profiles[64], and (b) with dissolution input option 2 compared to observed mean profiles (Table 5.7).

5.4.2.3. Oral administration in the fed state

Entocort® administration in the fed state was simulated using the fed state parameters within the Simcyp® PBPK model and by integrating the results of the *in vitro* dissolution profile in healthy conditions of the fed state (Section 5.3.1.1). As for the fasted state, two different dissolution options (option 1 and 2) are shown in Figure 5.5 and compared against observed PK data (Table 5.7). Both simulations successfully predicted budesonide exposure by

meeting the external validation criterion (Table 5.8). The simulations with both dissolution options performed similar in terms of the prediction of $AUC_{0\text{-inf}}$, but the simulation with option 2 was superior in predicting C_{max}.

Compared to the fasted state, the observed T_{max} was 1.77 h higher in the fed state which was also reflected in the predictions with an increase in T_{max} of 1.98 h and 3.72 h between fasted and fed state simulations with option 2 and option 1, respectively. Additionally, the observed C_{max} and $AUC_{0\text{-inf}}$ were slightly higher in the fed state compared to the fasted state. This was also reflected in both simulations and could relate to the higher drug release rate in the fed state (Section 5.4.1) or increased GI transit times resulting in a longer time frame available for absorption.

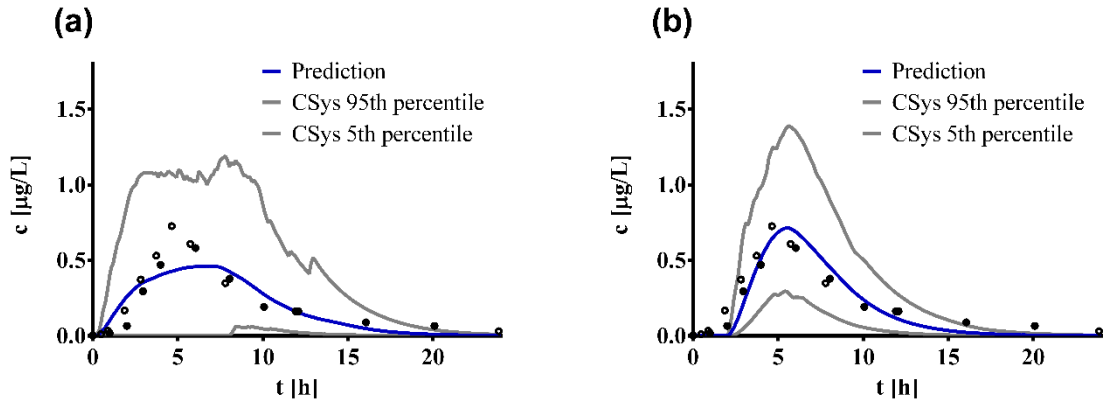

Figure 5.5: Simulation of budesonide plasma concentration for healthy subjects after administration of 3 mg Entocort® in the fed state with dissolution input (a) option 1 (triggering pH and a Weibull function), and (b) option 2 (discrete dissolution input) compared to observed mean profiles (Table 5.7).

Table 5.8: Overview of predicted and observed PK parameters and calculated fold error.

	Predicted			**Observed[f]**			**Ratio**		
	C_{max} [µg/L]	**$AUC_{0\text{-}inf}$ [µg/L*h]**	**T_{max} [h]**	**C_{max} [µg/L]**	**$AUC_{0\text{-}inf}$ [µg/L*h]**	**T_{max} [h]**	**C_{max} pre/obs**	**$AUC_{0\text{-}inf}$ pre/obs**	**T_{max} pre/obs**
Healthy									
IV administration	2.50	1.61	0.12	2.23	1.69	0.08	1.12	0.95	1.50
Oral solution	1.18	3.94	1.09	0.92	2.84	0.68	1.28	1.39	1.60
Entocort®, fasted state	0.44[a]/0.62[b]	3.47[a]/3.54[b]	3.00[a]/3.00[b]	0.54	5.19	3.57	0.81[a]/1.15[b]	0.67[a]/0.68[b]	0.84[a]/0.84[b]
Entocort®, fed state	0.46[a]/0.72[b]	4.37[a]/4.28[b]	6.72[a]/5.52[b]	0.63	5.61	5.34	0.73[a]/1.14[b]	0.78[a]/0.76[b]	1.26[a]/1.03[b]
Crohn's disease									
Entocort®, fasted state	0.62[c]/0.57[4]/0.43[5]	5.41[c] /5.25[4]/3.56[5]	3.24[c]/3.48[4]/3.12[5]	0.60	5.73	3.63	1.03[c] /0.95/0.72	0.94[c] /0.92/0.62	0.89[c] /0.96/0.86
Entocort®, fed state	0.88[c] /1.03[d]/0.76[e]	5.46[c] /5.98[d] /4.24 [e]	6.12[c] /6.00[d] /5.52 [e]	0.81	7.32	6.51	1.09[c] /1.27[d]/0.94[e]	0.75[c] /0.82[d]/0.58[e]	0.94[c] /0.92[d]/0.85[e]

[a] Dissolution input option 1: Triggering pH with Weibull function, [b] Dissolution input option 2: Discrete profile, [c] Low level CD population, [d] High level CD population, [e] Healthy volunteer population, [f] Mean value of all studies weighed by number of subjects and normalised to 3 mg budesonide administration, pre: predicted, obs: observed.

5.4.3. Impact of pathophysiological differences in CD on budesonide performance investigated with PSA

Figure 5.6 depicts the effect of hepatic CYP3A4 abundance in female and male subjects, intestinal CYP3A4 abundance and human serum albumin concentration in female and male subjects on C_{max} and AUC of budesonide after Entocort® administration in the fasted state as investigated with PSA.

The reduction of hepatic CYP3A4 abundance had a substantial impact on C_{max} and AUC with an enzyme reduction of 75% resulting in an increase of C_{max} by approximately 250% or 222% and of AUC by 267% or 239% in females and males, respectively. Since CYP3A4 contributes to 79% to the hepatic metabolism of budesonide and the hepatic extraction ratio is approximately 0.60, the lower CYP3A4 enzyme abundance results in a reduced hepatic clearance.[36, 72]

The intestinal CYP3A4 abundance investigated in the range of 91-149% of healthy intestinal CYP3A4 abundance had a lower impact on C_{max} and AUC with 84-103% and 85-103% of the parameters in the healthy simulation, respectively.

A reduced concentration of human serum albumin by up to -44% and -37% is expected to result in a reduction of budesonide C_{max} by up to 40% and 32% and of AUC by up to 40% and 34% in female and male subjects, respectively.

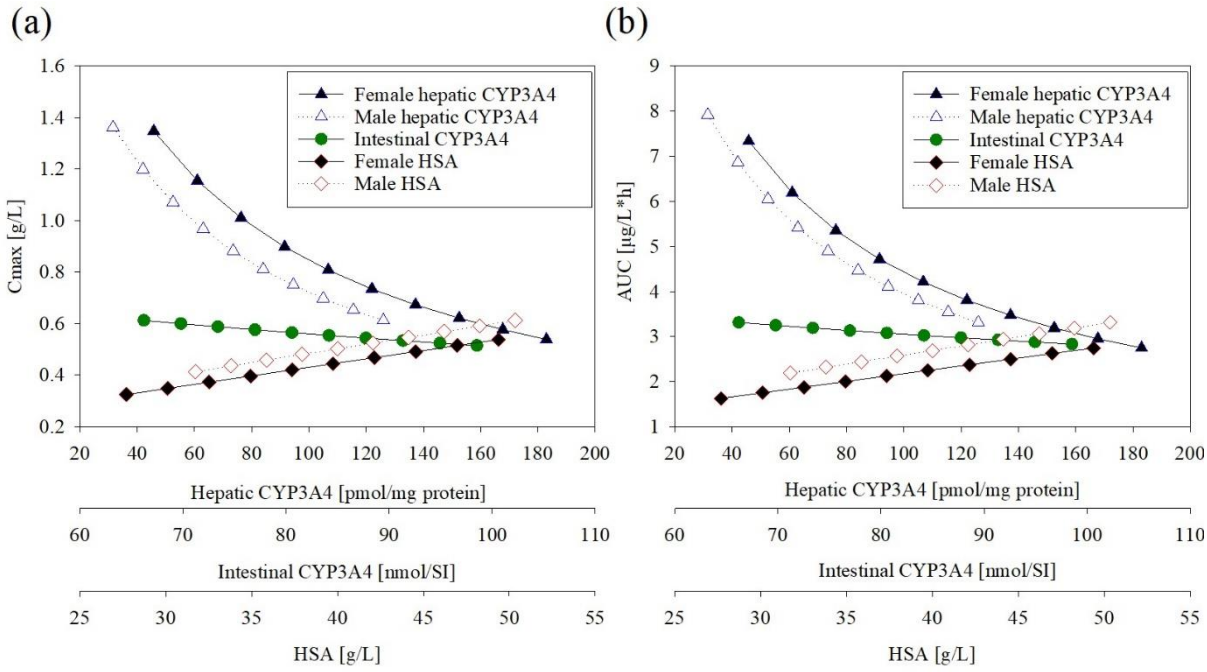

Figure 5.6: Parameter sensitivity analysis of the hepatic CYP3A4 abundance in male and female subjects, the intestinal CYP3A4 abundance and the human serum albumin concentration in male and female subjects on (a) C_{max} and (b) AUC.

The effect of gastric pH on the C_{max} and AUC of budesonide after Entocort® administration in the fasted state as investigated with PSA is illustrated in Figure 7. An increased gastric pH was shown to only impact on C_{max} and AUC when exceeding the triggering pH of the formulation (pH 5.5). The impact of a pH >5 was shown to be very limited with a decrease of C_{max} by 3.6%, an increase in AUC by 5.0% and a decrease of T_{max} from 4.1 h to 3.7 h. Therefore, the risk of altered budesonide performance from Entocort® in CD patients with increased gastric pH, either due to their co-treatment (e.g., proton pump inhibitors) or condition, is expected to be very low.

The GI transit time determines the time during which the drug is exposed to the intestinal membrane and therefore, available for absorption. A difference in GI transit times due to CD can consequently have an impact on drug product performance. The impact of gastric residence time and small intestinal transit time on C_{max} and AUC of budesonide after Entocort® administration in the fasted state as investigated with PSA is shown in Figure 5.7. Changes in gastric residence time from 0.5 h to 2.5 h showed no impact on the C_{max} and AUC of budesonide, while the T_{max} increased from 3.36 h to 5.40 h when increasing the gastric residence time from 0.5 h to 2.5 h. Since Entocort® has a triggering pH of 5.5 which is usually not exceeded by gastric pH, the gastric residence time mainly determines the onset of drug release rather than the extent.

The small intestinal transit time (SITT) had a very low impact on C_{max} but an increase in SITT from 3.0 h to 6.5 h resulted in an increase in AUC by 27% due to the longer presentation of budesonide to the absorptive GI membranes.

Consequently, gastric and intestinal transit times are expected to have only a limited effect on budesonide performance in comparison to the previously investigated factors. Due to the regional permeability differences of budesonide in the intestine, differences in transit times of specific compartments of the small intestine (opposed to the total SITT) could influence budesonide performance. These differences are expected to be in the same range as the impact of total SITT and were not further investigated.

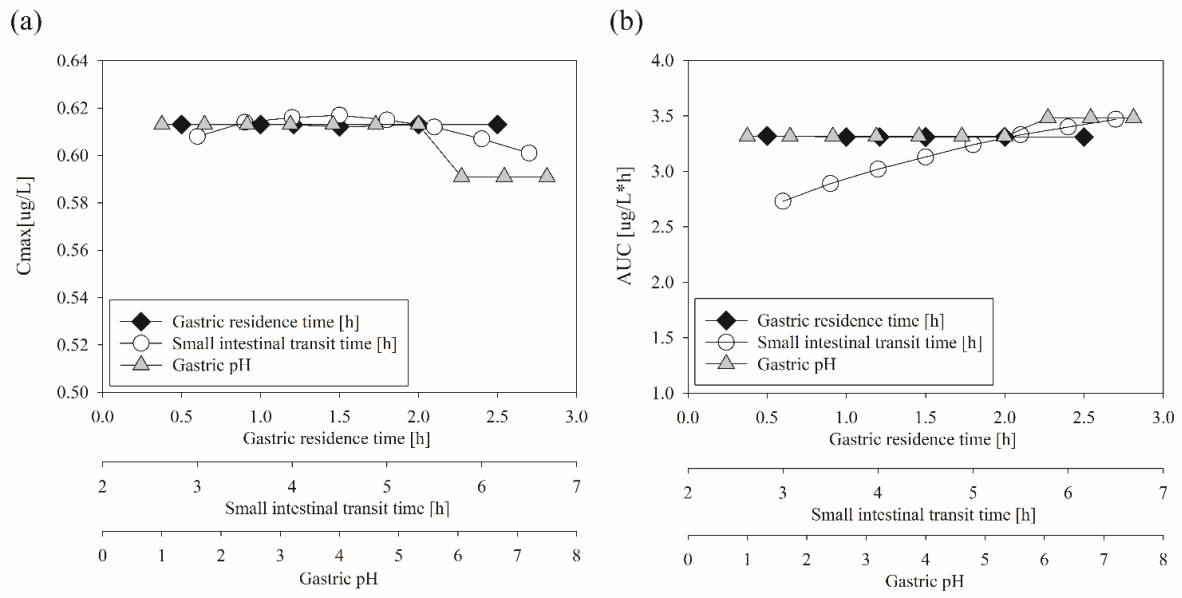

Figure 5.7: Parameter sensitivity analysis of the gastric mean residence time and the small intestinal transit time on (a) C_{max} and (b) AUC.

As shown in Figure 5.8, the lower available surface area had a very limited effect on budesonide performance. A reduction of the plicae circulares fold expansion by 84% resulted in a reduction of C_{max} in the range of 3-11% and AUC in the range of 3-7% in the different parts of the ileum. Only when reducing the plicae circulares fold expansion in all parts of the ileum by 84%, the impact would be higher with a reduction of C_{max} by 54% and AUC by 34%.

Due to the high permeability of budesonide, even profound changes in surface area are not representing a major risk for budesonide absorption. Additionally, CD affects the GI tract in a discontinuous manner, and it is unclear whether inflamed and ulcerated parts of the GI tract are unavailable for absorption.

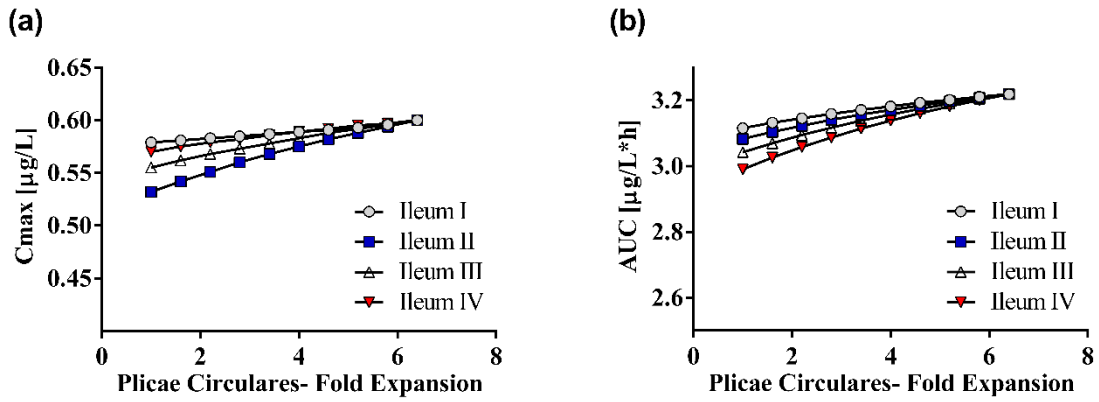

Figure 5.8: Parameter sensitivity analysis of the plicae circulares fold expansion in the four different parts of the ileum on (a) C_{max} and (b) AUC.

5.4.4. PBPK predictions for CD patients

Predicted and observed budesonide plasma concentration profiles after Entocort® administration in CD patients in the fasted and fed state are shown in Figure 5.9 and the respective PK parameters are presented in Table 5.8.

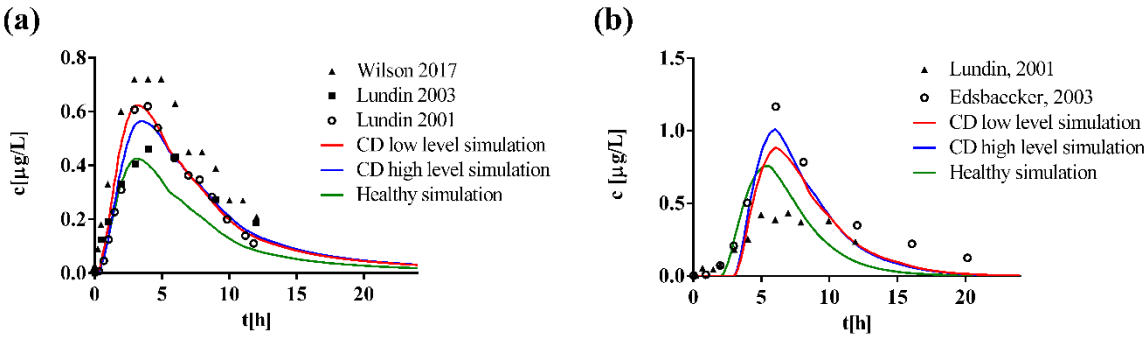

Figure 5.9: Simulation of budesonide plasma concentration in CD patients after administration of 3 mg Entocort® in the fasted state with dissolution input option 1 (a), and in the fed state with dissolution input option 2 (b) compared to observed mean profiles (Table 5.7).

In the fasted state, the highest exposure of budesonide was predicted for the CD low level population followed by the CD high level population and the simulation for healthy volunteers. Compared to the simulation for healthy volunteers and the observed PK parameters, the CD low level simulation improved the prediction of C_{max} by 25% and AUC by 32% and the CD high level simulation improved the prediction of C_{max} by 23% and AUC by 30%. It should be noted that a similar budesonide bioavailability in CD patients compared to healthy subjects after Entocort® administration in the fasted state was observed in one of

four studies, while all other studies showed an increased exposure of budesonide compared to healthy subjects (Table 5.7).

In the fed state, the exposure of budesonide was highest for the CD high level simulation followed by the CD low level simulation and the simulation for healthy volunteers. Compared to the simulation with the healthy volunteer population, simulations with the CD populations were closer to the observed AUC and improved the prediction by 17% and 24% for the low and high level population, respectively. In terms of C_{max}, the prediction of all three simulations was close to the observed value with the CD simulations being slightly higher (9-27%) and the healthy simulation being slightly lower (-6%). Also in the fed state, differences in the exposure of budesonide in CD patients in different studies were reported with one study showing a similar exposure compared to healthy subjects, while others show a substantial increase (Table 5.7).

The discrepancies between the PK studies in CD patients could be related to a different disease states of the patients or their concomitant medication. The inflammation process has been shown to decrease CYP3A4 activity in different inflammatory conditions such as rheumatoid arthritis and in haemodialysis patients.[73, 74] A higher budesonide exposure related to reduced buccal CYP3A activity has also been observed in patients with oral chronic graft-versus-host disease.[62] This can be explained by the inflammation process, which induces the production of pro-inflammatory cytokines leading to a down-regulation of CYP3A4.[75] When patients are treated with medication preventing cytokine production, this effect could be reversed.[75] For example, it has been shown that the repeated administration of budesonide in patients with active CD resulted in a reduction of the initial budesonide C_{max} and AUC by 22% and 26%, respectively.[26]

5.5. Conclusion

The budesonide exposure was successfully predicted after intravenous and oral administration using the developed PBPK model. By using *in vitro* biorelevant dissolution/release tests with PBPK modelling successful PK simulations were achieved for a controlled-release formulation of budesonide (Entocort®) in healthy subjects in the fasted and fed state. Pathophysiological differences in CD patients were identified in literature and their impact on budesonide performance was investigated revealing the highest impact on the simulations for hepatic CYP3A4 enzyme abundance and HSA concentration. A higher budesonide exposure in CD patients compared to healthy subjects was reported in literature and successfully predicted with a PBPK population adapted to CD physiology.

Apart from patients with CD, the workflow presented in the current study can be used to predict drug product performance in patients with other GI diseases. Therefore, the following steps should be followed: i. Development of an *in vitro* dissolution methodology representative of the GI fluid composition and hydrodynamics in the investigated population; ii. Development and validation of a PBPK model in healthy subjects; iii. Identification of pathophysiological differences in the respective GI disease patients compared to healthy subjects; iv. Investigation of the impact of the identified differences on the PBPK simulations; v. Development of a population representative of the investigated population according to relevant differences; vi. Prediction of drug exposure in the GI disease population and when applicable validation with PK data.

A mechanistic modelling approach allows to consider pathophysiological differences in patients with GI diseases and can therefore, be used to predict the effect of GI diseases on drug product performance. This is especially helpful due to the sparse clinical data available for this patient population. PBPK models could indicate when GI diseases pose a risk for safety and efficacy and dose adjustments are needed. Further studies investigating the physiology of patients with GI diseases and the drug product performance of additional drugs are needed to further refine and validate the respective models.

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Chapter 6

Investigating the impact of Crohn's disease on the bioaccessibility of a lipid-based formulation with an *in vitro* dynamic gastrointestinal model

Abstract

Objectives

The aim of the study was to investigate the impact of Crohn's disease (CD) on the performance of a lipid-based formulation of ciprofloxacin in a complex gastrointestinal simulator (TIM-1, TNO) and to compare the luminal environment in terms of bile salt and lipid composition in CD and healthy conditions.

Key findings

CD conditions were simulated with a reduced concentration of porcine pancreatin and decreased amount of porcine bile in the TIM-1 system. The bioaccessibility of ciprofloxacin was similar in CD and healthy conditions considering its extent as well as its time course in the jejunum and ileum filtrate. Differences were observed in terms of the luminal concentration of triglycerides, monoglycerides and fatty acids in the different TIM-1 compartments, indicating a reduction and delay in the lipolysis of formulation excipients in CD. Quantitative analysis of bile salts revealed higher concentrations for healthy conditions (standard TIM-1 fasted state protocol) in the duodenum and jejunum TIM-1 compartment compared to published data in human intestinal fluids of healthy subjects, while bile salt concentration in CD conditions were similar to these. A lipidomics approach with UPLC-MS has proven to be a time-efficient method to semi-quantitatively analyse differences in fatty acids and bile salts levels between healthy and CD conditions.

Conclusions

The dynamic luminal environment in CD and healthy conditions after administration of a lipid-based formulation can be simulated using the TIM-1 system. For ciprofloxacin, an altered luminal lipid composition had no impact on its performance indicating a low risk of altered performance in CD patients. Human duodenal and jejunal bile salt levels are lower than the levels in corresponding TIM-1 compartments when using the standard TIM-1 fasted state protocol.

6.1. Introduction

Crohn's disease (CD), affecting approximately 1.6 million people in Europe, is a chronic auto-inflammatory disorder and one of the main types of inflammatory bowel disease (IBD).[1] CD commonly affects the terminal ileum but can be localised in any part of the gastrointestinal (GI) tract. The disease manifests as transmural ulcerations that are discontinuously spread in the GI tract. Additionally, CD patients often present extra-intestinal manifestations such as inflammations of the eyes (uveitis, episcleritis), skin diseases (erythema nodosum, pyoderma gangrenosum), spondyloarthritis or hepato-pancreato-biliary diseases.[2] For the patients, CD results in a lifelong treatment with anti-inflammatory drugs (e.g., mesalamine, steroids, azathioprine, cyclosporine). Additional to this treatment, IBD patients have shown a higher use of antidepressants, anxiolytics, oral bisphosphonates, cardiovascular medication, antibiotics, proton pump inhibitors and nonsteroidal anti-inflammatory analgesics compared to the general population.[3] The use of antibiotics to treat CD was not investigated in large clinical trials and therefore, a therapeutic effect could only be observed in studies with a small number of subjects.[4] However, antibiotics are often used for CD patients experiencing complications such as fistulas or abscesses.[5] In this case, ciprofloxacin is one of the treatment options and has been shown to be beneficial for the treatment of perianal fistulas.[6]

To exert its pharmacodynamic effect, a drug must overcome many challenges to reach its target site in the body including drug release from the formulation, dissolution in the GI fluids, permeation of the GI membrane and escaping gut and hepatic metabolism. All these processes are dependent on the physiology of the treated subject. For patients with CD, pathophysiological differences with possible impact on drug product performance were observed in terms of the composition of luminal contents, the abundance of metabolising enzymes, GI transit times and the microbiota.[7]

Most candidates from contemporary drug discovery programs are poorly water-soluble with dissolution rate-limited absorption and typically belong to BCS class II or IV.[8] Therefore, formulation scientists are challenged to use more complex formulation approaches. For example, a higher bioavailability can be achieved with a lipid-based formulation (LBF) approach by e.g., circumventing at least partially the drug dissolution step due to the higher drug solubility in the formulation vehicle or the promotion of lymphatic drug uptake. Several LBFs are already commercially available and the ever-increasing number of poorly soluble compounds might further increase their number in the future.[9]

Upon entering the GI tract, LBFs are subject to a dynamic environment with dispersion and digestion processes. Various excipients of LBFs such as acylglycerols, phospholipids, polysorbates (Tweens), polyethyleneglycol mono- and di-esters can be digested along the GI tract.[10] The enzymes involved in their hydrolysis include gastric lipase and classical colipase-dependent pancreatic lipase, hydrolysing mainly triacylglycerols and diacylglycerols.[10] Additionally, several other pancreatic enzymes such as pancreatic carboxyl ester hydrolase, pancreatic lipase-related protein 2 and pancreatic phospholipase A2 act on micellar substrates and possess a phospholipase activity.[10] For the drug, the continuous reorganisation of colloidal structures composed of luminal bile acids, cholesterol, phosphatidylcholine, on the one hand, and excipients and their digestion products, on the other hand, can induce a supersaturated state or precipitation of a drug.[10] This complexity highlights the need for *in vitro* systems considering these dynamic processes to evaluate the formulation performance of LBFs.

The digestion and dispersion process of LBF is most often investigated in pH-stat lipolysis models focusing only on the small intestine, the main absorption and digestion area, and using porcine pancreatin as enzymatic source.[11] Therefore, the contribution of gastric lipase, estimated to around 3-37% of triglyceride (TG) digestion, is often neglected.[12-16] This is especially a limitation for the simulation of pathological conditions with a deficiency of exocrine pancreatic enzymes, where gastric lipase is assumed to have a significant role in fat digestion.[15, 17] In recent years, modifications of the pH-stat lipolysis models have been developed to address this issue with a two-step one-compartment or a two-step two-compartment model.[11, 18]

The complex GI simulator TIM-1 (TNO, Zeist, Netherlands) mimics closely the GI tract by simulating biliary and pancreatic secretion, controlling luminal pH with bicarbonate secretion, removing drug/micellar components via ultrafiltration and simulating gastric lipid digestion. The *in vivo* predictive ability of TIM-1 has previously been shown in nutritional sciences and in pharmaceutical formulation performance.[19-24] Due to the high level of biorelevance of the TIM-1 system, its suitability for the evaluation of LBFs has been suggested.[11] However, the high lipophilicity of drugs in LBFs might limit its use due to drug binding to the TIM-1 membranes and filters possibly resulting in a low recovery of the investigated drug.[23] To the best of our knowledge, no LBFs have yet been tested in the TIM-1 model.

Ciprofloxacin is used for the treatment of bacterial infections and belongs to the antibiotic group fluoroquinolones. In terms of physicochemical characteristics, ciprofloxacin possesses a log P of 0.28, poor aqueous solubility and is a zwitterionic molecule (high solubility at pH<5, pH>10).[25, 26] Apart from tablets, it is available as lipid-based oral suspension for reconstitution and marketed as Ciproxin® 250 mg/ 5ml granules and solvent for oral suspension (Bayer plc, Reading, UK). Ciprofloxacin tablets have previously been tested in the TIM-1 simulator and shown high levels of drug recovery.[27]

The aim of this study was to investigate the performance of ciprofloxacin from an oral lipid-based suspension in a complex dynamic simulator of the upper GI tract, TIM-1, in healthy conditions and conditions representative of CD. In addition, differences in the digestion process of excipients of the LBF between healthy and CD conditions were investigated and relevant components (bile acids, cholesterol) of the mixed micelles in the TIM-1 matrix were measured.

6.2. Materials

The formulation Ciproxin® 250 mg/5 mL granules and solvent for oral suspension from Bayer Plc, Reading, UK was used. The water used was Milli-Q grade.

For the TIM-1 experiments, potassium chloride, acetic acid and sodium chloride were used from Fisher Scientific, Loughborough, UK. Calcium chloride di-hydrate, hydrochloric acid (37%), pancreatin from porcine pancreas, sodium acetate trihydrate, pepsin from porcine gastric mucosa, sodium citrate, lipase from Rhizopus oryzae, amylase from *Bacillus sp*., (hydroxypropyl)methyl cellulose (HPMC) (2%) in water, porcine bile extract, sodium bicarbonate (1.14 mol/L) in water and trypsin were purchased from Sigma-Aldrich, Gillingham, UK. Sodium hydroxide (1 M) in water was used from Merck KGaA, Darmstadt, Germany. Porcine bile was purchased from Triskelion (Hendrix Slaughter House, Druten, Netherlands).

For the High-Performance Liquid Chromatography (HPLC) analysis of ciprofloxacin, formic acid and sodium hydroxide were purchased from Fisher Scientific, Loughborough, UK and ciprofloxacin from USP, Rockville, MD, US.

For the Gas Chromatography-Flame Ionisation Detector (GC-FID) analysis, chloroform, octanoic acid, decanoic acid, cholesterol and a Lipid Standard, Mono-, Di-, & Triglyceride Mix containing 1,3-Diolein 10 mg, 1,2-Dioleoyl-rac-glycerol 10 mg, Glyceryl trioleate

10 mg, Monoolein 10 mg were purchased from Sigma Aldrich, Gillingham, UK. Hydrochloric acid 1 M was purchased from Fisher Scientific, Loughborough, UK.

For the HPLC-Charged Aerosol Detector (CAD) analysis, HPLC grade methanol, ammonium formate and formic acid were used from Fisher Scientific, Loughborough, UK. Triethylamine, glycochenodeoxycholic acid (GCDC) sodium salt, glycocholic acid (GC), taurodeoxycholic acid (TDC) sodium salt, taurochenodeoxycholic acid (TCDC) sodium salt and taurocholic acid (TC) sodium salt were purchased from Sigma-Aldrich, Gillingham, UK.

For Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) analysis, HPLC grade acetonitrile and acetic acid were used from Fisher Scientific, Loughborough, UK and ammonium acetate from Sigma-Aldrich, Gillingham, UK, respectively.

6.3. Methods

6.3.1. TIM-1 experiments

To investigate the effect of CD on the performance of a LBF, a complex *in vitro* GI model TIM-1 (TNO, Zeist, Netherlands) was used, which has previously been described.[22, 23, 28] The system has been used in a pharmaceutical context to predict drug product performance of formulations [22-24] and in food sciences to investigate e.g., the digestion of lipids.[19-21] An overview of the TIM-1 system is given in Figure 6.1. The human upper GI tract is simulated with four serial compartments representing the stomach, duodenum, jejunum and ileum. These compartments consist of two connected equal basic units with a glass jacket and a flexible silicone membrane inside. Mixing of the chyme and control of the luminal temperature is achieved by pumping tempered water around the flexible membranes. Peristaltic valve-pumps connect the different TIM-1 compartments and allow the control of the chyme's flow rate between the different compartments. The volume of the luminal contents is controlled with level sensors and the secretion of buffers. A predetermined pH curve can be programmed for each compartment, monitored with a pH probe in each TIM-1 compartment and controlled by secretion of either water, 1 M hydrochloric acid (only gastric compartment) or 1 M sodium bicarbonate solution. Additionally, secretions of gastric electrolytes, gastric enzymes, pancreatic and biliary juices are included.

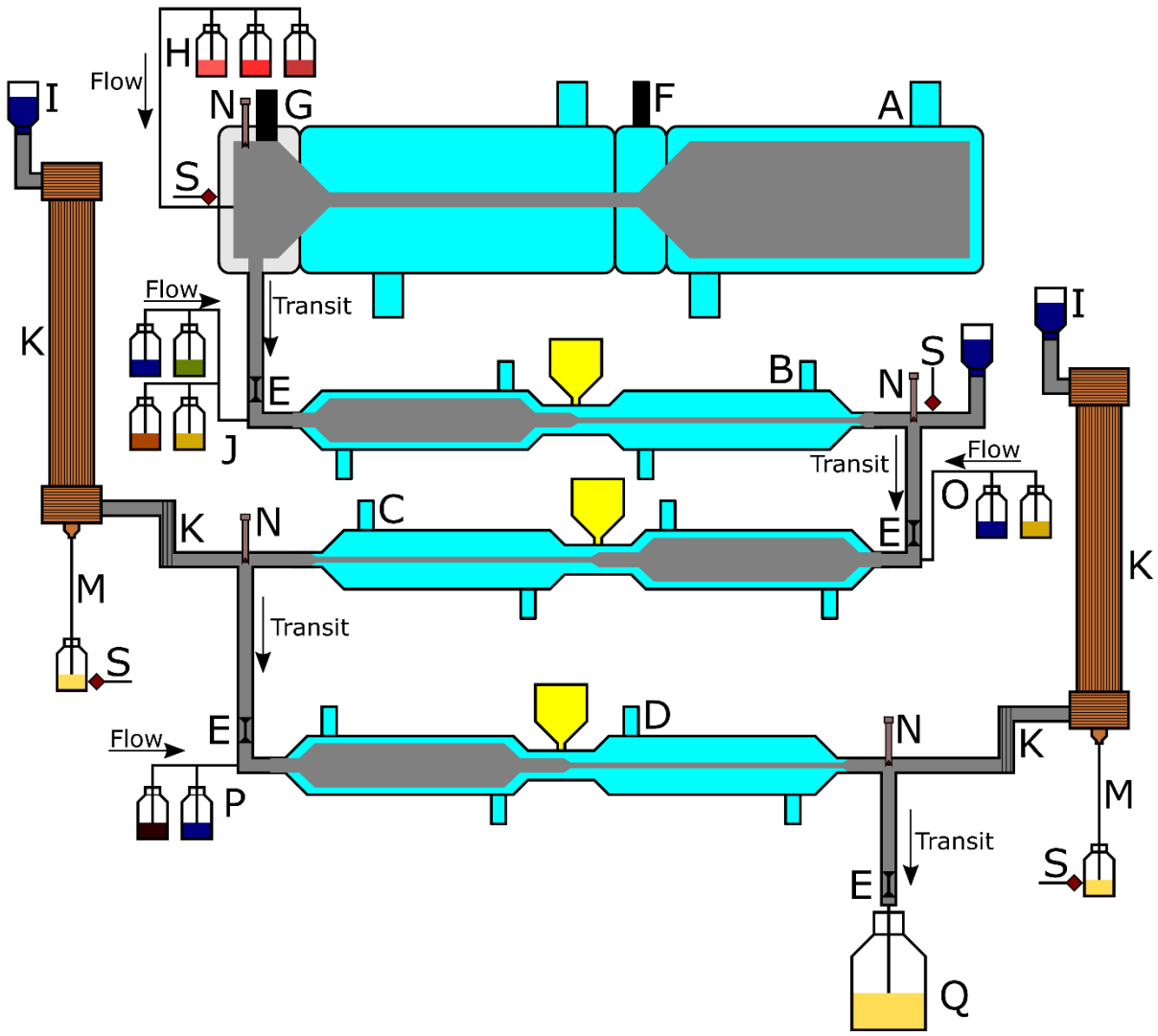

Figure 6.1: Overview of TIM-1 system. [A: Gastric compartment, B: Duodenum compartment, C: Jejunum compartment, D: Ileum compartment, E: Peristaltic valve, F: Dosing port, G: Pressure sensor, H: Gastric secretions, I: Level sensors, K: Filter system, L: prefilter, M: Filtrate (jejunum and ileum), N: pH-electrode, O: Jejunum secretions, P: Ileum secretions, Q: Ileum efflux, S: Sampling points].

6.3.1.1. Preparation of solutions, reagents and starting residues

Various solutions were prepared to perform the experiments with the TIM-1 system including 0.1 M sodium citrate buffer (pH 7.0) and 1 M sodium acetate buffer (pH 5.0). Gastric electrolyte solution (GES) was prepared by dissolving 8 g/L sodium chloride, 1.7 g/L potassium chloride and 0.16 g/L calcium chloride di-hydrate in water. HPMC 0.4% & bile 0.04% gastric solution was prepared by dissolving 0.4 g/L bile extract in water, subsequently adding 4.0 g/L HPMC and stirring the solution overnight. Gastric enzymes solution contained 1 mL 1 M sodium acetate buffer, 6000 units lipase, 1440000 units pepsin, 42000 units amylase and 299 mL GES. Small intestinal electrolyte solution (SIES) was

prepared by dissolving 7 g/L sodium chloride, 0.35 g/L potassium chloride and 0.1 g/L calcium chloride di-hydrate in water and adjusting the pH to 7.0 with 1 M sodium hydroxide solution. Pancreatic solution was prepared by dissolving pancreatin powder in water, centrifuging the solution for 20 min at 12.500 G at 4ºC and using the supernatant for the experiment. The bile solution used consisted of prefiltered pig bile in SIES. The concentration of the pancreatic and bile solution varied according to the experimental conditions as detailed below (Section 6.3.1.2).

At the beginning of the experiments, the gastric compartment was filled with 30 g gastric start residue, consisting of 15 g gastric enzyme solution and 15 g HPMC 0.4% & bile 0.04% gastric solution. The duodenum compartment was filled with 60 g of a solution consisting of 15 g SIES, 15 g pancreatin solution, 30 g bile solution and 2 mg trypsin in 1 mL SIES. The jejunum compartment was filled with a mixture of 35 g SIES, 35 g pancreatin solution and 70 g bile solution. The ileum compartment was filled with 140 g SIES.

6.3.1.2. Experimental conditions

Ciprofloxacin was selected as model drug for the studies with its lipid-based formulation Ciproxin® oral suspension, since another more lipophilic compound was tested initially but failed in pretesting experiments due to binding to membranes and filters of the TIM-1 system. This limitation may restrict the use of TIM-1 for the evaluation of LBFs of more lipophilic compounds.[22]

The Ciproxin® suspension was prepared according to the patient leaflet (brown bottle with granules was emptied into a large white bottle with diluent, turned horizontally and shaken for 15 s) and stored in a refrigerator until further use. At the start of each experiment, the bottle with the formulation was turned horizontally, shaken for 15 s, and 10 mL of the formulation were added with a syringe to the dosing port of the gastric compartment. According to the patient leaflet, a drink of water may be taken after Ciproxin® administration and therefore, water was added to the gastric compartment according to the experimental conditions shown in Table 6.1.

The Ciproxin® oral suspension consists of granules dispersed in an oily diluent consisting of miglyol 575, lecithin, sucrose and strawberry flavouring.[29] The medium chain TGs in Miglyol 575 are a mixture of octanoyl and decanoylglycerides. In this study, we selected the fasted state protocol for the TIM-1 experiments since it has been shown that a small quantity of medium chain TGs does not lead to substantial gallbladder contraction and therefore, does

not induce concentrations of biliary components representative of a fed state in the intestinal lumen.[30] Therefore, average physiological conditions of the GI tract in the fasted state were simulated in terms of pH, temperature, GI transit times and hydrodynamics, GI volumes, electrolyte concentrations and secretions of enzymes, biliary and pancreatic juice.

The pH in the gastric compartment was set to drop from 3.0 to 1.7 within 30 min.[27] The pH of the duodenum, the jejunum and the ileum compartment were 6.3±0.2, 6.5±0.2 and 7.4±0.2, respectively. The volume of bicarbonate solution secreted to maintain the specified luminal pH in the intestinal compartments was automatically reported by the TIM-1 system. The temperature was maintained at 37 ºC.

Gastric emptying was set according to the equation of Elashoff with a halftime of 20 min and a b-value (shape factor) of 1.0.[31] To simulate the house keeper wave, the total content of the gastric compartment was manually emptied and introduced into the duodenum compartment after the first 60 min. GI volumes were 55 mL, 130 mL and 130 mL for the duodenum, jejunum and ileum compartment, respectively.

The secretions to the gastric compartment included gastric enzyme solution, hydrochloric acid and water at a total secretion rate of 1.0 mL/min. The duodenal secretion consisted of bile solution, pancreatin solution and SIES. The jejunal secretion consisted of 10% V/V bile solution in SIES and the ileal secretion was only SIES.

To mimic the absorption of the dissolved or solubilised drug and digestion products, the "lipid membrane configuration" mode was selected.[23] Therefore, two hollow fibre polysulfone filtration units with a cut-off size of 50 nm and a surface area of 0.3 m^3 (Plasma Flux P1 dry, Fresenius Medical Care, Bad Homburg, Germany) were used. Before the experiment, the filters were saturated with 10 L of water and subsequently preconditioned by filtering a mixture of 50 mL porcine bile, 25 mL SIES and 25 mL pancreatic solution. As a next step, the filters were connected to the jejunum and ileum compartment. The drug analysed in both filtrates was considered the bioaccessible fraction of the drug within a given time period. The bioaccessibile fraction refers to the drug available for absorption through the gut wall.[32] Due to the use of a syringe instead of the supplied measuring spoon to administer the Ciproxin® formulation, the ciprofloxacin dose was slightly higher and the bioaccessible amount of ciprofloxacin was therefore, normalised to the total amount of ciprofloxacin recovered from the TIM-1 system (luminal samples, filtrates, ileal efflux, residues, washing solution).

Considering the lipolysis in the TIM-1 system, lipase from *Rhizopus oryzae* was used to simulate human gastric lipase, since human gastric lipase is not commercially available. *In vitro* experiments with lipase from *Rhizopus oryzae* showed a significantly higher lipid digestion compared to the *in vivo* lipid digestion by human gastric lipase.[33] Currently, there is still a lack of suitable substitutes for human gastric lipase due to differences in terms of the pH-optimum, the substrate affinity and the stereo selectivity of microbial and animal lipases.[11, 33] To simulate pancreatic lipases, porcine pancreatin was used as enzymatic source, which has previously been shown to be a good substitute for human pancreatic juice.[34]

Three different experimental conditions were used including healthy, CD and healthy blank TIM-1 experiments as shown in Table 6.1. In healthy conditions, the bile solution consisted of 20.0% v/v pig bile in SIES and the pancreatin solution of 7.0% w/v porcine pancreatin extract in water. The healthy blank run was performed without any formulation and with the same conditions as defined for healthy subjects.

In CD, pathophysiological changes can affect the composition of the GI fluids and hepatobiliary manifestations are common extraintestinal symptoms.[7] In terms of LBFs, differences in lipase activity and bile concentration could impact drug product performance. In CD patients, the pancreatic lipase activity was decreased to 28-80% of the activity in healthy subjects.[35-37] Additionally, the bile acid pool in CD patients was reduced to 38-58% of the size in healthy subjects.[38-40] To investigate the impact of these differences, CD conditions were simulated in the TIM-1 system (Table 6.1). The amount of porcine pancreatin was reduced to 28% of the concentration in healthy conditions, assuming a worst-case scenario. The bile concentration was reduced to 43% of the porcine bile concentration in healthy conditions, corresponding to an indirect approach by using the median reduction in studies investigating the bile acid pool.[38-40]

Table 6.1: Overview over experimental conditions of TIM-1 studies with ciprofloxacin.

Experimental conditions (number of replicates)	**Healthy (n=2)**	**CD (n=2)**	**Healthy blank (n=1)**
Setup	Lipid setup – ultrafiltration		
Prandial state	Fasted state		
Drug product	Ciproxin® oral suspension (10 mL)		-
c (porcine bile)	20.0% v/v pig bile in SIES	8.6% v/v pig bile in SIES	20.0% v/v pig bile in SIES
c (porcine pancreatin)	7.0% w/v in water	2.0% w/v in water	7.0% w/v in water
Experimental time [h]	5.0	5.0	4.0
Water added to the gastric compartment [mL]	230	230	240

SIES: Small intestinal electrolyte solution

6.3.1.3. Sampling and drug analysis

Samples were collected every 30 min for 5 h from the jejunal and ileal filtrate (drug available to permeate the intestinal membrane) and the ileal effluent (drug entering the colon). Additionally, 5 mL samples were taken directly from the gastric compartments at three different time points (0, 30, 60 min) and from the duodenal compartment every 30 min for 5 h. The collected samples were subsampled and stored at -18ºC for further analysis. After completion of the experiment, the residues were collected, the system was cleaned with 0.1 M sodium hydroxide solution and residues in the compartments and washing solution were analysed for remaining ciprofloxacin quantification.

For the HPLC analysis, all TIM-1 samples were diluted with 0.1 M sodium hydroxide solution and filtered through 1.0 µm PTFE syringe filters (Sigma-Aldrich, Gillingham, UK). Ciprofloxacin was quantified according to a published method with a Waters Acquity UPLC equipped with a Waters Xevo Triple Quadrupole Mass Spectrometer (Waters Corporation, Milford, MA, US).[27] A Waters Acquity UPLC BEH300 C18 column (2.1 x 200 mm, 1.7 µm) was used and set to a temperature of 40°C. The flow rate was 0.6 mL/min and 3 µL of sample were injected. The mobile phase A consisted of 0.1% Formic acid in water and the mobile phase B of 0.1% Formic acid in acetonitrile. A gradient elution mode was used as shown in Table 6.2. The Triple Quadrupole mass spectrometer was operated with a cone voltage of

45ºC, a source temperature of 500ºC, a desolvation gas flow rate of 800 L/h and a cone gas flow rate of 80 L/h. All samples were measured in positive ion electrospray mode and photodiode array detection was set to 210-400 nm (4.8 nm resolution). Multiple reaction monitoring was used for the parent and daughter m/z of 332.2 and 288.2, respectively.

Table 6.2: Mobile phase gradients used for HPLC-MS analysis of ciprofloxacin, HPLC-CAD analysis of bile salts and UPLC-MS analysis of lipids and bile salts.

	Time [min]	**% Mobile Phase A**	**% Mobile Phase B**
HPLC-MS analysis of ciprofloxacin	0.00	100	0
	12.00	0	100
	12.01	100	0
	15.00	100	0
HPLC-CAD analysis of bile salts	0.00	40	60
	25.00	10	90
	25.10	40	60
	30.00	40	60
UPLC-MS analysis of lipids and bile salts	0.00	65	35
	9.00	5	95
	10.00	5	95
	10.01	65	35
	12.00	65	35

6.3.2. Analysis of formulation and matrix components

6.3.2.1. GC-FID for lipid analysis

Lipid components were extracted as previously described.[20] Briefly, 900 µl chloroform and 100 µl of 0.1 M hydrochloric acid were added to 100 µl of sample in a vial, the mixture was vortexed for 1 min and the bottom layer was directly analysed by GC-FID. The analysis was performed on an Agilent 6890N network gas chromatograph (Agilent Technologies, Santa Clara, CA, US) equipped with an injector series 7683B and a flame ionisation detector. The

column used for the separation was a TG-5MT (Thermo Fisher Scientific, Loughborough, UK) with a length of 15 m, a diameter of 0.25 mm and a film thickness of 0.10 µm. Helium was used as carrier gas. The column was set to a constant pressure of 30.00 psi. Sample injection (1 µl) was performed from the bottom layer of the sample with a split ratio of 5:1 (split/splitless) on the column with an injector temperature of 300°C. The initial oven temperature was set to 60°C for 2 min, followed by an increase of 10°C/min during 34 min and a hold time of 2 min at 400°C resulting in a total run time of 38 min. The detector temperature was kept constant at 350°C. Empower® 3 (Waters Corporation, Milford, MA, US) was used for data collection.

For fatty acids (FA) and cholesterol, chromatographic peaks were identified by comparing retention time with those of known standards, resulting in a retention time of 3.6 min for octanoic acid, 6.0 min for decanoic acid and 20.6 min for cholesterol. For monoglycerides (MG) and TGs, chromatographic peaks were identified with an Agilent 5975 MS (Santa Clara, CA, US) [data not shown] with retention times of 20.0 min, 21.2 min, 22.3 min and 23.4 min for TGs and 9.2 min, 9.5 min and 11.3 min for MGs. Quantification of TGs was performed against Glyceryltrioleate, MGs against monoolein and for cholesterol, octanoic acid and decanoic acid against their standards.

6.3.2.2. HPLC-CAD for bile salt analysis

For the bile salt analysis, an Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, US) with a degasser (G1379B), binary pumps system (G1312B), autosampler (G1367C), thermostatted column compartment (G1316B) with a Corona Charged Aerosol Detector (CAD) (ESA Biosciences Inc., Chelmsford, MA, US) was used. A modification of a previously published method was used.[41] A Waters Halo C18 column (150 mm × 3 mm, 2.7 µm) was maintained at 30°C. The mobile phase A consisted of 20 mM ammonium formate with 0.5% formic acid and 0.2% triethylamine. The mobile phase B was methanol. A gradient method was used according to Table 6.2 with a flow rate of 0.5 mL/min. The TIM-1 samples were appropriately diluted with mobile phase (mobile phase A: mobile phase B 40:60 V/V) and a volume of 20 µL was injected. The CAD was used with a response range of 100 pA full scale. The retention time of TC, GC, TCDC acid and GCDC were 7.4 min, 10.2 min, 10.8 min and 14.0 min, respectively. Bile acids were quantified against their known standards (TC, GC, TCDC, GCDC) except for the bile acid with a retention time of 8.1 min which was quantified against GCDC due to having the same molecular weight, which had previously been determined by HPLC-MS (data not shown).

6.3.2.3. UPLC-MS for lipid and bile salt analysis

A lipidomics approach with UPLC-MS was used as semi-quantitative tool to identify the magnitude of changes considering FAs and bile salts in CD compared to healthy conditions in a time-efficient way. Therefore, TIM-1 samples from healthy conditions (n=1) and CD conditions (n=1) after administration of the Ciproxin® suspension were analysed. The samples were diluted with acetonitrile in a ratio of 1:3 (sample:acetonitrile). Additionally, a quality control (QC) sample was prepared by mixing 50 µL of each sample and diluting the mixture with acetonitrile in a ratio of 1:3 (sample:acetonitrile). The injection of a QC sample after every 6 TIM-1 samples was used to assure reproducibility. Three dilutions of the resulting QC sample with acetonitrile (2x, 5x and 10x) served to confirm the linearity of the peaks of interest over the respective range. TIM-1 samples were randomised for the UPLC-MS analysis.

The analysis was performed with a G6550A Agilent Q-TOF LC/MS System (Agilent Technologies, Santa Clara, CA, US) with a 6550 iFunnel Q-TOF equipped with a HiP-ALS autosampler (G4226A), a binary pump (G4220A) and a thermostatted column compartment (G1316C). A previously published method was used with an Acquity UPLC BEH C8 column (2.1 mm x 100 mm, 1.7 µm) maintained at 60°C.[42] The mobile phase A consisted of 50 mM ammonium acetate (pH 5.0) and acetonitrile was used as mobile phase B. A gradient according to Table 6.2 was applied with a flow rate of 0.6 mL/min.

All samples were measured in negative ion electrospray mode with Dual Agilent Jet Stream Electrospray Ionisation (Dual AJS ESI). The gas temperature was set to 250°C with a flow rate of drying gas of 15 L/min, a sheath gas temperature of 220°C and a sheath gas flow rate of 10 L/min. The nebulizer was set to 40 psig, the fragmentor to 400 V, the collision energy to 5 V and capillary voltage to 4000 V. A nozzle voltage of 1000 V was applied. Two different reference masses were used for the negative ESI (112.99 and 1033.99).

For the data analysis, the data was processed using XCMS online platform (https://xcmsonline.scripps.edu) with a metabolomics workflow including feature detection, retention time correction and alignment.[43] The following parameters were used for data processing. For feature detection, the centWave method was used with a maximal tolerated m/z deviation in consecutive scans of 10 ppm, a signal to noise ratio cut-off of 6, a peak width in the range of 10 to 60 s, a minimum m/z difference for peaks with overlapping retention times set to 0.01, a prefilter intensity of 10000, the prefilter peaks set to 3 and noise to 100. To align the retention time across samples, the method obiwarp was used with a prof

step of 1. For the grouping, density was used as method with a bandwidth of 5, a width of overlapping m/z slices of 0.015 and the minimum fraction and minimum number of samples necessary in at least one of the sample groups for it to be considered as a valid group were set to 0.5 and 1, respectively.

6.3.3. Light microscopy

Microscopic images of the TIM-1 samples from the different compartments of the model and at different time points were taken with a Nikon Labophot 2 microscope (Nikon, Tokyo, Japan) equipped with an Olympus DP12 camera (Olympus, Tokyo, Japan) and a TV lens C-0.45x (Nikon, Tokyo, Japan). After mixing each sample with a pipette, several drops of the TIM-1 sample were transferred onto microscopy slide and a cover slip was placed on top of the preparation. A 40x objective lens was used resulting in a total magnification of 400x. Z-Stacking was used to get a greater depth of field for the resulting images by taking approximately five pictures at different focus distances.

6.4. Results and discussion

6.4.1. Bioaccessibility of ciprofloxacin

The bioaccessibility of ciprofloxacin after administration of the Ciproxin® suspension in the TIM-1 system in healthy and CD conditions is presented in Figure 6.2.

The total bioaccessibility of ciprofloxacin was 82.6% and 86.4% in healthy and CD conditions, respectively, suggesting a similar drug product performance in CD patients compared to healthy subjects. The reduced levels of pancreatic enzymes and bile in CD conditions are therefore, not expected to impact on the performance of Ciproxin® oral suspension, most likely due to the hydrophilic nature of ciprofloxacin. Furthermore, the lipid excipients in the Ciproxin® formulation are most likely not needed for solubility enhancement and intended for another purpose (e.g., taste masking, dispersant, stability).

The high ciprofloxacin bioaccessibility was in accordance with previous TIM-1 studies with other formulations of this drug (immediate-release and extended-release tablets) and a high human bioavailability of 70-80%.[27, 29] The maximum amount of bioaccessible ciprofloxacin per time period was observed at 0.5-1.0 h with 25.7% in healthy conditions and 23.7% in CD conditions, respectively. Pharmacokinetic studies with the Ciproxin® suspension showed a slightly higher time to maximum plasma concentration (T_{max}) of 1.1-1.5 h.[29] For the first 2.0 h after administration of the formulation, the cumulative bioaccessible amount of ciprofloxacin was high, with 68.4% for the oral suspension in healthy conditions and 84.4%

for the previously investigated immediate-release tablet.[27] A similar performance of the oral suspension compared to the immediate-release tablets has been shown in a clinical study demonstrating their bioequivalence.[29]

Ciprofloxacin behaves as a BCS class I drug *in vivo* as indicated by a study using physiologically-based pharmacokinetic (PBPK) modelling despite its common classification as BCS class II/IV compound.[25] Additionally, a limited effect of differences in simulated GI fluids (e.g., pH) on ciprofloxacin performance was also revealed.

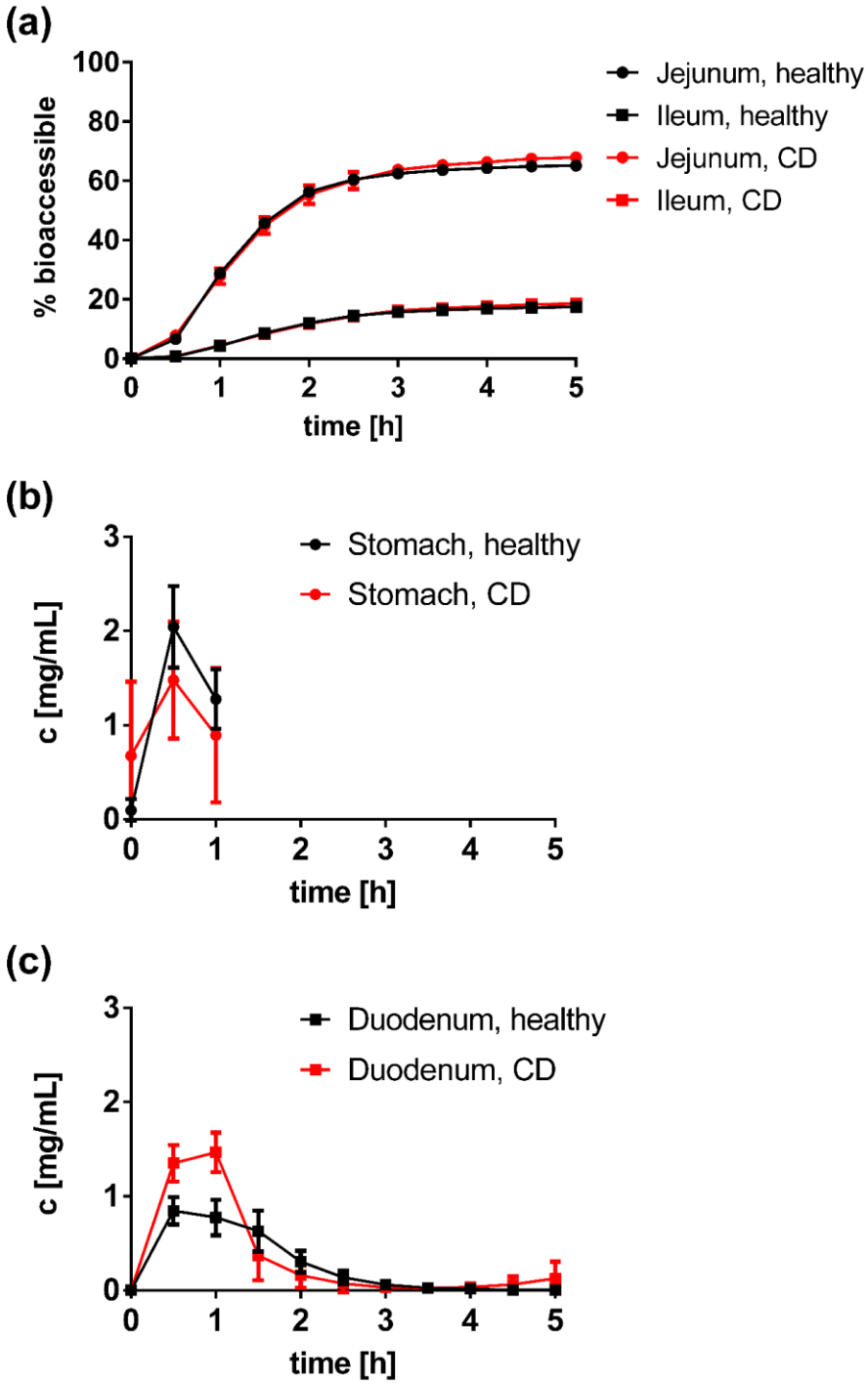

Figure 6.2: Bioaccessibility of ciprofloxacin in the jejunum and ileum compartment of TIM-1 in healthy and CD conditions (a), and ciprofloxacin concentration in the gastric compartment (b) and duodenum compartment (c).

6.4.2. Formulation and matrix components

6.4.2.1. Lipids

The digestion of excipients from a LBF can be followed in the different compartments of TIM-1, as shown in Figure 6.3, by the reduction of TGs and the increase of MGs and FAs over time as measured with GC-FID.

For triglycerides, a higher concentration in the gastric compartment was observed at time point 0.0 h in CD compared to healthy conditions. Since the concentration of gastric lipase is similar in healthy and CD conditions, no difference was expected. The observed difference could possibly be attributed to the gastric content not being well mixed at the start of the experiment and the low number of replicates (n=2). While at 0.5 h the TG concentration is higher in CD compared to healthy conditions, at 1.0 h the opposite is the case. This could be due to variations in the emptying of the gastric content and mixing as suggested by the high variability observed (coefficients of variation between 12-57%). In the duodenum, higher TG concentrations were observed for CD conditions after 0.5 h and 1.0 h, indicating a slower TG hydrolysis due to the reduced concentration of porcine pancreatin. After 2.0 h, no TGs were detected for both experimental conditions in all TIM-1 compartments.

For monoglycerides, the concentration in CD conditions reached only approximately one fifth of the concentrations observed for healthy conditions during the first 2.0 h considering all TIM-1 compartments. In both experimental setups the duodenum compartment showed the highest MG concentrations, followed by the jejunum and ileum. While in healthy conditions no MGs were detected after 3.0 h, in CD conditions MGs in the jejunum and ileum compartment were observed from 2.0-2.5 h until the end of the experiment. This indicates that the lipid hydrolysis in healthy conditions is complete after 3.0 h. In contrast, this process is slowed down in CD conditions and not complete within the 5.0 h of the experiment.

In terms of fatty acids, during the first 2.0 h, the total FA concentration in all TIM-1 compartments was approximately 5-times higher in healthy conditions compared to CD conditions. Similarly to MGs, the highest FA concentrations were observed in the duodenum followed by the jejunum and ileum for both setups. In the healthy setup, no FAs were observed after 3.5 h. Considering CD conditions, FAs in the jejunum and ileum compartment were observed starting from 1.5 h until the end of the experiment. Therefore, in healthy conditions the lipid hydrolysis of the TGs of the formulation is mainly located in the duodenum and jejunum and expected to be complete within 3.5 h. In CD conditions, the

lower FA concentrations and their delayed observation indicate a slower and unfinished digestion process.

Consequently, the different concentrations of lipids in CD compared to healthy conditions indicate that the drug is exposed to a different GI luminal environment in CD patients compared to healthy subjects.

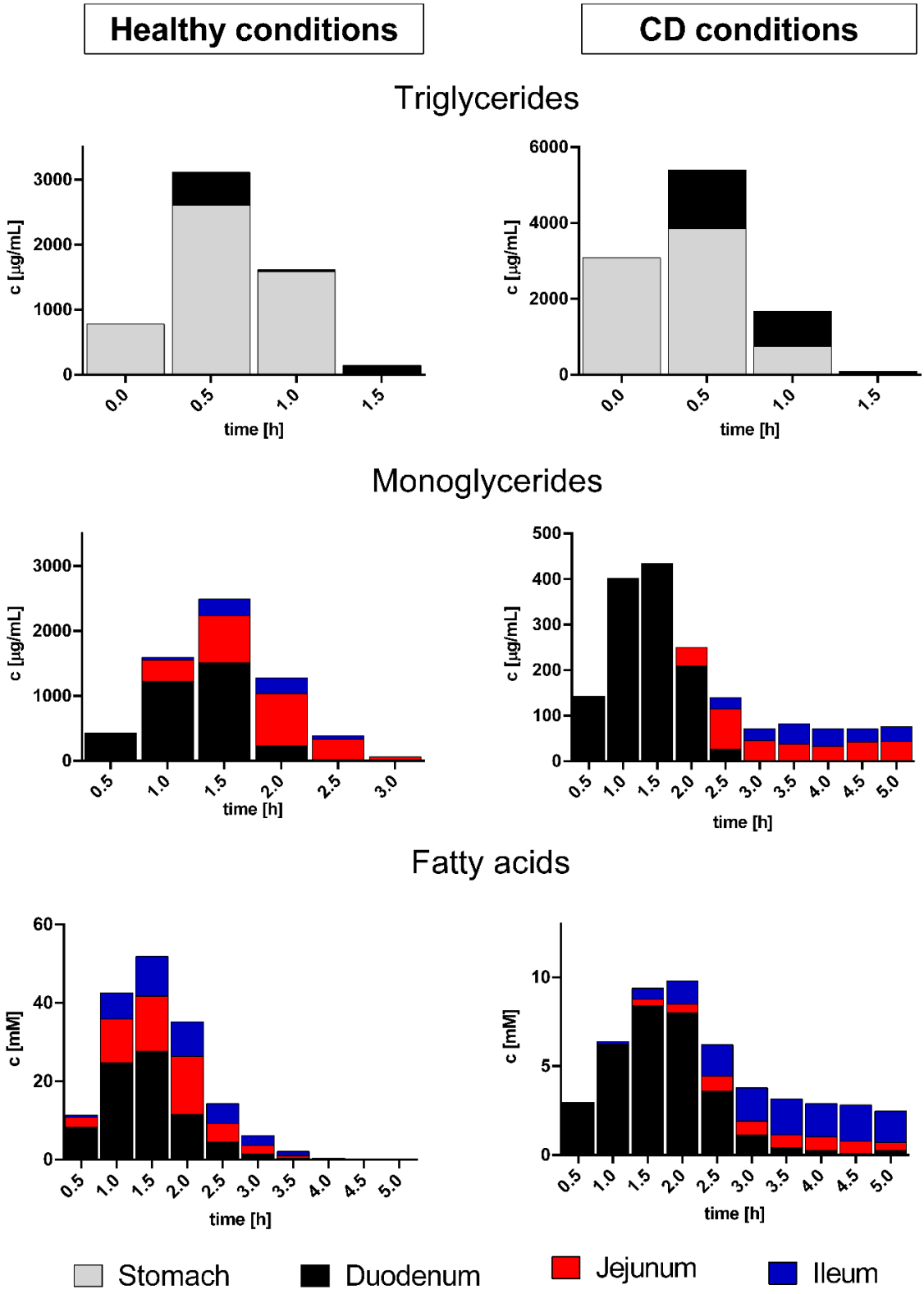

Figure 6.3: Analysis of lipid components in different compartments of TIM-1 in healthy (left) and CD conditions (right) including triglycerides (top), monoglycerides (middle) and fatty acids (bottom).

The ratio of the intensity of the FAs (octanoic and decanoic acid) in CD to healthy conditions, in the different compartments of the TIM-1, as assessed with semi-quantitative analysis using UPLC-MS is shown in Figure 6.4.

For both FAs, a lower concentration was observed in CD conditions compared to healthy conditions in the first two hours, with approximately one half of the FA concentration in the duodenum compartment and one quarter in the jejunum and ileum compartment. For octanoic acid, the concentration in CD conditions was higher compared to healthy conditions after 2.5 h in the duodenum compartment and after 3.0 h in the jejunum and ileum compartment. For decanoic acid, higher concentrations in CD conditions were observed after 2.5 h in the duodenum compartment, after 3.5 h in the ileum compartment and after 4.0 h in the jejunum compartment. Consequently, the UPLC-MS results are consistent with a delayed hydrolysis of TGs in CD conditions. Considering the total intensity of the FAs over all time points, in CD conditions only 65% and 61% of the intensity in healthy conditions was observed for octanoic acid and decanoic acid, respectively. This again suggests a lower extent of TG hydrolysis in CD. Therefore, the semi-quantitative UPLC-MS lipidomics approach has proven to be a useful quick tool to assess the differences in luminal FA concentrations.

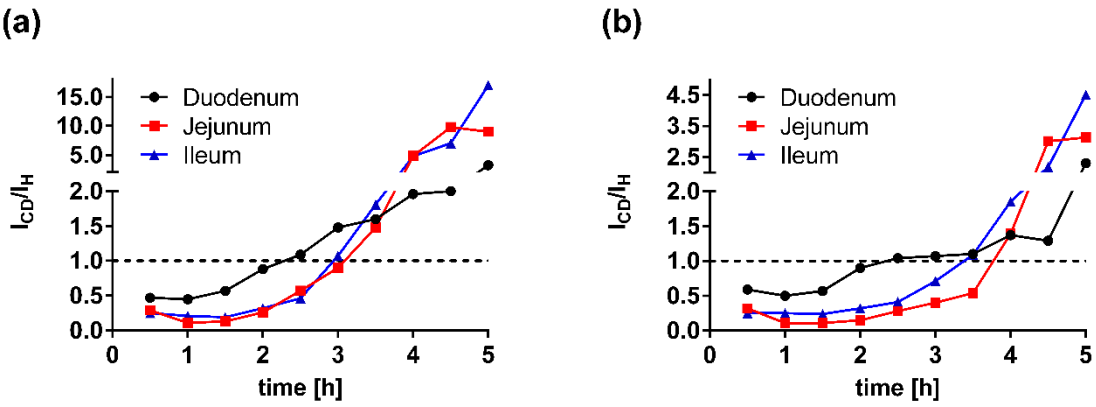

Figure 6.4: UPLC-MS intensity of fatty acids (n=1) illustrated as ratio of intensity in CD to healthy conditions for octanoic acid (a) and decanoic acid (b).

Cholesterol is an excipient of the Ciproxin® suspension but also a biliary component and therefore, present in the TIM-1 matrix. Since no cholesterol was observed in the gastric compartment, the observed cholesterol in the small intestinal TIM-1 compartments is

expected to be mainly from the biliary secretions (porcine bile). In Figure 6.5a, the mean cholesterol concentration over the 5.0 h time course of the experiment is shown in the different TIM-1 compartments and experimental setups. For the CD conditions, the cholesterol concentration is less than half of the concentration observed for healthy conditions, as expected due to the lower concentration of porcine bile in CD conditions. In terms of the biorelevance of the TIM-1 conditions, the mean duodenal and jejunal cholesterol concentrations in healthy conditions correspond to the range observed in human intestinal fluids that has been reported between 0.08 mM and 1.80 mM (mean cholesterol concentration).[30, 44-47] The time course of the cholesterol concentration in the different TIM-1 compartments is shown in Figure 6.5b. In the duodenum compartment, a lower concentration of cholesterol is observed in the first hour of the experiment, most likely due to the transfer of the gastric content to the duodenum compartment in the first hour until the housekeeper wave. In contrast, higher concentrations of cholesterol are observed for the first hour in the jejunum and ileum compartment, indicating a higher cholesterol concentration due to the preconditioning of the filter with a solution containing porcine bile or a higher concentration in the starting residues of both compartments.

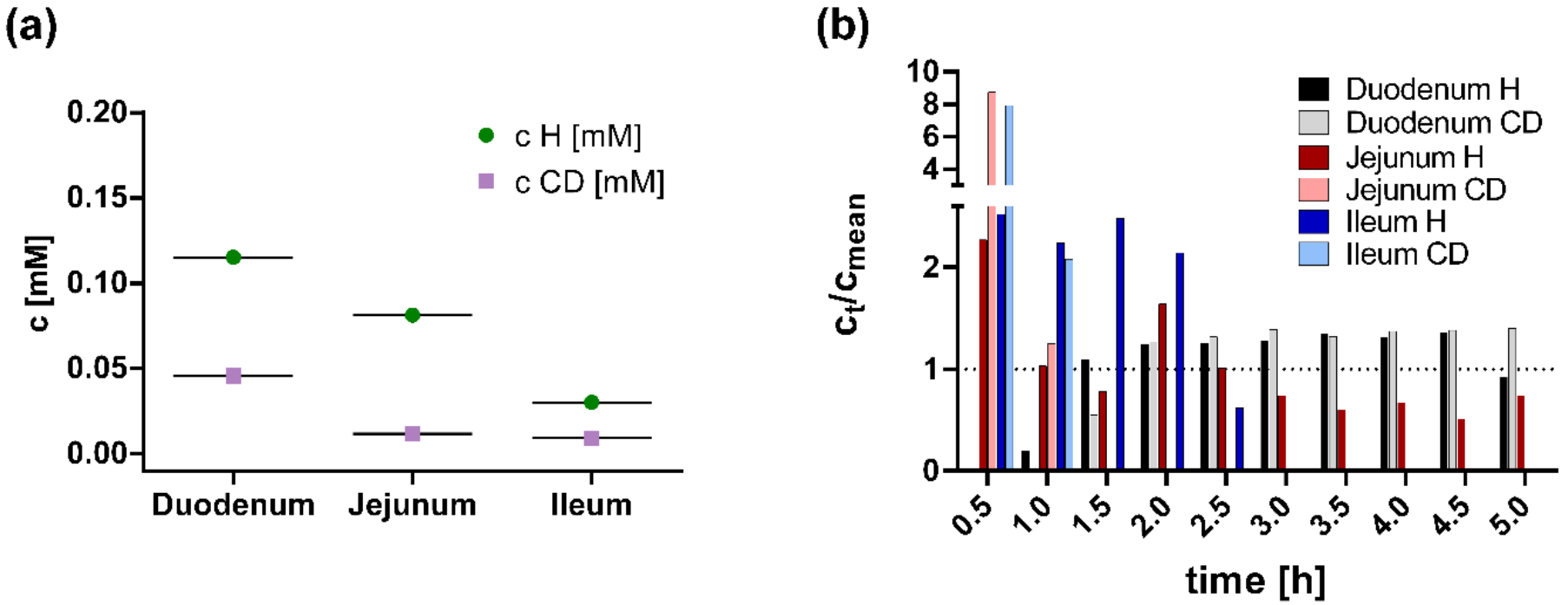

Figure 6.5: Concentration of cholesterol in different TIM-1 compartments in healthy and CD conditions shown as mean value over 5 h (a) and time course (b). [H: Healthy, CD: Crohn's disease].

6.4.2.2. Secretion of bicarbonate solution

The volume of bicarbonate solution secreted in the different TIM-1 compartments to maintain the pre-set pH in the different experimental conditions is shown in Figure 6.6. In healthy and CD conditions, more bicarbonate solution was secreted compared to the blank TIM-1 run in all compartments, indicating an impact of formulation components on pH. The digestion of TGs results in a release of FAs, which in turn provokes a pH reduction and, consequently, can trigger the secretion of bicarbonate solution. In the duodenum and jejunum compartment, more bicarbonate solution was secreted in healthy compared to CD conditions, possibly due to more FAs being released in healthy conditions (Section 6.3.2.1). In the jejunum compartment, the bicarbonate secretion slightly increased after 3 h in CD conditions, which agreed with increased FA concentrations observed at later time points (Section 6.3.2.1). Another point for consideration is that there is no direct relationship between the volume of bicarbonate solution secreted and the amount of FAs released in the compartments. For example, the concentration of FAs in the duodenal samples was higher compared to the jejunal samples in healthy conditions, while the total bicarbonate secretion was slightly higher in the jejunum. This highlights that other formulation factors and TIM-1 matrix components are also influential to the bicarbonate secretion.

The control of the bicarbonate secretion in TIM-1 is comparable to the use of sodium hydroxide in the pH stat method, another *in vitro* method for the evaluation of LBFs. For the pH stat method, the degree of lipid digestion is determined by the sodium hydroxide necessary for the neutralization of the FAs released by enzymatic lipid hydrolysis.[11] In comparison to the pH stat method, additional factors including various secretions and the compartmental transfer of formulation and matrix components can influence the pH in TIM-1 and therefore, the bicarbonate secretion. Additionally, it is difficult to assess the total digestion of the formulation in TIM-1 due to the constant removal of lipids e.g., MGs via filtration. It should be considered that in the case of formulations with long chain FAs possessing a higher pKa, the bicarbonate secretion might not be indicative of their release due to their presence in the undissociated form at luminal pH values of TIM-1.[48]

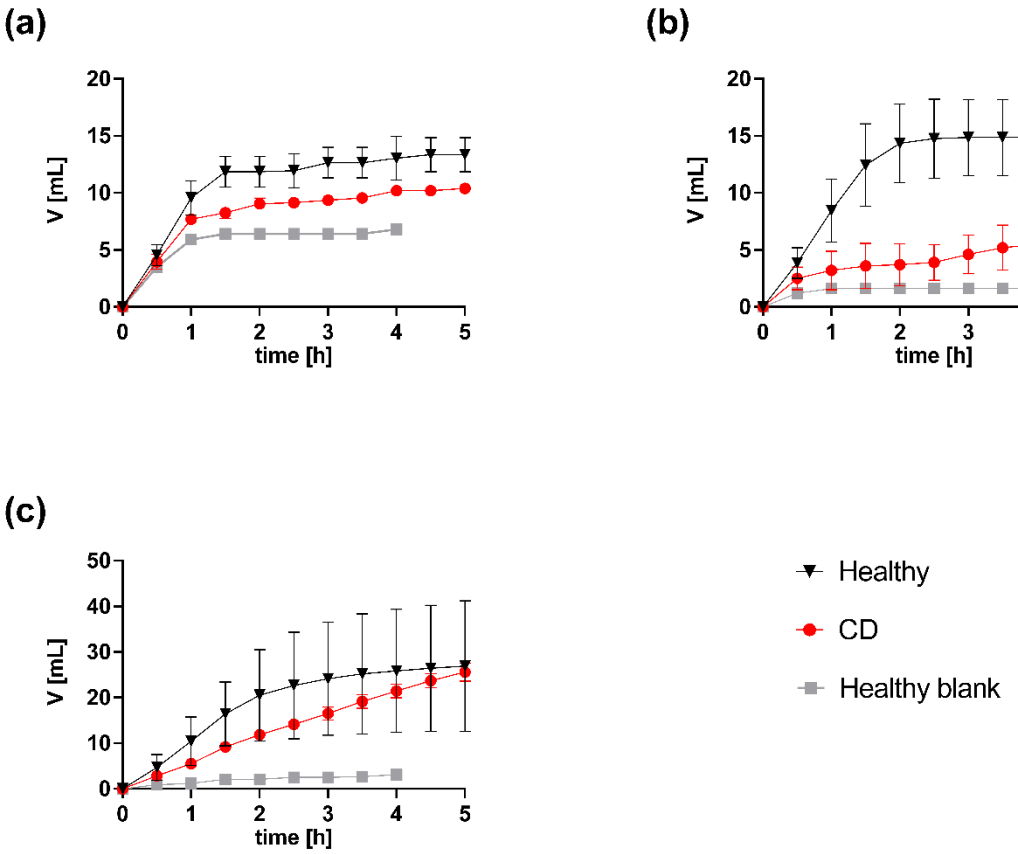

Figure 6.6: Secretion of bicarbonate solution in the duodenum compartment (a), the jejunum compartment (b) and the ileum compartment (c) in healthy and CD conditions with Ciproxin® suspension and healthy blank conditions.

6.4.2.3. Bile salts

The total bile salt concentrations over time in the different TIM-1 compartments and experimental conditions are shown in Figure 6.7.

Apart from the first two time points (0.5 h and 1.0 h), the bile salt concentration in the different TIM-1 compartments was stable over the remaining run time of 4 h. For the duodenum compartment, the difference in the beginning is most likely due to initial transfer of luminal content from the stomach to the duodenum compartment until the housekeeper wave after the first hour. In contrast, the higher bile salt concentration in the beginning in the jejunum and ileum compartment is likely due to the starting residues or initial preconditioning of the filters. Similar bile salt concentrations were observed in the different TIM-1 compartments for the healthy conditions with Ciproxin® formulation and the blank run.

For the healthy conditions, the average duodenal total bile salt concentration was 7.43 mM, the jejunal total bile salt concentration was 6.00 mM and the ileal total bile salt concentration was 3.14 mM. For the CD conditions, the average duodenal total bile salt concentration was 3.27 mM, the jejunal total bile salt concentration was 3.10 mM and the ileal total bile salt concentration was 2.28 mM. As expected, the reduced bile salt concentration in CD conditions (lower concentration of porcine bile) was reflected in all compartments with a reduced total bile acid concentration.

In comparison to human intestinal fluids, the duodenal bile salt concentration of the healthy experimental setup was significantly higher with 187% of the mean observed value in 13 different studies in healthy subjects.[16, 44, 47, 49-58] In contrast, the total bile salt concentration of the CD experimental setup was much closer to the concentration in human duodenal fluid (82% of value observed in healthy subjects). Similarly in the jejunum compartment, the total bile salt concentration in the healthy experimental setup was doubled the mean concentration in human jejunal fluid, as observed in 10 different studies, while the total bile salt concentration in CD conditions was similar (103% of the concentration in human jejunal fluids).[44, 45, 57, 59-67] Considering the ileum compartment, in both experimental setups the total bile salt concentration was 28- to 46-fold higher compared to the mean concentration in the human distal ileum in the fasted state as investigated in one study.[68] It should be taken into account that the high bile salt concentrations during the first hour have a high impact on the mean value of the ileum compartment. For example, when only the last two hours of the experiment are considered, the ileal total bile salt concentration in CD conditions was only 10-fold higher compared to the observed concentration in the human distal ileum.

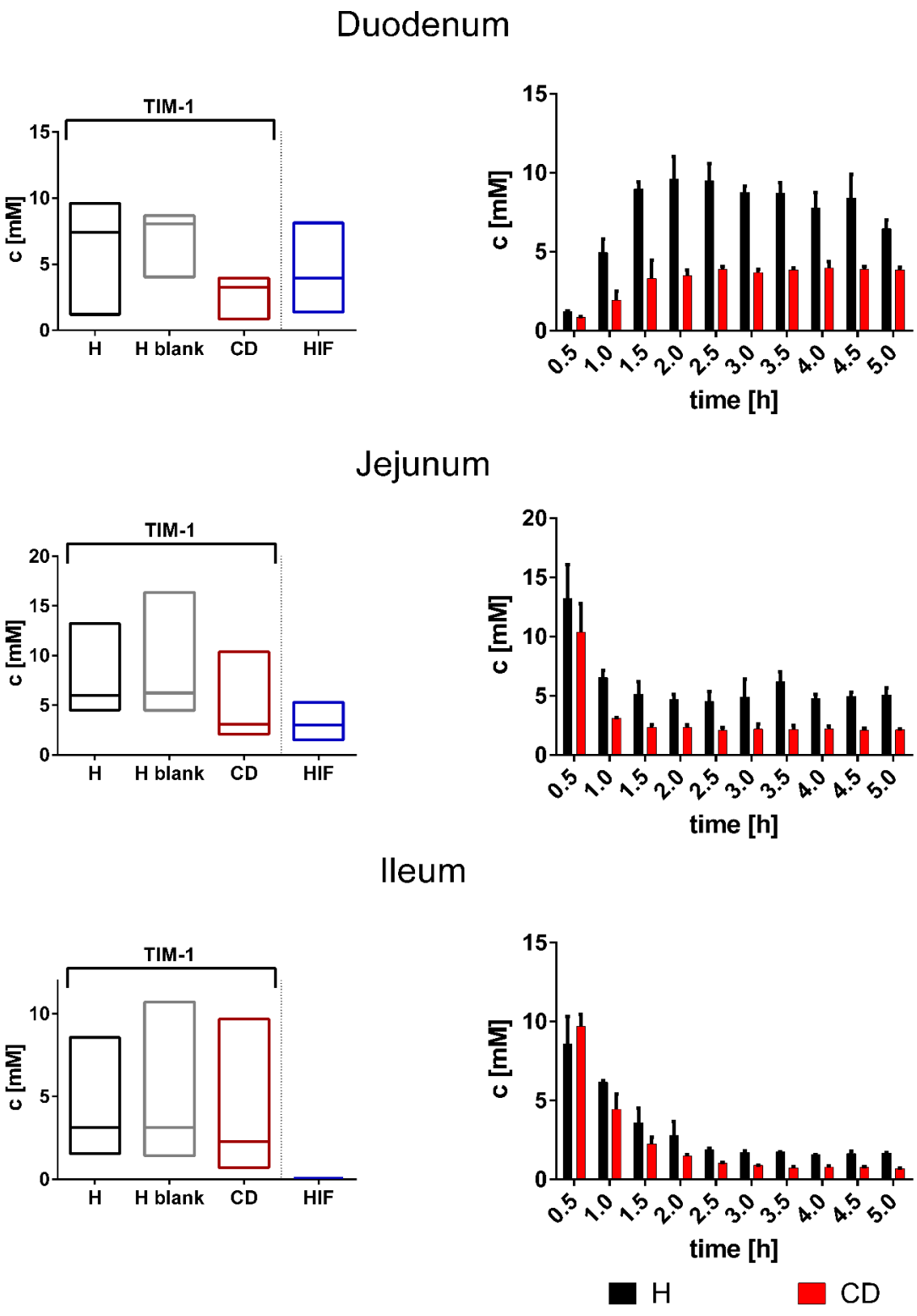

Figure 6.7: Overview of total bile salt concentration in TIM-1 in healthy and CD conditions with mean concentrations over time plus range in different compartments of the TIM-1 in comparison to human intestinal fluids (left), and total bile acid concentrations at different time points during TIM-1 run (right). [H: healthy conditions with Ciproxin®, H blank: healthy conditions without formulation, CD: CD conditions with Ciproxin®, HIF: Human Intestinal Fluids].[16, 44, 45, 47, 49-67]

In terms of the specific bile salt concentrations, Figure 6.8 gives an overview of the percentage of specific bile acids in the TIM-1 duodenum and jejunum samples in healthy conditions in comparison to human intestinal fluids.[44, 46, 49-52, 57, 59, 60] The bile salt with the retention time of 8.3 min and the molecular weight of 449.6 g/mol (data not shown, same molecular weight as GCDC) is in the following assumed to be Glycohyodeoxycholate (GHDC), which has been reported as a major component of porcine bile.[69]

Due to the similar source of bile salts (porcine bile), the duodenum and jejunum compartment showed similar percentages of the specific bile salts, with GHDC as most prominent component followed by GCDC and TC. TCDC and GC had the lowest percentages, both accounting for less than 6% of the total bile salt concentration.

In comparison to human duodenal fluids, a similar percentage of TC and GCDC was present in the duodenal TIM-1 samples compared to the percentage observed in human duodenal fluids. For TCDC and GC, the percentage in the TIM-1 samples of the duodenum compartment is lower compared to human duodenal fluids. GHDC, which accounted for approximately one third of the bile salt concentration in TIM-1 samples of the duodenum compartment, was not present in human duodenal fluids, instead another glycine conjugate, GDC, was present in human duodenal fluid. Considering the proportion of glycine to taurine conjugates, glycine conjugated bile salts are slightly more prevalent in TIM-1 samples with 71% to 29% compared to 59% to 40% in human duodenal fluids. Similar differences were observed when comparing the specific bile salt composition of TIM-1 samples of the jejunum compartment with human jejunal fluids.

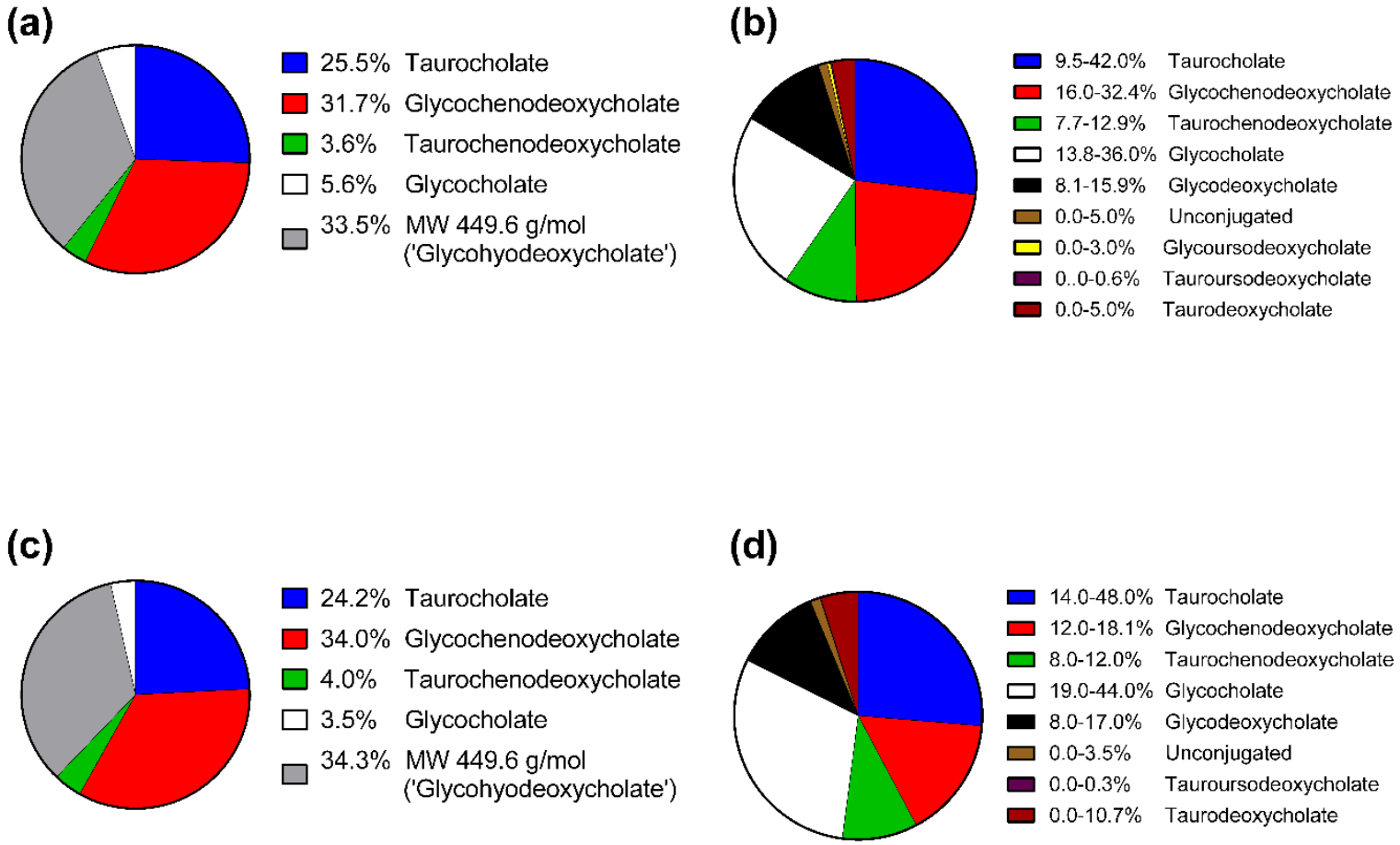

Figure 6.8: Mean bile salt composition of TIM-1 in the duodenum compartment (a) and the jejunum compartment (c) in comparison to the bile acid composition of human intestinal fluids from the duodenum (b) and the jejunum (d) as reported in literature.[44, 46, 49-52, 57, 59, 60]

The ratio of the intensity of specific bile salts in CD to healthy conditions in the different compartments of the TIM-1 as assessed with UPLC-MS is shown in Figure 6.9.

In the duodenum and jejunum compartment, the ratio of bile salts in CD to healthy conditions is stable after 1.5 h, with CD conditions showing approximately 50% of the bile salt intensity of healthy conditions. During the first hour of the experiment, the concentration of bile salts in CD conditions is closer to the bile salt concentration in healthy conditions, most likely due to the starting residues or preconditioning of the filters. In the ileum compartment, the bile salt concentration in CD conditions compared to healthy conditions was initially lower than in the duodenum and ileum. However, the overall bile salt concentration in CD conditions in the ileum was also approximately half of the concentration in healthy conditions. The lower concentration of porcine bile in the CD conditions (43% of healthy conditions) was therefore, approximately reflected in the bile salt concentrations in all TIM-1 compartments. The presented semi-quantitative UPLC-MS analysis of luminal bile salt concentrations can consequently, be used to monitor the difference between two different experimental setups in a time-efficient way.

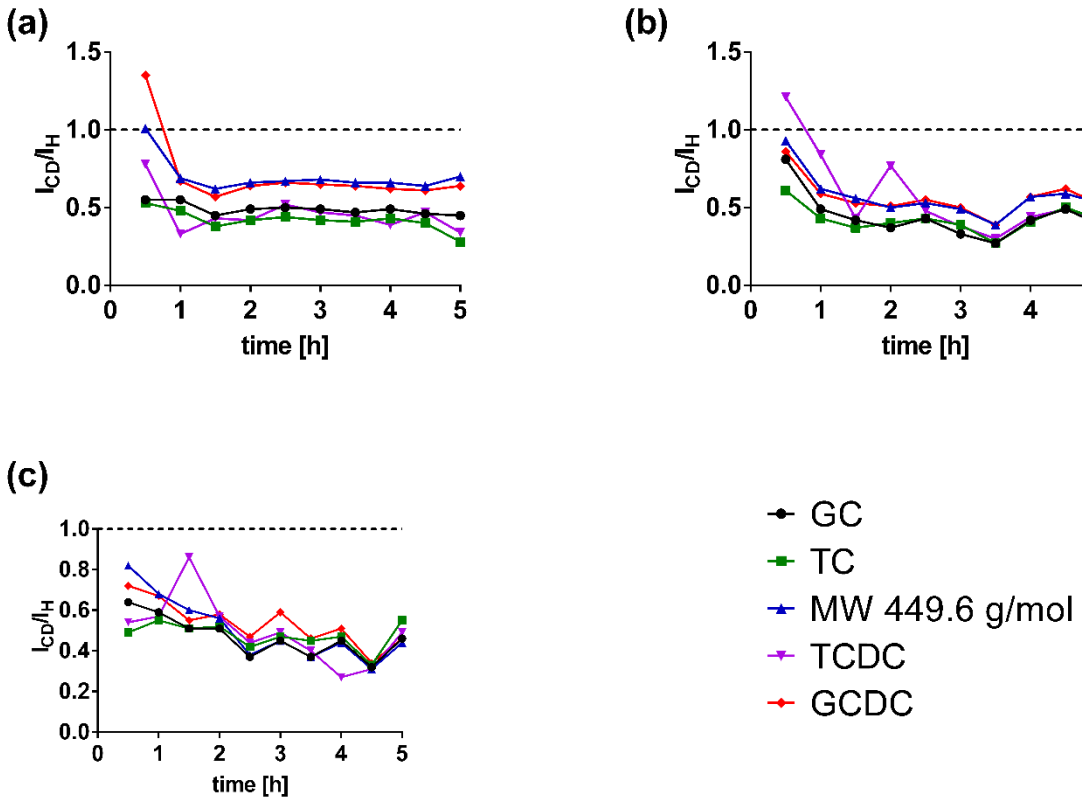

Figure 6.9: UPLC-MS intensity of specific bile salts in TIM-1 illustrated as ratio of the intensity in CD to healthy conditions in the duodenum compartment (a), in the jejunum compartment (b) and in the ileum compartment (c). [GC: Glycocholic acid, TC: Taurocholic acid, TCDC: Taurochenodeoxycholic acid, GCDC: Glycochenodeoxycholic acid].

6.4.3. Light microscopy

The contents of the gastric and duodenal compartment were examined with light microscopy as shown in Figure 6.10. In the stomach compartment, the emulsion droplets showed a polydisperse particle size distribution with similar droplet sizes for the different time points. In the duodenum compartment, the emulsion droplets were bigger during the first hour and their diameter decreased subsequently. Differences between healthy and CD conditions were not observed.

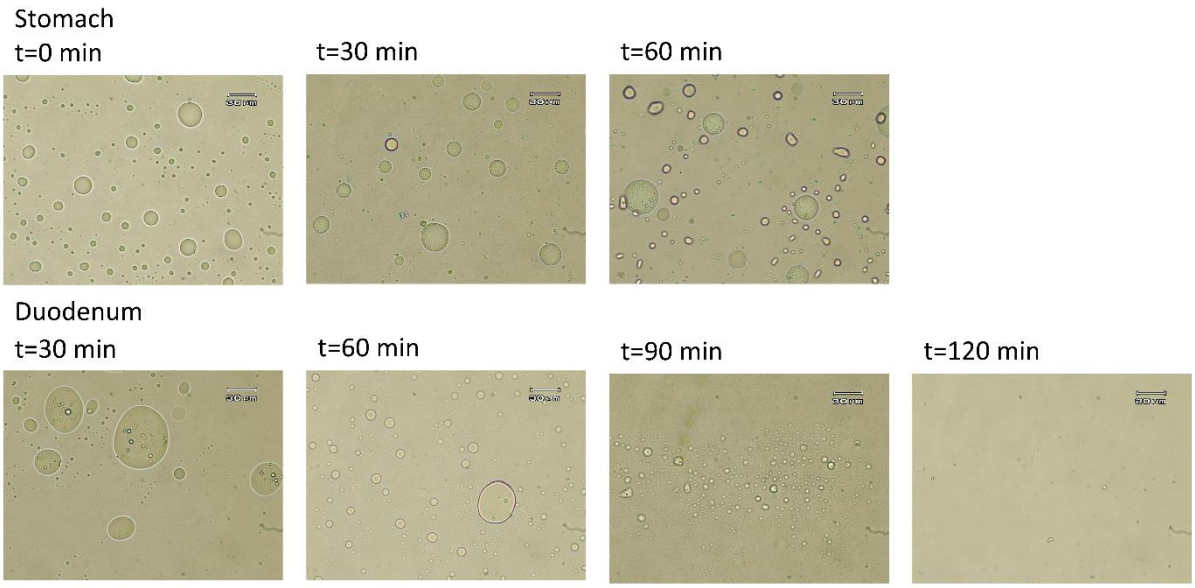

Figure 6.10: Light microscopy pictures of the contents of the gastric and duodenal compartment after administration of Ciproxin® oral suspension in healthy conditions (scale bar is 30 µm).

6.5. Conclusion

The performance of Ciproxin® oral suspension was not impacted by CD conditions, most likely due to the low lipophilicity of ciprofloxacin. The digestion of excipients of a LBF can be followed in the TIM-1 system. By comparing the lipolysis of the medium chain TGs in healthy and CD conditions, reduced FA and MG concentrations were observed in CD conditions during the first hours, followed by higher concentrations at the end of the experiment. This indicates a delayed and reduced digestion process in CD conditions. Consequently, the GI luminal environment is expected to be different in CD patients compared to healthy subjects, suggesting a possible impact on the performance of LBFs in CD.

For more lipophilic compounds, differences in drug product performance of LBFs are expected due to the differences observed in the luminal environment, which suggests an increased risk of altered drug product performance in patients with CD. However, the use of the TIM-1 system for the investigation of LBFs could be restricted due to drug binding to silicone membranes and filters of the TIM-1 system, as previously reported and as observed for another model drug in pretesting experiments.[22]

In terms of the biorelevance of the TIM-1 conditions, bile acid concentrations were higher in healthy TIM-1 fasted state conditions compared to reported concentrations in human

intestinal fluids. Interestingly, the conditions defined for CD patients showed similar bile salt concentrations compared to human intestinal fluids of healthy subjects. Cholesterol concentrations in healthy conditions were in the range of the levels observed in human intestinal fluids.

6.6. References

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Conclusions and Future Directions

Conclusions

Pathophysiological changes in patients with GI diseases such as CD, UC, CED can impact on drug product performance. In the absence of clinical studies, *in vitro* and *in silico* tools can be used to indicate the direction and magnitude of these changes on the bioavailability of drugs in GI disease patient populations.

To predict differences in drug solubility and dissolution *in vitro*, biorelevant media simulating the GI fluids of different GI compartments and prandial states in patients with CD, UC and CED have been developed. The developed GI disease media were compared to biorelevant media based on healthy subjects and differences in media characteristics were mainly observed in terms of surface tension, osmolality and buffer capacity. These results suggest a possible impact on drug or formulation performance due to altered wetting behaviour, osmotic pressure or ionisation. Drug solubility of six drugs with different physicochemical properties was determined in CD, UC and CED media and compared to healthy biorelevant media.

For CD, differences in drug solubility considering gastric CD media were related to drug ionisation. In fasted state intestinal and colonic fluids of CD patients, drugs with moderate to high lipophilicity are at risk of a lower drug solubility.

For UC, drugs with a high lipophilicity are at risk of altered drug solubility in UC intestinal fluids, especially in patients with low concentrations of bile salts and lecithin. In fasted and fed state colonic fluids of UC patients, major differences in drug solubility are expected due to drug ionisation indicating an increased risk for weak acids and bases. Additionally, a higher solubility is indicated for neutral compounds in fasted state colonic fluids of UC patients due to higher concentrations of soluble proteins. Decreased concentrations of lecithin in fed state colonic fluids of UC patients pose a risk of altered solubility to neutral compounds.

For CED, differences in the intestinal fluid composition compared to healthy subjects are expected to only pose a minimal risk to hydrophilic compounds. The highest impact of differences in CED was observed for neutral compounds with moderate to high lipophilicity indicating a higher solubility in most cases as a result of increased surfactant concentrations (bile salts, lecithin). Additionally, it was shown that the concrete influence of cholesterol,

lecithin and bile salts on drug solubility was specific to each investigated drug indicating a complex interplay between drugs and media.

For drugs with solubility- or dissolution rate-limited absorption, the described differences of drug solubility between healthy and GI disease media are likely to result in altered performance in these patients. Apart from drug dissolution, drug product performance can also be affected by differences in permeability, distribution, gut wall/hepatic metabolism and elimination. Therefore, pathophysiological differences considering all ADME processes need to be considered to identify all drugs at risk of altered drug product performance in patients with GI diseases. Results from solubility and dissolution experiments can be integrated in PBPK models offering the opportunity to integrate ADME processes mechanistically and to consider the special physiology of patient populations in order to predict a drug's plasma concentration profile *in vivo*.

This approach was pursued by investigating the performance of budesonide in patients with CD. An *in vitro* dissolution methodology was developed representative of conditions in healthy subjects and CD patients. For a controlled-release budesonide formulation (Entocort®), a similar performance was observed in the fasted state, while the release of budesonide was lower in the fed state in CD conditions compared to healthy conditions. By integrating these *in vitro* biorelevant dissolution profiles in PBPK models, the budesonide plasma concentration profile after administration of a controlled-release formulation of budesonide was successfully predicted for healthy subjects in the fasted and fed state. Pathophysiological differences in CD patients were identified in literature and included a reduced hepatic CYP3A4 activity, altered intestinal CYP3A4 activity, decreased concentration of human serum albumin, increased gastric pH and altered GI transit times. The impact of these differences on budesonide performance was investigated with the PBPK model and revealed the highest impact on the simulations for hepatic CYP3A4 enzyme abundance and human serum albumin concentration. A PBPK model with a population adapted to CD physiology successfully predicted the increased exposure of budesonide in CD patients compared to healthy subjects as reported in several PK studies. This mechanistic modelling approach has highlighted the importance of considering all ADME parameters in patients with GI diseases. Despite the location of CD in the GI tract, the main impact on budesonide performance was observed due to differences in metabolism and distribution, while differences in the GI tract showed only a minor impact.

For lipid-based formulations, *in vitro* tools need to additionally consider the enzymatic digestion of lipid excipients to simulate the dynamic luminal environment to which the formulation is exposed in the GI tract. Since in CD patients a reduced pancreatic lipase activity has been reported, differences in the performance of lipid-based formulations are expected. To consider such differences, a dynamic GI simulator (TIM-1, TNO) was used to simulate conditions in CD and healthy subjects and used to test the performance of a lipid-based formulation of ciprofloxacin (Ciproxin® oral suspension). While the lipid digestion process was delayed and reduced in CD, the performance of the formulation was not affected. This was most likely due to the low lipophilicity of ciprofloxacin indicating a different purpose for the addition of lipid excipients in this formulation. The altered luminal environment suggests an impact on the performance of LBFs in CD in cases when lipid excipients are needed for drug solubilisation. For those lipophilic compounds, however, drug binding to membranes and filters of the TIM-1 system could limit the application of this *in vitro* tool.

Future Directions

The research portrayed within this thesis presented several *in vitro* and *in silico* tools to predict drug product performance in patients with GI diseases with the aim to improve the drug therapy of this patient population. In the future, this aim can further be achieved by additional characterisation of the physiology of GI disease patients, by extending the presented tools to further GI diseases and by investigating more drugs and formulations to target the refinement of developed *in vitro/in silico* tools.

Considering the physiology, only a very limited amount of studies has been performed to characterise e.g., the luminal fluid composition, GI hydrodynamics, metabolic enzyme activities and distribution processes in patients with GI diseases. Further studies assessing those processes would help to increase the confidence in the developed *in vitro* and *in silico* tools and indicate when modifications are needed. Additionally, including a large number of patients in those studies would help to identify interpatient variability and allow to differentiate between findings specific to disease states or disease location when looking at specific patient populations.

In terms of the extension to further GI diseases, the development of biorelevant media comprised three different GI diseases including CD, UC and CED, while the focus for *in vitro* dissolution testing and PBPK modelling was on CD patients. The extension of those approaches to other GI conditions would allow for the prediction of formulation performance

in these patients and could further guide prescribers for the drug therapy of the affected patients.

Testing more drugs and formulations with the developed tools can be used for validation purposes and is expected to increase the confidence in the developed methods. Considering drug solubility in GI disease biorelevant media, the power of the developed statistical models could be increased by investigating a higher number of drugs with different physicochemical properties. The improved statistical models would further indicate the drugs that are at risk of altered performance in GI disease patients and therefore, narrow down the number of drugs for which *in vitro* studies are needed. For *in vitro* dissolution studies, different types of formulations and drugs should be investigated. This should especially include drugs at high risk of altered performance such as immediate-release formulations of BCS class II drugs with reduced drug solubility in biorelevant media of GI disease patients. Regarding the developed PBPK model, the developed CD patient population should also be used for the investigation of additional drugs and could further be validated with PK data in CD patients.

In terms of the impact of enzymatic differences in CD, an altered luminal dynamic environment compared to healthy subjects is expected after the administration of lipid-based formulations. Further studies are needed to investigate compounds with a high lipophilicity, for which drug product performance is expected to be impacted by those changes.

This declaration concerns the article entitled:									
Impact of gastrointestinal disease states on oral drug absorption - implications for formulation design - a PEARRL review									
Publication status (tick one)									
draft manuscript		**Submitted**		**In review**		**Accepted**		**Published**	✓
Publication details (reference)	Effinger A, O'Driscoll CM, McAllister M, Fotaki N. Impact of gastrointestinal disease states on oral drug absorption - implications for formulation design - a PEARRL review. J Pharm Pharmacol. 2019;71(4):674-98. https://doi.org/10.1111/jphp.12928 (https://onlinelibrary.wiley.com/doi/10.1111/jphp.12928)								
Candidate's contribution to the paper (detailed, and also given as a percentage).	The candidate contributed considerably in: **Formulation of ideas:** The ideas were developed by Dr N Fotaki (the main supervisor) (60%), co-supervisors (Prof CM O'Driscoll, Dr M McAllister) (5%) and the candidate (35%). The initial idea of the review paper belonged to Dr Fotaki. The candidate presented its suggestions for the review paper content according to the literature review, which were then reviewed and modified by Dr Fotaki and the co-supervisors. **Design of methodology:** The methodology was developed by Dr Fotaki (50%), industrial supervisors (5%) and the candidate (45%). **Experimental work/Literature review:** The literature review was carried out by the candidate (100%). **Presentation of data in journal format:** Data was formatted and presented in the paper by the candidate (40%) and its main supervisor Dr N Fotaki (50%). Data was processed and presented graphically by the candidate and reviewed and corrected by the main supervisor. Feedback was also provided by the co-supervisors (10%).								
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.								
Signed	A. Effi					**Date**	27 Sep 19		

This declaration concerns the article entitled:									
Investigating the impact of Crohn's disease on the bioaccessibility of a lipid-based formulation with an *in vitro* dynamic gastrointestinal model									
Publication status (tick one)									
draft manuscript	✓	**Submitted**		**In review**		**Accepted**		**Published**	
Publication details (reference)	Not applicable yet								
Candidate's contribution to the paper (detailed, and also given as a percentage).	The candidate contributed considerably in: **Formulation of ideas (60%):** The ideas were developed by Dr N Fotaki (the main supervisor), co-supervisors (Prof CM O'Driscoll, Dr M McAllister) and the candidate. The initial idea of the research paper belonged to the candidate. Further suggestions were given by Dr N Fotaki and the co-supervisors. **Design of methodology (50%):** The methodology was developed by Dr N Fotaki, co-supervisors, the candidate, Irena Tomaszewska, Mark Taylor, Steve Gomersall and James Heaton. **Experimental work (70%):** TIM-1 experiments were performed by the Pfizer TIM-1 team including Irena Tomaszewska, Kieran L Smith, Inese Sarcevica, Sudesha Wanigaratne, Aidan Harper, Sam L Young and the candidate at equal contribution. The analysis of bile salts with HPLC-CAD, lipids with GC-FID, lipids and bile salts with UPLC-MS was performed by the candidate with initial support and help for the method development from Mark Taylor, Steve Gomersall and James Heaton, respectively. The light microscopy pictures were taken by the candidate with initial support from Neil Dawson. **Presentation of data in journal format (65%):** Data was formatted and presented in the paper by the candidate and its main supervisor Dr N Fotaki. Data was processed and presented graphically by the candidate and reviewed and corrected by the main supervisor. Feedback was also provided by the co-supervisors.								
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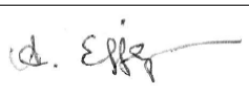
Signed					**Date**	30.09.2019			