National and Kapodistrian University of Athens

Characteristics of contents of proximal colon: Implications for drug absorption and in vitro simulations

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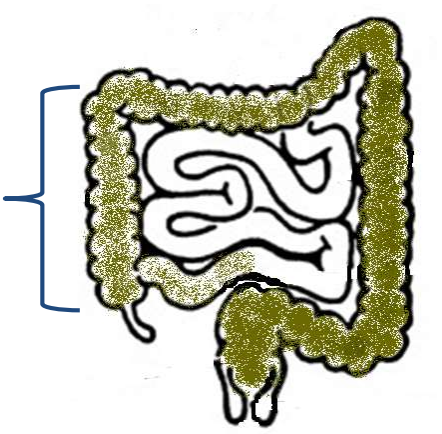
COLOTAN

EU H2020 MSCA ETN COLOTAN Kick-off Event
18 June 2021

Proximal / Right / Ascending Colon
(usually includes Cecum)



Vertzoni et al. (2018)

At distal regions, drug dissolution/release and approach to the mucosa are expected to be limited, due the limited fluid volume* and the increased bacterial content**, and systemic drug absorption can be problematic

Schiller et al. 2005*; Murray et al. 2017*; Rajilic-Stojanovic et al. 2007**; Sousa et al. 2008**

OUTLINE

- Methodologies for characterizing the contents of proximal colon

- Characteristics of contents of proximal colon

- Implications for drug absorption

- In vitro simulations for evaluating the impact of luminal characteristics on drug absorption from the proximal colon

Characterizing the contents of proximal colon...

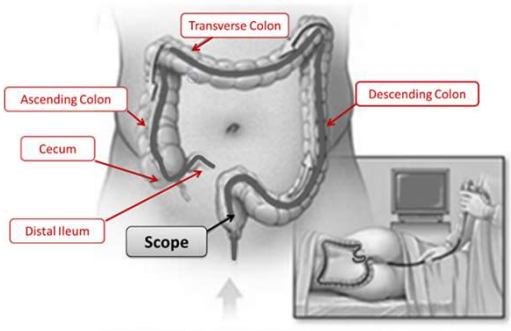
Available experimental techniques? Prandial conditions? Population?

Imaging techniques, e.g. Telemetric capsules, Magnetic Resonance Imaging

- little or no bowel preparation
- pH and fluid volumes have been investigated

Direct sampling techniques, e.g. Colonoscopy

- pH, volume of contents, buffer capacity, viscosity, presence of solubilizing agents, osmolality
- Preparation of distal colon is required



Characterizing the contents of proximal colon...

Sampling from the proximal colon in the framework of drug (product) disposition studies requires preparation of the distal colon without disturbing the physiological state of proximal colon at sampling time

KULeuven (Lemmens et al. 2021)
One-time enema of 250ml of water appears sufficient to cleanse the left hemicolon

NKUA (Diakidou et al. 2009)
(After evaluating various methodologies)
50h and 44h prior to colonoscopy 10mg bisacodyl [bis(p-acetoxyphenyl)-2-pyridylmethane] (BIS, Dulcolax®, mild laxative) is orally administered to prepare primarily the descending and sigmoid colon

BIS for distal colon preparation?

After oral administration BIS is rapidly converted to the active metabolite bis(p-hydroxyphenyl)-2-pyridylmethane (BHPM) and its action is initiated by activating protein kinase C releasing prostaglandin E2 and, thereby, inducing net fluid secretion (in vitro & human data)

Effects on the intralumenal physiology have been shown to be reversible (rat and human data).

Mucus secretion occurs only at BHPM concentrations of at least 1 μg/ml
Sodium and fluid secretion occurs at intracolonic BHPM concentrations of ≥5 μg/ml (rat data)

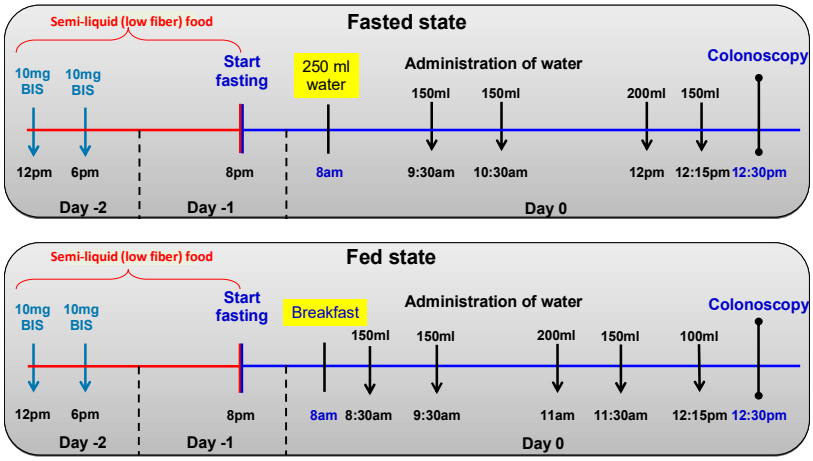
With the NKUA protocol for colon preparation, after overnight fasting and 5h after a glass of water, BHPM at proximal colon is ~ 0.5 μg/ml
Also, stools consistency and osmolality of contents at the proximal colon are used as supporting evidence

A useful methodology, especially when considering sensitive populations

Diakidou et al. Pharm. Res 2009

Characterizing the contents of proximal colon...

The two protocols applied at NKUA for sampling in the framework of drug (product) disposition studies



These protocols may also be useful for mapping intraindividual variability even when not aiming at simulating the conditions during typical BA/BE studies

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Fluid volumes in the young healthy adult large intestine

Table 1. Gastrointestinal fluid volumes as determined by magnetic resonance imaging (MRI) under fasting conditions and 1 h after a meal ($n = 12$)

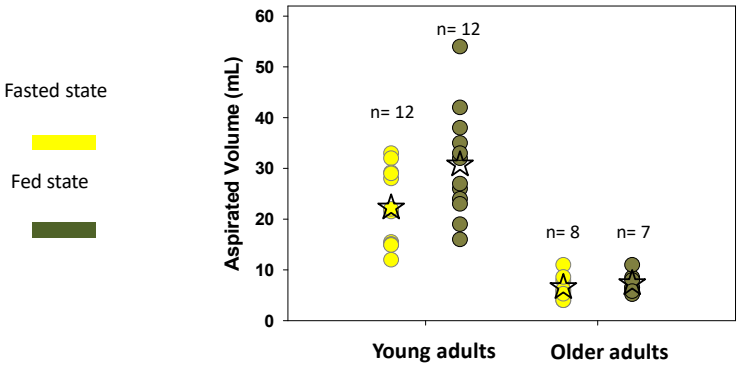
	Stomach [volumes (mL)]*	Small intestine [volumes (mL)]	Large intestine [volumes (mL)]
Fasting			
Minimum	13	45	1
Maximum	72	319	44
Median	47	83	8
Mean (s.d.)	45 (18)	105 (72)	13 (12)
Fed			
Minimum	534	20	2
Maximum	859	156	97
Median	701	39	18
Mean (s.d.)	686 (93)	54 (41)	11 (26)

* The volume of the stomach after the meal represents the filling volume (not only fluid).

Schiller et al. 2005

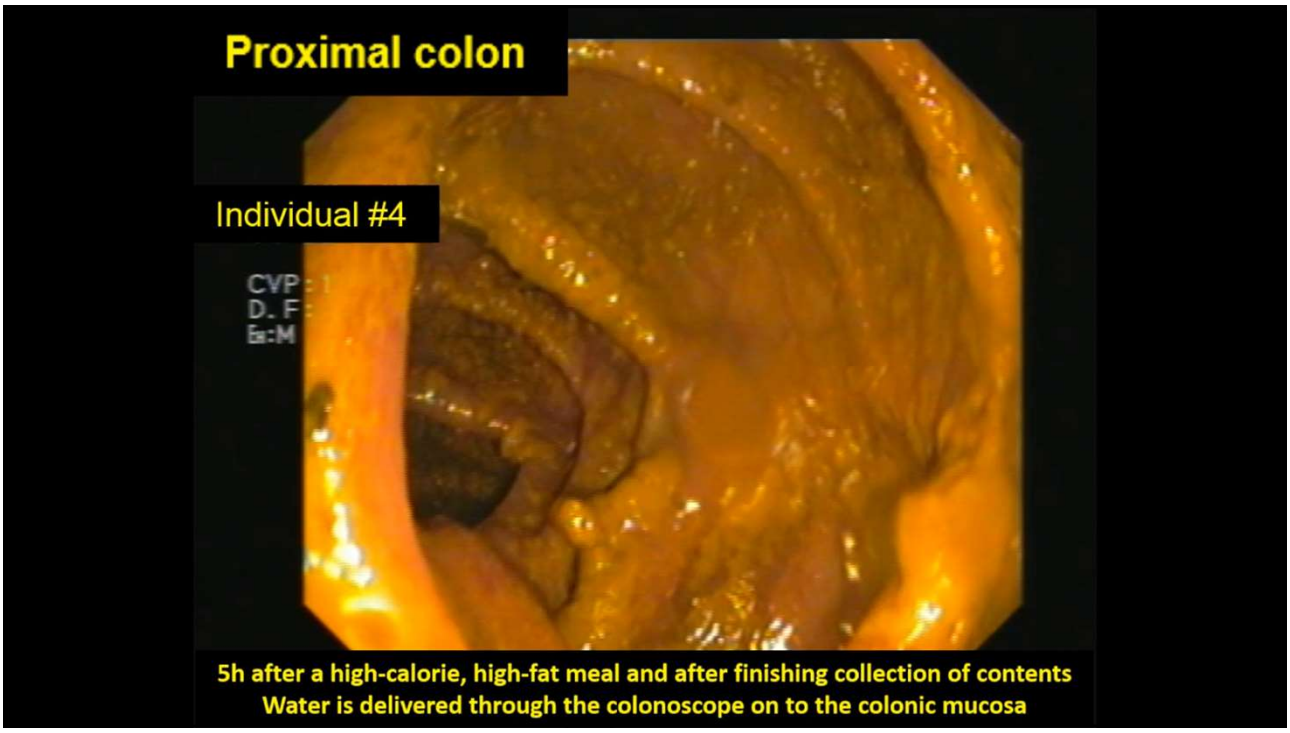
For the fasted large intestine similar values have been reported by Murray et al. (2017)

Total aspirated volumes from the proximal colon: healthy young adults vs. older adults (65-74y)



Age effect is significant at both prandial states

Vertzoni et al. 2020



Total aspirated volumes from the proximal colon: healthy young adults vs. older adults (65-74y)

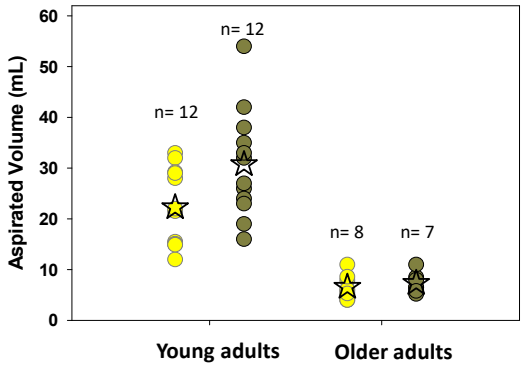
In older adults the proportion of contents adhered on the mucosa ranged from 2/3 to 9/10 of total contents

Regardless of the age, in the fed state, the mucosa of proximal colon was always “wet”, no pockets of contents were observed

Total aspirated volumes from the proximal colon: healthy young adults vs. older adults (65-74y) vs. UC adults in relapse

Fasted state

Fed state



Age effect is significant at both prandial states

Vertzoni et al. 2020

Fasted state / UC adults in relapse: Treatment with prednisolone significantly increases the volume of aspirated contents from the proximal colon 37.4(14.4)ml vs. 19.3(6.3)ml (n=12)

Vertzoni et al. 2010a

Table 1 Intestinal luminal pH studies using radiotelemetry capsules in healthy volunteers

Study	Patients	Small bowel pH		Caecum/right colon pH	Left colon/ rectal pH
		Proximal	Distal		
Watson, 1972[2]	2 normals+7 misc. GI disorders	5.5–7.0	6.5–7.5	5.5–7.5	6.5–7.5
Bown, 1974[3]	11 normals	5.9	7.5	6.0	7.0
Evans, 1988[4]	66 normals	6.6	7.5	6.4	7.1
Fallingborg, 1989[5]	39 normals	6.4	7.3	5.7	6.6
Raimundo, 1992[6]	7 normals	6.6	7.4	6.7	N/A
Fallingborg, 1998[8]	13 normals	6.4	7.4	5.8	N/A
Sasaki, 1997[9]	4 normals	6.8	7.7	6.8	7.2
Press, 1998[10]	12 normals	6.7	7.5	6.1	6.1
Ewe, 1999[11]	13 normals	6.5	7.6	6.2	7.0

N/A, data not available.

Early studies: Food consumption during the study was not monitored

Nuggent et al. 2001

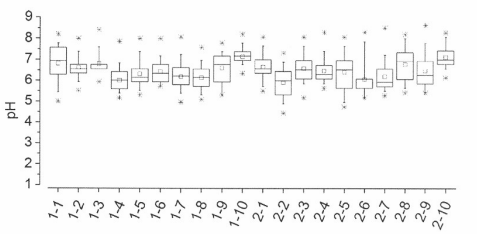
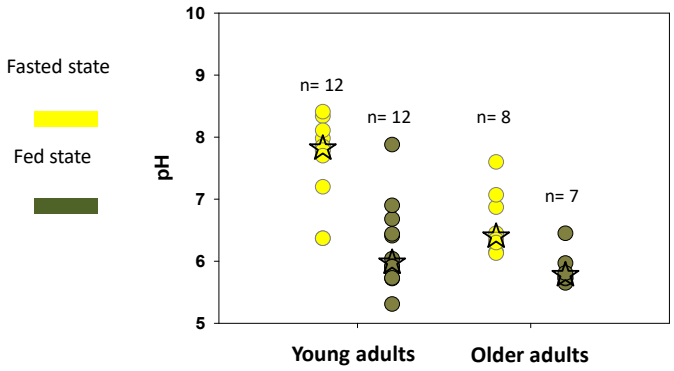


Figure 6. Box plots (box: 50%, whisker: 5%–95%, square: mean, asterisks max/min) of colonic pH values for both studies.

Koziolek et al. (2014):
Colonic pH fluctuated 5-8
Lunch was consumed 4h after ingesting the capsule in the fasted state

pH of contents measured immediately upon aspiration from proximal colon: healthy young adults vs. older adults (65-74y)



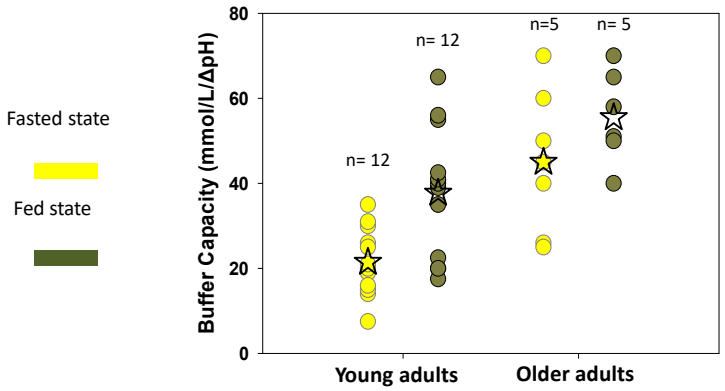
In the fasted state,
age effect is significant

- Decreased absorption of nutrients in the small intestine adults (?)
- 2 older adults on aspirin treatment (decreased mucosal prostaglandins, decreased bicarbonate production)

Vertzoni et al. 2020

Fasted state: No pH drop from distal small intestine to the proximal colon (not shown)

Buffer capacity (HCl) of contents measured immediately Upon aspiration from proximal colon: healthy young adults vs. older adults (65-74y) vs. UC adults in relapse



Age effect is significant
at both prandial states

Vertzoni et al. 2020

Fasted state / UC adults in relapse:
Increased buffer capacity [32.0(18.1)] compared with young healthy adults

Vertzoni et al. 2010a

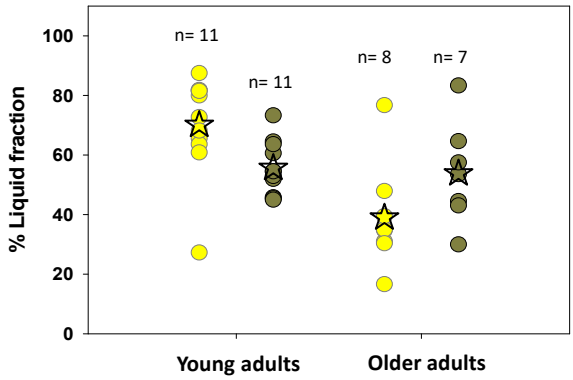
Size of non-liquid particles diameter collected after ultracentrifugation of contents of proximal colon of young adults

	Fasted state	**Fed state**
Number of volunteers	5	6
Volume Mean Diameter (D[4,3], μm) ± SD*	73.60 ± 89.22	262.64 ± 51.64

*For each volunteer, 1-4 random samples were measured, i.e. n=9-14

Reppas et al. 2015

% Liquid fraction after ultracentrifugation of contents: healthy young adults vs. older adults (65-74y)



In the fasted state, impact of age is significant

Vertzoni et al. 2020

PChem characteristics of the liquid fraction of contents of proximal colon

Young adults (upper row) – Older adults (65-74y) (lower rows) – UC adults in relapse

	Fasted state	Fed state
Osmolality of aqueous fraction (mOsmol/kg)	**81 (102)**	224 (125)
	299 (49)	264 (76)
Short Chain Fatty Acids concentration in the aqueous fraction (mM)	**31 (15)**	**48 (24)**
	88 (36)	**78 (20)**
Long Chain Fatty Acids concentration in the aqueous fraction (μM)	120 (83)	**225 (201)**
	468 (464)	**490 (205)**
Cholesterol (μM)	4756 (6104)	2297 (1969)
	1703 (1674)	1882 (1325)
Phosphatidylcholine (μM)	349 (415)	311 (137)
	362 (210)	539 (393)

In line with the increased buffer capacity in older adults

Vertzoni et al. 2020

Fasted state / UC adults in relapse:
SCFAs are signficiantly lower [23.2(14.9)] compared with young healthy adults

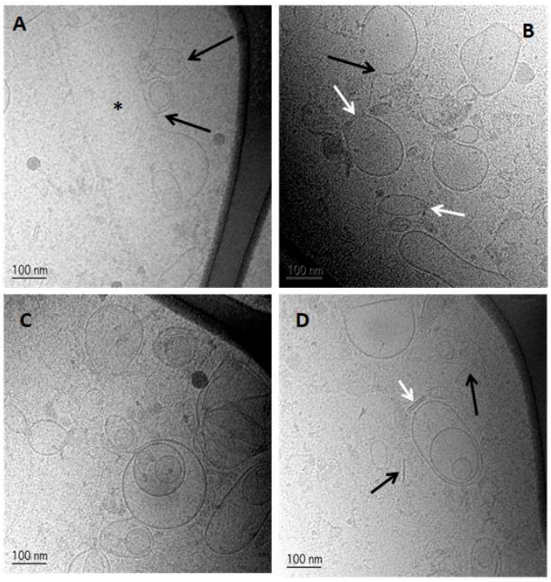
Vertzoni et al. 2010a

Colloidal structures in the liquid fraction of contents of proximal colon

Colloidal structures have been observed,
e.g. fasted adults with UC

Black dots: Micelles

A. Vesicles (arrows) attached to plate – like structures (*)
B. Open bilayers (arrows)
C. Vesicles
D. Discoidal structures



Müllertz et al. (2013)
Tran et al. (2017)

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Characteristics of contents affect drug's availability for transport towards the mucosa

When transport towards the mucosa of proximal colon is of interest?

After oral administration of
- colon-targeting products
- (most of) extended release products
- immediate release products of drugs whose absorption is incomplete in the "upper intestine"

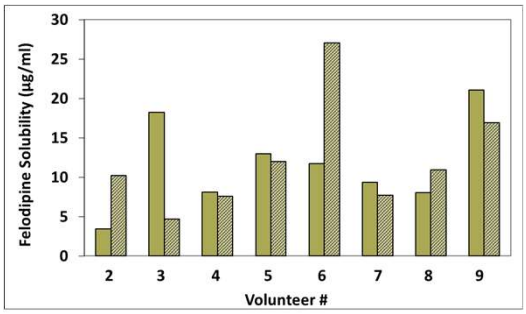
Characteristics of contents affect drug's availability for transport towards the mucosa

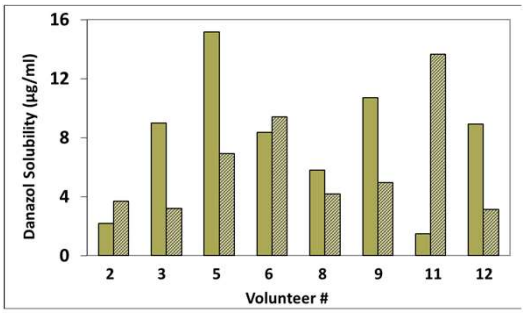
Why?	Potential mechanisms?
Affect the amounts of drug that are apparently dissolved in the liquid contents and, therefore, available to be transported towards the mucosa	Affect on the capacity of liquid contents to accomodate the drug (apparent solubility) Affect the Release/Dissolution rates May lead to decreased concentrations, due to bacterial degradation of the drug

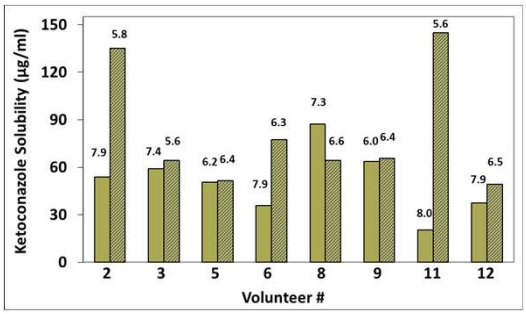
Apparent drug solubility in liquid contents of proximal colon of young healthy adults

Fasted state

Fed state







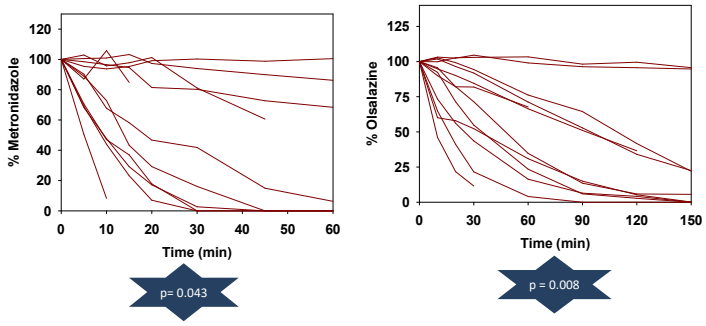
High intraindividual variability; pH is key for ionizable drugs

High interindividual variability

Vertzoni et al. 2010b

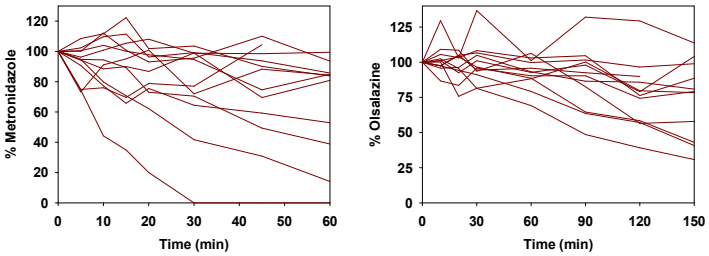
Ex vivo drug bacterial degradation profiles in contents of proximal colon of young healthy adults

Fasted state



Food remnants decrease the degradation rates cecum/ascending colon

Fed state



High interindividual variability

Karatza et al. 2017

Evaluating bacterial drug degradation in the lower intestine

- Prior assessment of potential chemical degradation is required

- Data presented in the previous slide were collected with experiments which lasted couple of hours only

- Impact of handling and storage of bacterial sample (including the storage in the anaerobic station) should have been evaluated prior to experimentation (Karatza et al. 2016)

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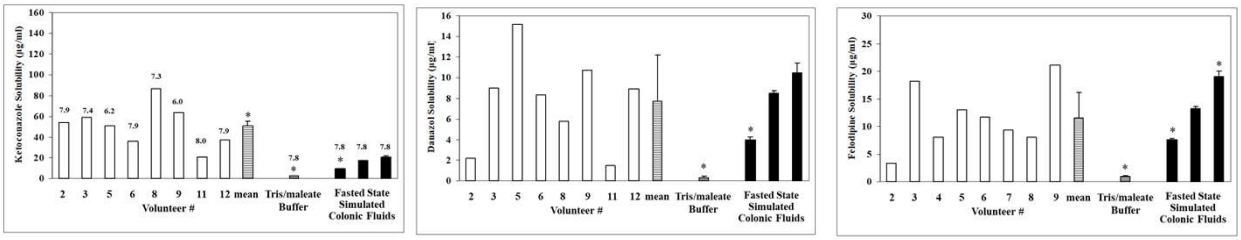
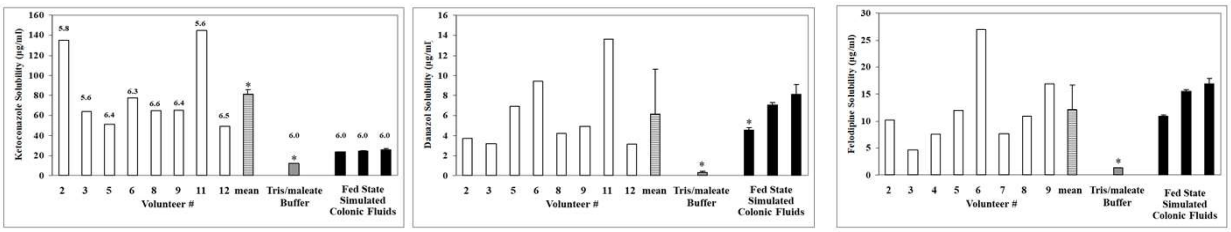
Estimating drug solubility in liquid contents of proximal colon of young healthy adults by using biorelevant media

Fasted state



Fed state



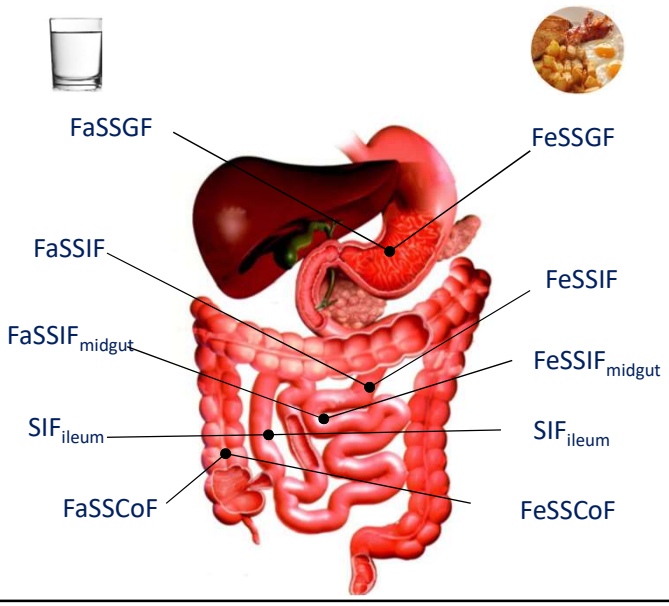
Vertzoni et al. 2010b

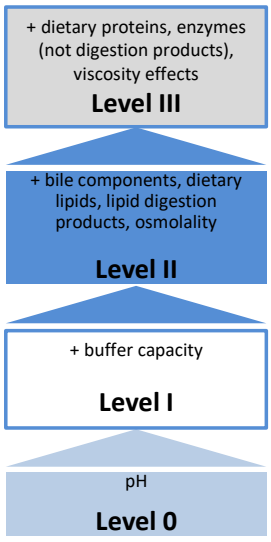
Evaluating dissolution in the lower intestine of young healthy adults

Total aspirated volume from proximal colon is ~5 times bigger than total aspirate volume from distal ileum (Reppas et al. 2015)

It is important to know whether a non-disintegrating / matrix type is considered (Garbacz et al. 2008)

Composition of luminal contents: What level of biorelevance is needed?





Markopoulos, Andreas et al. 2015

Simulating the composition of contents in the lower intestine of young healthy adults: Fasted state

		SIF_{ileum}-V2	FaSSCoF
Sodium cholate (mM)		-	0.15
Lecithin (mM)		-	0.3
Sodium oleate (mM)		-	0.1
Maleic acid (mM)		120	75.8
Tris (mM)		-	45.4
NaOH (mM)		240.6	120
pH		8	7.8
Buffer capacity [(mmol/L)/ΔpH)]		7.6	14.4
Osmolality (mOsmol/kg)		275	217
Level I/II	Volume (mL)	40	200
Level III	Liquid volume (mL)	39.9	180.4
	Microcrystalline cellulose (g)	2.18 (250 µm)	31.34

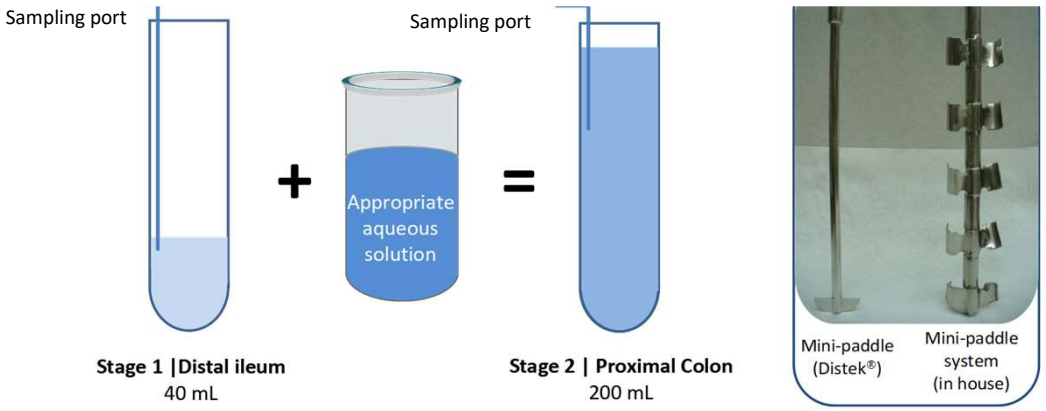
Georgaka et al. 2017

Simulating the composition of contents in the lower intestine of young healthy adults: Fed state

		SIF_{ileum}-V2	FeSSCoF-V2
Sodium cholate (mM)		-	0.6
Lecithin (mM)		-	0.5
Sodium oleate (mM)		-	0.2
Glucose (mg/ml)		-	4.8
Maleic acid (mM)		120	65
Tris (mM)		-	65
NaOH (mM)		240.6	16.5
pH		8	6.0
Buffer capacity [(mmol/L)/ΔpH)]		7.6	38
Osmolality (mOsmol/kg)		275	170 (Level I) 207 (Level II)
Level I/II	Volume (mL)	40	200
Level III	Liquid volume (mL)	36.1	171.2
	Microcrystalline cellulose 250 µm (g)	6.27	42.00

Georgaka et al. 2017

Evaluating dissolution in the lower intestine of young healthy adults using commercially available dissolution system and equipment and a two-stage methodology: Non-matrix, disintegrating drug products



Level I or Level II / mini-paddle: 75rpm (Stage 1) / 40rpm (Stage 2)

Bergstrand et al. 2012

Level III / mini-paddle system: 100rpm

Georgaka et al. 2017

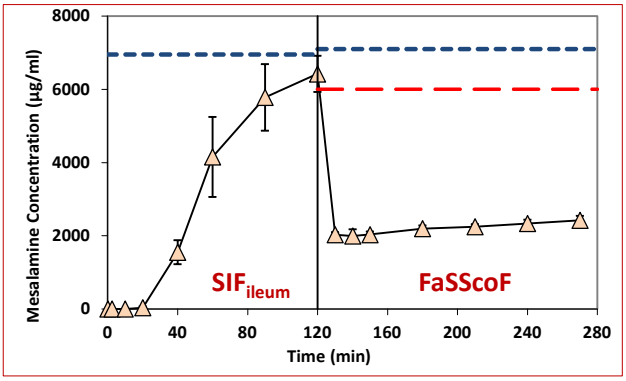
Asacol® tablets (400g/tab) (Eudragit S coating – dissolves at pH ≥7)

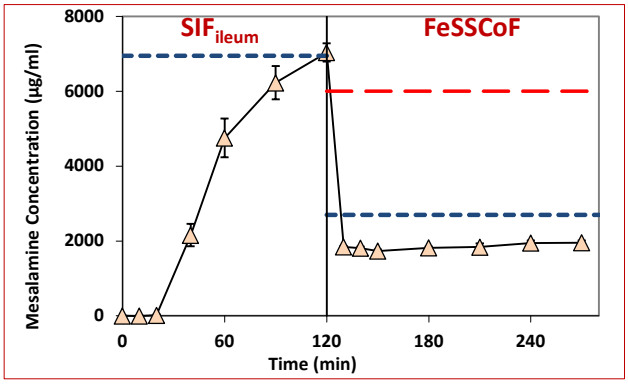
Mesalamine, MW 153.1; pKa_1: 2.30 (acidic) / pKa_2: 5.69 (basic); logP -1.5 to 0.8; BCS III; Dose: 1200 mg

▲ Level I biorelevant media

--- Equilibrium solubility in Level II biorelevant media

—— Nominal concentration if the entire dose would be dissolved





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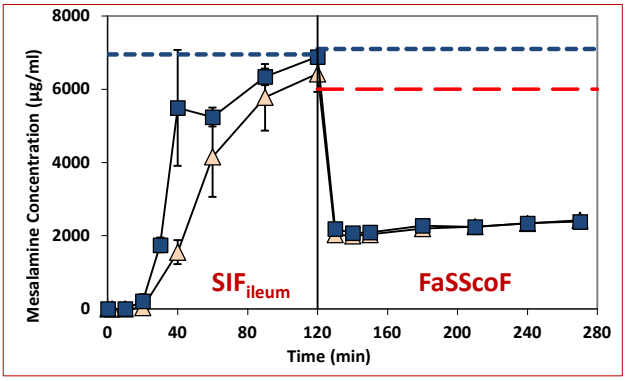
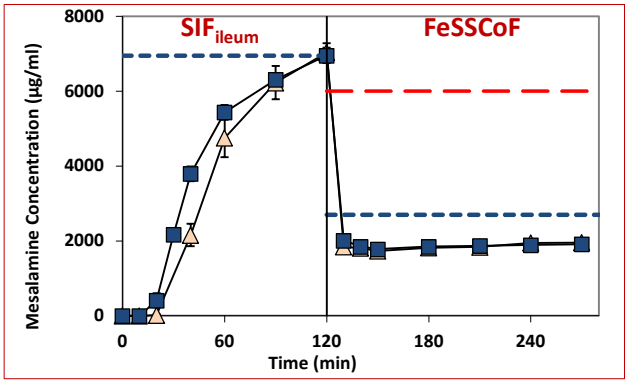
Level I biorelevant media
Level II biorelevant media
Equilibrium solubility in Level II biorelevant media
Nominal concentration if the entire dose would be dissolved





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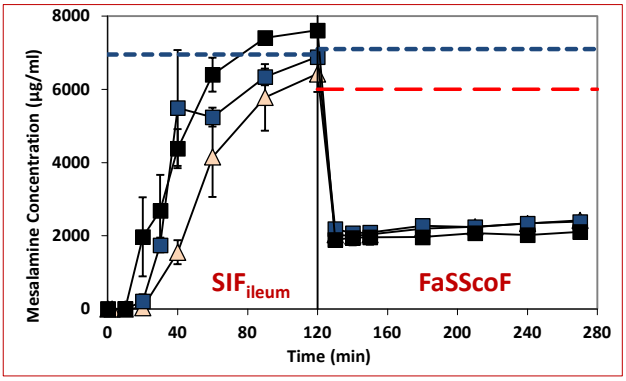
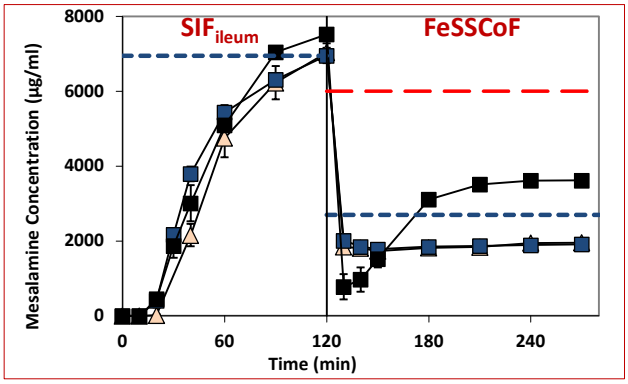
Level I biorelevant media
Level II biorelevant media
Level III biorelevant media
Equilibrium solubility in Level II biorelevant media
Nominal concentration if the entire dose would be dissolved



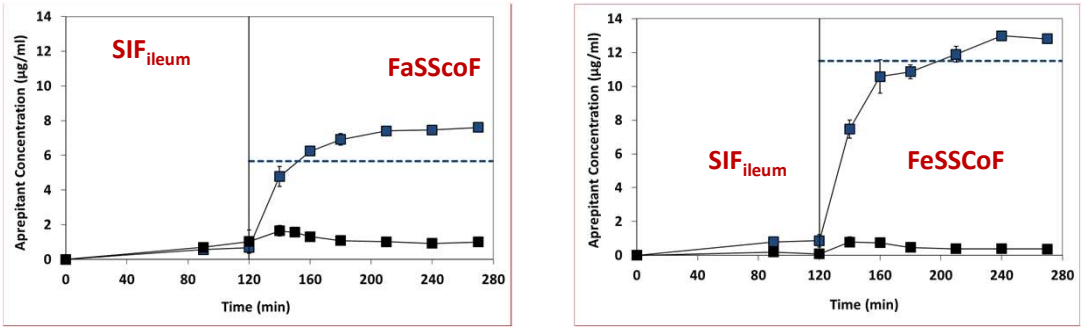


The impact of solid particles on dissolution in the lower intestine was found to be clinically insignificant for Asacol® tablets
The impact of food on mesalamine dissolution is expected to be limited in line with human data in plasma

Micronized aprepitant

MW 534.4; pKa: 9.7 (acidic); logP 4.8; BCS II/IV; Dose: 100 mg

■ Level II biorelevant media
■ Level III biorelevant media
- - - Equilibrium solubility in Level II biorelevant media



Solubilizing agents seem to interact with the particles included in the media for Level III simulation and solubilizing capacity of liquid content is decreased for this highly lipophilic API

Level of simulation of luminal environment in the lower intestine was important for dissolution of this highly lipophilic API; is it important for plasma levels?

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults ex vivo using adult fecal material

Based on
- P_{eff} human ileal and colonic permeability values of BCS highly permeable APIs
- Residence times in the regions

Assuming that
- degradation is clinically important when ≥20% reduction in absorption from distal ileum or proximal colon occurs
- degradation and absorption occurs according to 1st order kinetics

30min and 95min seem to be reasonable point estimates of maximum bacterial degradation half-lives, in order bacterial degradation in distal SI and in proximal colon, respectively, to be clinically important

Vertzoni et al. 2018

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults ex vivo using adult fecal material

Based on
- the ileal and cecal metronidazole and olsalazine degradation profiles with clinically relevant half-lives

Simulated ileal bacteria (SIB) consists of 5.5% (w/v) stools in normal saline

Simulated colonic bacteria (SCoB) consists of 8.3% (w/v) stools in normal saline

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$t_{1/2,SIB}$<30min

Table 2
Mean (SD) values for the bacterial degradation half-life (min) of model compounds tested in simulated intestinal bacteria (SIB) and in simulated colonic bacteria (SCoB).

	Simulated ileal bacteria (SIB)	Simulated colonic bacteria (SCoB)
Levodopa	Stable[a]	Stable[a]
Budesonide	202.7 ± 2.6	147.0 ± 7.6
Nitrendipine	26.9 ± 3.4	16.7 ± 1.6
Nimodipine	73.2 ± 7.8	48.7 ± 2.7
Rivaroxaban	Stable[a]	Stable[a]
Sulfasalazine	13.42 ± 0.45	7.897 ± 0.090

[a] Experiments in SIB or SCoB were not performed as these compounds did not reveal clinically important bacterial degradation (levodopa) or were stable (rivaroxaban) in the comparatively more concentrated bulk fecal material.

$t_{1/2,SCoB}$<95min

Data in line with available literature data in humans

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$$\frac{t_{1/2,SIB}}{t_{1/2,SCoB}} \approx \frac{Stool\ content\ in\ SCoB}{Stool\ content\ SIB} \approx 1.5$$

i.e. bacterial degradation in SIB could be evaluated from data in SCoB

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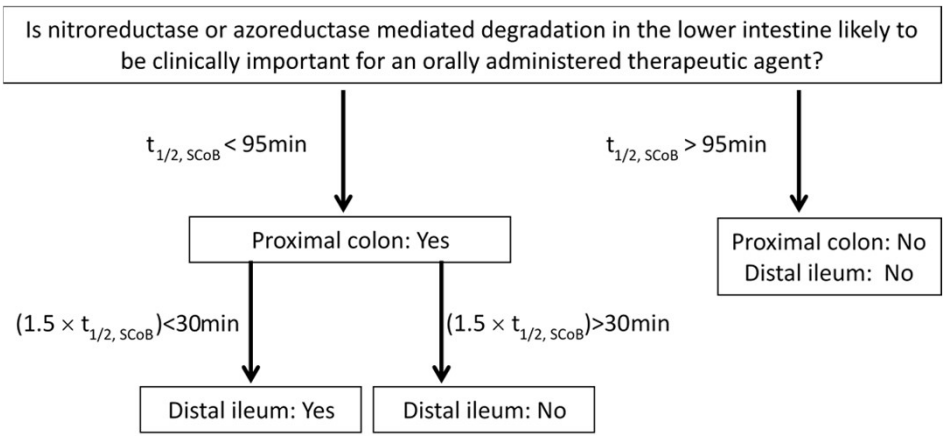

Currently, investigating the usefulness of SCoB in simulating sulfoxide reduction

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Innovative Tools for Oral Biopharmaceutics