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Tablet formulation with dual control concept for efficient colonic drug delivery

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Abstract

Aim of this study was to develop a tablet formulation for targeted colonic drug release by implementing two control mechanisms: A pH-sensitive coating layer based on Eudragit® FS 30 D to prevent drug release in the upper gastrointestinal tract, combined with a matrix based on plant-derived polysaccharide xyloglucan to inhibit drug release after coating removal in the small intestine and to allow microbiome triggered drug release in the colon. *In vitro* dissolution tests simulated the passage through the entire gastrointestinal tract with a four-stage protocol, including microbial xyloglucanase addition in physiologically relevant concentrations as microbiome surrogate to the colonic dissolution medium. Matrix erosion was monitored in parallel to drug release by measurement of reducing sugar equivalents resulting from xyloglucan hydrolysis. Limited drug release in gastric and small intestinal test stages and predominant release in the colonic stage was achieved. The xyloglucan matrix controlled drug release after dissolution of the enteric coating through the formation of a gummy polysaccharide layer at the tablet surface. Matrix degradation was dependent on enzyme concentration in the colonic medium and significantly accelerated drug release resulting in erosion-controlled release process. Drug release at physiologically relevant enzyme concentration was completed rapidly within the bounds of colonic transit time. The dual control concept was applicable to two drug substances with different solubility, providing similar release rates in colonic environment containing xyloglucanase. Drug solubility mechanistically affected release, with diffusion of caffeine, but not of 5-ASA, contributing to the overall release rate out of the matrix tablet.

Keywords

Colonic delivery, 5-aminosalicylic acid, dissolution testing, erosion-controlled, colonic microbiome, xyloglucan

Abbreviations used

5-ASA, 5-aminosalicylic acid

API, active pharmaceutical ingredient

CCR, controlled colonic release

IBD, inflammatory bowel disease

IR, immediate release

1. Introduction

To date, colon targeting is primarily needed for the local treatment of inflammatory bowel diseases (IBD) [1,2] with its two major types Crohn's disease and ulcerative colitis. The first can potentially affect any part of the gastrointestinal tract but often involves the ileocecal region [3], while the latter extends proximally from the rectum and remains restricted to the colon [4]. First-line therapy for the treatment of IBD is the administration of non-steroidal anti-inflammatory agents such as 5-aminosalicylic acid (5-ASA) or corticosteroids such as budesonide. Immunosuppressants and biologics are used as second and third line options, respectively [5].

Drug applied to the large intestine via the rectal route with enema and suppositories does not reach further than the transverse colon [6,7]. When oral formulations are intended to target the colon, premature drug release in the upper gastrointestinal tract and loss of drug in the feces must be prevented. Differences between segments of the gastrointestinal tract with respect to transit time, pressure, pH or bacterial count have been exploited to devise strategies for colonic drug delivery [8].

Delayed-release formulations such as the Pulsincap™ [9] rely on the reported small intestine transit time of 3±1 h [10], but failed due to the highly variable gastric retention [11]. Combination of delayed-release coating with an enteric coating still produced variable onset of drug release despite the increased complexity of the formulation, with drug diffusing through the swollen coating layer or tablets disintegrating only shortly before defecation [12–14]. Moreover, most of the employed manufacturing technologies were not applicable to large scale [15].

As there is a pH drop between distal ileum and proximal colon [16,17], enteric coating layers must dissolve before the dosage form passes through the ileocecal valve. However, high inter- and intra-individual variability has been shown for pH profiles along the entire gastrointestinal tract [17], depending furthermore on food intake and disease state. This variability has led to premature drug release in the upper gastrointestinal tract [8] or defecation of intact dosage forms [18–22]. Hindered coating dissolution due to decreased pH in the proximal colon might be even more pronounced in ulcerative colitis patients sometimes showing significantly lowered colonic pH [23,24]. The problem of insufficient drug release in particular was addressed by the use of two coating layers, such as in the DuoCoat® technology in which rapid water uptake by the inner coating creates a buffered environment, accelerating dissolution of the outer layer and concomitant drug release from the core [25]. Double-coated dosage forms showed a reduced lag-time before drug release when compared to dosage forms with a single enteric coating both *in vitro* [25,26] and *in vivo* [18,27], and coated multi-unit particulate systems allowed for a more rapid drug release upon

initiation of coating dissolution due to their high surface to mass ratio. However, drug delivery from both dosage forms remained dependent on reaching a threshold pH for coating dissolution and the timing of it in view of transit and residence times in the gut [21]. A majority of current commercial oral 5-ASA formulations such as Asacol®, Mezavant® (Lialda® in the US) and Salofalk® rely on a single pH-sensitive polymer coating. These exhibited large variability of drug dissolution in a recent clinical study [28].

Other colonic delivery systems relied on the abrupt rise of bacterial count in the large intestine, with the enzymatic machinery acting as release trigger. A prominent example is the 5-ASA prodrug sulfasalazine, which releases its active drug moiety only when azoreductases of the colonic microbiome cleave the diazo bond [29]. No premature drug release occurs with this strategy, but sulfapyridine, which is cleaved off the prodrug and rapidly absorbed into systemic circulation, leads to severe side effects [30]. Furthermore, this strategy is not applicable to other drug substances, as specific structural features are required for prodrug linkage [8].

The enzymatic trigger has also been utilized in connection with bacterially degradable matrix formers and coating substances. However, upon swelling or even dissolution of typically used polysaccharides in the upper gastrointestinal tract, the enzymatic trigger for drug release in the large intestine becomes void. Therefore, polysaccharides have been combined with inert substances or modified chemically. The starch constituent amylose, for example, was used in combination with ethylcellulose or zein for reduced gastric swelling [31,32], while bacterial fermentation of amylose promoted release under colonic conditions. Pectin was further combined with HPMC and chitosan [33,34] or a calcium pectinate matrix was coated with an enteric dissolving film [35]. For both amylose and pectin, however, the polysaccharide content in the film coating leads to increased drug release in both the small and the large intestine making optimization difficult, while the matrix system released drug already in the small intestine. Furthermore, large film thicknesses were required in most cases, leading to large tablets with poor patient acceptability.

Chitosan capsules coated with HPMC phthalate [36–38] showed accelerated release in the presence of rat cecal contents, but degradation of chitosan possibly resulted from lowered pH rather than from bacterial degradation [37] and *in vivo* experiments in rats showed drug release before colonic arrival [36,38]. Matrix tablets based on chemically modified chitosan showed improved gastric resistance, but failed to prevent drug release along the small intestine [39]. Guar gum was used for both matrix tablets and compression coats. However, the amount of polysaccharide employed was very critical for achieving colonic drug delivery and both, premature release in the upper gastrointestinal tract [40,41] and delayed tablet disintegration *in vivo* [40] or incomplete drug release in the presence of rat cecal contents *in vitro* [42] were observed. In order to prevent insufficient guar gum degradation in the large

intestine, probiotics were co-administered with guar-gum coated sulfasalazine spheroids [41], thereby acknowledging insufficiency of the bacterial enzymatic trigger as sole drug release mechanism.

The Phloral™ technology, also used in Opticore™ colonic targeting dosage forms, combines both pH and bacterial count as trigger mechanisms in the form of a pH-sensitive polymer coating with integrated high amylose starch together with at least one inner coating layer [43,44]. This technology was successfully commercialized, being the only new technology to reach the market in the last decade and was shown in a scintigraphy study to provide accurate tablet disintegration in the colon [46]. Bacterial enzymatic digestion of the modified starch in the large intestine, however, requires starch pretreatment under tightly controlled temperature conditions [43], and manufacturing complexity rises as the number of different coating layers to be applied increases. Also, premature drug release along the upper gastrointestinal tract upon dissolution of the enteric coating due to pH variation is still possible. Yet such premature release was reduced *in vitro* by adding a slowly dissolving, inner coating layer [45].

A combination of three different triggers was implemented in the CODES™ delivery system. The immediate release core containing drug and lactulose was coated with three different film layers [47,48]: An outer enteric coating, an intermediate hydrophilic layer allowing for time-dependent dissolution during small intestinal passage, and an inner acid-soluble coating dissolving after colonic bacteria-elicited conversion of released lactulose to short-chain fatty acids. However, drug release mediated solely by pH changes was shown [48], rendering the enzymatic trigger superfluous. An effect of formulation parameters on t_{max} could be demonstrated *in vivo* [48] but the CODES™ system has not been further pursued.

In summary, while most of the marketed products utilize enteric coating as sole pH-based release control, research has introduced multiple coatings and additional mechanisms such as enzymatic trigger and matrix systems to satisfy the need for a more reliable control that provides no or very low premature drug release in the upper gastrointestinal tract and complete drug release during colonic passage. The combination of specific polysaccharide-based matrix formulations with an enteric coating, however, has not been studied in detail so far.

The purpose of the present study was to develop a new oral formulation for controlled colonic release (CCR) tablets to achieve selective and efficient drug delivery to the colon based on a dual control concept: A pH sensitive Eudragit® FS coating layer is used to prevent drug release in the stomach and upper small intestine. Additionally, a matrix based on the plant-derived polysaccharide xyloglucan is used to prevent drug release after the coating has dissolved in the small intestine before the tablet reaches the colon, which is then digested by

microbial enzymes triggering drug release in the large intestine. Xyloglucan is a hemicellulose with a backbone consisting of β-1,4-linked glucose moieties and variable length sidechains consisting mainly of xylose and galactose [49]. It is susceptible to enzymatic degradation by xyloglucanase of the colonic microbiome cleaving the polysaccharide backbone, ultimately leading to matrix dissolution and drug release. Sustained drug release properties of xyloglucan-based formulations – predominantly employing a de-galactosylated form of the polysaccharide – have been investigated for other targeting sites [50–52], and preliminary results about colonic microbiome degradation were reported in rat cecal contents [53,54], but to the best of the authors' knowledge, no enteric coated colonic delivery formulation based on a native xyloglucan matrix has been proposed to date.

CCR tablet formulations are tested *in vitro* in environments simulating the passage through the entire gastrointestinal tract. Typically employed in the treatment of IBD, the anti-inflammatory agent 5-ASA is used as active pharmaceutical ingredient (API) embedded in the xyloglucan matrix. Caffeine is further used as a model drug to study the effect of API solubility on release characteristics. Dissolution experiments are additionally conducted with CCR tablet cores to assess the role of the matrix in the dual control concept. The dissolution and enzymatic degradation of the matrix polysaccharide is finally monitored to obtain insights into the kinetic control of the release process. CCR tablets with these two APIs have been used in a comparative evaluation of colonic delivery performance *in vivo* as reported in a prospective manuscript.

2. Materials and methods

2.1. Materials

5-Aminosalicylic acid (5-ASA) was purchased from AK Scientific, Union city, USA. Xyloglucan 3S was ordered from DSM Gokyo food & chemical, Tokyo, Japan. Caffeine, polyvinylpyrrolidone (PVP) K30, triethyl citrate, calcium chloride, hematin, histidine, iron (II) sulfate heptahydrate, magnesium chloride, and vitamin K3 were supplied by Sigma Aldrich, St. Louis, USA. Primojel (sodium starch glycolate type A) was obtained from DFE Pharma, Goch, Germany. Vivapur 101 (microcrystalline cellulose) was provided from JRS Pharma, Rosenberg, Germany. Pharmacoat 603 (hydroxypropyl methylcellulose HPMC 3 mPa·s) was acquired from Shin-Etsu, Tokyo, Japan. Magnesium stearate, talc and granulac 200 (lactose monohydrate) were purchased from Hänseler Swiss Pharma, Herisau, Switzerland. Eudragit® FS 30 D was kindly donated by Evonik, Essen, Germany. Hydrochloric acid 1 M, potassium dihydrogen phosphate, sodium hydroxide 32%, iron (III) oxide red, D-(+)-glucose, ammonium sulphate, sodium chloride, L-cysteine (free base), and vitamin B12 were ordered from Carl Roth, Karlsruhe, Germany. Iron (III) oxide monohydrate yellow was supplied by Strem Chemicals Inc., Newburyport, USA. Sodium hydroxide 1 M was acquired from Honeywell-Fluka, Morristown, USA. Xyloglucanase (nominal activity 1000 U/mL) was purchased from Megazyme, Irishtown, Ireland. p-Hydroxybenzhydrazide (PAHBAH) was obtained from TCI, Tokyo, Japan.

2.2. Methods

2.2.1. Preparation of tablet cores

Controlled colonic release (CCR) tablet cores containing 200 mg of either 5-ASA or caffeine were prepared after fluid bed granulation. A powder blend containing 33.3% API and 66.7% xyloglucan 3S was granulated in a GPCG2 fluid bed apparatus (Glatt, Binzen, Germany) using a 10% (w/w) PVP K30 solution. Batch size was 600 g for 5-ASA and 1200 g for caffeine while other granulation parameters can be found in **Table S1** in supplementary information.

Immediate release (IR) 5-ASA tablet cores were prepared with a powder blend containing 33.3% 5-ASA, 20% microcrystalline cellulose, 41.7% lactose and 5% HPMC. The blend was granulated in a high-shear mixer (Diosna, Osnabrück, Germany) with batch size of 450 g using deionized water at an impeller rotation rate of 250 rpm and a chopper rotation rate of 2200 rpm.

All granule formulations were characterized for their API content (Jasco V-630 Spectrophotometer, Jasco, Tokyo, Japan, n = 3), bulk and tapped density (Erweka SVM 102, Erweka, Heusenstamm, Germany, n = 1), true density (AccuPyc II 1340 gas pycnometer, Micromeritics, Unterschleissheim, Germany, n = 3), and particle size (SympaTEC Helos / KF, SympaTEC VIBRI, SympaTEC RODOS, all three from Sympatec GmbH, Clausthal-Zellerfeld, Germany, n = 3).

Tablets containing 200 mg of either 5-ASA or caffeine were produced after addition of lubricant and – where applicable – disintegrant on a single-punch tablet press (Korsch XL 1, Korsch AG, Berlin, Germany) with concave punches having a diameter of 12 mm and a curvature radius of 9 mm. Total tablet core weight was between 590 and 644 mg and the complete tablet composition for all three formulations can be taken from **Table S2** in supplementary information.

Tablet cores were characterized for their physical properties including weight, height, diameter, and breaking force (MT50, Sotax, Aesch, Switzerland, n = 10).

2.2.2. Preparation of coated tablets

Tablet cores were coated to produce CCR tablets or enteric coated tablets. Coating was carried out in a drum coater (GMPC 1 Mini-coater, Glatt, Binzen, Germany) to a coating amount corresponding to 4.0 to 4.7% weight gain or a l-value of 7.1 to 8.7 mg/cm^2.

Triethyl citrate as plasticizer, talc as anti-tacking agent and iron (III) oxide as pigment were homogenized in deionized water for 10 minutes (IKA® T18 basic Ultra-Turrax®, dispersing tool S18N-10G, IKA®, Staufen, Germany) and the suspension was slowly added under stirring to a 30% (w/w) Eudragit® FS 30 D dispersion and stirring was continued for another hour. Final solids proportion in the coating suspension amounted to 20%. Coating composition can be taken from **Table S2** in supplementary information**Error! Reference source not found.**.

Batch size was 600 g, whereby 150 g tablet cores were filled up with placebo tablets, and coating process parameters are given in **Table S3** in supplementary information.

Coating amount was calculated by dividing the total film weight by the surface area of uncoated tablets.

2.2.3. Dissolution testing

Dissolution experiments took place in a USP 2 paddle apparatus (Sotax AT7, Sotax, Aesch, Switzerland) at 100 rpm paddle speed and 37 ± 0.5°C media temperature.

Coated tablets were tested using a four-stage protocol reflecting gastric, upper and lower small intestinal, and colonic environments (**Table 1**). Full media change took place after the gastric and the small intestinal stages while pH adjustment within the small intestinal stages took place by addition of the necessary volume of 32% NaOH. Xyloglucanase concentrations in the colonic media ranged from 0 to 1 U/mL by dilution of the enzyme stock solution (nominal activity 1000 U/mL) in the buffer. Dissolution experiment was continued until tablet disintegration and an increment between measured concentration of consecutive sampling points of generally less than 1.15%.

CCR tablet cores were tested only in the colonic medium. The starting point of these experiments corresponds to t=6 h of CCR tablets.

At predefined time points, 4.6 mL samples were withdrawn and replaced with an equal volume of fresh media. During the colonic stage of dissolution testing, the withdrawn sample was divided into an unfiltered part for measurement of reducing sugar equivalents (section **2.2.3.2.**) and into a filtered part for UV/Vis quantification of released drug.

Sink conditions were ensured during the complete dissolution testing. Reported solubility of 5-ASA is 6.5-10.2 mg/mL at pH 1 [40,55] and between 3.9 and 17.5 mg/mL at pH 6.8 [40,55,56]. Caffeine has a pH-independent aqueous solubility of 22 mg/mL at room temperature [57].

Dissolution experiments were conducted at least in triplicate and results are expressed in % of drug and reducing sugar equivalents detected in solution at the given sampling time normalized to the final measured value in each vessel corresponding to the end of the dissolution process.

Table 1 *Dissolution testing setup*

Dissolution media	Volume (mL)	Duration (h)	Simulation of	pH
HCl 0.1 M	900	2	Stomach	1.2
0.1 M KH_2PO_4	900	2	Upper small intestine	6.5
0.1 M KH_2PO_4	900	2	Lower small intestine	6.8
0 to 1 U/mL xyloglucanase in 0.1 M KH_2PO_4	200	>9 until completion	Colon	6.8

2.2.3.1. Drug release quantification

The withdrawn samples were filtered through a 0.45 µm nylon filter (Infochroma AG, Goldau, Switzerland) and the amount of released API was determined spectrophotometrically (Jasco V-630 Spectrophotometer, Jasco, Tokyo, Japan): 5-ASA was quantified at 303 nm during the acid stage and at 331 nm for all phosphate buffer stages. Filtered caffeine samples were analyzed at 272 nm. No significant loss of drug was observed after filtration over the selected filter membrane while excipients did not interfere at the employed wavelength (data not shown). Drug quantification took place based on an external calibration curve in the range of 1-50 µg/mL and 1-24 µg/mL of 5-ASA and caffeine concentrations, respectively.

2.2.3.2. Reducing sugar equivalents measurement

Xyloglucan release was quantified based on the concentration of reducing sugar equivalents measured in solution. Reducing sugar equivalents can be detected in native xyloglucan and are additionally formed by enzymatic cleavage of the β-1,4-glycosidic bonds of the xyloglucan backbone.

A 5% PAHBAH stock solution in 0.5 M HCl was prepared and diluted on demand to 1% with 0.5 M NaOH. This working solution was kept on ice for maximally one day. Immediately after sampling, 20 µL of the unfiltered sample from the colonic stage of dissolution experiments were pipetted into 240 µL ice-cold PHABAH working solution, ensuring enzyme inactivation due to the pH-shift [58].

The sample-PAHBAH mixtures were kept light-protected on ice until the start of the reducing sugar equivalents measurement, for which 120 µL were heated for 5 minutes at 95°C and subsequently cooled for 10 minutes to 4°C in a PCR thermocycler (Peqlab Primus 96 advanced, VWR, Radnor, USA). The absorbance of 100 µL of the yellowish complex was subsequently measured at 410 nm (Epoch 2 microplate reader, BioTek Instruments Inc., Winooski, USA).

In addition to immediate analysis, withdrawn samples of the colonic stage were analyzed again after off-line xyloglucan hydrolysis for one hour at room temperature. For this off-line xyloglucan hydrolysis, samples from dissolution experiments with 0.1 and 1 U/mL xyloglucanase were kept as is, whereas 10 µL of a 10 U/mL xyloglucanase solution were added to 150 µL of the unfiltered sample from dissolution experiments with 0 U/mL enzyme. Subsequent to the one hour off-line xyloglucan hydrolysis at room temperature, all samples were processed as above for measurement of reducing sugar equivalents.

Reducing sugar equivalents were calculated based on an external calibration curve made with glucose solutions with concentration ranging from 0.1 to 4 mM and measured daily under identical conditions as the samples of the dissolution experiment.

2.2.3.3. Statistical analysis

Release profiles of API and reducing sugar equivalent of the same experiment were compared point by point with a paired two-sided t-test while profiles of different formulations were compared with a two-sided t-test assuming unequal variances at a level of significance of $p \leq 0.05$ (Excel, version 2205, Microsoft, USA).

2.2.4. Xyloglucanase activity measurements in human feces

Xyloglucanase activity in human feces was estimated by incubating a fecal slurry with xyloglucan in solution and measuring the viscosity reduction of the solution. Viscosity measurement was carried out with a SV-A1 Vibro viscometer (A&D Company, Tokyo, Japan).

Feces samples of four healthy volunteers were collected in sterile microbiological feces tubes with screw cap-integrated spatula and stored at 4°C. The samples were diluted to 0.33 g/mL with minimal medium (consisting of 13.6 g/L KH_2PO_4, 0.875 g/L NaCl, 1.125 g/L $(NH_4)_2SO_4$, 0.5 g/L L-cysteine (free base), 1.9 µM Hematin, 0.2 mM Histidine, 0.1 mM $MgCl_2$, 0.4 mg/L $FeSO_4 \cdot 7\ H_2O$, 1 mg/L Vitamin K3 (menadione), 8 mg/L $CaCl_2$, 5 µg/L Vitamin B12) and homogenized by shaking to a slurry. Fifty µL of the slurry were transferred to 5 mL of a pre-warmed 1% w/v xyloglucan solution prepared in minimal medium. The enzymatic reaction took place at 37°C and was stopped after 15 minutes by adding 100 µL of 18% HCl to the mixture, and the viscosity of the sample was determined.

The measurement was calibrated by incubating 5 mL of a pre-warmed 1% w/v xyloglucan solution in minimal medium with 100 µL xyloglucanase solution with concentrations ranging from 3 to 50 mU/mL and following the same protocol as for the feces samples. The data points were approximated by an empirical function (**Eq. 1**)

$$\eta = \eta_0 - \frac{A}{A_{0.5} + A}(\eta_0 - \eta_\infty) \qquad \textit{Eq. 1}$$

where η is measured sample viscosity, A is xyloglucanase activity, $A_{0.5}$ is xyloglucanase activity at half-maximal viscosity, η_0 and η_∞ represent viscosity at zero and theoretically infinite enzyme activity, respectively. Parameter values were estimated by fitting the function to the measured data points (**Fig. S5** in supplementary information).

3. Results

3.1. Granules and tablets characteristics

Granule characteristics are summarized in **Table S4** in supplementary information.

Tablet characteristics for both uncoated and coated tablets are shown in **Table 2**. Tablet core weight was adapted to contain 200 mg API. Tablet porosity as well as coating amount of CCR caffeine tablets was adapted to yield similar dissolution profiles as CCR 5-ASA tablets (see also paragraph **4.2.**).

Table 2 *Tablet characteristics before and after enteric coating represented as average ± standard deviation (n = 10)*

	CCR 5-ASA tablet cores (uncoated)	CCR 5-ASA tablets (coated)	CCR caffeine tablet cores (uncoated)	CCR caffeine tablets (coated)	5-ASA tablet cores (IR) (uncoated)	Enteric coated 5-ASA tablets (coated)
Tablet weight (mg)	590.7 ± 1.2	619.3 ± 1.7	643.6 ± 5.5	674.9 ± 6.4	629.7 ± 3.0	652.3 ± 4.6
Tablet height (mm)	6.79 ± 0.02	6.91 ± 0.01	6.98 ± 0.05	7.13 ± 0.03	6.97 ± 0.02	7.08 ± 0.01
Tablet diameter (mm)	12.08 ± 0.01	12.19 ± 0.01	12.05 ± 0.02	12.12 ± 0.01	12.03 ± 0.02	12.09 ± 0.01
Tablet breaking force (N)	53.7 ± 1.6	78.0 ± 11.3	102.4 ± 11.4	158.6 ± 9.5	50.4 ± 2.2	79.7 ± 6.5
Tablet porosity (%)	24.8 ± 0.3	-	18.4 ± 1.1	-	24.3 ± 0.52	-
Tablet tensile strength (N/mm^2)	0.76 ± 0.03	-	1.33 ± 0.17	-	0.65 ± 0.03	-
Coating weight gain[1] (%)	-	4.0	-	4.7	-	4.6
Coating amount[1] (l-value in mg/cm^2)	-	7.1	-	8.7	-	8.2

[1]Determined by weighing ten tablets

3.2. Xyloglucanase activity measurement in human feces

Table 3 summarizes the xyloglucanase activity determined in the feces of healthy volunteers based on viscosity reduction of a xyloglucan solution. Lowest enzyme concentrations were found in the feces of a volunteer used to convenience food while the highest activity belonged to a vegetarian.

Table 3 *Xyloglucanase activity in the feces of healthy volunteers*

	Xyloglucanase activity (mU/g)	Dietary habits
Donor 1	23	Convenience food
Donor 2	366	Vegetarian
Donor 3	137	Omnivore
Donor 4	103	Omnivore

3.3. Dissolution testing

3.3.1. CCR 5-ASA tablets

Fig. 1 depicts the drug release profile of CCR 5-ASA tablets in four different dissolution media reflecting transit through the gastrointestinal tract (see also **Table 1**). Three different enzyme concentrations ranging from 0 to 1 U/mL are used in the final stage representing the colonic environment.

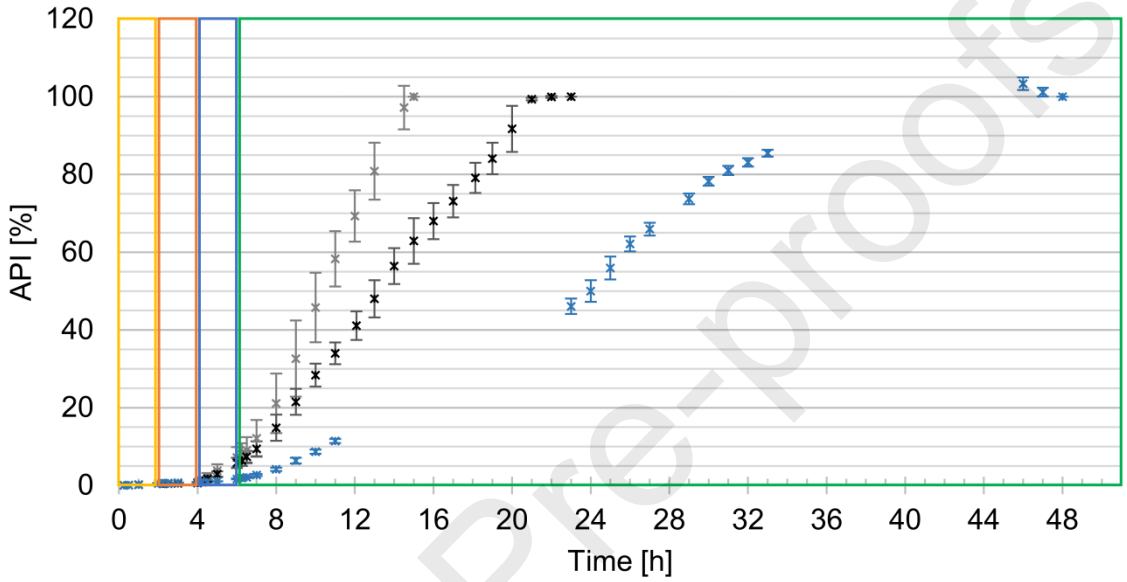

Fig. 1 *API release from CCR 5-ASA tablets as a function of time for different xyloglucanase concentrations in the colonic stage (1 U/mL: grey, 0.1 U/mL: black, 0 U/mL: blue). Data points and error bars represent average and standard deviation (n = 3). The yellow box illustrates dissolution in 900 mL pH 1.2, orange and blue boxes denote dissolution in 900 mL pH 6.5 and 6.8, respectively, the green box denotes dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment.*

Almost no API release (<1%) was measured during the first four hours of dissolution testing at pH 1.2 and pH 6.5. Drug release started upon increase of dissolution medium pH to 6.8. Overall, less than 7% of the dose was released after a total of six hours dissolution testing with some variation between the different experiments. Release in the colonic test stage depended strongly on xyloglucanase concentration. At 0 U/mL xyloglucanase, drug release was slow requiring a total of 46 hours for complete dose release. In the presence of 0.1 U/mL enzyme, drug release was markedly accelerated and was completed within 21 hours. Finally, total 5-ASA release occurred within only 15 hours at 1 U/mL xyloglucanase concentration. A seemingly linear release profile was observed in the colonic stage at the highest enzyme concentration while especially in the absence of enzyme an S-shaped profile was obtained.

In parallel to API release, erosion of the matrix was monitored by measuring the amount of xyloglucan in terms of reducing sugar equivalents released in the medium during the colonic test stage. **Fig. 2a** depicts the amount of 5-ASA and reducing sugar equivalents released

from CCR tablets for enzyme concentrations of 0.1 and 1 U/mL. The profiles of released API and reducing sugar equivalents were identical ($p > 0.05$ throughout the experiment) for both xyloglucanase concentrations. Continued off-line incubation of these samples for one hour at room temperature did not result in an increase of reducing sugar equivalents (data not shown). This is evidence that enzymatic digestion of xyloglucan was complete at the time of sampling of the dissolution media in the presence of xyloglucanase.

In the absence of xyloglucanase, reducing sugar equivalents immediately after sampling (light blue triangles in **Fig. 2b**) remained mostly below 10%. Only when these samples were subsequently incubated off-line with enzyme, a strong increase in reducing sugar equivalents was found (dark blue triangles). This suggests that in the absence of enzyme, xyloglucan was released from the tablets in an intact form. Profiles of released drug and reducing sugar equivalents after off-line enzymatic digestion were not statistically different in 13 out of the 21 timepoints ($p > 0.05$).

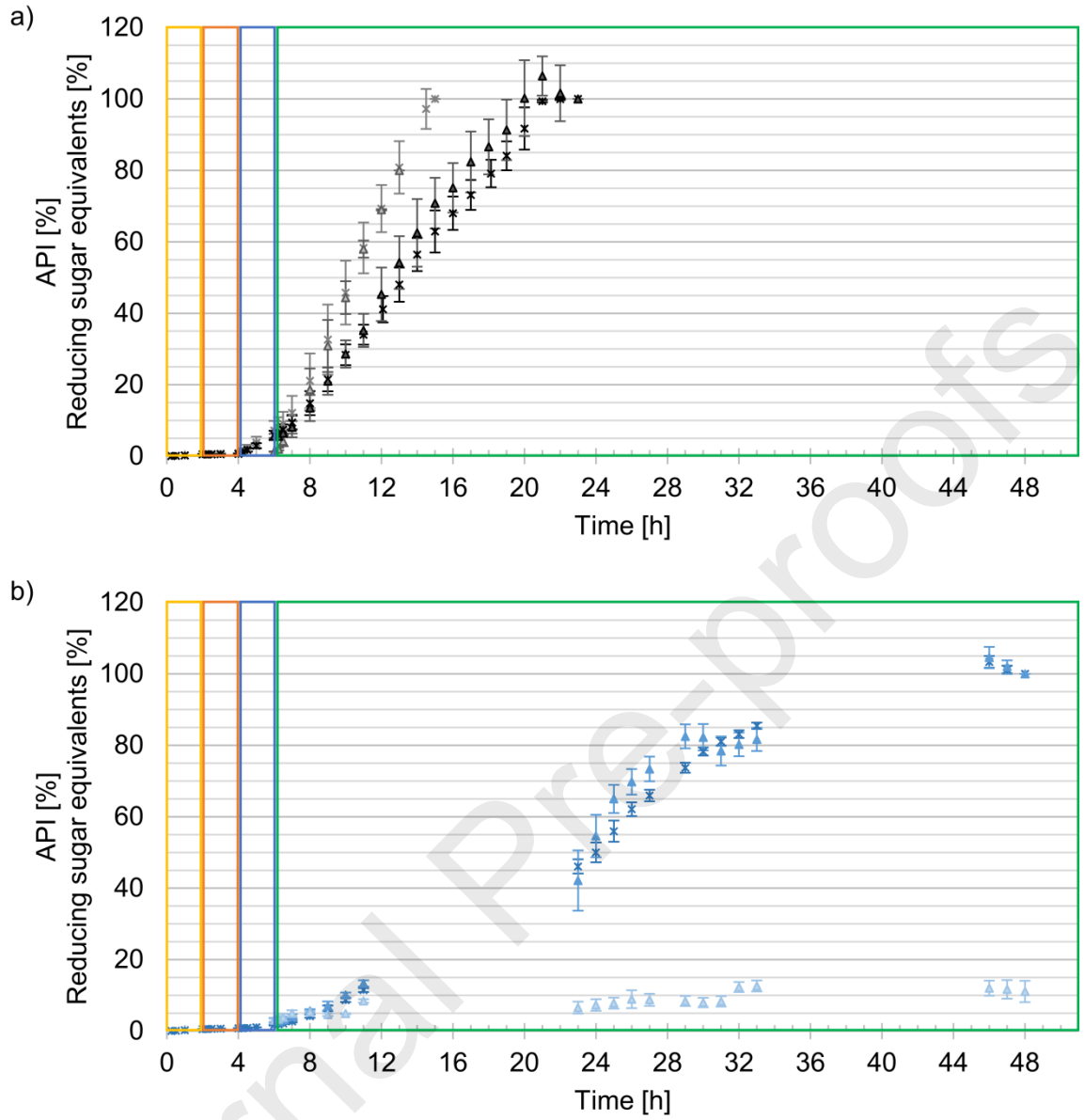

Fig. 2 *Release of API (crosses) and reducing sugar equivalents (triangles) from CCR 5-ASA tablets as a function of time. Panel a) 1 U/mL (grey) and 0.1 U/mL (black) xyloglucanase concentration in the colonic stage. Panel b) 0 U/mL xyloglucanase concentration in the colonic stage. Reducing sugar equivalents immediately after sampling (light blue) and after off-line enzyme incubation (dark blue). Data points and error bars represent average and standard deviation (n = 3). The yellow box illustrates dissolution in 900 mL pH 1.2, orange and blue boxes denote dissolution in 900 mL pH 6.5 and 6.8, respectively, the green box denotes dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment.*

3.3.2. CCR caffeine tablets

Fig. 3 depicts the drug release profile of CCR caffeine tablets in four different dissolution media reflecting transit through the gastrointestinal tract. Three different enzyme concentrations ranging from 0 to 1 U/mL are used in the final stage representing the colonic environment. Practically no release (<1%) was observed in the pH 1.2 and pH 6.5 stages while drug release started upon pH increase to pH 6.8. Some variation in the release profile between different experiments was evident leading in one instance to a maximum of 13% dose release after a total of six hours of dissolution testing. Further drug release in the colonic test stage was strongly dependent on the xyloglucanase activity. Complete dose dissolution was observed after a total of 46, 25, and 16.5 hours for enzyme concentrations of 0, 0.1, and 1 U/mL, respectively. The differentiation in the dissolution profiles between 0 and 0.1 U/mL enzyme concentration became evident at later times of the measurement.

Similar profiles were obtained for CCR caffeine tablets and CCR 5-ASA tablets in the first three stages of the dissolution test simulating stomach and small intestine (**Fig. 1** and **Fig. 3**). A rather limited amount of both drugs was released before the start of the colonic stage. It should be kept in mind that tablet properties were specifically adjusted as discussed in paragraph **4.2.** to achieve this similar behavior of CCR tablets for both drugs.

At 1 U/mL enzyme level, identical ($p > 0.05$ for all sampling timepoints in the colonic medium) and nearly linear release profiles were obtained for caffeine and for 5-ASA. At xyloglucanase concentration of 0.1 U/mL, caffeine and 5-ASA release exhibited a similar S-shaped profile, the caffeine curve being shifted towards longer times. The largest difference between the two drugs was observed in the absence of enzyme, where caffeine release was faster than that of 5-ASA over most of the colonic test stage, yet both drugs yielded a similar S-shaped profile.

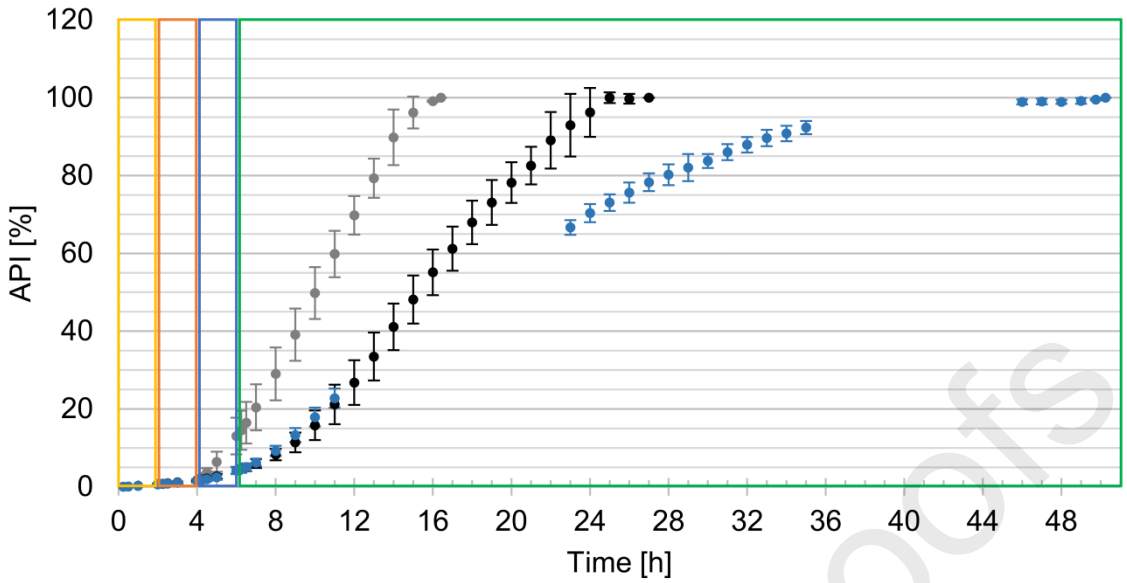

Fig. 3 *API release from CCR caffeine tablets as a function of time for different xyloglucanase concentrations in the colonic stage (1 U/mL: grey, 0.1 U/mL: black, 0 U/mL: blue). Data points and error bars represent average and standard deviation (n = 3). The yellow box illustrates dissolution in 900 mL pH 1.2, orange and blue boxes denote dissolution in 900 mL pH 6.5 and 6.8, respectively, the green box denotes dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment.*

Contrasting CCR 5-ASA tablets, the reducing sugar equivalents release of CCR caffeine tablets in the colonic test stage in the presence of 1 U/mL xyloglucanase concentration was lower ($p < 0.05$) than API release (**Fig. 4a**) up to the 13-hour point of dissolution testing. At 0.1 U/mL enzyme level, reducing sugar equivalents data were also below the caffeine release data at dissolution time points of less than 17 hours ($p < 0.05$), while the later parts of the curves were not statistically different (**Fig. 4a**). As in the CCR 5-ASA experiment, continued off-line incubation of these samples resulted in no increase of reducing sugar equivalents (data not shown), indicating that enzymatic digestion of xyloglucan was complete at the time of sampling.

In the absence of enzyme in the colonic test stage, reducing sugar equivalents were less than 10% over the entire duration of the experiment (light blue triangles in **Fig. 4b**). After subsequent off-line incubation of these samples with xyloglucanase, strong increase of reducing sugar equivalents was detected (dark blue triangles) in agreement with the CCR 5-ASA experiment, suggesting that release of xyloglucan from the tablets at 0 U/mL enzyme concentration took place in its intact form. Notably however, the reducing sugar equivalents data after off-line enzymatic digestion were below the drug release data ($p < 0.05$) up to the 25-h point of dissolution testing and clearly contrasted the result obtained for CCR 5-ASA tablets at this enzyme level.

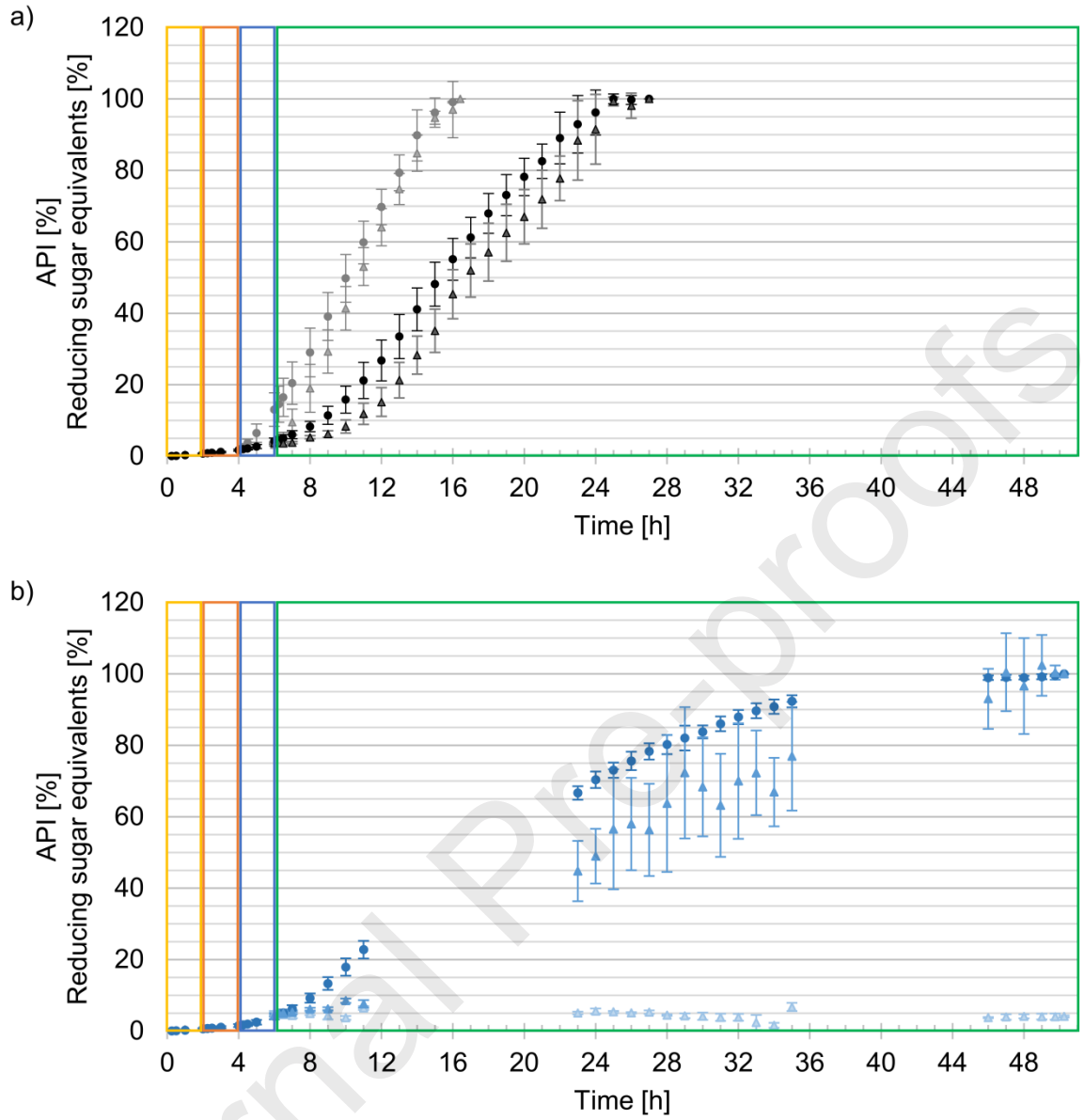

Fig. 4 *Release of API (circles) and reducing sugar equivalents (triangles) from CCR caffeine tablets as a function of time. Panel a) 1 U/mL (grey) and 0.1 U/mL (black) xyloglucanase concentration in the colonic stage. Panel b) 0 U/mL xyloglucanase concentration in the colonic stage. Reducing sugar equivalents immediately after sampling (light blue) and after off-line enzyme incubation (dark blue). Data points and error bars represent average and standard deviation (n = 3). The yellow box illustrates dissolution in 900 mL pH 1.2, orange and blue boxes denote dissolution in 900 mL pH 6.5 and 6.8, respectively, the green box denotes dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment.*

3.3.3. CCR 5-ASA tablet cores

Release profiles of CCR 5-ASA tablet cores in the colonic test stage is shown in **Fig. 5**. A burst drug release was observed at first. Such rapid initial API release was not found for CCR 5-ASA tablets upon transition to the colonic test stage. After this initial burst release, identical ($p > 0.05$) and linear release profiles were observed for CCR 5-ASA tablet cores and CCR 5-ASA tablets in the presence of 1 U/mL xyloglucanase, and API release was complete within 9 hours. In the absence of enzyme on the other hand, complete drug release required 41 hours, corresponding to the drug release from CCR 5-ASA tablets in the colonic stage although the shape of both release profiles was dissimilar. While the CCR tablets yielded an S-shaped release curve, a concave levelling off shape of curve was obtained for tablet cores.

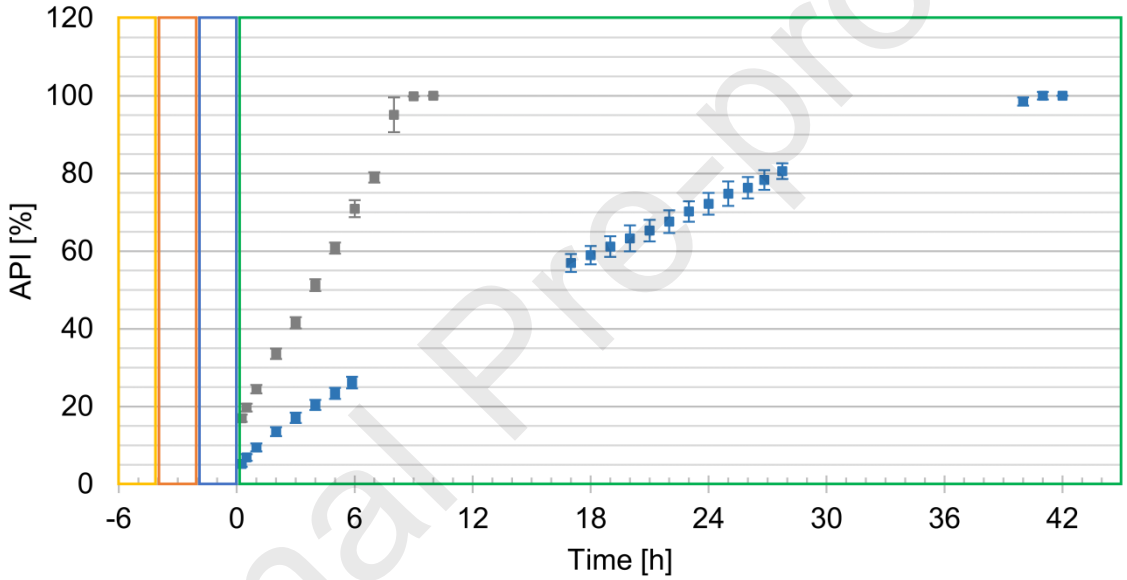

Fig. 5 *API release from CCR 5-ASA tablet cores as a function of time for different xyloglucanase concentrations in the colonic stage (1 U/mL: grey, 0 U/mL: blue). Data points and error bars represent average and standard deviation (n = 3). Only data in the green box are reported, denoting dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment. The yellow, orange, and blue boxes contain no measurements and are shown for consistency with the previous figures.*

At 1 U/mL enzyme concentration, the reducing sugar equivalents release curve was identical ($p > 0.05$) to that of the API (**Fig. 6a**) after the initial burst. Reducing sugar equivalents remained low immediately after sampling at 0 U/mL enzyme concentration (**Fig. 6b**). Only after subsequent off-line incubation of these samples, higher concentrations were detected, and the release profiles of both API and reducing sugar equivalents were identical ($p > 0.05$) after the initial burst. This relationship between API and reducing sugar equivalents release for tablet cores was in agreement with the observations made for CCR 5-ASA tablets at all enzyme concentrations.

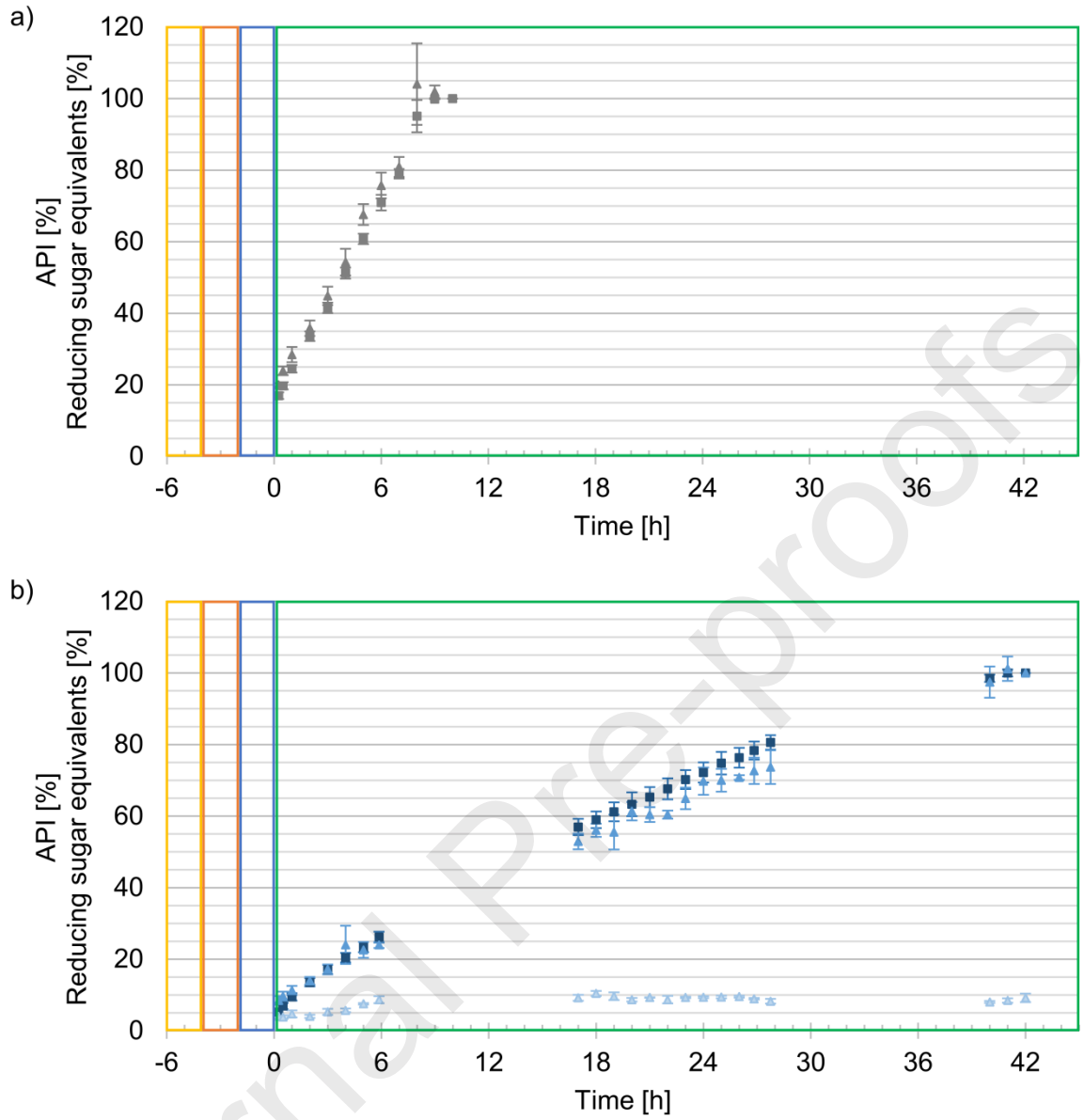

Fig. 6 *Release of API (squares) and reducing sugar equivalents (triangles) from CCR 5-ASA tablet cores as a function of time. Panel a) 1 U/mL (grey) xyloglucanase concentration in the colonic stage. Panel b) 0 U/mL xyloglucanase concentration in the colonic stage. Reducing sugar equivalents immediately after sampling (light blue) and after off-line enzyme incubation (dark blue). Data points and error bars represent average and standard deviation (n = 3). Only data in the green box are reported, denoting dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment. The yellow, orange, and blue boxes contain no measurements and are shown for consistency with the previous figures.*

3.3.4. CCR caffeine tablet cores

Release profiles of CCR caffeine tablet cores in colonic media is shown in **Fig. 7**. Unlike CCR caffeine tablets, a burst drug release was observed at first in the colonic test stage at all enzyme concentrations. Following this burst, identical ($p > 0.05$) and nearly linear drug release profiles were obtained for CCR caffeine tablet cores similar to CCR caffeine tablets in the presence of 1 U/mL xyloglucanase. Release profiles from CCR caffeine tablet cores at 0.1 U/mL enzyme concentration had a concave levelling off shape and were shifted toward longer times compared to CCR caffeine tablets, that exhibited an S-shaped profile. In the absence of enzyme, release profiles of CCR caffeine tablets and tablet cores differed in the same fashion in the shape of the curve upon entry in the colonic test stage as with 0.1 U/mL enzyme. Complete API release was measured after 40, 19, and 10 hours in experiments employing 0, 0.1, and 1 U/mL xyloglucanase, respectively, corresponding to drug release measured for CCR caffeine tablets in the colonic test stage.

When comparing CCR tablet cores of both drugs, no difference between caffeine and 5-ASA was observed at 1 U/mL enzyme level. In the absence of enzyme however, caffeine release occurred faster than release of 5-ASA over most of the colonic stage, in agreement with the observation made for CCR tablets.

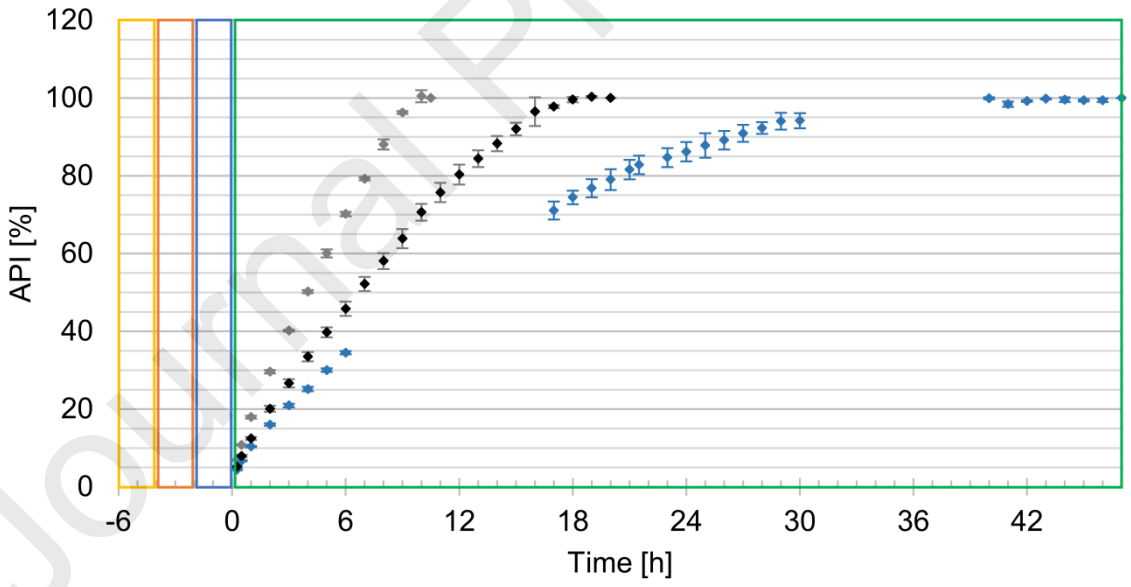

Fig. 7 *API release from CCR caffeine tablet cores as a function of time for different xyloglucanase concentrations in the colonic stage (1 U/mL: grey, 0.1 U/mL: black, 0 U/mL: blue). Data points and error bars represent average and standard deviation (n = 3). Only data in the green box are reported, denoting dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment. The yellow, orange, and blue boxes contain no measurements and are shown for consistency with the previous figures.*

Identical caffeine and reducing sugar equivalent profiles ($p > 0.05$) were obtained after four hours in experiments with 1 U/mL enzyme concentration in the colonic test stage (**Fig. 8a**). At 0.1 U/mL xyloglucanase level, reducing sugar equivalents remained below the caffeine

data points in the early part of the curve ($p < 0.05$), while from 11 h onwards the curves were not statistically different ($p > 0.05$) (**Fig. 8a**). In the absence of enzyme, finally (**Fig. 8b**), the profile of API was not matched by the profile of reducing sugar equivalents after the one-hour off-line incubation of the samples ($p < 0.05$ for all sampling timepoints until 40 h). This relationship between API and reducing sugar equivalents release was qualitatively in agreement with the observations made for CCR caffeine tablets at all enzyme concentrations.

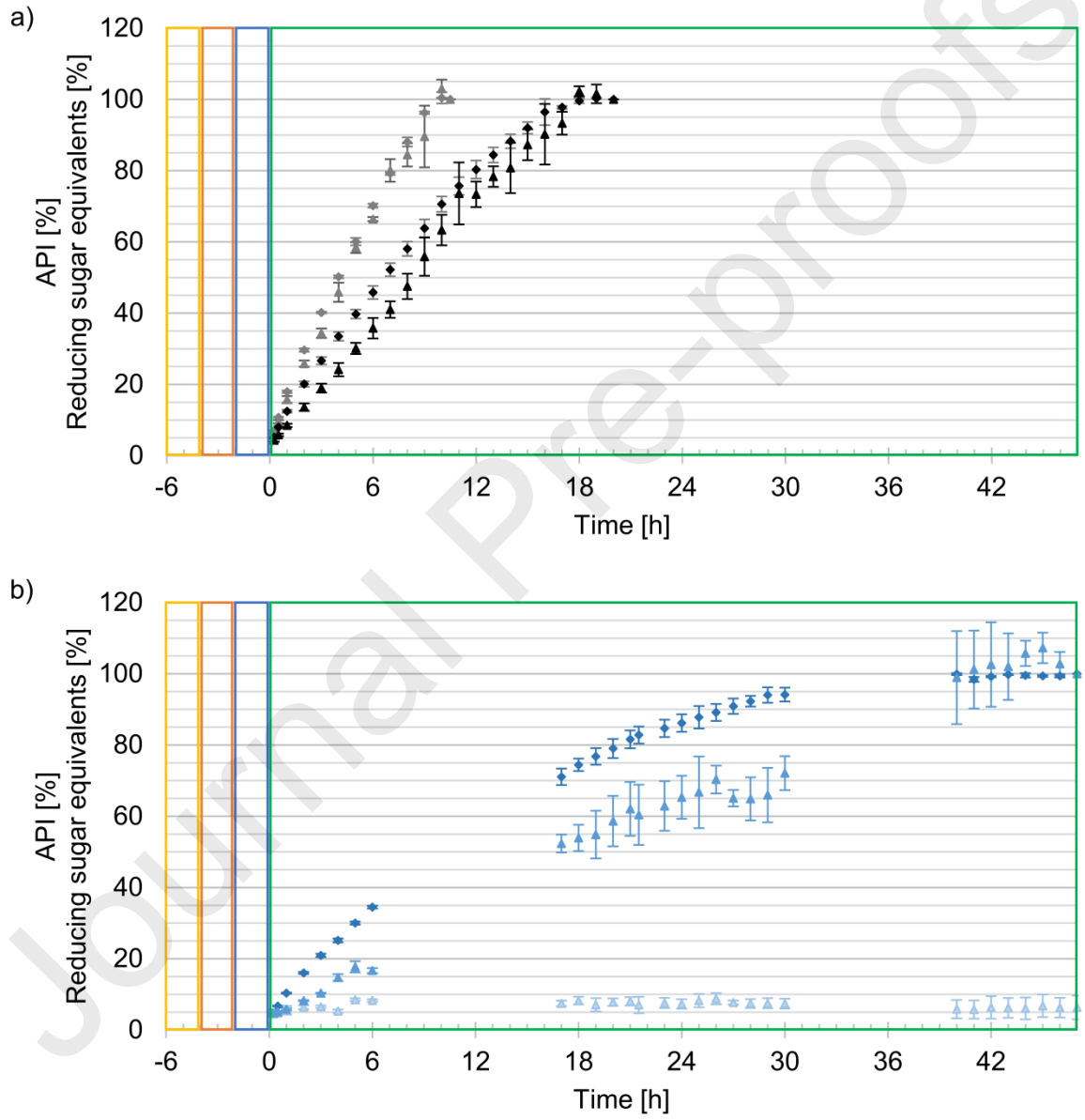

Fig. 8 *Release of API (diamonds) and reducing sugar equivalents (triangles) from CCR caffeine tablet cores as a function of time. Panel a) 1 U/mL (grey) and 0.1 U/mL (black) xyloglucanase concentration in the colonic stage. Panel b) 0 U/mL xyloglucanase concentration in the colonic stage. Reducing sugar equivalents immediately after sampling (light blue) and after off-line enzyme incubation (dark blue). Data points and error bars represent average and standard deviation (n = 3). Only data in the green box are reported, denoting dissolution in 200 mL pH 6.8 and a xyloglucanase concentration between 0 and 1 U/mL depending on the experiment. The yellow, orange, and blue boxes contain no measurements and are shown for consistency with the previous figures.*

3.3.5. Enteric coated 5-ASA tablets

Enteric coated 5-ASA tablets without xyloglucan exhibited an immediate drug release after transition from the gastric to the upper small intestinal test stage and complete drug release at the end of the lower small intestinal stage at pH 6.8 (**Fig. S2**). Variable coating disruption was observed early in the intestinal stage, resulting in wide error range of drug release.

4. Discussion

4.1. Dissolution testing methodology

Selection of media volume, buffer species, and stirring rate was based on the USP monograph for delayed release 5-ASA formulations [59], as was the duration of the gastric test stage. Duration of small intestinal test stage was based on the reported range of 3 to 4 hours for small intestinal transit time [60] and was equally divided into upper and lower small intestinal transit of two hours each. Stomach pH was taken from the USP monograph, and pH of 6.5 was selected to represent the upper small intestinal region, this pH value being within the reported physiological range for both duodenum and jejunum [16,61]. For the test stage simulating the lower small intestine, pH 6.8 was selected which is below the lowest bounds of the range of small intestinal pH values reported in the literature for this intestinal segment [16,62]. This rather low pH value was chosen deliberately to ensure that if dissolution of the enteric coating of the developed formulation takes place in the *in vitro* test it will also take place in all cases *in vivo* before tablets reach the colon taking into account the intra- and interindividual pH variability in the lower small intestine. The 200 mL of medium used for the colonic test stage was the smallest volume for which the paddle of the USP 2 apparatus is fully submerged. No pH decrease was implemented in the simulated large intestinal segment although the ascending colon is characterized by a pH drop compared to the terminal ileum, which might even be larger in active ulcerative colitis patients [23,24]. However, because of the requirement of the test that the enteric coating dissolves at the rather low pH chosen for the lower small intestinal stage, a further decrease of test pH would not be indicative of formulation performance. Furthermore, xyloglucan dissolution and xyloglucanase activity – being responsible for enzymatic digestion of xyloglucan – are not pH-dependent in the used pH or the physiological pH range in the colon [58,63]. Purified xyloglucanase was employed as microbiome surrogate in the colonic test stage, as use of microbial cultures containing for example, *B. ovatus*, *C. japonicus*, or *Paenibacillus KM21* which express the enzyme does not easily allow for standardized test conditions. The employed xyloglucanase concentrations ranged from 0 to 1 U/mL. Measurements of xyloglucanase activity in human feces samples (**Table 3**) gave values ranging from 0.02 to 0.37 U/g feces, which despite intraindividual variability were in good agreement with values derived from the literature [64] and notwithstanding the rather small sample size can be referred to as a first validation of the enzyme concentrations used in the *in vitro* dissolution test. Since feces collection in this study did not take place under fully anaerobic conditions and although it is not certain whether this would affect the determined xyloglucanase activity, the reported result can be considered as low limit of the actual enzymatic activity in human large intestine. Potential variation of enzymatic activity in the state of IBD is discussed below.

4.2. Drug release

The enteric coating film applied onto the CCR tablets successfully prevented premature drug release for both drug substances in the test stages simulating upper gastrointestinal tract. Coating dissolution started at the tablet edges at pH 6.8 which is below the dissolution threshold of pH 7 stated by the coating manufacturer [65]. Different *in vitro* tests in the literature have described Eudragit® FS 30 D coating dissolution at pH values as low as 6.8 [12] and as high as 7.5 [66]. Given these reports, the result of the present work is plausible and verifies dissolution of the used enteric coating in the lower small intestinal environment.

The objective of formulation development was a minimal and equally low drug release for both drug substances during the small intestinal stages. This was accomplished since release in gastric and small intestinal stages remained in the range of ten percent for CCR 5-ASA and CCR caffeine tablets. For a decreased premature release of caffeine, a reduced tablet core porosity as well as a thicker Eudragit® FS 30 D coating layer (**Table 2**) were required. These formulation adjustments were necessary due to the increased solubility of caffeine compared to 5-ASA at relevant pH values and have been mechanistically discussed in the literature in terms of reduced water influx [67,68] and retardation of edge disruption of the coating [69], both impeding drug release.

After coating removal, drug release from the CCR tablets occurred in a controlled fashion for both drug substances. This is evident by the limited amount of drug release at the end of the six hours of the dissolution test representing the end of the small intestinal transit and the low rate of release in the colonic test stage in the absence of xyloglucanase. These results demonstrate the advantage of combining an enteric coating layer with a matrix tablet with respect to preventing premature drug release. By comparison, commercial products relying solely on enteric coating did exhibit premature drug release. Measurements carried out with the present test protocol show that release of these products started at pH 6.5, corresponding to entry of the dosage form in the small intestine, and is completed within six hours (see **Fig. S3** in supplementary information). As these formulations contain disintegrant, significant drug release occurs upon coating dissolution, potentially leading to drug wastage and compromised therapy when this happens before entry into the large intestine. Enteric coated 5-ASA tablets of the present work with the same coating film as CCR tablets but containing no xyloglucan exhibited immediate drug release after the gastric stage (**Fig. S2** in supplementary information) and provide further evidence of the release controlling property of xyloglucan.

The dual control concept employed in the developed CCR tablets successfully elicited efficient drug release in the colonic test stage as most of the dose (>87%) was released in this medium. The presence of xyloglucanase in this medium markedly accelerated drug

release from the CCR tablets in a concentration-dependent fashion with time until complete dose dissolution following the sequence 1 U/mL < 0.1 U/mL < 0 U/mL for 5-ASA and caffeine. This demonstrates that xyloglucanase triggered drug release. At 1 U/mL xyloglucanase concentration, release of both API was completed within ten hours and release rate was constant over time laying between 20 mg/h and 24 mg/h. Xyloglucanase level of 0.1 U/mL led to a time-average release rate of roughly 13 mg/h for both tested drugs despite the shift of the profile of CCR caffeine tablets towards longer times (probably due to experimental variability) and allowed for complete drug release within 19 hours. Interestingly, the ten-fold increase in enzyme concentration led to a roughly two-fold increase in drug release rate. Large interindividual and prandial state-dependent variability of colonic passage time in healthy man ranging from 2 to 45 hours has been reported [10,70], that was more extreme in case of inflammatory bowel disease [24,71], for which prolongation of median transit time was detected by telemetric devices despite the prevailing diarrhea and substantially increased number of bowel movements [71]. In view of this uncertainty, which is augmented by the dependence of transit time on the size of the studied object [72], it is imperative to guarantee accelerated controlled drug release in the colon. The release time measured for the CCR tablets and an enzymatic activity as low as 0.1 U/mL is comparable to the average colonic transit time of 20 hours (95% CI = 13.4 to 27.2 h) deduced by meta-analysis of available literature data [60]. This enzymatic activity was discussed above to be physiologically relevant and may be deemed sufficient for achieving efficient colonic delivery. Notably, fiber-rich diets were shown to increase enzymatic activity towards the respective polysaccharides [73,74], while a change in colonic microbiome composition due to e.g. antibiotics use [75] or disease state including IBD [76,77] might reduce xyloglucanase activity. However, due to the broad dietary prevalence of xyloglucan, a complete absence of enzyme activity towards this hemicellulose is seen as unlikely [78]. Moreover, the fact that a ten-fold difference of enzymatic activity in the present study resulted in a difference in drug release rate of only about two-fold mitigates the risk of possible delivery failure due to variation of enzyme concentration.

4.2.1. Function of xyloglucan in drug release

The controlled drug release of the CCR tablets after dissolution of the enteric coating may be attributed to the xyloglucan matrix and its behavior upon contact with aqueous media. Cross-sections of tablet cores illustrate the formation of a hydrated highly viscous gummy layer at the surface of the tablet surrounding the matrix and persisting for many hours (**Fig. 9**). The formation of this gummy layer appears to play a role in controlling drug release from the xyloglucan matrix. This release control mechanism is studied in detail in an upcoming manuscript.

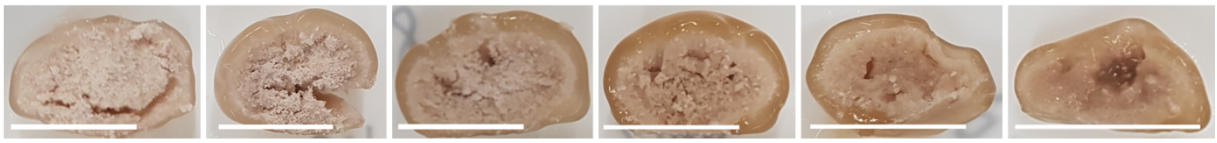

Fig. 9 *Cross-section of a CCR 5-ASA tablet cores after 2, 5, 8, 16, 20, and 24 hours in phosphate buffer pH 6.8 in USP 2 apparatus at 37°C and 100 rpm. The scale bar represents 10 mm.*

To further elucidate the role of the xyloglucan matrix for the dual release control concept and assess potential influence of the enteric coating on the behavior of xyloglucan, drug release from CCR tablet cores was studied. Only the colonic dissolution step was carried out, as drug release from the CCR tablets predominantly took place in this stage, in which release was controlled by the xyloglucan matrix. An initial burst release was observed in all experiments and was attributed to the inexistence of the gummy layer surrounding the CCR tablet core in the beginning of the measurement. A slowing down of drug release after this burst was observed, particularly for experiments with 0 and 0.1 U/mL xyloglucanase concentration, which presumably occurred after this gummy layer was formed. Subsequently, release duration from the CCR tablet cores was comparable to those of CCR tablets in the colonic stage for both drug substances and at all tested enzyme levels. However, the form of the release curve of CCR tablets being S-shaped at 0 and 0.1 U/mL enzyme concentration was notably different from the one of the CCR tablet cores. This is probably because in CCR tablets, water ingress through the intact enteric coating layer conceivably prepares for the gummy layer formation ahead of time, resulting in no burst release upon coating dissolution. This illustrates the synergistic effect between the enteric coating layer and the xyloglucan matrix employed in the CCR tablet formulation for drug delivery control. These effects could not be deciphered at 1 U/mL enzyme concentration because of the fastness of release. It should be pointed out that none of the other polysaccharides studied previously for colonic delivery showed this behavior, as they either lacked the retardation effect on drug release in the absence of coating presumably because they lacked the unique property of xyloglucan to form a gummy layer on the surface of the tablet, or displayed insufficient response to microbial degradation [35,37,39–41,48].

Robustness of the developed CCR concept and the role of the xyloglucan matrix for controlled colonic delivery was demonstrated by varying the drug load of 5-ASA in CCR tablets. Predominant drug release in the colonic test stage is evident at a considerably increased drug load of up to 66% (**Fig. S4** in supplementary information) whereas release rate at 1 U/mL xyloglucanase concentration increased to some extent with increasing drug load. These results show that the performance of the xyloglucan matrix is in principle not impaired by a wide variation of this relevant formulation parameter.

4.3. Xyloglucan matrix erosion and release control

The PAHBAH method used in this work, slightly adapted from [79], detects hemiacetal groups produced upon glycosidic bond cleavage of the xyloglucan backbone as reducing sugar equivalents in addition to reducing groups detectable in intact polysaccharide. Advantages of this method include the absence of interference by the two employed drug substances, its simplicity, and the tailorable calibration range.

In dissolution experiments with 0.1 and 1 U/mL xyloglucanase in the colonic test medium, release profiles of 5-ASA and reducing sugar equivalents were identical while for CCR caffeine tablets, reducing sugar equivalents were mostly lagging API release. Hydrolysis of xyloglucan was shown to be complete at the time of sampling. However, it was not possible to determine whether hydrolysis is completed at the surface of the tablet or whether xyloglucan is first dissolved in the medium to be subsequently hydrolyzed in bulk by the enzyme. Since, on the other hand, reducing sugar equivalents concentration in the medium increased at a higher rate when xyloglucanase concentration was increased, hydrolysis of xyloglucan taking place predominantly at the surface of the tablet may be concluded. Therefore, release of 5-ASA at these enzyme concentrations is shown to be controlled by enzymatic tablet erosion alone, while diffusion additionally affected release rate of caffeine.

Complete tablet disintegration and drug release did not require enzymatic action, although enzyme significantly accelerated the process. The progressive erosion of the tablet matrix even in the absence of xyloglucanase confirms xyloglucan dissolution taking place in aqueous media [80] and demonstrate that intact polysaccharide was released from the matrix tablets. The much slower erosion observed in the absence of enzyme compared to when xyloglucanase was present in the media further supports the view that enzymatic action in the latter case took place at the surface of the tablet. Reducing sugar equivalents measured after off-line enzyme incubation and drug profiles of release were practically identical for CCR 5-ASA tablets, demonstrating erosion-controlled drug release also when no enzyme was present in the medium. Tablet erosion in this case took place by dissolution of intact xyloglucan. In contrast, for CCR caffeine tablets, the measured reducing sugar equivalents after off-line enzyme incubation remained below the drug throughout most of the release process. The different behavior of the two drugs was probably because of the higher solubility of caffeine in water compared to 5-ASA, leading to higher diffusion rate in the gummy layer surrounding the tablet. This is consistent with the faster release measured for caffeine compared to 5-ASA in the absence of xyloglucanase. Thus, caffeine diffusion and tablet erosion both contributed as release controlling mechanisms.

The above observations discussed for CCR tablets are applicable also to tablet cores of both drugs at the respective enzyme concentrations. This indicates that the function of xyloglucan

and the role of matrix erosion for controlling drug release are in principle independent of the enteric coating. The importance of tablet erosion as drug release controlling mechanism is attributed to the formation and maintenance of the gummy layer at the surface of the tablet. This is postulated to be the process by which the xyloglucan matrix exerts its control effect on drug release.

5. Conclusion

This *in vitro* work demonstrates the validity of the dual control principle for colonic delivery, consisting of enteric coating and xyloglucan matrix, the latter preventing drug release after dissolution of the coating in small intestinal environment and being digested by microbial xyloglucanase thus triggering rapid drug release under simulated large intestinal conditions. A synergistic effect between enteric coating and xyloglucan matrix is shown with respect to prevention of premature drug release. Mechanistically, a gummy layer of hydrated xyloglucan formed on the surface of the matrix appears to control drug release while enzymatic xyloglucan degradation elicits significant acceleration of drug release by matrix erosion. These processes can provide selective, efficient colonic delivery within the known range of variability of physiological parameters including the state of disease, substantiating the therapeutic relevance of the concept.

The dual control concept was applicable to two drug substances with different solubility providing similar release rates in colonic environment containing xyloglucanase. Diffusion of caffeine – but not of 5-ASA – out of the matrix tablet contributed to the overall rate of release.

Colonic drug delivery of the CCR tablets is tested in a preclinical *in vivo* study in a follow-up manuscript. Also, the function of the gummy layer at the tablet surface, as well as the mechanisms underlying drug release from the CCR tablet cores are systematically analyzed.

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Declaration of interest

The authors declare no competing interests. This work was supported by BRIDGE project nr. 20B2-1_180977 of the Swiss National Science Foundation and Innosuisse.

CRediT

Viviane Doggwiler: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Michael Lanz**: Conceptualization, Methodology, Resources. **Valeria Paredes**: Methodology, Investigation. **Georg Lipps**: Conceptualization, Project administration, Funding acquisition. **Georgios Imanidis**: Conceptualization, Writing – review & editing, Project administration, Funding acquisition, Supervision.

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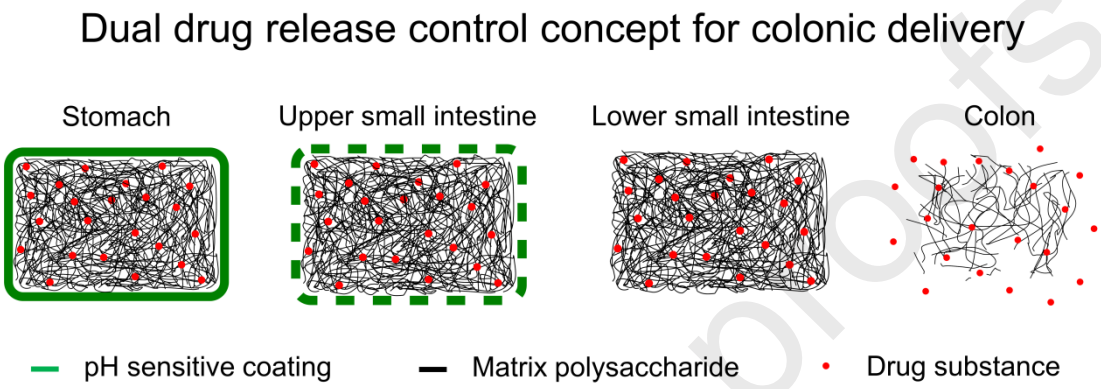

CRediT

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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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