ColoPulse tablets in inflammatory bowel disease

Formulation, potential application and evaluation

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CHAPTER 1

General introduction

1. Colon-specific drug delivery

From a patient point of view oral drug delivery is the preferred route of administration compared to parenteral administration. Dosage forms suitable for oral drug delivery can be divided in solid and liquid dosage forms. The application of liquid dosage forms is desirable when patients have difficulty swallowing tablets or when variable dosages are prescribed. However, these dosage forms are relatively complex to be administered and calculation errors occur easily. Furthermore, liquid dosage forms are less stable compared to solid dosage forms. Finally, release and absorbance of the active substance starts in the stomach immediately upon administration and modified release is not possible. Therefore solid dosage forms are pharmaceutically preferred when fixed dosages have to be administered. The most common examples of solid dosage forms are tablets and capsules. Their release profile can be modified using different techniques, which results in release in a specific segment of the gastrointestinal tract or in a combination of segments. In figure 1 a schematic overview of the different gastrointestinal segments is shown.

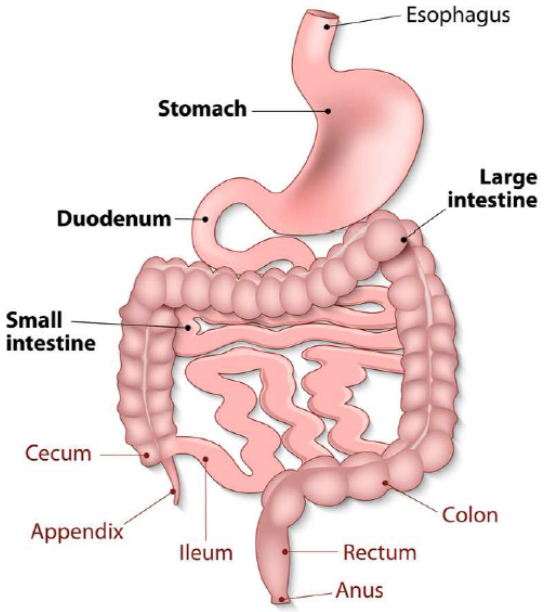

Figure 1: Schematic overview of the gastrointestinal tract. Source: shutterstock.com

Each gastrointestinal segment has specific characteristics based on pH, content, motility, microflora etc. Oral drug delivery to the colonic region has been given interest over the past 40 years, because it has several potential pharmacotherapeutic advantages compared to conventional oral, parenteral and rectal administration. For example, site-specific treatment of diseases located in the colon is more effective when drug release occurs in the affected area, because a higher concentration of drug substance is obtained at the desired site of action resulting in less systemic exposure and adverse events. Furthermore for major gastrointestinal diseases such as inflammatory bowel disease the development of biologicals starts with parenteral application, despite the fact that patients prefer needle-free administration. Colon-specific oral delivery of this treatment is more patient friendly and could therefore improve patient satisfaction combined with less visits to the out-patient clinic [1-3]. In the literature colon-specific oral delivery is considered an alternative for the current parenteral administration of macromolecular and peptide drugs due to the relatively neutral pH of the ileo-colonic region combined with the relatively low proteolytic activity of the colon compared to the small intestine [4]. However due to challenges caused by technological and safety issues only a few peptide formulations for oral delivery have been approved so far or are currently under investigation [5].

Since one of the first publications in 1982 addressing colon specific release from a capsule containing sulphapyridine and coated with a pH-responsive polymer coating [6], several strategies to deliver an intact molecule to the colon have been described. They include systems with release depending on gastrointestinal pH, microflora, time, intraluminal pressure, bioadhesion in a specific organ, osmotic pressure or a combination of such approaches. Dosage forms may contain a single dose, but also multiple unit dosage forms containing (coated) microspheres or nanoparticles have been described [7-10]. We developed an oral solid dosage form for site-specific release in the ileo-colonic region based on gastrointestinal pH: ColoPulse dosage forms. Patients with inflammatory bowel diseases could potentially benefit from colon specific release of active substances for local treatment of their disease when located in the ileo-colonic region. Therefore we focused our current research on ColoPulse dosage forms in this patient group.

2. ColoPulse dosage forms: a summary

The first ColoPulse dosage forms were capsules developed in 2008 by Schellekens et al [11]. These dosage forms are characterized by a high and pulsatile release of content into the ileo-colonic region. They differ from other available modified release dosage forms by the incorporation of the

superdesintegrant Ac-Di-Sol® in a pH-responsive Eudragit S coating in a non-percolating lattice. This patented formulation (WO2007/13794 A1) results in fluid penetration and disruption of the coating once the pH threshold of 7.0 has been reached, which occurs *in vivo* in the ileo-colonic region. In figure 2 a schematic overview of the release process from a ColoPulse dosage form is shown [11].

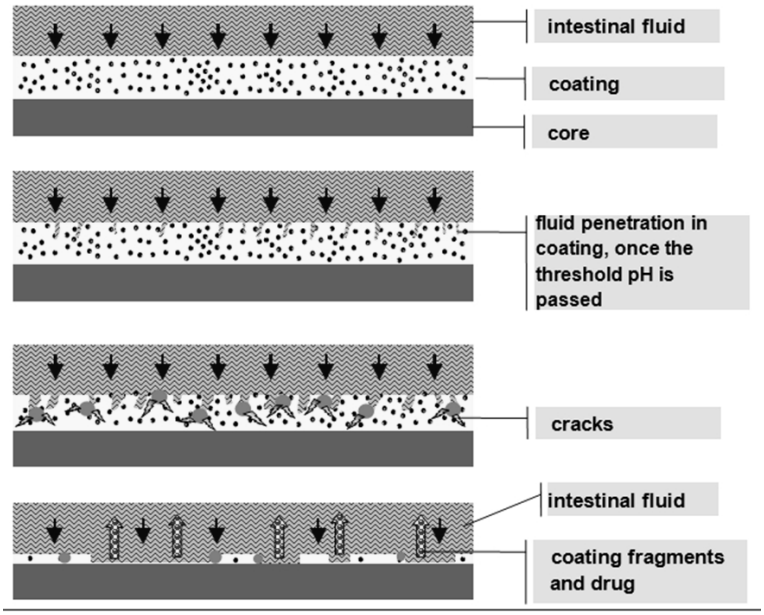

Figure 2: Overview of the mechanism and release from a ColoPulse dosage form

The *in vitro* release profile of ColoPulse dosage forms can be studied using a abbreviated dissolution test named Gastro-Intestinal Simulation System (GISS). This test simulates four compartments of the gastrointestinal tract i.e. stomach, jejunum, distal ileum and proximal colon [12] by varying pH, composition of the dissolution fluid and residence time in the dissolution vessel. With UV measurements of the active substance or caffeine as a marker substance, release from the dosage form can be studied. A typical example of an *in vitro* obtained release profile is illustrated in figure 3. This figure shows that the release occurs fast, pulsatile and soon after pH 7.0 has been reached.

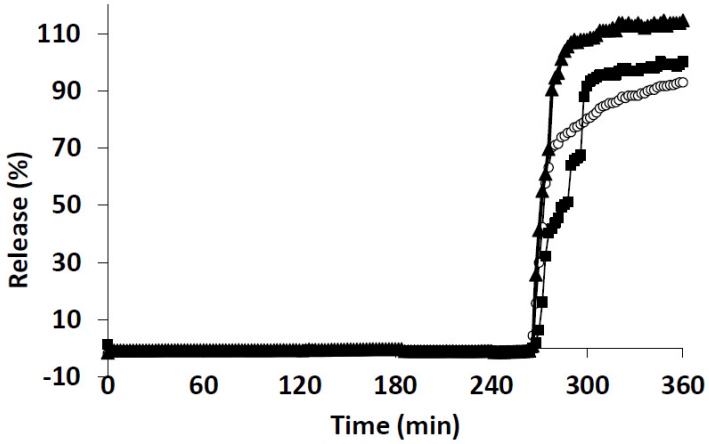

Figure 3: Typical release profile from a ColoPulse 25 mg caffeine tablet, coat thickness 15.3 mg/cm^2 (n = 3)

The first human studies with ColoPulse capsules, reported between 2008-2010 in a total of 20 subjects, showed the expected ileocolonic, pulsatile release profile [11,13,14]. In two of these studies a stable isotope of urea was used and the experiments were performed on different days. However, due to possible physiological variation in urea metabolism the performance of a bioavailability study on different days was considered less desirable. Therefore an optimized study design to be able perform better and faster bioavailability studies for colon-specific dosage forms would be very appropriate.

Despite the promising results in healthy volunteers, the influence of food as well as time of food intake on release characteristics remains to be investigated. So far, most data were obtained with a standardized breakfast three hours after administration of a ColoPulse capsule, which is not feasible in daily practice. Also the performance of ColoPulse dosage forms in the first aimed patient group to study, patients with Crohn's disease, remains to be investigated. This patient group is in need of new possibilities to treat their chronic disease, because of several disadvantages related to their current therapy as described below. It cannot be excluded that their disease state will affect the release characteristics

of a ColoPulse dosage form. Therefore more insight and knowledge of the *in vivo* performance of ColoPulse dosage forms in healthy volunteers and in Crohn's patients is necessary before clinical studies with active substances can be performed in the near future.

3. Infliximab in inflammatory bowel diseases

Infliximab, a chimeric murine-human monoclonal antibody against tumor necrosis factor alpha (anti-TNF-α), is one of the monoclonal antibodies currently available for treating patients with Crohn's disease and ulcerative colitis. It has proven to be effective in the treatment of both diseases [15,16]. Neutralization of soluble and transmembrane TNF-α in combination with apoptosis induction and local anti-inflammatory and immunomodulatory effects in the bowel mucosa by downregulating the formation of adhesion molecules in the lamina propria are considered as the most important mechanisms of action of infliximab [17,18].

Currently infliximab is administered by intravenous infusion in fixed doses of 5-10 mg/kg and at fixed intervals [19]. There is increasing interest in therapeutic drug monitoring of intravenous administered infliximab in order to optimize clinical outcome and to maintain remission. Several publications indicate that infliximab serum trough concentrations are related to higher rates of remission and mucosal healing [20-22]. In view of this, the development of a pharmacokinetic model for infliximab could therefore be relevant in predicting serum trough concentrations and help to facilitate the proposal of strategies for dose optimization in the induction and maintenance phase. This can be described as "precision medicine". However, most studies on model development are performed in controlled patient groups. To fill the gap, we aimed to develop a model based on data obtained in a real life out-patient setting that can be used in daily clinical practice.

It should be realized that, even with optimized treatment, one of the main concerns with systemic exposure of infliximab is the development of anti-drug-antibodies, which are associated with a shorter duration of response and an increased risk of infusion reactions [23,24]. Furthermore intravenous administration of infliximab is associated with serious systemic adverse events, for example infectious complications. From a patient perspective, intravenous therapy is considered as a serious burden (i.e. hospital visits, needle stick punctures) compared to daily oral therapy. Because of the above-mentioned problems related to systemic treatment with infliximab, the availability of new treatment strategies beside intravenous administration would be welcome. Local treatment seems to be a suitable approach, because it will result in lower systemic exposure.

Our vision on this topic matches with the current opinion in the international literature. In a recent review, Moroz et al. [4] concluded that oral delivery to gastrointestinal targets is currently more promising than systemic delivery because of the accessibility and the lack of intestinal permeability enhancement. They also described that in the treatment of inflammatory bowel diseases targeting TNF-alpha through luminal application is a promising alternative to systemic treatment with anti-TNF-alpha antibodies (e.g. adalimumab, infliximab, golimumab and certolizumab pegol). This will probably reduce current disadvantages of intravenous therapy related to systemic immunosuppression, the development of neutralizing antibodies and administration related problems. The described approach corresponds exactly with the potential application of a ColoPulse tablet because release from a ColoPulse tablet occurs at the site of inflammation without the use of permeation enhancers. The concept of local delivery is also supported by the results of the Atlas study as described by Yarur et al. [25]. The authors suggested that local tissue inflammation characterized by high levels of TNF serves as a sink for anti-TNF and that patients with high serum anti-TNF levels have active disease, because tissue levels of anti-TNF are insufficient to neutralize local TNF production.

A limited number of small-scale open-label, non-placebo controlled studies are available describing local injections of infliximab in patients with active or fistulating Crohn's disease [26,27]. However, this treatment also requires several hospital visits. To circumvent this we aim to introduce a completely new strategy and to develop a ColoPulse infliximab tablet for the potential application in inflammatory bowel diseases.

4. Aim of the thesis

Several pharmaceutical challenges are combined in this thesis, all aimed at the improvement of the treatment of patients with inflammatory bowel diseases. The first objective of this thesis is to obtain more insight and knowledge about *in vitro* and *in vivo* behavior of a ColoPulse tablet. The second objective was to lay down a foundation for future research with ColoPulse tablets with a focus on the pharmacokinetics and the formulation of an oral dosage form of the monoclonal antibody infliximab.

5. Outline of the thesis

Chapter 2: in this chapter we present a quality by design study to get more insight into the properties of ColoPulse tablets and the influence of various critical process parameters on release parameters lag time, pulse time and total release. This information will be helpful in the selection of suitable active

substances to be formulated in a ColoPulse tablet and will promote more efficient development.

Chapter 3: in this chapter we describe the formulation of a ColoPulse infliximab tablet with the potential application to study the effect of local treatment with ColoPulse infliximab tablets in patients with Crohn's disease. A stability indicating profile was established and a stability study was performed during a period of 16 months using three different storage conditions.

Chapter 4: the objective of the retrospective study presented in this chapter was to develop a pharmacokinetic model for therapeutic drug monitoring of intravenously administered infliximab in patients with inflammatory bowel diseases. We discuss the development of the model and the potential use of it in optimization of infliximab dosing strategies.

Chapter 5: stable isotopes can be used in bioavailability testing of dosage forms. However conventional bioavailability testing based on concentration-time graphs is not applicable to topical treatment of intestinal segments. We performed a proof-of-concept study to determine the feasibility of the combination of two stable isotopes of urea, 13C-urea and 15N2-urea, and non-invasive sampling techniques (i.e breath and urine) to study the release profile and bioavailability of colon-specific drug delivery systems.

Chapter 6: release from a ColoPulse tablet is triggered by pH. In previous studies with ColoPulse dosage forms, release in the ileo-colonic region was shown, but this was never correlated to gastrointestinal pH. In this chapter we describe a prospective study and investigate the *in vivo* relationship between gastrointestinal pH and the release profile of ColoPulse tablets using real-time *in vivo* pH measurements in healthy volunteers.

Chapter 7: more data about the performance of ColoPulse tablets are necessary to study ColoPulse tablets containing an active substance in Crohn's patients in the future. In this chapter we describe a crossover study that was performed in healthy volunteers and in Crohn's patients. The results of both groups were compared and the influence of food as well as time of food intake on the release from a ColoPulse tablet was investigated using the non-invasive study design with ^{13}C-urea and $^{15}N_2$-urea as described in chapter 5.

Chapter 8: the outcome of the research in this thesis is discussed and future perspectives are presented.

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CHAPTER 2

Influence of critical process parameters on the release characteristics of ColoPulse tablets: a quality by design approach

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Abstract

ColoPulse tablets are characterized by a pulsatile and colon-specific drug release. Release starts in the ileo-colonic region after pH 7.0 has been reached. The influence of the critical process parameters pKa of active substance, coating thickness, exposure to pH 6.8 and type of coating solvent on drug release from a ColoPulse tablet was investigated using a quality by design approach. Release parameters lag time, pulse time and total release were determined using an abbreviated dissolution test. The results indicate that acetone is the preferred coating solvent and that pKa of the model substance and coating thickness affect drug release. Application of a 2.7 mg/cm^2 hydroxypropylmethylcellulose seal coating on the tablet core did not solve the problem of poor release for strongly acidic substances. Substances with a pKa from 6 up to approximately 11 combined with a coating thickness of 11 - 17.5 mg/cm^2 will display the desired release profile. Substances with pKa < 3 and > 11 are in general not suitable for use in the current ColoPulse formulation. The knowledge obtained will facilitate the selection of suitable active substances to be formulated in a ColoPulse tablet and will make further rational development of ColoPulse tablets more feasible and efficient.

1. Introduction

Colon-specific delivery of drugs is particularly of interest in the (local) treatment of inflammatory bowel diseases. Drugs may have a poor bioavailability in the ileo-colonic region due to destabilization by gastric acid, degradation by digestive enzymes or inactivation by binding to bile salts when formulated in immediate release solid dosage forms. Moreover, (unwanted) absorption in a higher part of the gastrointestinal tract will reduce the amount of drug substance available for a local effect in more distal parts of the intestine.

The colonal environment exhibits a low proteolytic activity compared to both stomach and small intestine and, combined with a long residence time, makes it a potentially suitable delivery site for (proteinaceous) drugs intended for topical treatment [1].

In the literature several strategies for colon-specific delivery have been described. They are mainly based on physiological parameters like pH, gastric emptying, residence time, intraluminal pressure and microflora [1,2]. The ColoPulse technology is an example of a pH responsive system. ColoPulse tablets contain a coating consisting of Eudragit S100 in which the super-disintegrant croscarmellose is dispersed in a non-percolating manner yielding a high and pulsatile release of the active substance in the ileocolonic region. Release from a ColoPulse tablet is triggered by the physiologically occurring increase in pH from 5.5 to 6.8 in the upper small intestine to 7.5 in the ileo-colonic region and starts at a pH over 7.0 [3,4].

A recent *in vivo* study showed no difference in bioavailability and site of release from a ColoPulse tablet between healthy volunteers and patients with Crohn's disease in remission [5]. That study was done with ColoPulse tablets containing stable isotopes of urea by which it was possible to measure release in different parts of the intestine. A logical next step in the development and application of ColoPulse technology is the formulation of tablets containing an active drug substance used in daily patient care. Infliximab and a combination of mesalazine and budesonide are considered as good candidates in this context [6,7].

From a pharmacotherapeutic point of view it is desirable that the ColoPulse technology can be applied as platform technology enabling rapid development of oral formulations with different active substances. However, it was found in an earlier study that the composition of the tablet core influences the *in vitro* release pattern of active substances from a ColoPulse tablet. When using citric acid as model substance the release was < 10% compared to tablets containing the neutral or alkaline substances sodium benzoate or sodium bicarbonate. Furthermore, it was observed that coating thickness influences the release

pattern from a ColoPulse tablet [8]. To overcome this problem the application of an additional seal coating layer of material that does not affect release, (i.e. hydroxypropylmethylcellulose (HPMC)) was suggested.

Knowledge about critical process parameters (CPP) influencing degradation of the coating will be helpful in the selection of suitable active substances to be formulated in a ColoPulse tablet. This provides insight in and understanding of the design space of possible ColoPulse formulations and will make rational development and formulation of ColoPulse tablets more feasible and efficient.

The aim of the present study was to obtain more insight in the influence of critical process parameters on the release pattern of ColoPulse tablets based on a quality by design approach [9]. Furthermore the influence of application of a 2.7 mg/cm^2 HPMC coating to prevent stabilization / destabilization of the coating by the tablet core was investigated.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG) 6000, acetone, caffeine, magnesium stearate, lactose monohydrate, silicon dioxide, primojel, talc, citric acid, monobasic sodium phosphate, dibasic sodium phosphate, sodium hydroxide, HPMC 4000 mPa.s (BUFA, the Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany) were obtained via a certified wholesaler (Spruyt-Hillen, the Netherlands). Ethanol 96% and water for injections were obtained from Fresenius Kabi (Germany). Oxalic acid was obtained from Sigma Aldrich (Germany). All ingredients were of pharmacopoeial grade (Ph Eur and/or USP) except oxalic acid (chemical grade).

2.2. Methods

2.2.1. Experimental design

The pKa of active substance (X1), coating thickness (X2), exposure to H 6.8 (before switch to pH 7.5) (X3) and coating solvent (X4) were identified by an expert team as critical process parameters (CPP) highly influencing the drug release from a ColoPulse tablet. The design space was calculated using Design-Expert® software (version 8.4.4.1 Statease®) for Windows. A central composite design was applied for the response surface methodology (RMS). The numeric process variables X1-X3 were varied over five levels. For each parameter a

minimum and a maximum was determined (table 1). Factor X4 was tested as a categorical factor level (acetone - ethanol). This yielded a total of 2 x 20 runs including 6 replicates of the center points as controls. Furthermore for each coating solvent 6 axial and 8 factorial points were assigned. Runs were performed randomly to prevent bias. For each run, tablets were prepared with the assigned model substance, coating thickness Eudragit S and coating solvent. In table 2 a summary of the combination and levels of CPP, the order of runs and type of design points is shown.

Table 1: Critical Process Parameters (CPP) for ColoPulse coating

Factor	Description	Units	Type	Subtype	Min	Max
X1	Model substance	pKa	Numeric	Continuous	1.3	15.7
X2	Coating thickness	mg/cm^2	Numeric	Continuous	4.0	21.0
X3	Time exposed to pH 6.8	min	Numeric	Continuous	30	450
X4	Coat Solvent	n.a.	Categoric	Continuous	Acetone	Ethanol

2.2.2. Tablet core

Five different core tablet formulations (A-E) were prepared (table 3) based on five different model substances (instead of active substances) chosen on the basis of their pKa. All tablets contained caffeine as a marker substance for dissolution testing. The mixture was prepared by blending caffeine, the model substance and excipients, except magnesium stearate, in a Braun blender for two minutes. After adding magnesium stearate blending was continued for one minute. Subsequently the powder was compacted using an eccentric tablet press (HOKO KJ 2) to 9 mm biconvex tablets with a weight of 350 mg. Tablets met all quality control criteria except for content of caffeine due to disturbance of the analysis caused by extreme high pH (table 4) of dibasic sodium phosphate and sodium hydroxide. However, for both substances, no problems with flowability of the powder mixture were noticed and all other parameters showed a relative small standard deviation. Therefore it was assumed that the caffeine content would be within the requested limits of 90-110%.

Table 2: Summary of experimental design: combination and levels of CPP, order of runs and type of design points

Combination	Run	CPP				Type design point
		pKa model substance	Coating thickness (mg/cm^2)	Time pH 6.8 (min)	Coating Solvent	
1	11	7.1	12.5	240	Ethanol	Center
2	31	12.3	7.5	360	Acetone	Factorial
3	3	3.1	17.5	120	Ethanol	Factorial
4	39	15.7	12.5	240	Acetone	Axial
5	12	7.1	12.5	240	Acetone	Center
6	13	7.1	12.5	240	Ethanol	Center
7	32	12.3	17.5	360	Ethanol	Factorial
8	4	3.1	17.5	360	Ethanol	Factorial
9	14	7.1	12.5	240	Acetone	Center
10	15	7.1	20.9	240	Ethanol	Axial
11	40	15.7	12.5	240	Ethanol	Axial
12	33	12.3	17.5	120	Acetone	Factorial
13	34	12.3	17.5	360	Acetone	Factorial
14	35	12.3	7.5	360	Ethanol	Factorial
15	5	3.1	17.5	120	Acetone	Factorial
16	36	12.3	7.5	120	Ethanol	Factorial
17	16	7.1	12.5	36	Ethanol	Axial
18	17	7.1	12.5	240	Acetone	Center
19	18	7.1	20.9	240	Acetone	Axial
20	19	7.1	4.1	240	Acetone	Axial
21	37	12.3	7.5	120	Acetone	Factorial
22	6	3.1	7.5	360	Ethanol	Factorial
23	20	7.1	12.5	240	Ethanol	Center
24	38	12.3	17.5	120	Ethanol	Factorial
25	21	7.1	12.5	444	Ethanol	Axial
26	7	3.1	17.5	360	Acetone	Factorial
27	8	3.1	7.5	360	Acetone	Factorial
28	1	1.3	12.5	240	Ethanol	Axial
29	22	7.1	12.5	240	Ethanol	Center
30	23	7.1	12.5	240	Acetone	Center
31	24	7.1	12.5	240	Ethanol	Center
32	2	1.3	12.5	240	Acetone	Axial
33	25	7.1	12.5	240	Acetone	Center
34	26	7.1	4.1	240	Ethanol	Axial
35	27	7.1	12.5	36	Acetone	Axial
36	28	7.1	12.5	444	Acetone	Axial
37	29	7.1	12.5	240	Ethanol	Center
38	9	3.1	7.5	120	Acetone	Factorial
39	10	3.1	7.5	120	Ethanol	Factorial
40	30	7.1	12.5	240	Acetone	Center

Table 3: Composition of the tablet cores

		A oxalic acid	B citric acid	C monobasic sodium phosphate	D dibasic sodium phosphate	E sodium hydroxide
pKa Model substance		1.3	3.1	7.1	12.3	15.7
Model substance		100 mg	100 mg	100 mg	100 mg	100 mg
Marker substance	Caffeine	25 mg	25 mg	25 mg	25 mg	25 mg
Excipients	Avicel PH 102	85 mg	85 mg	85 mg	85 mg	85 mg
	Lactose	123 mg	123 mg	123 mg	123 mg	123 mg
	Primojel	14 mg	14 mg	14 mg	14 mg	14 mg
	Silicon dioxide	0.7 mg	0.7 mg	0.7 mg	0.7 mg	0.7 mg
	Magnesium stearate	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg
Batch size		500	500	500	500	500

Table 4: Quality control data of the tablet cores presented as means and (standard deviation) where applicable

Parameter	Specification	A oxalic acid	B citric acid	C monobasic sodium phosphate	D dibasic sodium phosphate	E sodium hydroxide
Tablet weight	350 mg (n = 20)	348.6 mg (3.4)	348.0 mg (5.5)	351.4 mg (5.9)	351.2 mg (5.4)	353.3 mg (4.9)
Weight variation	RSD < 4.0% (n = 20)	1.0%	1.6%	1.7%	1.6%	1.4%
Friability	< 1.0% after 100 rotations (n = 1)	0.2%	0.3%	0.2%	0.3%	0.0%
Resistance to crushing	80-150 N (n = 20)	91.3 N (9.4)	107.7 N[a] (7.2)	82.7 N (8.5)	122.8 N (7.8)	87.2 N (16.8)
Disintegration time	All < 15 min (n = 6)	All < 15 min	All < 15 min	All < 15 min	All < 15 min	All < 15 min
Content (caffeine)	90-110% (n = 10)	104.6 (7.4)	97.3 (5.5)	109.9 (10.2)	111.9 (10.5)	46.4 (12.1)

[a] n = 3

2.2.3. Tablet coating

HPMC coating

The tablet core was coated with a HPMC seal coating with the intention to act as a barrier between the core and the subsequently applied ColoPulse coating. HPMC was dissolved in a mixture of water for injections and ethanol 96% in a ratio of 85:15 (v/v) yielding a 5% (w/v) solution. The coating was applied on the different tablet cores using a conventional continuous spray-coating process performed in an in-house build mini rotating drum until a weight gain of approximately 7 mg per tablet was reached. This corresponded with a coating of 2.7 mg/cm^2 HPMC which was proven not to influence the dissolution behavior

of the tablet core (data not shown). Coating conditions were: rotation speed of 60 rpm, temperature of approximately 35°C, continuous coating.

ColoPulse coating

After the HPMC coating had dried, a ColoPulse coating was applied according to the schedule in table 2 (4.1, 7.5, 12.5, 17.5, 20.9 mg/cm^2) using the above described spray-coating equipment. Coating conditions were: rotation speed of 60 rpm, temperature of approximately 35°C, discontinuous coating. The coating suspension was composed of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol:talc in a ratio of 7:1:3:2 (w/w/w/w). The solvent was a water/acetone or a water/ethanol 3:97 mixture (w/v) according to the schedule in table 2. During each run 70 randomly taken tablets were coated followed by curing for 2 hours at 40°C. Coating thickness was determined and expressed as the amount of Eudragit S100 applied per cm^2 using the following formula

$$Coating\ thickness = \frac{weight\ coated\ tablet - weight\ uncoated\ tablet}{surface\ uncoated\ tablet}\ x\ fraction\ eudragit\ S100$$

2.2.4. Release characteristics

The release characteristics of a ColoPulse tablet are reflected by the lag time ($t_{5\%}$) and the pulse time. Therefore lag time (Y1), pulse time (Y2) and total release (Y3) were identified as most suitable critical quality attributes (CQAs) (table 5). The lag time is the time point at which the tablet starts to release the active substance and is defined as the time at which 5% of the marker substance caffeine is released. The pulse time reflects the pulsatile release characteristics and is defined as the period between the lag time ($t_{5\%\ release}$) and the time 70% is released ($t_{70\%\ release}$). These parameters were measured in an abbreviated dissolution test with a total duration 2.5 – 9.5 hours (depending on the time at phase II (pH 6.8). In this dissolution test, known as the Gastro-Intestinal Simulation System (GISS) which was described previously by Schellekens et al. [10], the pH is varied over time to simulate the different stages during passage of the gastrointestinal tract. In the current study an abbreviated GISS version was applied that existed only of the phases II (jejunum) and III (distal ileum). Phase I (stomach) and phase IV (proximal colon) were excluded. The specifications of the abbreviated test are given in table 6. In this study only the phases II and III were used, since the ColoPulse system is expected to show its typical performance characteristics in these phases and not in the first of fourth phase. The release of caffeine from the tablets was measured with in-line UV

spectroscopy at a wavelength of 273 nm. For each run n = 3 tablets from the same batch were tested simultaneously in three separate vessels of the abbreviated GISS.

Table 5: Critical Quality Attributes (CQAs) for release from a ColoPulse tablet

Factor	Description	Units	Specification
Y1	Lag time after switch to phase III	Minutes	> 240 minutes
Y2	Pulse time	Minutes	≤ 60 minutes
Y3	Total release	Percentage	> 80%

Table 6: Specifications of the dissolution test (GISS)

Phase	Gastrointestinal Segment	Volume (ml)	Residence time (min)	pH	Osmolality (mosmol/kg)
I	Stomach	500	not applicable[a]	1.2 ± 0.10	150 ± 25
II	Jejunum	629	36-444	6.8 ± 0.20	250 ± 50
III	Ileum (distal)	940	2.0	7.5 ± 0.25	250 ± 50
IV	Colon (proximal)	1000	not applicable[a]	6.0 ± 0.25	250 ± 60

[a]Phase I (stomach) and phase IV (proximal colon) were excluded in this study

2.2.5. Statistical analysis

The Design-Expert® Software indicated for each CQAS which model was favorable to use for the analysis (i.e linear or quadratic) based on their significance using an analysis of variance (ANOVA) F-test. A Box-Cox transformation was performed when suggested by the software. A model was considered suitable for analysis when R-squared was > 0.80, when the predicted R-squared was in reasonable agreement (difference < 0.2) with the adjusted R-squared and when the normal probability plot of residuals contained appeared to be randomly. Non-significant model terms were removed using backward elimination.

3. Results

The study was performed according to the study design summarized in table 2. A summary of the results of the combination of CPPs tested and their measured CQAs is given in table 7. In table 7 the CPPs are subsequently sorted on the basis of the pKa of the model substance and coating thickness for readability. An example of a typical dissolution profile for tablets containing different model substances obtained with the described abbreviated GISS (only phase II and III) is shown in figure 1. The coating thickness was 12.5 mg/cm^2 for all tablets. This figure shows that the release pattern of a ColoPulse differs when tablets

containing three model substances covering a wide range of pKa's are compared.

Table 7: CQAs lag time, pulse time and total release (mean and (SD), n = 3)

Run	Model sub-stance (pKa)	Coating thickness (mg/cm2)	Time exposed to pH 6.8 (min)	Coating solvent	Lag time (min)	Pulse time (min)	Total release (%)
28	1.3	12.5	240	Ethanol	-[a]	-[a]	4 (1.8)
32	1.3	12.5	240	Acetone	-[a]	-[a]	3 (0.4)
38	3.1	7.5	120	Acetone	155 (32.0)	-[a]	47 (2.8)
39	3.1	7.5	120	Ethanol	130 (7.2)	119 (15.1)	84 (3.1)
22	3.1	7.5	360	Ethanol	109 (26.7)	339 (25.8)	89 (6.4)
27	3.1	7.5	360	Acetone	94 (10.8)	324 (16.7)	108 (10.4)
3	3.1	17.5	120	Ethanol	212 (10.2)	-[a]	35 (5.5)
15	3.1	17.5	120	Acetone	194 (1.2)	-[a]	28 (2.7)
8	3.1	17.5	360	Ethanol	428 (2.3)	-[a]	22 (2.1)
26	3.1	17.5	360	Acetone	436 (11.4)	-[a]	27 (5.8)
20	7.1	4.1	240	Acetone	168 (4.6)	100 (5.7)	100 (10.7)
34	7.1	4.1	240	Ethanol	52 (3.1)	198 (1.5)	107 (7.5)
17	7.1	12.5	36	Ethanol	87 (7.2)	35 (6.2)	102 (6.1)
35	7.1	12.5	36	Acetone	74 (3.1)	17 (2.5)	103 (2.3)
1	7.1	12.5	240	Ethanol	269 (7.5)	69 (8.3)	89 (6.3)
5	7.1	12.5	240	Acetone	278 (7.5)	29 (20.1)	112 (22.8)
6	7.1	12.5	240	Ethanol	250 (6.0)	80 (8.1)	112 (3.3)
9	7.1	12.5	240	Acetone	274 (8.0)	25 (17.2)	111 (25.7)
18	7.1	12.5	240	Acetone	272 (3.5)	42 (5.5)	96 (6.1)
23	7.1	12.5	240	Ethanol	248 (7.1)	79 (2.3)	100 (7.8)
29	7.1	12.5	240	Ethanol	235[b] (7.1)	75[b] (1.7)	102[b] (7.8)
30	7.1	12.5	240	Acetone	270 (4.5)	45 (24.0)	97 (5.7)
31	7.1	12.5	240	Ethanol	222 (9.3)	97 (6.6)	113 (24.1)
33	7.1	12.5	240	Acetone	283 (2.0)	11 (1.5)	101 (4.3)
37	7.1	12.5	240	Ethanol	232 (0.6)	84 (14.5)	99 (9.1)
40	7.1	12.5	240	Acetone	278 (6.4)	39 (28.0)	97 (9.7)
25	7.1	12.5	444	Ethanol	374 (15.1)	103 (19.2)	112 (7.4)
36	7.1	12.5	444	Acetone	221 (33.8)	242 (46.0)	110 (9.4)
10	7.1	20.9	240	Ethanol	303 (1.2)	51 (0.7)	81 (18.2)
19	7.1	20.9	240	Acetone	298 (3.5)	13 (4.4)	104 (10.8)
16	12.3	7.5	120	Ethanol	54 (2.3)	20 (2.5)	85 (6.2)
21	12.3	7.5	120	Acetone	116 (18.9)	34 (2.3)	103 (9.1)
2	12.3	7.5	360	Acetone	195 (28.4)	115 (43.0)	106 (13.7)
14	12.3	7.5	360	Ethanol	48 (5.3)	30 (4.9)	100 (5.6)
12	12.3	17.5	120	Acetone	167 (1.0)	21 (1.0)	108 (9.6)
24	12.3	17.5	120	Ethanol	156 (4.6)	23 (10.1)	95 (2.6
7	12.3	17.5	360	Ethanol	175 (3.1)	31 (15.2)	113 (8.6)
13	12.3	17.5	360	Acetone	352 (28.8)	58 (26.0)	117 (20.2)
4	15.7	12.5	240	Acetone	41 (6.4)	42 (11.0)	100 (1.3)
11	15.7	12.5	240	Ethanol	20 (1.2)	60 (12.7)	93 (3.5)

[a] not measurable

[b] n = 2

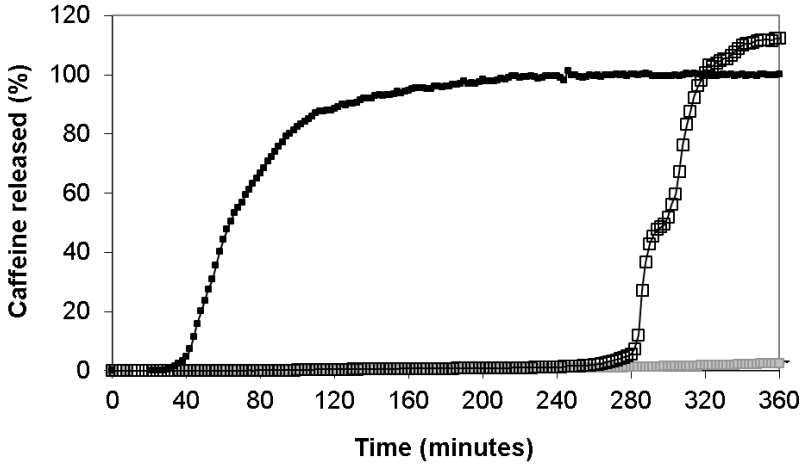


Figure 1: Example of dissolution profiles of ColoPulse tablets containing different model substances. Coating thickness is 12.5 mg/cm^2, time at pH below 6.8 is 4 hours, solvent is acetone

Oxalic acid (pKa 1.3)
Monobasic sodium phosphate (pKa 7.1)
Sodium hydroxide (pKa 15.7)

3.1. Model building

To study the influence of the CCPs on the CQAs a model was constructed for each CQA using the Design-Expert® software. All models had an R-squared > 0.80 (0.842, 0.837 and 0.885 for lag time, pulse time and total release, respectively). For all models the difference between the adjusted R-square and the predicted R-square was < 0.2 and the normal probability plot of residuals appeared to be random. However, models could not be build if the substance with lowest pKa of 1.3 (oxalic acid) was included and only partially for the substance with pKa of 3.1 (citric acid) because in 7 out of 10 runs no lag and/or pulse time could be observed (table 7). Therefore the suitability of the models for substances with an acid nature remains to be investigated, because the experimental design used in our study did not comprise enough data points in this area.

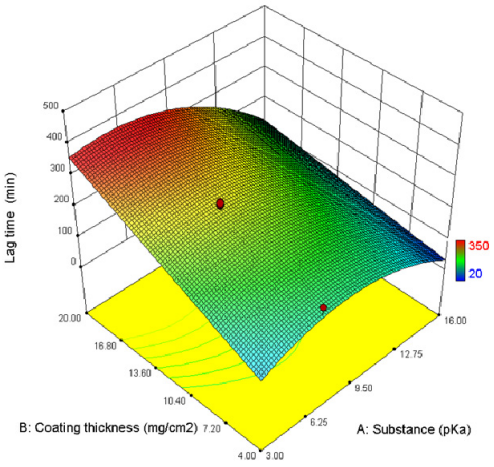
For the presented figures the time exposed to pH 6.8 was set at 4.0 hours because this corresponds to normal physiological conditions (oral to cecal transit time = 240 ± 88 min [11]) and the combined time at phase I and II in the original GISS [10].

3.2. Lag time

The lag time was determined using the abbreviated GISS. For tablets with oxalic acid as model substance no lag time and no pulse-time could be measured because the coating did not disintegrate and the tablets remained intact.

The 3D plot and the corresponding contour plot in figure 2 indicate that the lag time is influenced by the pKa of the model substance and the coating thickness. The lag time increased with increasing coating thickness and the lag time decreased with increasing pKa. In general, substances with a pKa > 6 and a coating thickness > 11 mg/cm ^{2} will yield an adequate lag time. Lag time has to be > the CPP "exposure to pH 6.8". This depends on both coating thickness and model substance. There was no difference in lag time between tablets coated with coating fluid containing acetone or ethanol.

(A)



(B)

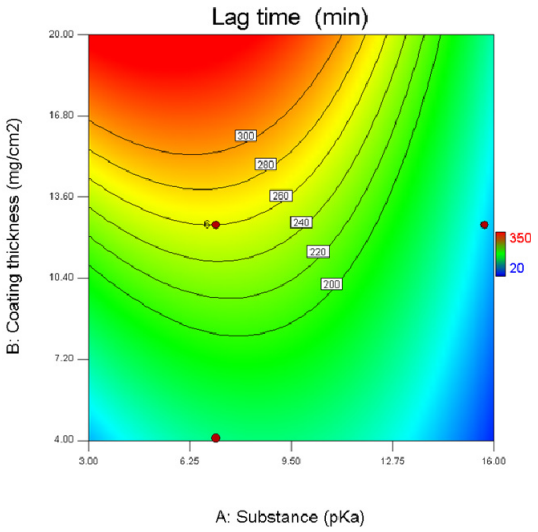


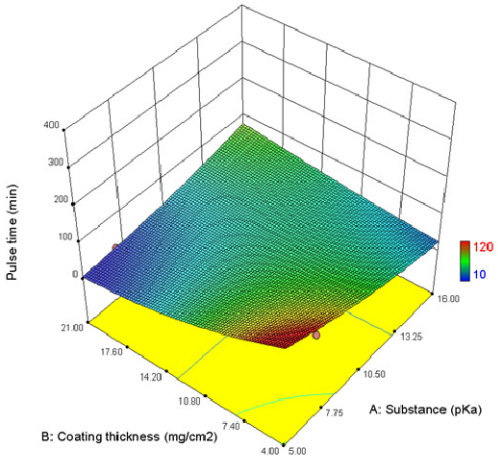
Figure 2: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on lag time (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

3.3. Pulse time

The pulse time of the different ColoPulse tablets was determined in the abbreviated GISS. As described above hardly any release was observed for tablets with oxalic acid as model substance. For tablets with citric acid as model substance, pulse time could not be determined due to incomplete total release (< 47%) in three out of four runs. In the other run a pulse time > 60 minutes (324 minutes) was measured.

The 3D plot and corresponding contour plot in figure 3 indicate that the pulse time is out of specification for substances with pKa approximately < 6, but, as explained, the model does not comprise enough design points between pKa 3 and 6. Because a coating thickness of 4 (very low) and 21 mg/cm^2 (very high) were both tested only once, results at the outside limits of the model should be interpreted with caution. In general, figure 3 shows that the pulse time increased with decreasing pKa. The pulse time increased with increasing coating thickness.

(A)



(B)

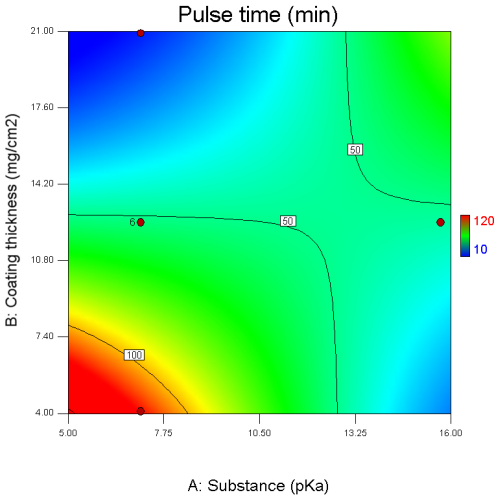


Figure 3: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on pulse time (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

Regarding pulse time, a difference was observed between the coating solvents ethanol and acetone. For example, for a marker substance with pKa 7, a coating thickness of 12.5 mg/cm^2 and 4 hours exposed to pH 6.8, pulse time appeared to be out of specification, i.e. > 60 minutes, for 6 out of 6 runs for tablets when ethanol was used as coating solvent. Under these conditions all tablets coated with acetone as coating solvent had a pulse time < 60 minutes. The difference

between ethanol and acetone, with longer pulse times for ethanol, is elegantly illustrated with the cube plots for both solvents (figure 4).

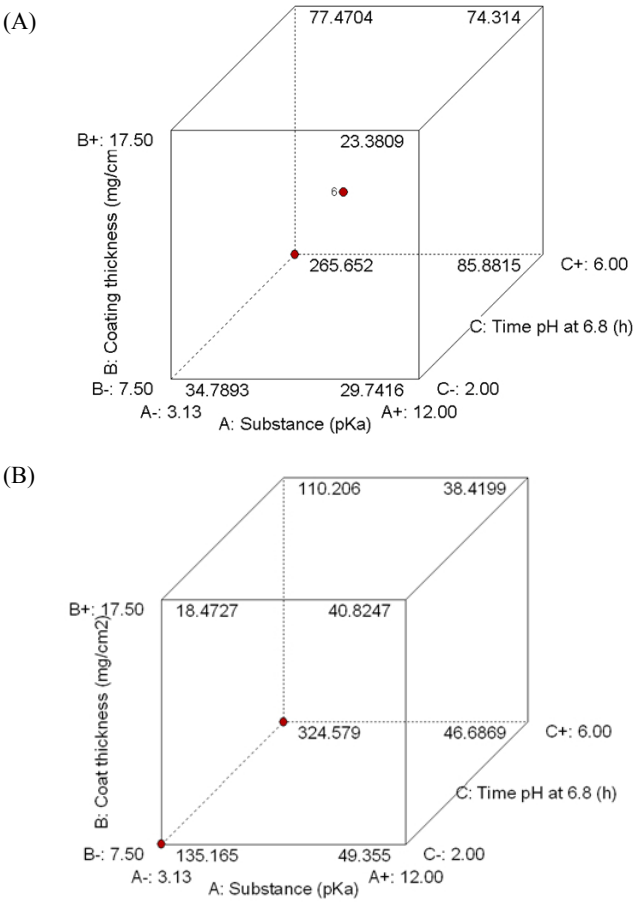


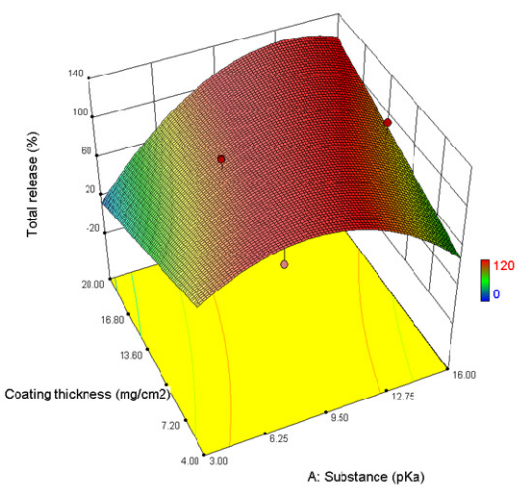
Figure 4: Cube plot of the effect of pKa, time exposed to pH 6.8 and coating thickness on pulse time for ColoPulse tablets coated with acetone (A) and ethanol (B).

3.4. Total release

Total release was determined as the percentage of caffeine released at the end of the dissolution test. The 3D plot and the corresponding contour plot in figure 5 show that the total release from a ColoPulse tablet with a model substance of pKa > 6 was influenced mainly by the model substance and not by coating thickness. Total release increased from 0% at pKa 1.3 to > 80% at pKa > 6.0.

The time exposed to pH 6.8 in the dissolution test had no influence on total release. However, coating thickness did have influence on release for substances with a pKa < 3. For these substances the total release increases when coating thickness decreased. There was no difference regarding lag time between tablets coated with acetone or ethanol.

(A)



(B)

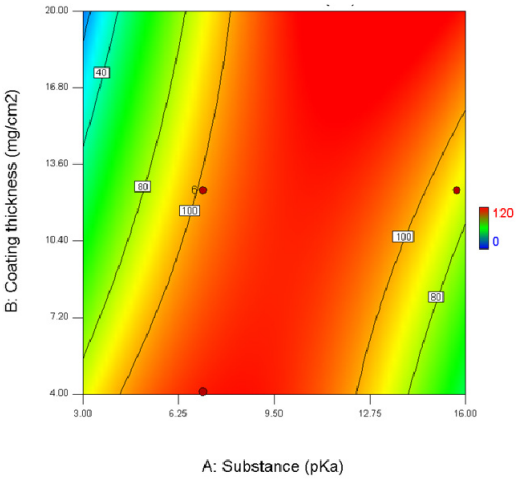


Figure 5: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on total release (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

4. Discussion

This is the first study in which the influence of selected critical factors (the CPP) on the performance of ColoPulse tablets is systematically studied using a quality by design approach. The results are in agreement with our previous findings [8] that the composition of the core as well as the coating thickness influence the release pattern of an active substance from a ColoPulse tablet, but the current study provides more insight in the extent of these phenomena. Moreover, information was generated on the effect of process and testing variables.

The application of a 2.7 mg/cm^2 HPMC coating between tablet core and ColoPulse coating did not act as the desired barrier to prevent any effect of the core on the coating performance. Neutral to weakly acidic and slightly alkaline drug substances appear to be excellent candidates for formulation in a ColoPulse tablet. In contrast, substances with low (< 3) and high pKa (> 11) were less or not suitable to be formulated into a ColoPulse tablet with the current formulation. A low pKa strongly reduced total drug release combined with extreme long pulse times to an unacceptable level. The suitability of a ColoPulse coating for substances with their pKa between 3 and 6 remains to be investigated. These results can be relevant for future development of ColoPulse tablets. Manallack describes that among the WHO essential medicines 78% of them has an ionizable group with a pKa in the range of 2-12 and in another dataset 50% of all substances containing a single acid group had a pKa between 3 and 6 [12].

4.1. Ethanol vs acetone

No influence of the coating solvent, ethanol or acetone, on the model parameters lag time and total release was found. However pulse time was > 60 min for a substantial number of the results when tablets were coated with ethanol as solvent. This indicates that acetone is the preferred coating solvent for application of a ColoPulse coating. The difference can possibly be explained by acetone being more volatile than ethanol (vapor pressure approximately 25 kPa versus 8 kPa, respectively, at 25ºC). This causes acetone to evaporate faster than ethanol during the coating process and subsequent curing of the tablets which results in less residual solvent in the coating. Therefore more research on coating and curing conditions has to be done before ethanol can be used as coating solvent to apply ColoPulse coating.

4.2. Lag time

For interpretation of the results regarding lag time, it has to be realized that *in vivo* the pH rises along the small intestine, will reach a value above pH 7.0 in the terminal ileum and drops relatively fast to a value around 6 after passage of the ileocecal valve occurs. After this pH drop (phase IV in the original GISS) (pulsatile) release of active substance from the ColoPulse tablet will be limited [4]. Furthermore it has to be taken into account that, from a technical point of view, application of a coating with a thickness up to 17.5 mg/cm^2 is practically feasible and results in a smooth coating combined with an acceptable coating time. A higher coating thickness bears practical difficulties, although the CQAs are within specifications. From the presented data it can be concluded that substances with a pKa from 6 up to approximately 11 in combination with a feasible coating thickness of 11 - 17.5 mg/cm^2 will result in an adequate lag time (250-300 minutes). For oxalic acid with a pKa of 1.3 only a 12.5 mg/cm^2 coating thickness was studied. From a previous study [8] it is known that substances with low pKa show relatively long lag times and poor total release. Therefore additional research to optimize the model for low pKa values was considered unuseful.

4.3. Pulse time

An *in vitro* pulse time of ≤ 60 minutes is relevant, because when tablets meet this specification it is known that the *in vivo* pulse time of a ColoPulse tablet is approximately 220 minutes in healthy volunteers and in Crohn's patients [5]. A longer *in vitro* pulse time is therefore not desirable. From the presented data regarding to pulse time, it can be concluded that substances with pKa < 3 are not suitable to be formulated in a ColoPulse tablet because of long pulse times and that coating thickness has only limited influence on pulse time for substances with pKa > 6.

4.4. Total Release

As expected total release was ≥ 80% in almost all runs with a measurable pulse time. For the marker substances with pKa 1.3 and 3.1 only limited release of caffeine was found in the majority of the runs. This is in agreement with the results published by Schellekens et al. [8]. In this paper it was described that substances with low pKa showed little total release of caffeine. It was hypothesized that attrition of tablets during the initial phase of the coating process produces some powder that is incorporated into coating. This creates a micro-environment with the uptake of a small amount of water in the coating resulting in stabilization or destabilization of the coating in case the core tablet contains an acidic or a basic compound, respectively. For this reason an HPMC

coating between core and ColoPulse coating was suggested as possible solution for this problem.

In our study tablets containing oxalic acid were clearly swollen at the end of the experiment and the appearance of the core could be described as somewhat granulated. The coating remained intact, except for the formation of some small pores that could also be visually observed during the GISS. For tablets based on citric acid more pores were seen, but again the coating remained merely intact. At the end of the experiment all tablets containing citric acid were clearly swollen, while the structure of the core could be described as a granulated gel. From these findings it can be concluded that the applied 2.7 mg/cm^2 HPMC coating did not exhibit the desired barrier function and did not prevent the over-stabilization of the coating. Possible explanations for this observation may be found in either the occurrence of minor amounts of acid in the HPMC layer due to attrition or in the permeation of minor liquid amounts through the ColoPulse coating in the early stages of the test, which led to diffusion of acid into the coating before phase III.

4.5. Future research

More research has to be done on the formulation of substances with high and low pKa in a ColoPulse tablet. Strategies could be to find a suitable other sealcoating that does not affect tablet dissolution, to increase the thickness of the HPM seal coating or to make the coating less porous for example by lowering the amount of PEG 6000 or a combination of these. Furthermore the influence of the percentage of the drug substance on the total tablet weight can influence this interaction. In the current study this percentage was 28.6%, which is relatively high. It is not unlikely that the described interaction is less relevant when this percentage is reduced by decreasing the amount of active substance or by increasing the amount of filler material. Another option for future research could be to “titrate” the tablet core i.e. add acidic excipients when the active compound is strongly basic and add basic excipients when the active compound is strongly acidic. However, this might influence the biopharmaceutical properties of the active substance.

5. Conclusion

The aim of the current study was to investigate the influence of the CPP pKa of active substance, coating thickness, time exposed to pH 6.8 and type of coating solvent on the performance of a ColoPulse tablet using a quality by design approach. The application of a 2.7 mg/cm^2 HPMC seal coating between tablet core and ColoPulse coating did not act as the desired barrier to prevent influence of active substances on the ColoPulse coating. All CPPs influenced the lag time,

pulse time and/or total release of a ColoPulse tablet. Acetone was the preferred coating solvent with the current settings and the pKa of the model substance and coating thickness both influenced release from a ColoPulse tablet. Based on this, active substances with a pKa from 6 up to approximately 11 in combination with a coating thickness of 11 - 17.5 mg/cm^2 will result in an adequate release profile.

Active substances with pKa < 3 are in general not suitable to be formulated in a ColoPulse tablet, because the coating is stabilized by the tablet core which prevents release from the tablet. Substances with pKa > 11 are in general not suitable, because destabilization of the coating by the tablet core leads to preliminary release of the content. The suitability of the models remains to be investigated in more detail for substances with a pKa between approximately 3 and 6, because the experimental design used in our study did not comprise enough data points in this area. The results of this study can be used in the formulation of new ColoPulse tablets and will make further rational development more feasible and efficient.

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CHAPTER 3

Development and potential application of an oral ColoPulse infliximab tablet with colon specific release: a feasibility study

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Abstract

The monoclonal antibody infliximab is one of the cornerstones in the treatment of Crohn's disease. Local delivery of infliximab would be an alternative to overcome the inherent disadvantages of intravenous therapy. For this purpose 5 mg infliximab tablets were developed. To stabilize the antibody during production and storage it was incorporated in a sugar glass containing the oligosaccharide inulin. To obtain colon-specific release a ColoPulse coating was applied. The tablets were stored for 16 months under different conditions based on ICH climatic zone I:

Condition 1: 25°C/60% RH closed vial

Condition 2: 25°C/60% RH open vial

Condition 3: 40°C/75% RH closed vial

With a panel of tests (i.e. HP-SEC, UV, CD) a stability indicating profile was obtained. Infliximab tablets were stable for up to four months when stored at temperatures varying from 25 - 40 °C. Tablets stored under condition 1 were most stable and displayed 16 months after production still a biological activity of 83% compared to a freshly prepared infliximab solution. This study is a first step in the development of a novel strategy in the treatment of patients with Crohn's disease.

1. Introduction

Crohn's disease is an autoimmune disorder of the gastrointestinal tract with huge impact on quality of life. The entire gastrointestinal tract can be affected, but in most cases inflammation is localized in the terminal ileum and the colon [1]. Currently the treatment of Crohn's disease is mainly symptomatic and aimed at the induction of remission and prevention of flare-ups. Pharmacotherapeutic treatment depends on patient characteristics and severity of disease. Several drugs are available to treat different stages of the disease, including 5-aminosalicylates, corticosteroids, methotrexate and thiopurines. Infliximab is considered a cornerstone in the treatment of moderate to severe Crohn's Disease, achieving rapid symptom relieve and maintaining remission [1,2].

Infliximab, a chimeric monoclonal antibody, was approved in 1998 by the Food and Drug Administration (FDA) for the treatment of Crohn's disease. It is now also used in other inflammatory diseases. The antibody binds to transmembrane and soluble tumor necrosis factor alpha (TNF-alpha) and exhibits its effect in Crohn's disease mostly by causing apoptosis of TNF-alpha expressing, activated T-lymphocytes and by neutralizing TNF-alpha [3,4].

At the start of therapy infliximab is administered by intravenous infusion at a dose of 5 mg/kg in the weeks 0, 2 and 6, followed by an infusion of the same dose every eight weeks. Although the antibody has proven to be effective in the treatment of Crohn's disease, it has several disadvantages when administered intravenously. Systemic exposure to infliximab is known to give rise to anti-infliximab antibodies resulting in infusion reactions, increased clearance and loss of response in a considerable number of patients. Furthermore, intravenous administration of infliximab is associated with serious adverse events, for example infectious complications, that can occur when the immune system of the patient is compromised. Also acute infusion reactions, hypersensitivity and anaphylactic shock have been reported [3]. Last but not least, regular intravenous therapy is perceived as a serious burden to the patient compared to chronic oral therapy. Because the mentioned clinical disadvantages are related to systemic exposure they may be overcome by a site-specific administration of (a lower dose of) infliximab. Small scale studies have shown a good clinical effect after local injections of infliximab [5-7].

As substitute for local administration by injections, the aim of this study is to develop an infliximab tablet with local release at the site of inflammation in patients with ileocolonic Crohn's disease.This aim is supported by the recent review of Moroz et al. [8]. They concluded that oral delivery to gastrointestinal

targets is currently more promising than systemic delivery because of the accessibility and the lack of intestinal permeability enhancement. They also stated that in the treatment of inflammatory bowel diseases capturing TNF-alpha in the intestinal lumen is a promising alternative to systemic treatment with anti-TNF-alpha antibodies (e.g. adalimumab, infliximab, and certolizumab pegol) with regard to systemic immunosuppression induced by these antibodies. In addition, oral administration is more patient friendly than the parenteral route as it is needle-free. The concept of local delivery of infliximab is also supported by the results of the Atlas study as described by Yarur et al. [9]. The results of this study suggest that local tissue inflammation characterized by high levels of TNF serves as a sink for anti-TNF. The authors stipulated that patients with high serum anti-TNF levels have active disease because tissue levels of anti-TNF are insufficient to neutralize local TNF production.

From several studies it appears that the colon is a suitable delivery site for proteinaceous drugs due to limited proteolytic activity (compared to higher parts of the gastrointestinal tract) combined with a relatively long residence time [10,11]. However, it remains a challenge to deliver a proteinaceous drug undamaged to the colon via oral administration. In the literature different strategies for colon targeting have been described. They include pH-responsive systems, time-based systems and systems triggered by the colonic flora, as well as combinations of such systems [12]. The ColoPulse technology is an example of a pH-responsive system which in several human studies was shown to deliver a drug site-specific into the ileocolonic region.

The ColoPulse system consists of a coating that dissolves at pH > 7.0, enabling drug release in the ileocolonic region. The release from the ColoPulse system is faster and more pulsatile than from other pH responsive systems because a superdisintegrant is incorporated in the coating [13]. In an *in-vitro* study release from several commercially available mesalazine drug products with a pH sensitive coating was tested in a modified dissolution test and in *vitro* colon selectivity was found to be suboptimal [14]. All formulations showed release of a substantial part of the active substance in the simulated stomach and jejunum. Using the same dissolution test for ColoPulse based dosage forms a different *in vitro* release profile was found with mainly release in the simulated jejunum / colon [15].

ColoPulse tablets containing ^{13}C-urea as a marker substance revealed no relevant difference in site-specific delivery and release kinetics between healthy volunteers and patients with Crohn's disease [16]. To gain insight in the influence of food and time of food intake on the release from a ColoPulse tablet a study was performed in healthy volunteers and in patients with Crohn's

disease in remission. There was no relevant influence of food and time of food intake on the release characteristics and bioavailability in both healthy volunteers and in Crohn's patients when a standardized breakfast 3 h after administration of a ColoPulse tablet was compared to a non-standardized breakfast after 1 h.

The results from another study in which the relationship between *in vivo* gastrointestinal pH measurements and *in vivo* release from a ColoPulse tablet was studied in healthy volunteers confirm that release from a ColoPulse tablet indeed occurs in the distal ileum and colon and after pH 7.0 is reached [17].

This leads to the conclusion that the characteristics of a ColoPulse tablet correspond with the described aim of developing a modified release tablet for local treatment with infliximab in patients with Crohn's disease, because release from a ColoPulse tablet occurs at the ileocolonic region and without the use of permeation enhancers.

Another major challenge in the development of an infliximab tablet is to maintain the protein's stability during manufacturing and shelf life. To prevent loss of activity, the protein can be stabilized by incorporating it into a sugar glass. This can be achieved by freeze-drying of a solution in which both the protein and a suitable sugar are dissolved [18,19].

Combining the incorporation of infliximab into a sugar glass matrix with subsequent formulation of a ColoPulse tablet is considered an alternative to overcome the problems and drawbacks related to intravenously administered infliximab. Our hypothesis is that orally administered infliximab could be a novel strategy in the treatment of Crohn's disease. This would be a significant step forward in therapy. However, until now no data about formulation and stability of such a dosage form are available. In this paper we describe a suitable formulation for infliximab tablets with stability data of more than one year after production. This will enable us to study the effect of local treatment with ColoPulse infliximab tablets in Crohn's patients in the near future.

2. Materials and methods

2.1. Materials ColoPulse infliximab tablets

Polyethylene glycol 6000, caffeine, colloidal anhydrous silica, sodium stearyl fumarate, talc (BUFA, the Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany), were obtained via a certified wholesaler

(Spruyt-Hillen, the Netherlands). Inulin 4.0 kD was obtained from Sensus (the Netherlands). Ethanol 96% and water for injections were obtained from Fresenius Kabi (Germany). Infliximab (Remicade®) vials 100 mg were obtained from MSD (the Netherlands). All ingredients were of pharmacopoeial grade (Ph Eur or USP).

2.2. Composition and production of ColoPulse infliximab tablets

2.2.1. Preparation of the sugar glass

A sugar glass was prepared by reconstitution of vials Remicade® containing 100 mg infliximab, sucrose, polysorbate 80, dibasic and monobasic sodium phosphate with 10 ml of a 17.5 wt% inulin solution in water. The infliximab-inulin solution was then transferred into vials of 4 ml each. Subsequently the vials were placed into liquid nitrogen to freeze the solution and the vials were placed in a Christ model Alpha 2–4 freeze-dryer (Salm and Kipp, the Netherlands). Primary drying was performed for 24 h at a shelf temperature of -35˚C and pressure of 0.220 mbar. Thereafter, secondary drying was done for 24 h at a shelf temperature of 20˚C and a pressure of 0.05 mbar. After freeze-drying samples were stored at 20˚C/45% relative humidity (RH) for at least 48 h until further use. The glass transition temperature (T_g) as determined using a DCS 2920 (TA instruments, Belgium) was 129.7°C. The water content as determined with a Karl Fischer titration using a 720 KFS Titrino (Metrohm, Switzerland) was 9.8%.

2.2.2. Production of 5 mg infliximab tablets

To obtain a powder with sufficient flowability the slugging technique was used to perform dry granulation. Tablets with a diameter of 5 cm were made using an ESH hydraulic press (Hydro Mooi, the Netherlands) with a compaction force of 10 kN and a compaction speed of 2.5 kN/s after which the tablets were passed through a 0.8 mm sieve.

Two batches of infliximab 5 mg tablets were produced (batch A 180 tablets and B 45 tablets). The quantitative composition of batches A and B is shown in table 1. The amount of sugar glass was corrected for the water content in the infliximab sugar glass. Batch A also contained 25 mg caffeine for quality control purposes and for dissolution testing. The sugar glass was mixed in a turbula mixer with the excipients and subsequently the powder was compacted with a Korsch eccentric press (Berlin, Germany) to yield biconvex tablets with a diameter of 9 mm and a weight of 350 mg. The instrument settings were adjusted in such a way that tablets with a crushing strength of 150-200 N were

obtained. The tablets were analyzed according to the European Pharmacopoeia 8th edition. The results of the quality control tests are shown in table 2.

Table 1: Composition of 5 mg infliximab tablet cores.

Substance	Function	Batch A	Batch B
Infliximab sugar glass	Active substance	131 mg	131 mg
Caffeine	Marker sub-stance	25 mg	0 mg
Microcrystalline cellulose	Excipient	175 mg	200 mg
Croscarmellose sodium	Excipient	14 mg	14 mg
Colloidal anhydrous silica	Excipient	1.8 mg	1.8 mg
Sodium stearyl fumarate	Excipient	3.5 mg	3.5 mg
Total weight		350 mg	350 mg
Number of tablets		180	45

Table 2: Quality control data of 5 mg infliximab ColoPulse tablets presented as mean (standard deviation) where applicable.

Parameter	Specification	Batch A (with caffeine)	Batch B (without caffeine)
Infliximab 5 mg cores (uncoated)			
Yield	n.a.[a]	147 tablets	42 tablets
Appearance	White biconvex tablet	Complies	Complies
Crushing strength	150-200 N	168 N	162 N
Friability	< 1% (Ph Eur)	0.109%	-[b]
Disintegration (cores)	All < 15 min (Ph Eur)	All < 15 min	-[b]
Uniformity of dosage units (caffeine, n = 10)	AV < 15 (Ph Eur)	13.9	-[b]
Content (caffeine)	90-110%	96.7% (4.9)	-[b]
Amount infliximab (mg) able to bind to TNF-alpha	4.5-5.5 mg	5.5 mg (0.4)	-[b]
Tablet weight (mg)	350 mg	348.1 mg (3.5)	346.4 mg (3.3)
Infliximab 5 mg ColoPulse tablets (coated)			
Coat thickness (mg/cm^2)	13-17 mg/cm^2	9.5 mg/cm^2	10.5 mg/cm^2
Lag time (n = 6)	> 240 min	215.2 min (8.8)	-[c]
Pulse time (n = 6)	< 60 min	30.5 min (10.7)	-[c]
Release at $t_{360\ min}$ (n = 6)	> 80%	94.5% (11,9)	-[c]

[a.] n.a., not applicable

[b.] Not determined due to small batch size

[c.] Not possible due to absence of caffeine

2.2.3. Coating of infliximab tablets

A coating was applied on 75% of the tablets of batch A and all tablets of batch B using the ColoPulse technology as described before [15]. In this study tablets were coated in a Strea 1 fluidized bed coater (Aeromatic, USA). Coating time was 50 minutes. The coating consisted of a mixture of PEG 6000 : Eudragit S100 : Ac-di-sol : Talc in a ratio of 1:7:3:2 (w/w/w/w). The solvent used was ethanol 96%. Coating thickness was determined and expressed as the amount of Eudragit S100 applied per cm^2 using the following formula:

$$Coating\ \ thickness = \frac{weight\ coated\ tablet - weight\ uncoated\ tablet}{surface\ uncoated\ tablet}\ x\ fraction\ \ eudragit\ S100$$

The coating thickness applied was 9.7 mg/cm^2 for batch A and 10.5 mg/cm^2 for batch B.

2.3. Stability study

2.3.1. Storage conditions

The tablets were stored under different conditions, based on the ICH guidelines, climatic zone I [20]:

- Condition 1: 25°C/60% RH closed vial
- Condition 2: 25°C/60% RH open vial
- Condition 3: 40°C/75% RH closed vial

The tablets were individually packed in a closed or open glass container. When applicable vials were hermetically closed with a screw cap. The above-mentioned storage conditions were created in an exsiccator. A saturated solution of potassium bromide was used to create an atmosphere of 60% RH and the exsiccator was then placed in a cabinet of 25°C during the study period of 16 months. An RH of 75% was reached using an exsiccator with a saturated solution of ammonium chloride; the exsiccator was placed in a cabinet of 40˚C during the study period of 16 months.

2.3.2. Stability indicating profile

For large proteins like infliximab (150 kD), there is not one single analytical method that can be used to assess the different characteristics and the stability of the protein. Therefore a stability indicating profile has to be established guaranteeing that potential changes in identity, purity and potency of the protein will be detected [21]. To achieve this the use of a panel of different analytical methods is necessary. The evaluation of the combined results will give

information about the characteristics and stability of the protein. The tests performed in the stability study with infliximab tablets are shown in table 3. The tablets were analyzed at t = 0, 1, 2, 3, 4, 5 and 16 months after production according to the schedule in table 3. Tablets at t = 0 were uncoated except for the dissolution test and Circular Dichroism (CD) analysis. At all other time points the tablets were covered with the ColoPulse coating. The analytical methods used are described shortly in 2.3.3 to 2.3.9. In all tests phosphate buffered saline (PBS) pH 7.4 containing 0.45% sodium chloride, 15 mM monobasic sodium phosphate dihydrate, 53 mM dibasic sodium phosphate dodecahydrate and water for injection was used for dilutions. Solutions were filtrated as described in the individual method sections to remove insoluble parts of the coating.

Table 3: Test schedule stability study infliximab 5 mg ColoPulse tablets. Tests are marked with X and each test was performed for all storage conditions with n = 3 unless otherwise specified:
Condition 1: 25°C/60% relative humidity closed vial
Condition 2: 25°C/60% relative humidity open vial
Condition 3: 40°C/75% relative humidity closed vial

Test	Aim	t = 0 month	t = 1 month	t = 2 months	t = 3 months	t = 4 months	t = 5 months	t = 16 months
ELISA	Content	X [a]		X		X		X
SDS-PAGE	Molecular mass/degradation products	X[a]		X		X		X
Bio-assay	Biological activity				X			X
Dissolution	Dissolution profile	X[f]	X	X	X			X[b]
Circular Dichroism	Secondary and tertiary structure	X		X[c]	X	X		X[d]
UV	Aggregation	X[a]				X		X
HP-SEC	Aggregation				X[e]		X[e]	X

[a] uncoated
[b] only for storage condition 1 and 3
[c] only for storage condition 1
[d] n = 2 for storage condition 3 Far UV
[e] n = 1 for each storage condition
[f] n = 6

2.3.3. ELISA

An ELISA was used to gain more insight in content changes of the amount of infliximab able to bind TNF-alpha in the ColoPulse tablets. Tablets of batch A were dissolved in 5.0 ml PBS to yield a concentration of 1.0 mg/ml (n = 3 for each storage condition) followed by filtration through a 0.22 μm Millex® GP filter. As control a fresh infliximab solution was prepared using the same method. Solutions were further diluted with albumin 200 mg/ml (Sanquin, the Netherlands) to yield a final infliximab concentration of 5 μg/ml in polypropylene vials. Controls of Remicade® with final concentrations of 1.0, 2.5, 5.0 and 10.0 μg/ml (n = 5) in PBS, prepared as described above, were analyzed to validate the method of sample preparation and were included in every run. Accuracy and the coefficient of variation were calculated. The ELISA was performed by Sanquin, the Netherlands according to a protocol described by Vande Casteele et al. [22].

2.3.4. SDS-PAGE

The molecular mass of the different subunits and possible soluble aggregates or smaller sized degradation products of infliximab in the tablets were monitored using SDS-PAGE. SDS PAGE separates proteins according to their molecular weights. Possible (soluble) aggregates present in the tablet can thus be detected as well as smaller size degradation products. Coated tablets of batch A were dissolved in 10.0 ml PBS (n = 3 for each storage condition) followed by filtration through a 0.22 μm Millex® GP filter. This procedure was also applied to prepare a fresh infliximab solution from Remicade®. SDS-PAGE was performed by using a vertical Bio-Rad Mini-Protean 3 gel electrophoresis system (Bio-Rad, USA) under reducing (dithiothreithiol, Invitrogen, The Netherlands) and non-reducing conditions according to the manufacturer's instructions using a 10% gel. The gel was loaded with appropriate volumes containing 10 μg infliximab and 7.5 μl of a control (Precision Plus Protein Standard all blue, Bio-Rad, USA). After running the gel was colored with Coomassie Brilliant Blue staining solution (Bio-Rad, USA) according to the manufacturer's instructions.

2.3.5. Bioassay

A bioassay was used to determine the TNF-alpha neutralizing activity of infliximab. Coated tablets of batch A were dissolved in 5.0 ml PBS to yield a concentration of 1.0 mg/ml followed by filtration through a 0.22 μm Millex® GP filter (n = 3). This procedure was also applied to prepare a fresh infliximab solution from Remicade®. PBS was used as negative control. The bioactivity of

infliximab tablets was determined with a TNF-alpha sensor assay using HeLa 8D8 cells that express Green Fluorescence Protein (GFP) under control of an NF-κβ response element. GFP expression was quantified by flow cytometry [23]. In this bioassay TNF-alpha stimulates the expression of GFP and expression of GFP is inhibited by infliximab by neutralisation of TNF-alpha. The GFP expression of cells incubated with PBS was set at 100% (TNF-alpha inhibition 0%) and the GFP expression of cells incubated with infliximab solution was set at 0% (TNF-alpha inhibition 100%).

2.3.6. CD Spectroscopy

The secondary and tertiary structure of infliximab was analyzed by far-UV and near-UV CD, respectively [24]. Coated tablets of batch B (without caffeine) were dissolved in 5.0 ml PBS to a concentration of 1.0 mg/ml followed by filtration through a 0.22 µm Millex® GP filter (n = 3 for each storage condition). This solution was used to record the near-UV spectrum. 100 µl of the solution was further diluted with PBS to a concentration of 0.025 mg/ml for recording of the far-UV spectrum. The same procedure was carried out to prepare a fresh infliximab solution in PBS.

The samples were placed in a 10 mm quartz cuvet and the UV-spectra were recorded using a Jasco-J-850 CD spectrometer (Jasco, Italy). The UV spectra were collected in a continuous scanning method for the range 205-250 nm for the far-UV and 250-350 nm for the near-UV at a scanning speed of 50 nm/min, a response time of 2 sec, a bandwidth of 2 nm and an accumulation of 5 scans. The spectra were background corrected for the solvent, smoothed with Savitzky-Golay using a convolution width of 9 and converted to specific ellipticity.

2.3.7. UV Spectroscopy

To gain insight in the degree of insoluble aggregates of infliximab in the tablets, the aggregation index was determined using UV spectroscopy. Coated tablets of batch B were dissolved in 5 ml PBS to yield a concentration of 1.0 mg/ml followed by filtration through a 0.22 µm Millex® GP filter (n = 3 for each storage condition) and were measured on the same day (t = 0, 4 and 16 months) or the next day (t = 1 month). A freshly prepared infliximab solution of 1.0 mg/ml in PBS made from Remicade® was used as a control.

UV spectroscopy was performed with a Unicam UV 500 spectrophotometer (Thermo Spectronic, United Kingdom). Samples were measured in a quartz cuvet with path length of 10 mm and UV absorbance was measured at 280 and 340 nm. The absorbance was not corrected for the absorbance of Eudragit S100 at these wavelengths, because this test was intended to follow the development

of soluble aggregates over time and to compare results between the different storage conditions.

The aggregation index (AI) was calculated from the UV spectra using the following formula, which was described in the literature [25]:

$$AI = \frac{Absorbance_{340\ nm}}{(Absorbance_{280\ nm} - Absorbance_{340\ nm})} * 100$$

2.3.8. High Performance Size Exclusion Chromatography

High Performance Size Exclusion Chromatography (HP-SEC) was used to determine the degree of soluble aggregates of infliximab in the tablets [26]. HP-SEC was performed on a LaChrom Elite HPLC system with UV detection (Hitachi, US). A Superdex 200 10/300 GL column was used in combination with an eluent containing 0.02M sodium dihydrogen phosphate dihydrate and 0.02M sodium hydrogen phosphate dihydrate in sodium chloride 0.9%, pH 7.0. Coated tablets of batch B were dissolved in 5 ml PBS to yield a concentration of 1.0 mg/ml followed by filtration through a 0.22 µm Millex® GP filter (n = 3 for each storage condition). An infliximab solution of 1.0 mg/ml in PBS made from Remicade® was used as a control. 20 µl were injected onto the column and separation was performed at a flow rate of 0.7 ml/min. The absorption was measured at 220 and 280 nm.

2.3.9. Dissolution test

The pulsatile release properties of a ColoPulse tablet are reflected by the lag time and the pulse time. Caffeine was used as a marker substance to confirm that ColoPulse infliximab tablets met the specified release specifications and, for example, did not release active substance at a pH < 7.0. The lag time is the time point at which the tablets start to release the active substance and was defined as the time at which 5% of caffeine was released. The pulse time reflects the pulsatile release characteristics and was defined as the period between the lag time and the time at which 70% of caffeine was released. These parameters were established in a dissolution test with a total duration of 360 minutes during which the pH was varied in time to simulate the different stages of the gastrointestinal tract as described before. This dissolution test known as Gastro-Intestinal Simulation System (GISS) was described before by Schellekens et al. [14].

The dissolution profile of the coated tablets of batch A (n = 3 for each storage condition, n = 6 for t = 0) was determined using the GISS. The test was

performed as described previously [14]. In short, the different parts and residence times of the gastrointestinal tract were simulated by varying the pH, the volume and the osmolality of the dissolution medium in four different phases. A summary of the specifications of this dissolution test is shown in table 4.

The release of caffeine from the tablets was measured with UV spectroscopy at a wavelength of 273 nm. At the end of the dissolution test a visual check on disintegration of the tablet was performed.

Table 4: Specifications of the dissolution test (GISS).

Phase	Gastrointestinal Segment	Volume (ml)	Residence time (h)	pH	Osmolality (mosmol/kg)
I	Stomach	500	2.0	1.2 ± 0.10	150 ± 25
II	Jejunum	629	2.0	6.8 ± 0.20	250 ± 50
III	Ileum (distal)	940	0.5	7.5 ± 0.25	250 ± 50
IV	Colon (proximal)	1000	1.5	6.0 ± 0.25	250 ± 60

2.4. Statistical procedures

Where applicable the results were evaluated by descriptive statistics with SPSS version 22. The center was characterized by the mean and the dispersion by the standard deviation (SD), CV and range. A paired samples t-test and an independent samples t-test (two tailed, $\alpha = 0.05$) were used to compare the results between different time-points and different storage conditions, respectively. Results were considered significant when $p < 0.05$.

3. Results

Infliximab 5 mg ColoPulse tablets met all current quality control criteria at t = 0 as shown in table 2, except for coating thickness and the related lag time. Despite the coating thickness being lower than expected (which affects mainly the lag time), the stability study was performed, because a reduced thickness of this type of coating is unlikely to influence protein stability in the tablet core. The results of all different tests, together describing the characteristics and stability indicating profile, are described below. A summary of the results at t = 16 months can be found in table 5. The appearance of the tablets showed no difference over time or between different storage conditions.

Table 5: Summary of results at t = 16 months presented as mean (standard deviation). N of each test can be found in table 3.

Test	Aim	Requirement	Result condition 1	Result condition 2	Result condition 3
ELISA	Content	≥ 4.5 mg	4.8 mg (0.64)	3.0 mg (0.26)	1.2 mg (0.21)
SDS-PAGE	Molecular mass/ degradation products	No difference compared to freshly prepared infliximab solution	No difference	No difference	No difference
Bio-assay	Biological activity	> 80%	83.4% (17.0)	72.9% (12.6)	12.2% (7.9)
Dissolution	Dissolution profile	Lag time > 240 min Pulse time < 60 min Release at $t_{360\ min}$ > 80%	246 min (8.3) 10.0 min (5.0) 102% (5.0)	Not available	255 min (4.0) 9.3 min (3.5) 107% (3.5)
Circular Dichroism	Secondary and tertiary structure	No difference compared to freshly prepared infliximab solution	No difference	Differences observed	Differences observed
UV	Aggregation	Not applicable	17.5 (4.7)	25.0 (9.4)	46.6 (11.2)
HP-SEC	Aggregation	No aggrega-tion/degradation	No aggrega-tion/degradation	Aggregation	Aggregation + degradation

3.1. ELISA

The suitability of the sample preparation for ELISA was tested with controls made from Remicade® with final infliximab concentrations of 1.0, 2.5, 5.0 and 10.0 µg/ml. The mean accuracy of the method was -0.6% (range -6.1 - 6.0%) and the mean CV was 18.3% (range 16.1 - 22.0%) depending on the concentration. The sample preparation method was judged suitable for determination of the content of infliximab 5 mg ColoPulse tablets.

The amount of infliximab able to bind to TNF-alpha of 5 mg infliximab tablets stored under different storage conditions at t = 0, 2, 4 and 16 months is shown in figure 1. At t = 0 the mean amount was 5.5 mg (n = 3, SD 0.42). After 2 and 4 months no difference was found between the different storage conditions and all measurements, except for condition 2 at t = 4 months (5.8 mg), were within the specifications of 4.5 - 5.5 mg.

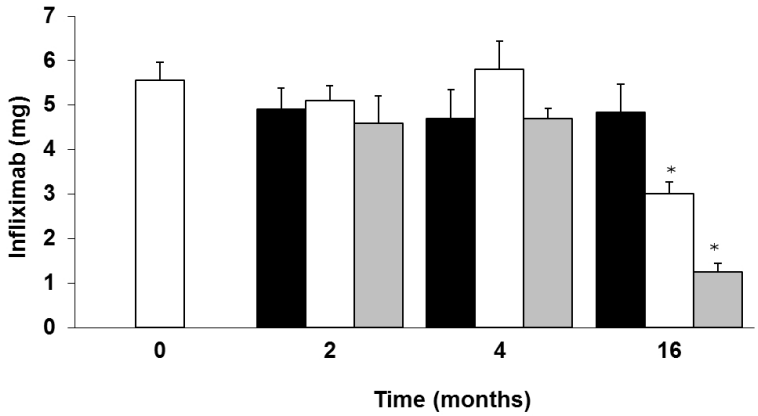


Figure 1: Mean amount of infliximab able to bind to TNF-alpha and standard deviation of infliximab 5 mg ColoPulse tablets (n = 3) at different storage conditions. Analysis at t = 0 was performed with uncoated tablets.
* indicates a statistically significant difference ($p < 0.05$) compared to t = 0

condition 1: 25°C/60% RH closed vial
condition 2: 25°C/60% RH open vial
condition 3: 40°C/75% RH closed vial

The amount of infliximab able to bind to TNF-alpha at t = 16 months under storage conditions 2 and 3 decreased to a mean value of 3.0 mg and 1.2 mg, respectively. The mean amount at t = 16 months under condition 1 closed vial was 4.8 mg (n = 3, SD 0.66, 87.3% of T = 0). The results did not differ significantly between t = 0 and t = 16 months for tablets stored at this condition ($p = 0.187$, paired samples t-test).

3.2. SDS-PAGE

In figure 2 the result of a representative gel (1 out of 3) at t = 16 months is shown. Under non-reducing conditions two fragments with a molecular weight of approximately 100 and 25 kD were found. Also two lightly colored bands corresponding to protein fragments of about 55 and 50 kD were found. Under reducing conditions two fragments were found of approximately 50 and 25 kD. Also a lightly colored band corresponding to a protein fragment of about 55 kD was found. There was no difference between the position of fragments at the different time points (t = 0, 2, 4 and 16 months) and the position of the fragments was identical to that of the fragments obtained using an infliximab control solution.

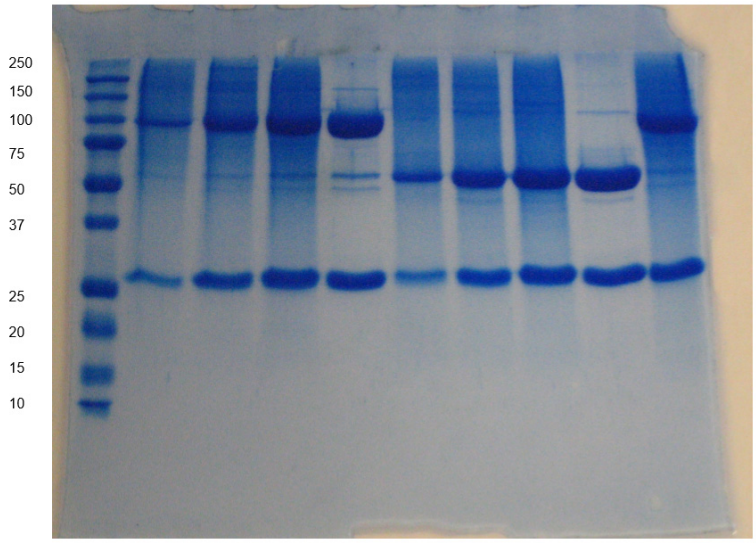


Figure 2: SDS-PAGE at t = 16 months. Lanes from left to right: ladder/non-reducing conditions (tablets storage condition 1/2/3/infliximab solution) / reducing conditions (tablets storage condition 1/2/3/infliximab solution) / extra: non-reducing tablet condition 3

3.3. Bioassay

In figure 3 a summary of the bioassay results is presented. At t = 16 months storage under conditions 1 - 3 shows a mean of 83.5, 72.9 and 12.2% TNF-alpha inhibition, respectively, compared to a fresh infliximab control solution. At t = 3 months this was 115.5, 96.3 and 95.2%, respectively. There was a significant difference in TNF-alpha inhibition between the freshly prepared infliximab solution and infliximab incorporated in tablets stored under condition 1 and 3 when results at t = 3 months and 16 months were compared ($p = 0.045$ and $p = 0.001$, respectively, paired samples t-test).

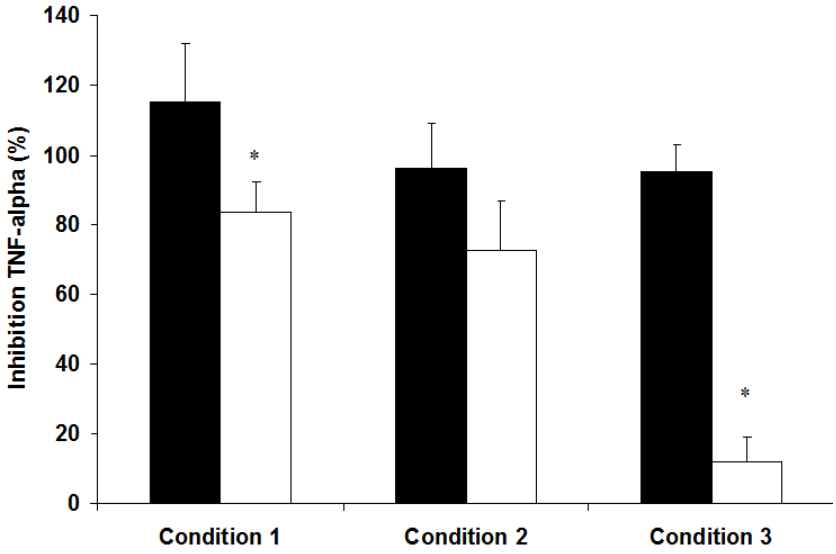
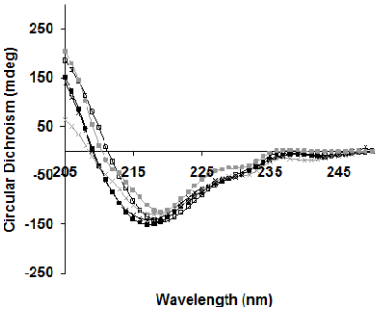


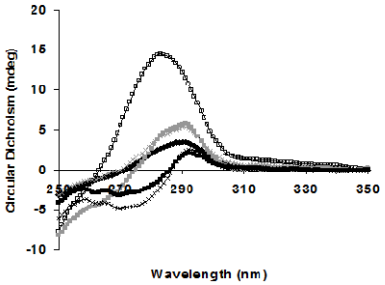
Figure 3: Bioassay: Mean TNF-alpha inhibition after 3 months (■) and 16 months (□) storage at different conditions (n = 3). TNF-alpha inhibition of a freshly prepared 1 mg/ml infliximab solution was set at 100%. * indicates a statistically significant difference

3.4. CD spectroscopy

The far-UV and near-UV CD spectra are shown in figure 5 for all storage conditions and all time points. The shape of the far-UV CD spectra, representing the secondary structure of a protein, shows little change over time during storage under conditions 1 and 2. During storage under condition 3, however, substantial changes are visible. The shape of the near- UV CD spectra, representing the tertiary structure of a protein, shows little difference between t = 2 (condition 1 only), 3 and 4 months for all storage conditions. However, for t = 16 months differences were observed, mostly for storage condition 2 and 3.

(A)


Circular Dichroism (mdeg)
250
150
50
-50
-150
-250
205
215
225
235
245
Wavelength (nm)


Circular Dichroism (mdeg)
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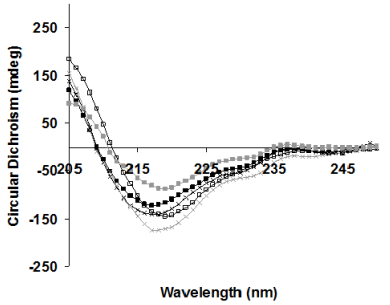
15
10
5
0
-5
-10
290
310
330
350
Wavelength (nm)

(B)


Circular Dichroism (mdeg)
250
150
50
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-250
205
215
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235
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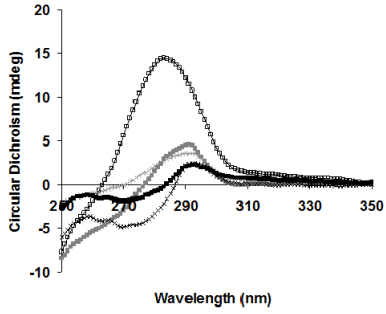
Wavelength (nm)


Circular Dichroism (mdeg)
20
15
10
5
0
-5
-10
270
290
310
330
350
Wavelength (nm)

(C)

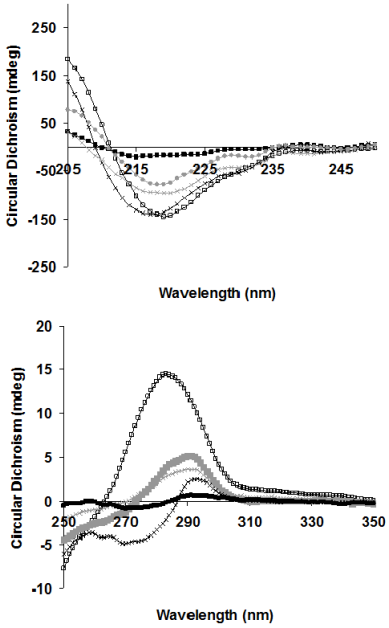


Figure 5: Far and near UV CD spectra of infliximab 5 mg tablets (mean of n = 3) at different storage conditions and time points

□ t = 0 (uncoated) / ♦ t = 2 months (only condition 1) / X t = 3 months / ■ t = 4 months 4 / ■ t = 16 months / X Infliximab standard (spectra from analysis t = 16 months)

5A: condition 1: 25°C/60% RH closed vial

5B: condition 2: 25°C/60% RH open vial

5C: condition 3: 40°C/75% RH closed vial

3.5. UV Spectroscopy

Using UV spectroscopy the aggregation index of infliximab in the tablets was calculated from the absorption at 280 and 340 nm. The results of these measurements are presented in figure 6. At t = 16 months the aggregation index increased to a mean value of 17.5, 25.0 and 46.7 for storage conditions 1, 2 and 3, respectively. For all conditions the aggregation index increased during storage. At both time points the aggregation index of condition 1 was lower than that of condition 2 and condition 2 showed a lower aggregation index than condition 3.

This could also be observed when condition 2 and 3 were compared.

Over time absorption at 280 nm decreased and absorption at 340 nm increased (data not shown). This confirms that the measured aggregates could be attributed to infliximab and not to other solid substances like inulin which is not likely to degrade.

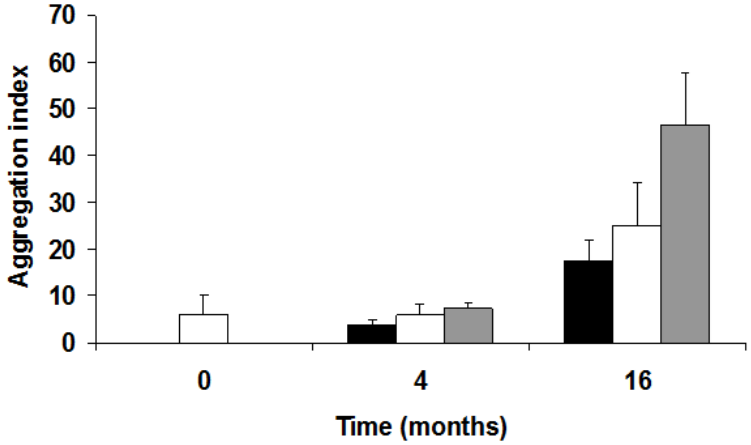


Figure 6: Mean aggregation index and standard deviation of infliximab 5 mg tablets (n = 3) at different storage conditions.
■ condition 1: 25°C/60% RH closed vial
□ condition 2: 25°C/60% RH open vial (t = 0: core)
■ condition 3: 40°C/75% RH closed vial

3.6. High Performance Size Exclusion

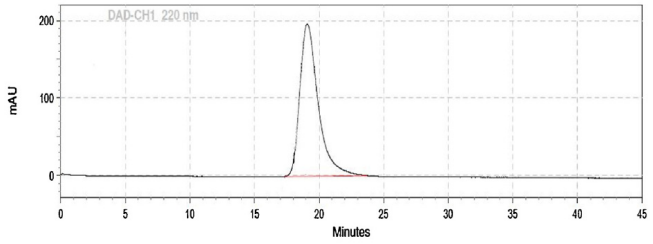
Additional information about soluble aggregates of infliximab was obtained using HP-SEC at t = 3, 5 and 16 months of storage. At t = 3 and 5 months the chromatograms of the samples stored under condition 1, 2 and 3 were comparable to each other (data not shown). In figure 7 representative results of t = 16 months are shown for all storage conditions. The infliximab peak has a retention time of approximately 19 minutes.

For condition 1 some small extra peaks could be observed at all time points when results were compared with a freshly prepared infliximab solution as measured at 220 nm and at 280 nm. Further experiments (data not shown) pointed out that these peaks could be attributed to the Eudragit of the ColoPulse coating. Eudragit showed at 220 nm a small peak between 11 and 14 minutes. At 280 nm absorption of Eudragit could be seen at approximately 11 and 25 minutes.

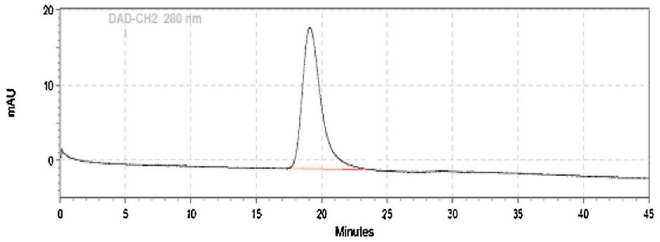
Tablets stored at condition 2, t = 16 months showed several extra peaks (compared to condition 1) before the infliximab peak at 280 nm. These could possibly be attributed to the formation of soluble aggregates.

The chromatogram of the samples stored under condition 3 for 16 months showed several differences when compared with the freshly prepared infliximab solution. The large peaks before and after the protein peak, indicate substantial aggregation and degradation, respectively.

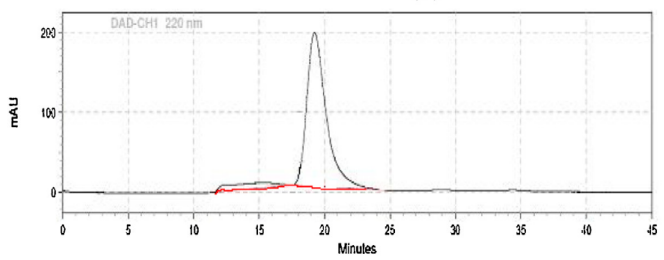
(A) **220 nm**



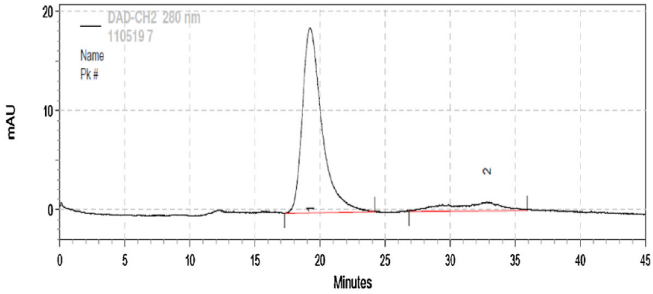
280 nm



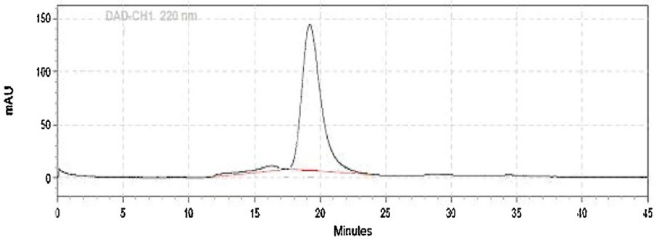
(B) **220 nm**



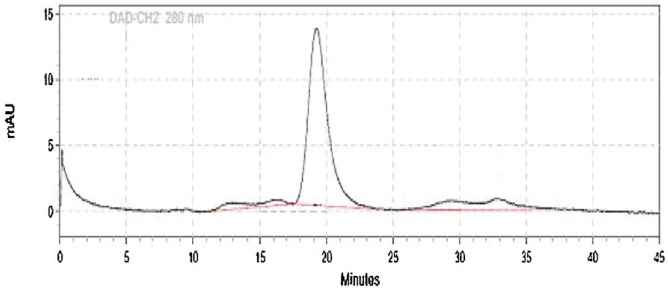
280 nm



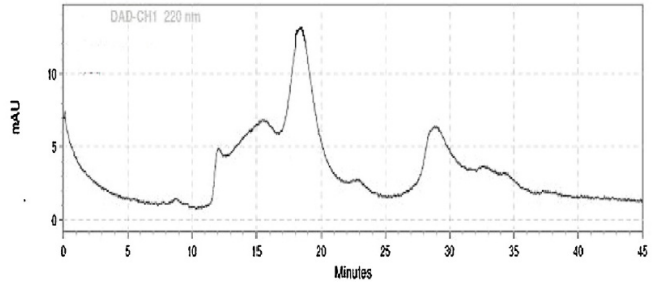
(C) **220 nm**



280 nm



(D) **220 nm**



280 nm

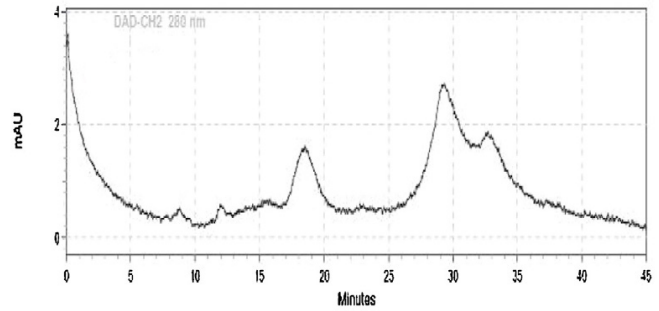


Figure 7: HP-SEC representative chromatograms at t = 16 months for an infliximab solution (A) and for all storage 1 and 3 of infliximab 5 mg tablets (B-D). Measurements were performed at a wavelength of 220 en 280 nm.
7A: infliximab solution
7B: condition 1: 25°C/60% RH closed vial
7C: condition 2: 25°C/60% RH open vial
7D: condition 3: 40°C/75% RH closed vial

3.7. Dissolution

The dissolution profile after production of the ColoPulse tablets and at t = 16 months is shown in figure 4. All tablets met the criteria for release and pulse time at all time points. The mean lag time at t = 0 was 215 min (range 201-224 min) which did not meet the specification of > 240 min. This was also observed at time points t = 2 and 3 months (range 215-263 min). At t = 16 months mean lag time (both storage conditions combined) was 250.7 min (range 237-259 min). All tablets had disintegrated at t = 360 minutes as visually determined.

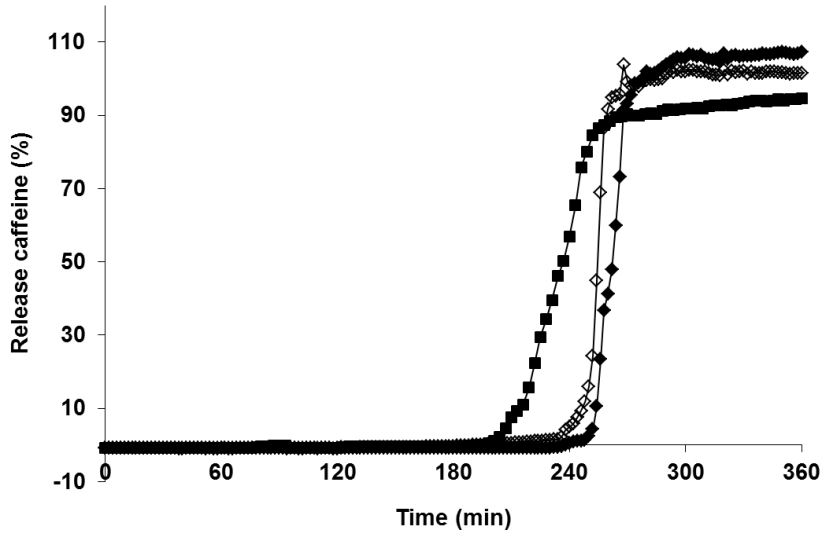


Figure 4: Dissolution profiles of ColoPulse infliximab tablets at t = 0 (■) and t = 16 months (♦, condition 1 and ◊ condition 3). The graph shows the mean of n = 6 (t = 0) and n = 3 (t = 16 months) tablets.

4. Discussion

To the best of our knowledge, this is the first study in which the feasibility to formulate an oral infliximab tablet has been explored. Infliximab 5 mg tablets stored under ICH condition I showed no relevant loss of content, potency and change in structure up to 4 months after production. At t = 16 months major differences were observed for storage under accelerated (extreme) conditions (40°C/75% RH). However, after 16 months infliximab from a ColoPulse tablet stored under normal conditions (25°C/60% RH, closed vial) still displayed a mean biological activity of 83% compared to a fresh infliximab solution.

4.1. Selection of infliximab dosage

In the literature no information is available yet about the optimal dosage of orally administered infliximab for topical therapy in patients with Crohn's disease in the ileocolonic region. In most publications the dosage described for local injections varies between 10 – 40 mg [5-7]. This corresponds on average with 2.5 – 10.0% of the intravenous dosage for a patient of 80 kg, however, the frequency of local administration of infliximab every 2 – 6 weeks [5–7] is higher than intravenous administration which is given once every eight weeks.

The colon is a suitable absorption site for protein and peptide drugs due to the absence of digestive enzymes and a relatively low proteolytic activity besides providing a longer residence time than in the small intestine. However, intestinal transport and diffusion of protein drugs is difficult to predict [10]. But the fact that intravenously administered infliximab exhibits its effects in the lamina propria (part of the mucosa) [27] suggests that infliximab is able to migrate across the intestinal epithelium.

Our hypothesis is that a daily (low) oral dose of infliximab will be as effective as intravenously administered infliximab 5 mg/kg every 8 weeks, because of the continuous exposure of the intestinal mucosa to infliximab. Furthermore the clinical effect of infliximab is also related to trough levels [28] and with our proposed design continuous low (local) trough levels can be reached during therapy. Based on the assumption that local bioavailability will be around 75% due to enzymatic degradation, degradation by the colon flora and loss with feces, a daily dosage range of 10 – 20 mg infliximab was chosen for our planned study. Considering this, tablets with 5 mg infliximab were selected because of being a suitable amount per dosage unit, also in view of envisaged need of dosing flexibility.

4.2. Tablet formulation and quality control

Infliximab tablets were compounded using the commercial product Remicade®. The formulation of Remicade® is mainly based on sucrose and the appearance of the freeze dried powder can best be described as a porous cake. This formulation as such is not suitable for the production of tablets due to the poor flowability of the powder. Furthermore the protein should be further stabilized to make long-term storage of the tablets possible, preferable at room temperature.

Flowability was improved using dry granulation of Remicade® followed by sieving. To stabilize a protein and to protect it from degradation during tablet production and storage it could be formulated into a sugar glass. In a sugar glass the mobility of molecules is strongly reduced when the environmental temperature is considerably below the glass transition temperature (T_g), which enhances stability of the protein [29]. Therefore a sugar glass with a high T_g is preferable for production of ColoPulse infliximab tablets. Sucrose as present in Remicade® has a relatively low glass transition temperature (T_g) of 60ºC [30] when anhydrous and, since water acts as a plasticizer, this will be much lower when it contains some residual or absorbed moisture. This makes a formulation with sucrose considerably less suitable for formulation of infliximab tablets. A low T_g may lead to problems during tablet production because the RH of the environment should be low which may lead to static charging of the powder

resulting in poor flowability. In addition, a low T_g may result in compaction induced crystallization [31] which reverses the protective effect of the sugar glass. Several publications describe the oligosaccharide inulin as promising to stabilize proteins due to its high glass transition temperature amongst other physicochemical characteristics [30,32]. For this reason infliximab was incorporated in inulin resulting in a sugar glass with a T_g of approximately 130ºC.

The quality control results of the tablet cores of batch A (formulation with caffeine) met all criteria as described before. Due to the explorative character of this study not enough material was available to perform a comparable control of batch B (formulation without caffeine). This formulation was used for various UV-based analytical procedures (UV, CD, HP-SEC) as caffeine, which shows UV absorption, would have disturbed these measurements. The aim of these tests was mainly to detect and follow protein aggregation during storage. Based on the assumption that the tablet mixture was homogeneous and the fact that the theoretical weight complied with the criteria, batch B was considered suitable for the mentioned aim.

4.3. Influence of relative humidity and temperature

There are only few publications available about the short term stability of infliximab after reconstitution. No loss of biological activity occurred during a study period of two weeks at 4ºC for a 400 mg/L solution in sodium chloride 0.9% [33]. In another study the immunoreactivity of infliximab at two different concentrations (50 and 0.069 µg/L) in 0.9% sodium chloride remained stable over the six-week study period at 4ºC [34]. Data on long term storage or storage at room temperature are not available at all, but it is obvious that at higher temperatures infliximab will be less stable [35].

In our study tablets were stored for 16 months at 25ºC, which is a convenient and normal storage condition when products are used in patient care. The temperature of 40ºC was chosen as a challenging storage condition, but this condition does not reflect daily practice. The results obtained under this condition could be used to gain more insight in stability when tablets are for a short period stored under stress conditions, for example during transport.

The results of the different tests show that incorporation into an inulin sugar glass matrix prevents infliximab from degradation up to four months of storage under all storage conditions, which is already relatively long. At the next time point t = 16 months, samples showed clear degradation of the antibody when stored at 40ºC/75% RH but less for storage at 25ºC/60% RH in a closed vial. A possible explanation for the degradation despite the high T_g of 130°C is that the

T_g was lowered by the relatively high RH at 40°C resulting in crystallization of inulin and subsequent degradation of infliximab. The T_g and appearance of the tablet core should be closely monitored in the further development of this tablet.

Comparing the test results of condition 1 and 2 (closed and open vial, respectively) an improved stability was found when the tablets were stored in a closed vial. This emphasizes that the packaging and instructions for storage are extremely important for protein formulations and should also be considered carefully in the further development of this tablet formulation.

4.4. Summary of stability indicating profile

A panel of different methods of analysis was used to study the stability of 5 mg infliximab ColoPulse tablets during production and long-term storage. Combining the results of the different analysis for each storage condition leads to the conclusion that tablets stored under condition 1 showed the least differences between the time points, while tablets stored under condition 3 showed the most. This was expected because storage at high temperature and high humidity is a very challenging condition for an antibody, even when stabilized in a sugar glass. The observed pattern could be found for each test.

5. Conclusion

Based on the presented results it can be concluded that formulation of 5 mg ColoPulse infliximab tablets is technically feasible. Incorporation of infliximab in a sugar glass matrix based on inulin resulted in a tablet that was stable for up to four months when stored at temperatures varying from 25 - 40 °C. 16 months after production tablets stored at 25°C in a closed vial still displayed a mean biological activity of 83% compared to a fresh infliximab solution. To obtain a stable oral formulation of infliximab suitable for use in clinical studies, packaging and refrigerated storage will be investigated in a follow-up study. However, the currently obtained results are a first step in the further development of a safe, effective and more patient friendly dosage form of infliximab for the treatment of patients with Crohn's disease.

Acknowledgements

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CHAPTER 4

Population pharmacokinetics of infliximab in patients with inflammatory bowel disease: potential implications for dosing in clincial practice

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Abstract

Background

Infliximab (IFX) is effective in the treatment of inflammatory bowel diseases (IBD). Currently IFX is administered at fixed doses and intervals, however costs are high and optimization is necessary. Several publications indicate that IFX should be dosed on trough levels ≥ 3.0mg/L. For optimizing IFX dosing, the use of a pharmacokinetic model is important. Population pharmacokinetics of IFX have been described earlier, however these models were not used for dose optimizing.

Aim

To develop a pharmacokinetic model for IFX in IBD patients that can be used for dose-optimization of IFX and to predict serum trough levels in this population.

Methods

An observational retrospective study was performed in 42 IFX treated IBD patients. Serum samples were drawn before infusion at T = 0, 2, 6, 14, 22 and 54 weeks and analysed for IFX and antibodies against IFX (ATI). Relevant covariates were recorded and a population pharmacokinetic model was developed.

Results

Individual plots created using the final model showed good correspondence between observed and model predicted values. Serum levels were influenced by ATI, disease activity, sex and albumin. Our results show that in patients without ATI target trough levels ≥ 3.0mg/L can be achieved by increasing dosing intervals from 8 to 12 weeks combined with a dose-increase. This results in a reduction of 33% in concomitant costs.

Conclusion

In IBD patients without ATI, trough level dosing based on longer intervals can reduce IFX-therapy related visits to the hospital with one third. Trough level based dose intensification should always be justified by disease activity parameters.

1. Introduction

Infliximab (IFX), a chimeric mouse-human monoclonal antibody against tumor necrosis factor alpha (anti–TNF–α) has proven to be effective in the treatment of inflammatory bowel diseases (IBD) i.e. Crohn's disease (CD) and ulcerative colitis (UC) not responding to conventional therapy. IFX is effective in inducing and maintaining remission of luminal and fistulizing CD and UC [1–4]. Anti-TNF-α agents are administered at fixed doses and fixed intervals derived from dose finding studies [4,5]. Measuring IFX and antidrug antibodies against IFX (ATI) levels is not common practice and until recently dose-intensification was only based on clinical evaluation of the disease which appeared to be often inaccurate [6-8].

In the literature several studies demonstrated a relationship between IFX drug concentrations, the presence of ATI and clinical outcome [9]. Episodic IFX treatment in patients with CD has been associated with a higher rate of ATI as compared with scheduled maintenance therapy [10]. Patients on IFX therapy who develop ATI have a threefold higher increased risk of loss of response to therapy compared to those who do not develop ATI's [11].

Several publications indicate that low IFX trough levels (variating from 2 – 3.5 mg/L) may increase the risk of flare of disease symptoms and inflammation. Low trough levels are associated with (clinical) parameters like high C-reactive protein (CRP) levels, male sex and higher albumin levels [12-16]. IFX levels ≥ 3 mg/L at the start of a maintenance regime appeared to be predictive for sustained response to IFX [9,17]. It has also been demonstrated that the achievement of endoscopic healing requires even higher IFX trough levels [18].

Therefore therapeutic drug monitoring (TDM) based on IFX trough levels and antidrug antibody level measurements has the potential to play an important role in the optimization of anti-TNF-α treatment [6,19]. Currently several studies designed to dose IFX based on trough levels are ongoing. To be able to predict the IFX serum levels and optimize the IFX dose based on serum level the use of a pharmacokinetic model is of great importance. Furthermore the costs in IBD therapy are mainly driven by anti-TNF- α therapy and a strategy to optimize IFX therapy and avoid unnecessary treatment is desirable [20].

The population pharmacokinetics (popPK) of IFX have been described earlier for patients with ankylosing spondylitis, rheumatoid arthritis and IBD [21–23]. However these models were not used to predict serum trough levels or for dose optimizing of IFX. In this paper we describe a retrospective study that was performed to develop a pharmacokinetic (PK) model for IFX in IBD patients in

an out-patient setting that can be used for dose-optimization of IFX and to predict serum trough levels in this population.

2. Materials and Methods

2.1. Study design

This study was an observational retrospective single center study of IFX treated CD and UC patients in the years 2007-2012 conducted at the Gastroenterology department of the University Medical Center Groningen. The study population comprised patients who were treated with IFX infusion at week 0, 2, 6 as induction phase followed by a maintenance phase for at least 40 weeks in a dosage of 5 mg/kg every eight weeks. Serum samples analyzed for IFX trough levels were routinely collected before infusions at weeks 0, 2, 6, 14, 22 and 54. Samples at t = 54 were also analyzed for ATI. Patient files were reviewed by an investigator and possible relevant covariates were recorded: clinical (Harvey Bradshaw index (HBI) [24], Global physician scale (GPA), Mayo score, Montreal classification, extension of disease, disease duration, concomitant immunosuppressive drugs, prior IFX use, smoking, UC/CD, weight) and laboratory parameters (CRP, albumin, leukocytes). Data from the HBI were treated as continuous data. Of the initially identified possible covariates, only covariates for which values were available for at least half of the patients (21) were included in the statistical analysis. The results were divided in two periods: induction (week 0 – 6) and maintenance (week 14, 22 and 54).

2.2. Analysis of infliximab and ATI levels

In order to measure IFX trough and ATI levels the samples were sent to the laboratory for monoclonal therapeutics, Sanquin Diagnostics, Amsterdam, the Netherlands.

IFX trough levels were measured using an in house developed Enzyme-linked immunosorbent assay (ELISA). The lower limit of quantification was 0.002 mg/L. ATI levels were measured using a radioimmunoassay. Both methods are extensively described previously [25-27].

2.3. Model development

A popPK model was developed incorporating the full dosing history and concentration measurements of all patients. A two-compartment model, also used in literature, was used as starting model [21–23,28]. Due to the small size of the study cohort, it was not attempted to re-evaluate the model structure for the PK model, but model parameters were re-estimated. After initial model fitting, visual inspections of concentration-time plots showed several potential

outlying data points. Therefore, using conditional weighted residuals (CWRES) from the base model, data points with CWRES > 3.5 (corresponding to values outside the 99.95% confidence interval (CI) for normally distributed data), were labeled as outlier and removed from the dataset.

A stepwise covariate modeling (scm) procedure was implemented on the base model for the parameters *clearance (CL)* and *central volume of distribution (V_c)*. It was not attempted to estimate covariates on parameters describing peripheral distribution (*peripheral volume of distribution (V_p) and inter-compartimental clearance* (*Q)*) as these could only be estimated with moderate precision. In the first "forward" inclusion step of the scm, covariates were added to the base model in a stepwise fashion based on statistical significance ($p < 0.05$). In a second "backward elimination" step, covariates were then removed from the final model obtained in the first step, if removal of the covariate did not result in a significantly ($p < 0.01$) worse fit. For both continuous and binary covariates, only linear models were considered. In the scm, continuous covariates were centered respective to the median value. For missing time-invariant covariates, the median covariate value was imputed, while for missing time-varying covariates the last known value was carried forward (LOCF), if any earlier observation was available for the individual. An effect of a covariate of less than 25% was deemed clinically irrelevant. Therefore, after the scm procedure, only covariates with a relative absolute effect size of > 0.25 were retained in the full model. For continuous covariates the relative effect was defined as the estimated coefficient multiplied by 2 standard deviations of the covariate values in the patient population. A plot was implemented showing the effect of the statistically significant covariates on PK parameters, as well as the uncertainty around the estimate ("clinical relevance plot").

Final model evaluation was performed using a visual predictive check (VPC). Relative standard errors for the parameter estimates were obtained from the covariance step in NONMEM. For the final full model, a bootstrap analysis using 1000 samples was implemented to obtain non-parametric estimates of uncertainty in parameter estimates (95% CI).

2.4. Simulation

Monte Carlo simulations were implemented to study expected drug concentration profiles for a clinical patient population. Simulation results were stratified by covariates that were identified as significant / relevant in the covariate analysis. Simulations were performed for three dose levels (300, 400, 600 mg), for three dosing schedules in the maintenance phase (dosing every 8, 12, or 16 weeks) and using an initial loading phase (dosing at 0, 2, 6 weeks). Patients were assumed to be on steady state after 3 doses in the maintenance

phase. Simulation of the Harvey Bradshaw index in patients was done using a parametric distribution. The current data best supported a log-normal distribution, with mean 1.96 and standard deviation 0.53 (both on log-scale).

2.5. Software

Models were implemented in NONMEM (version 7.2, ICON Development Solutions), with Pirana, PsN and R/Xpose as modeling environment [29]. The first order conditional estimation method with random effects interaction was used throughout the analysis. Data handling, generation of plots, and simulations were performed using R (version 3.0.0 or higher, http://cran.r-project.org/).

2.6. Endpoints

The primary objective of this retrospective study was to develop a pharmacokinetic model for IFX in IBD patients that can be used to optimize IFX dosing in an out-patient setting.

3. Results

Data from 42 individuals were available. All patients were still considered as responders to IFX-therapy at T = 54 weeks and were in clinical remission. For most patients, six samples were available for analysis (t = 0 included), which resulted in a total of 188 IFX serum levels available for analysis. None of the IFX measurements were below the lower limit of quantification for the IFX assay. After initial model fitting, five measurements were identified as outliers and were removed from the dataset. A summary of patient demographics and covariate values is shown in table 1. Median CRP levels at baseline and at week 54 were 5 mg/L (range 5 – 105) and 5 mg/L (range 5 – 38), $p = 0.138$, respectively. The median GPA score at baseline was 2 (range 1 – 3) and 0 (range 0 – 2) at week 54. The Harvey Bradshaw index declined from median of 6 (range 3 – 24) at baseline to median of 2.5 (range 0 – 9) at week 54, $p < 0.001$. There were no significant differences between HBI, CRP, and albumin between males and females and between smokers and non-smokers.

Table 1: Patient demographics and covariates at baseline.

Time-invariant	Median (range)/numbers in cat.	Missing	Time-varying*	Median (Range)	Missing
Weight (kg)	75 (51 - 145)	-	Albumin (g/L)	41 (33 - 50)	1
Age (year)	44 (19 - 80)	-	CRP (mg/L)	5.0 (5.0 - 105)	-
Smoking	31-/11 +	-	Leukocytes (x 10^9/L)	5.8 (2.6 - 16)	-
Sex	22 F / 20 M	-	TNF-alfa (ng/L)	1191 (885 - 1667)	22
HBI	6 (3 - 24)	8			
GPA *	2 (1 - 3)				
Prior IFX use	40- / 2+	-			
Disease type (UC / CD)	8 / 34	-			
Baseline medication		-			
- Thiopurines	16- / 26+	-			
- Steroids	23- / 19+	-			
- Mesalazine	32- / 10+	-			
- Methotrexate	34- / 8+				

*GPA = Global physician assessment score (0 = normal, 1 = mild disease, 2 = moderate disease, 3 = severe disease)

Table 1b: Baseline immunosuppressive medication, dose and duration expressed as median (range)

Medication	Number of subjects	Dose (mg)	Duration (months)
Azathioprine	20	150 (50 - 300)	25 (3 - 360)
6-Mercaptopurine	6	88 (75 - 100)	5 (1 - 13)
Methotrexate	8	15 (12.5 - 25)	9 (1 - 72)
Budesonide	11	6 (3 - 9)	7 (1 - 108)
Prednisolone	5	30 (10 - 50)	1 (1 - 7)
5-aminosalicylic acid	10	3550 (3000 - 4000)	12 (3 - 204)
Baseline: no medication	4	-	-
Baseline: mono therapy	20	-	-
Baseline: combination therapy	18	-	-

3.1. IFX trough and ATI levels

Figure 1 and table 2 show a summary of the measured IFX through levels. All patients had detectable IFX trough levels however a large interindividual variation was observed. In the induction phase 77% of the female and 95% of the male patients had an IFX trough level ≥ 3 mg/L after 3 infusions which decreased to 46% in the female and 30% in the male patients at T = 54 weeks in the maintenance phase. Two patients had developed ATI at week 54. Median IFX concentrations at week 2 and week 54 were 34 (range 4 – 62) and 3 (range 0 – 25), $p < 0.001$, respectively. There was a significant correlation for CRP at baseline and IFX trough level at week 2 ($R = 0.408$, $p = 0.010$), but not for IFX trough level at week 54. There was no significant difference in IFX trough levels at week 2 and 54 between smokers and non-smokers.

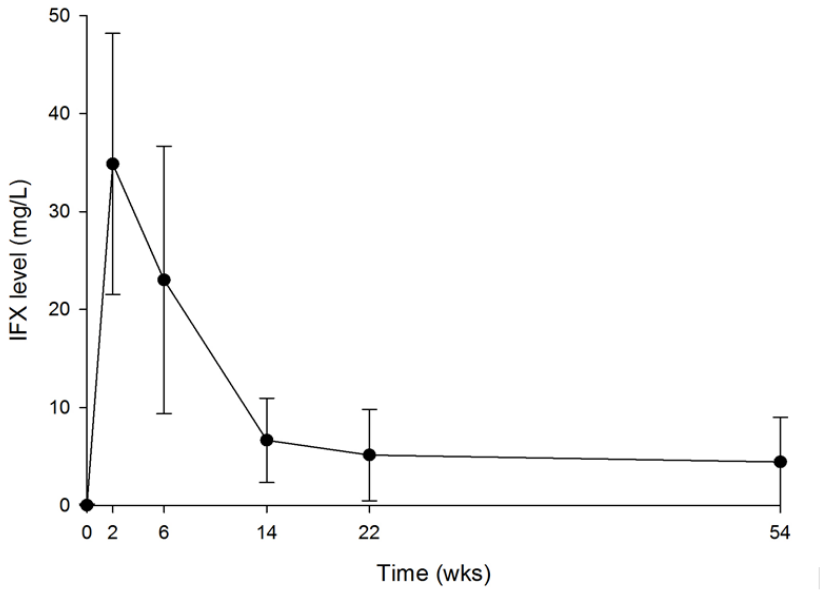

Figure 1: Summary IFX trough levels based on the available serum samples of 42 patients (mean±SD).

Table 2: Percentage of patients with IFX trough levels above 2.0 mg/L.

Week	No of samples	Sex	% ≥ 2.0 mg/L
0	42	Male	0
		Female	0
2	42	Male	100
		Female	100
6	40	Male	95
		Female	82
14	41	Male	75
		Female	91
22	42	Male	70
		Female	77
54	32	Male	55
		Female	64

3.2. Model development

Reasonably good fit was obtained with the base model. Even when using the parameter estimates from Fasanmade et al [23] but re-estimating only residual error magnitude, the estimates for the residual error components were lower than reported in the original publication, and evaluation of individual plots revealed reasonable fit. However, re-estimation of the model parameters of the base model resulted in a very significant improvement in fit ($p < 0.001$). Parameters were estimates with good precision (%) as is shown in table 3. Especially the parameters describing drug distribution were considerably different from those reported by Fasanmade et al [23]. A 40% increase in CL in the maintenance phase was observed compared to the induction phase. There was no significant difference in CL between patients with UC en CD (34 vs 42%).

Table 3: Parameter estimates for final population kinetic model of IFX.

Parameter	Parameter	Estimate (precision) mate(RSE%)	Unit	Range
CL	Clearance	0.199 (6%)	L.day-1	(0.161 - 0.228)
Vcc	Central volume of distribution	4.94 (10%)	L	(3.030 - 5.800)
Q	Inter-compartmental clearance	0.0618 (23%)	L.day-1	(0.038 - 0.104)
Vp	Volume of peripheral compartment	3.13 (32%)	L	(1.360 - 5.940)
ωCL	BSV[a] in CL	18.0% (18%)		(7.7 - 27%)
ωVc	BSV in Vc	17.1% (31%)		(1.5 - 33%)
σprop	Proportional error magnitude	21.7% (30%)		(8.0 - 32%)
σadd	Additive error magnitude	0.98 (18%)	mg/L	(0.61 - 1.54%)
θperiod	Increase of CL in maintenance phase	+40% (11%)		(15% - 94%)
θATI	Effect of ATI on CL	+72% (35%)		(24% - 136%)
θsex	Effect of sex on CL	+35% (34%)		(12% - 59%)
ΘHBI	Effect of HBI on V	-3.6 (28%)	HBI point-1	(-7.5 - -0.4)

[a] BSV = between subject variability

3.3. Covariate model

Treatment period was implemented manually as a covariate before implementation of the scm, and showed a significant improvement in fit ($p < 0.001$). In the forward step of the scm, 4 additional covariates were identified as statistically significant (ATI, SEX, and Albumin (ALB) on CL, and HBI on volume of distribution (V)), which were also retained in the model during the backward elimination step. The covariate effect sizes could be estimated with reasonable precision (11-35%). The largest relative effect size was found for ATI on CL, as can be seen in figure 2. In contrast with other popPK studies for monoclonal antibodies [21 - 23,28], we did not find a relationship between bodyweight and CL or V. In fact the model showed worse fit when any of the earlier reported relationships for weight were implemented in the model. The sex of the patient was found to be a significant predictor in our study. The CL for male patients was estimated to be 35% higher than in females, a finding which has also been reported by others [13].

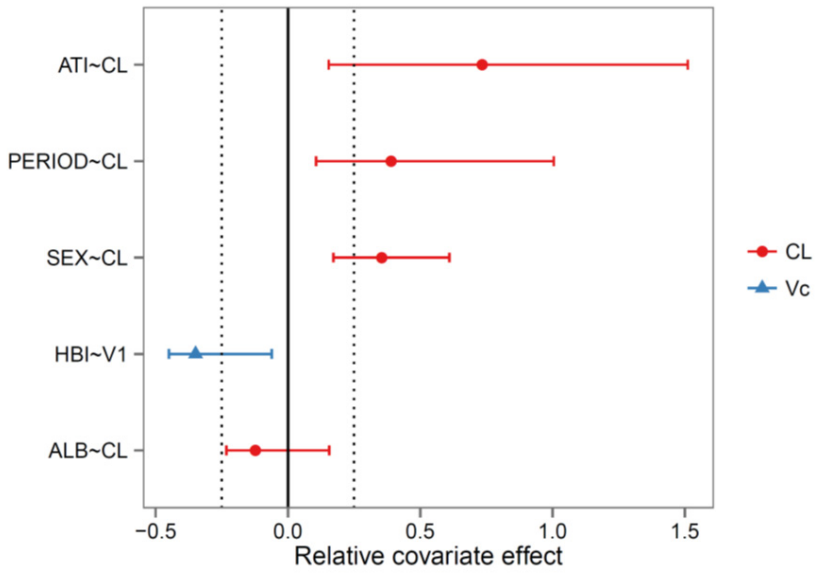

Figure 2: Plot of estimated effect magnitude on CL and V with the horizontal lines indicating the 95% CI around the estimated effect magnitude (dot). The dotted lines indicate the pre-specified clinical (ir-)relevance criterium. For continuous covariates the relative effect was defined as the estimated coefficient multiplied by 2 standard deviations of the covariate values in the patient population.

Albumin was found to be a significant predictor of PK, having a negative effect on CL, which corroborates findings by others. However, in our analysis we found the effect to have only a small clinical relevance, i.e. lower than our defined threshold of 25% relative magnitude, and the bootstrap analysis also showed that the confidence interval included 0. The covariate was therefore removed from the model.

For V, a significant and clinically relevant effect was found for the HBI at baseline, a higher value resulting in lower values of V. The final full model was thus defined as:

$$CL_i = CL_{pop} \cdot 1.345^{SEX} \cdot 1.722^{ATI} \cdot 1.40^{PERIOD} \qquad [1]$$

$$V_i = V_{pop} \cdot 0.964 \cdot (HBI - 6) \qquad [2]$$

in which SEX is defined as 0 for males and 1 for females, ATI is 0/1 for the presence of antibodies against IFX, and PERIOD is 0 for induction phase and 1 for the maintenance phase, HBI is the HBI at baseline.

Individual plots created using the final full model showed good correspondence between observed and model predicted values, as can be seen in figure 3 which compares the population prediction with the observed IFX concentrations for a few randomly selected patients. Goodness-of-fit plots of conditional weighted residuals versus time and predictions and of individual predictions versus observations revealed no trends, indicating an unbiased model fit (data not shown). Shrinkage in empirical Bayes estimates (EBEs) was only 3% for inter-individual random effects in CL and V, and 11% for the residual errors. The visual predictive check (figure 4) for the full model indicated that the model was able to describe the population mean PK profile as well as the between subject variability adequately, as all observed quantiles (5%, 50%, 95%) were contained within their respective prediction interval in every bin.

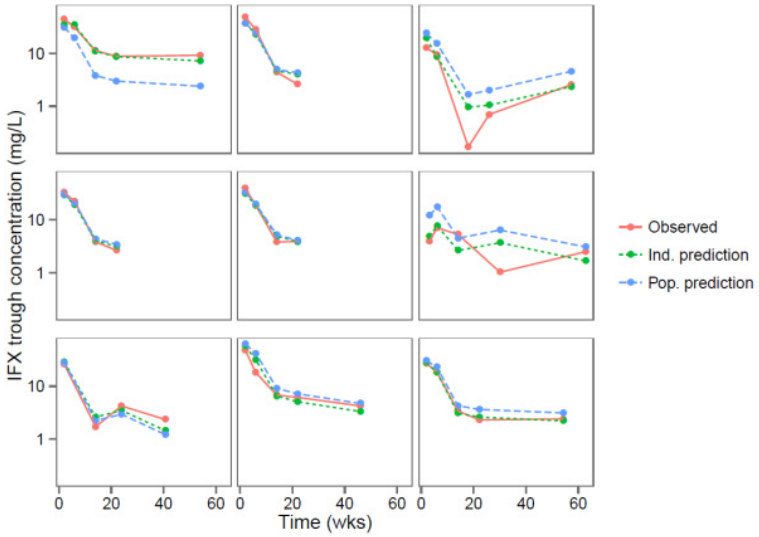

Figure 3: Model predictions (both population and individual) and observations plotted for nine representative patients. Note that for the predictions not the continuous time-course is shown, but only the expected trough concentrations.

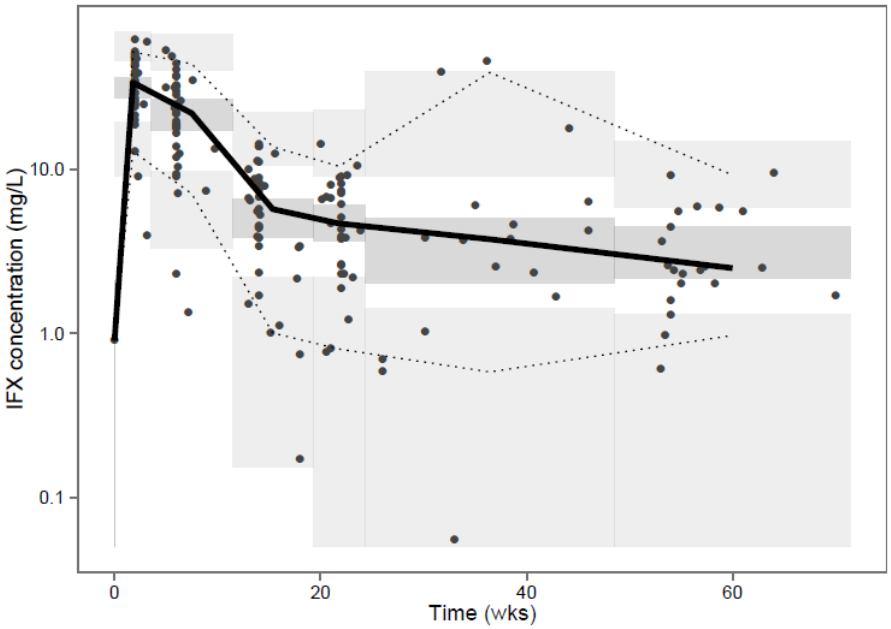

Figure 4: Visual predictive check of final model. The solid line indicates the observed median values per time interval, while the dotted lines indicate the observed 5th and 95th percentile of the observed data. The shaded areas represent the prediction interval for the median (dark grey), and the 5th and 95th percentile (light grey).

3.4. Simulation

The expected time course of IFX concentrations (in a patient population similar to our cohort) is shown in figure 5, assuming every 8 week dosing in the maintenance phase. This shows that at 400 mg or 600 mg, the majority of patients that do not show ATI are expected to have trough concentrations higher than 3 mg/L [9,17]. However, a majority of patients (either male or female) that do show ATI are expected to experience trough concentrations below the threshold.

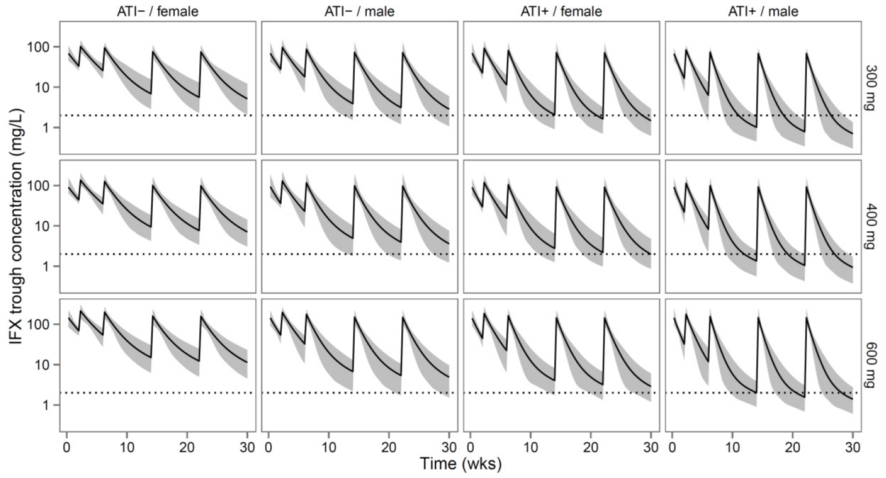

Figure 5: Monte Carlo simulation of patient population for "every 8 week" dosing, stratified by dose level and covariates ATI and sex. The horizontal dashed line shows the minimum trough level aim (3.0 mg/L).

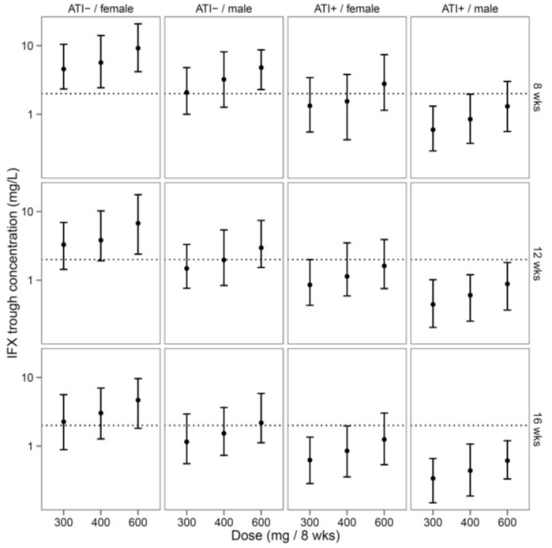

Figure 6: Monte Carlo simulation of patient population for 8, 12 and 16 week dosing, stratified by covariate. The horizontal dashed line shows the minimum trough level aim of ≥ 3.0 mg/L

Results from the simulation studying dosing regimens with longer dosing intervals are summarized in figure 6, which shows the expected distribution of trough levels for different dosing regimens, stratified by dose and patient characteristic, indicating that dosing every 16 weeks invariably results in the majority of patients showing trough concentrations lower than the threshold, even in those patients not showing ATI. Dosing every 12 weeks shows improved profiles, i.e. in patients that do not show ATI, this regime is expected to result in adequate levels in most patients. Table 4 summarizes the fractions of patients that are expected to have trough levels under the 3 mg/L threshold.

Table 4: Expected fraction of patient population below 3.0 mg/L at T = 54 weeks, calculated by simulation. Dosing scenarios where expected fraction of patients under 3 mg/L is lower than 20% are bold.

Dose interval	Sex	ATI	300 mg*	400 mg*	600 mg*
q8 wks	Female	-	**14%**	**8%**	**0%**
		+	51%	48%	40%
	Male	-	42%	33%	14%
		+	53%	53%	52%
q12 wks	Female	-	32%	**19%**	**9%**
		+	53%	52%	49%
	Male	-	49%	44%	31%
		+	53%	53%	53%
q16 wks	Female	-	42%	31%	**12%**
		+	53%	53%	52%
	Male	-	52%	49%	42%
		+	53%	53%	53%

* = dosing per 8 wks, so 300mg = 450mg for q12 and 600mg for q16 regimens.

4. Discussion

Our study resulted in several interesting findings not reported in earlier PK analyses of IFX. A PK model was developed and considerably higher CL was observed during the maintenance phase compared to the induction phase and the HBI was identified as a predictor of V. Simulations from the developed model showed that dosing every 12 weeks instead of every 8 weeks can be considered in IFX treatment of patients with IBD, but only in those that do not show ATI. Considering the high percentage of patient in remission with trough levels ≤ 3.0 mg/L, dose intensification or modification in dose intervals should always be combined with clinical and/or endoscopic disease activity parameters.

Despite years of experience with the use of IFX in treatment of patients with IBD several questions still remain unanswered. These questions include which patient demographics or biomarkers are predictive of pharmacokinetics, whether PK is different between the induction and maintenance phase, and whether a longer dosing interval can result in similar adequate trough levels and similar effectiveness. Furthermore observational studies showed that approximately 25-40% of the patients experienced loss of response over time [30]. In some studies it was demonstrated that these patients would require an increase in dose or decrease in infusion interval [31,32]. Katz et al concluded that dose intensification leads to a response in 47% of CD patients who lost response to standard IFX dose, but concluded that halving the infusion intervals is probably not superior to dose-doubling [33]. Kopylov et al showed that shortening the dosing interval to 6 weeks appears to be at least as effective as doubling the dose to 10 mg/kg [34]. The conclusions of these studies were drawn without TDM and based on clinical parameters. Therefore the question remains which role TDM can play in optimization of IFX treatment.

To be able to perform adequate TDM a PK model was developed based on models published earlier for IFX [21-23,28]. Although this was a relatively small study, sufficient data were available to allow the development of a population PK model that could be used with confidence to perform simulations of several dosing regimens.

Not all findings from the model building process were in line with reports from previous studies. Except for the volume of distribution [35] the main PK parameters were significantly different from those reported by others, especially those describing distribution to peripheral tissue. This may be attributed e.g. to differences in patient populations, or different sampling schemes. Therefore it was attempted to re-estimate all PK parameters, including Q and V2, and the bootstrap analysis confirmed that most PK parameters could be estimated with reasonable precision (all < 35%). However it must be noted that for the simulation of expected trough levels, the distribution process is not the most important component, as trough samples are always taken in the “elimination phase” of the drug.

The covariate modeling procedure identified several statistically significant. Some of these were expected *a priori,* but not all. The relationship identified in other studies between CL and patient weight was not found in our analysis. Most likely we did not find such a relationship in our study because the patients in our dataset only spanned a limited range of weight (90% CI between 60-100, with a few outliers > 100kg). Other studies contained wider ranges of weights, some also including data from children. To illustrate, the relationship identified in [23]

predicted only a difference of 5.6% in CL for patients with weights ranging between 60kg and 100kg, so it is unlikely that this effect would have been identified in our cohort.

The sex of the patient was a significant predictor of PK in our study, with CL 35% higher in males than in females, which was found in a previous studies as well and of similar magnitude [23].

Our analysis did identify a relationship between albumin levels (at baseline) and CL. However, similar to weight, our population showed only a moderate amount of variability in albumin levels, in which the interquartile difference would only result in an 8.3% difference in CL according to the relationship in [23], which was likely too low a signal to be detected in our cohort.

In our cohort only two patients showed ATI, but the effect was still found to be significant. Due to the limited number of patients with ATI, probably due to the fact all subjects had concomitant medication for IBD treatment, the effect must be interpreted with some caution. However, ATI were also identified in other popPK reports as relevant predictor of PK. The effect that was found in our statistical analysis confirmed our expectations, but it is the magnitude of the effect that may require further study in a larger population. In a study by Fasanmade [(23)] an effect of ATI was also identified, although it was found to be lower (29.2% vs 72%, but within the 95% CI of our current estimate).

Finally, we identified the HBI as significant covariate on V, i.e. a higher HBI was correlated with a lower V. The HBI is a measure of disease state used in the diagnosis of Crohn's disease, and includes parameters like the general well-being of the patient, the presence of abdominal pain, and the number of stools per day. An effect of HBI on PK parameters has not been reported before, but to our knowledge, HBI has also never been tested as possible covariate in reported popPK analyses. Disease activity could influence effectiveness of biologicals such as IFX by increased utilisation or fecal losses due to mucosal ulcerations. We did not include faecal calprotectin as a covariate in our analysis because in most patients this parameter was not measured routinely during this retrospective study. The statistical significance and clinical relevance of disease activity (HBI or another disease activity score with or without a fecal marker such as calprotectin) needs to be confirmed in a prospective study in a larger patient population.

The simulations from our model predict lower trough levels in patients that develop ATI. Our simulations show that almost all of these patients will have a trough level below 3 mg/L, when dosed at 400 mg. In our dataset we had only two patients that developed ATI (on 400 and 500 mg), and these patients

showed trough levels in the range of 0.58 – 2.02 mg/L during the maintenance phase, corroborating our prediction that these patients are likely to show ineffective trough concentrations. In these patients a dose increase (or a decrease of the dosing interval) is warranted when disease activity is still present. However switching to another anti-TNF antibody is probably more cost effective [36].

Our simulations also showed that for patients without ATI, it may be considered to dose every 12 weeks instead of every 8 weeks: at dose relative to 400 mg/8 weeks, this is expected to result in adequate dose levels (> 3 mg/L) for a majority of female patients (81% at 12 weeks versus 92% at 8 weeks), while at 600 mg/8 weeks > 99% are expected to show concentrations above the threshold. Unfortunately for male patients our simulations predict that dosing every 12 weeks will result in about half of the population experiencing too low trough concentrations. If dosed at an even longer time interval (every 16 weeks) the majority of patients without ATI, either male (42 - 52%) or female (12 - 42%), are expected to show trough levels < 3 mg/L even if dosed at 600 mg, rendering this dosing schedule infeasible in clinical practice).

Dosing every 12 weeks instead of every 8 weeks will reduce concomitant costs to IFX therapy (laboratory, nurses, out-patient clinic etcetera) with 33% for each patient treated according to this strategy. However these aspects of therapy represent a minor part of the total costs in IBD patients [20]. More important is the fact that patients have to visit the hospital for IFX-related therapy only 4 times a year instead of 6 times. This is more convenient for the patients but also creates more capacity in the hospital which can be used for other purposes.

Anti-TNF-α therapy is expensive and therefore it is important to optimize the use of it. TDM can be used to optimize dosing regimens in patients with low, but also with high IFX levels, in order to obtain a cost-effective treatment. In this study all patients had detectable IFX trough levels and good clinical response with a significant decline of HBI, with many patients below a score of 4. However at t = 54 weeks only 46% of female and 30% of the male patients had IFX trough levels of > 3.0 mg/L. Unfortunately more accurate disease activity parameters such as endoscopy or fecal calprotectin were not available for most patients. It is tempting to speculate that these patients in remission with low trough levels would be good candidates for a stopping strategy. Therefore, trough level dosing should always be combined with clinical and/or endoscopic disease activity parameters in order to avoid unnecessary dose intensification.

5. Conclusion

The developed pharmacokinetic model could be used to optimize TDM of IFX in IBD patients, but it needs to be confirmed in a prospective clinical trial. Simulations from the model show that dosing every 12 weeks can be considered in the treatment of patients with IBD with IFX, but only in those that do not show ATI. This strategy reduces IFX-therapy related visits to the hospital with one third. Considering the high percentage of patients in remission with trough levels ≤ 3.0 mg/L, dose intensification or modification in dose intervals should always be combined with clinical and/or endoscopic disease activity parameters.

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CHAPTER 5

A non-invasive, low-cost study design to determine the release profile of colon drug delivery systems: a feasibility study

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Abstract

Purpose

Conventional bioavailability testing of dosage forms based on plasma concentration-time graphs of two products in a two-period, crossover-design, is not applicable to topical treatment of intestinal segments. We introduce an isotope dual-label approach (^{13}C- and $^{15}N_2$-urea) for colon drug delivery systems that can be performed in a one-day, non-invasive study-design.

Methods

Four healthy volunteers took an uncoated or a ColoPulse-capsule containing ^{13}C-urea and an uncoated capsule containing $^{15}N_2$-urea. In case of colon-release ^{13}C-urea is fermented and ^{13}C detected as breath $^{13}CO_2$. Absorbed ^{13}C-urea and ^{15}N-urea are detected in urine.

Results

^{13}C and ^{15}N in urine released from uncoated capsules showed a ratio of 1.01 ± 0.06. The $^{13}C/^{15}N$-recovery ratio after intake of a ColoPulse-capsule was constant and lower >12h post-dose (median 0.22, range 0.13-0.48). The $^{13}C/^{15}N$-ratio in a single urine sample at $t \geq 12$ h predicted the 24 h non-fermented fraction ^{13}C of < 26%. Breath $^{13}CO_2$ indicated delayed (> 3h) release and a fermented fraction $^{13}C > 54\%$.

Conclusions

Breath and urine ^{13}C and ^{15}N data describe the release-profile and local bioavailability of a colon delivery device. This allows non-invasive bioavailability studies for evaluation of colon-specific drug delivery systems without radioactive exposure and with increased power and strongly reduced costs.

1. Introduction

Investigation of the bioavailability is an early step in the clinical development of a new drug product or drug delivery system. The United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have released guidelines for bioavailability testing of drug products which aim for systemic exposure of the drug substance [1,2]. The conventional systemic bioavailability study design is a two-sequence, two-period crossover design, where blood pharmacokinetic parameters as the maximal concentration (C_{max}) and the area under the concentration–time curve (AUC) play a pivotal role. This conventional approach however is not applicable to drug delivery systems which aim for delivery of the drug substance in a specific intestinal segment for topical treatment. Examples are 5-aminosalicylic acid or immunosuppressant formulations such as budesonide for the treatment of inflammatory bowel disease. In these specific cases it is not relevant to investigate systemic bioavailability. To the best of our knowledge no international guidelines are available describing a consensus approach to evaluate local bioavailability. In the international literature a myriad of approaches are described to determine intestinal drug delivery. Pharmacokinetics is often combined with imaging technologies to localize release, such as endoscopy, radiology, gamma scintigraphy [3-5] or MRI [6,7]. Stable isotope technology is also mentioned in this context [8].

In two earlier studies we determined the local bioavailability and release profile of a coated capsule which acts as colon-specific drug delivery system (the ColoPulse-system) using stable isotope technology [9,10]. The ColoPulse system is characterized by a pulsatile release of its contents at pH > 7.0. The coating consists of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol (58.3%:8.3%:33.3% w/w) [11]. The first paper describes a proof-of-concept study in which it was shown that ^{13}C-urea was able to provide information on both the release kinetics of a ColoPulse-capsule and the gastro-intestinal segment of release. The second paper describes a single dose two-period crossover study in which ^{13}C-urea was used as the marker substance. In this study an uncoated capsule was taken on day 1 (as a reference) and a ColoPulse-capsule on day 8. The delivery in the colon by the ColoPulse-capsule was monitored by measuring the $^{13}CO_2$ response in breath produced by bacterial fermentation in the colon of ^{13}C-urea. Local bioavailability was determined by recovery of ^{13}C in breath. Total recovery was quantified by the sum of recoveries of ^{13}C in breath and blood or urine. A strong correlation (r = 0.943) was found between blood and urine kinetics, indicating that non-invasive urine sampling could replace blood sampling.

We hypothesized that investigation of local bioavailability and determination of the release profile of ColoPulse-capsules could be improved by application of a dual-label isotope strategy. This approach permits a one-day study design and non-invasive sampling. A ColoPulse-capsule containing ^{13}C-urea and an uncoated capsule containing $^{15}N_2$-urea (as a reference) are taken simultaneously. Release of ^{13}C- or $^{15}N_2$-urea in the small intestine (urease-poor region) from an uncoated capsule leads to the recovery of unaltered ^{13}C- or $^{15}N_2$-urea in urine. Release of ^{13}C-urea in the ileocolonic intestinal segment (urease-rich region) from a ColoPulse-capsule leads to *in situ* fermentation of ^{13}C-urea into $^{13}CO_2$ followed by exhalation of in breath. Local bioavailability in the colon can be described by the difference between kinetics of $^{15}N_2$- and ^{13}C-urea (figure 1). The differential kinetics of these isotopically labeled substances can potentially describe both release kinetics and the gastro-intestinal segment of release. Using this strategy, the clinical trial can be shortened to a one-period design and the sample load can be reduced by 50%. As a consequence the cost of a bioavailability trial is reduced. In addition, the influence of day-to-day variation in urea kinetics is eliminated, which increases the power of the study. Furthermore less subjects need to be included, which further reduces the cost of the clinical trial.

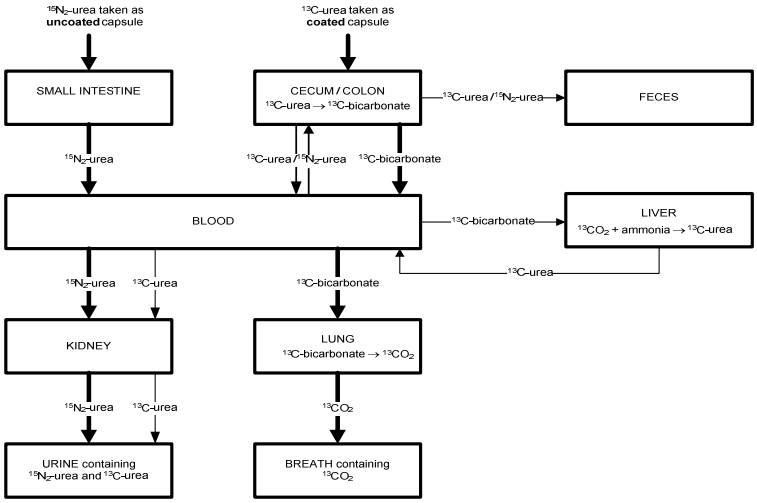


Figure 1: Absorption, metabolism and elimination of ^{13}C-urea and $^{15}N_2$-urea. The weight of the arrow symbolizes the importance of the kinetic process

In this paper we describe a proof-of-concept study to demonstrate the feasibility of the dual-isotope strategy to determine the release profile of ColoPulse-capsules in a one-day, non-invasive study design.

2. Materials and Methods

2.1. Chemicals, drug substances and drug products

Polyethylene glycol 6000, acetone, colloidal anhydrous silica (BUFA, The Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany), were obtained via a certified wholesaler (Spruyt-Hillen, The Netherlands). Hard gelatine capsules (size 2) were obtained from Lamepro (The Netherlands). Water for injections was obtained from Fresenius Kabi (Germany). All ingredients were of pharmacopoeial grade (Ph. Eur.). The stable isotope labelled ^{13}C-urea and ^{15}N-urea (AP 99%) was obtained from an FDA-controlled facility (Isotec, USA). Hard gelatine capsules containing 100 mg ^{13}C- or 50 mg $^{15}N_2$-urea were prepared according to the compounding procedures of the Laboratory of Dutch Pharmacists (LNA). The capsules were manually filled with a premix of ^{13}C-urea or $^{15}N_2$-urea and excipients. A coating was applied using the ColoPulse technology [11]. Coating thickness was calculated and expressed as the amount of Eudragit S-100 applied per cm^2. The coated capsules met established quality control criteria (table 1). The pulsatile release properties are reflected by the so-called pulse-time, defined as the period between the lag time (= $t_{5\%\ release}$) and $t_{70\%}$ release.

Table 1: Quality control data of the capsules

Parameter	Specification	Result
Variation of mass (capsules, uncoated, n = 20)	< 4%	1.52%
Variation of mass (capsules, coated, n = 20)	< 4%	1.59%
Coat thickness (mg Eudragit S/cm2) (n = 20)	Not applicable	9.8
Bursts or cracks in coating (n = 6)	None	None
Lag time (minutes) (n = 6)	> 180	220
Pulse time (minutes) (n = 6)	< 60	38
Release at t_{360min} (n = 6)	> 80%	107.9%

2.2. Subjects

Four healthy volunteers (one female, three males, age 30, 39, 50, 61 years) participated in the study. They had neither history of gastrointestinal diseases (ulcerative colitis, Crohn's disease, spastic colon, colon cancer, ileus, stoma,

stomach- and/or intestinal infection) nor of gastrointestinal surgery. They did not use antibiotics or drugs influencing the gastrointestinal transit time for at least 3 months before start of the study. A possible *Helicobacter pylori* infection was excluded. The study design was approved by the ethical committee of the University Medical Center Groningen and he study was performed according to principles of the Declaration of Helsinki.

2.3. Study Design

The clinical study consisted of two experiments. In the first experiment two uncoated capsules containing 50 mg $^{15}N_2$-urea and 100 mg ^{13}C-urea respectively were taken simultaneously in order to compare the kinetic behaviour of ^{13}C-urea and $^{15}N_2$-urea affected by absorption, distribution, metabolism and elimination. In the second experiment an uncoated capsule containing 50 mg $^{15}N_2$-urea and a ColoPulse-capsule containing 100 mg ^{13}C-urea were taken simultaneously. The second experiment aimed to give information on the release of ^{13}C-urea in the ileocolonic intestinal segment (urease-rich region) and of $^{15}N_2$-urea in the proximal small intestine (urease poor region) respectively. During the experiments the subjects feeding and drinking were standardized as described before [8,9]. The subjects were fasted on day 1 from 20:00 h. Only water and tea without sugar were allowed. In the morning on day 2, they received the capsules together with 200 ml apple juice. After a period of 3 hours a standardized breakfast consisting of a double sandwich was consumed in order to control the oro-cecal transit time. 5 and 10 hours after intake of the capsules lunch and dinner were allowed. There were no food-restrictions except foods enriched in ^{13}C like corn, cane sugar and pineapple. During the day only water and tea without sugar were allowed. The study ended at 8.00 h on day 3.

2.4. Sample collections and analysis

Breath samples were collected every 30 minutes from 30 minutes before up to 15 h after intake of the capsules and were analysed as described before [8,9]. Briefly, $^{13}C/^{12}C$ isotope ratios in the CO_2 of breath samples were analysed by using a validated breath ^{13}C-analyser (Thermo Fisher Scientific, Bremen, Germany) based on isotope ratio mass spectrometry (IRMS). Urine samples were collected during 24 h at prescribed intervals (0 - 4, 4 - 8, 8 - 12, 12 - 16 and 16 - 24 h) in 200 ml containers each containing 650 µl 6M HCl. Urine volumes were recorded and 20 ml samples were stored at -20^{o}C until analysis. The remaining urine was pooled and a 20 ml sample was stored at -20^{o}C. The pooled urine volume was considered as a the 24 h collection and used as gold standard for modeling (section 2.6).

Concentrations of total N and C were determined based on element analysis. Urine aliquots of 25 µl were combusted in an elemental analyzer SL^{TM} (SerCon, Crewe, UK) using copper oxide at 900ºC to NO_x and CO_2. NO_x was subsequently reduced to nitrogen gas over copper at 600ºC. Thereafter the ^{13}C and ^{15}N enrichments were measured online by IRMS (Tracer mass 20-20^{TM}, SerCon, Crewe, UK).

Data were expressed either as enrichment, as atom percent excess (APE) or as percentage of the dose ^{15}N or ^{13}C recovered (PDR). The method of urine sample preparation and IRMS-analysis was tested for accuracy (recovery), precision and linearity. This test was performed by spiking equal volumes (100 ml) of urine collected by one subject during 24 h with fixed amounts of $^{15}N_2$-urea (5 mg) and increasing amounts of ^{13}C-urea (0 - 10 mg).The theoretical ratios of $^{13}C/^{15}N$ in these samples were 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0.

2.5. Calculations

The percentage of the dose recovered (PDR) of ^{13}C and ^{15}N in each urine sample, the ratio of the PDRs from ^{13}C versus ^{15}N-ratio (the $^{13}C/^{15}N$-ratio), the fermented ($F_{fermented}$) and non-fermented ($F_{non\text{-}fermented}$) fraction of ^{13}C- urea were calculated as described before [10]. In short, the fermented fraction was calculated as the cumulative PDR as ^{13}C in breath over the 15 h time period. The non-fermented fraction was calculated as the ratio of the percentage of the dose recovered as ^{13}C and ^{15}N (ratio $^{13}C/^{15}N$) in the 24 h urine collection. Total recovery was expressed as $F_{non\text{-}fermented} + F_{fermented}$.

2.6. Statistical procedures and modeling

The results were evaluated by descriptive statistics. The center was characterized by the arithmetic mean and median. The dispersion was characterized by the coefficient of variation (CV) and range correspondingly. A Wilcoxon matched-pairs test (two tailed, $\alpha = 0.05$) was used to compare the ratio $^{13}C/^{15}N$-ratio in the 24 h urine collection with the calculated ratio from a single urine sample and 95% confidence intervals were established.

The correlation between the $^{13}C/^{15}N$-ratio in the 24 h urine collection and in a single urine collection collected at a time point $\geq$ 12 h post dose was investigated. The algorithm was obtained from the regression line. The correlation coefficient was calculated from the determination coefficient of the regression line.

2.7. Endpoints

The first endpoint of the study was to determine that ^{13}C-urea and $^{15}N_2$-urea exhibit the same kinetic properties in terms of absorption, distribution and elimination. The second was to show that a reduced PDR of ^{13}C-urea in urine is an indicator of bacterial fermentation of ^{13}C-urea delivered in the colon. The third endpoint was the total recovery of the ^{13}C-labeled atom to confirm that all elimination routes are covered by our sampling plan.

3. Results

3.1. Urine spiking experiment

The method of sample preparation and IRMS analysis showed a recovery of 98 ± 3.7% for the ^{15}N- (n = 7) and 94 ± 2.2% (n = 6) for the ^{13}C-isotope. The precision was 3.8% (n = 7) for ^{15}N and 2.6% (n = 6) for ^{13}C. Furthermore, the method was linear in a range of 0 to 100 mg ^{13}C-urea/L (slope = 0.95, $r^2 = 0.9987$). The ratio $^{13}C/^{15}N$ was linear in a range of 0 to 1.0 (slope = 0.98, $r^2 = 0.9999$) when the measured ratio was plotted against the theoretical value.

3.2. *In-vivo* experiment

3.2.1. Urine-data

In figure 2 the ratio of the PDRs of ^{13}C and ^{15}N measured in the urine samples is shown as a function of time. The $^{13}C/^{15}N$-ratio from uncoated capsules showed a mean ratio of 1.01 ± 0.06 (n = 20) during the first 24 h. The $^{13}C/^{15}N$-ratio after intake of the ColoPulse-capsule showed larger interindividual variation but remained constant in all subjects subject after 12 h post dose (median 0.22, range 0.13-0.48). The $^{13}C/^{15}N$-ratio in the 24 h urine collection after intake of the coated capsules (median 0.15, range 0.09-0.32) was lower than the ratio measured in the single urine samples after 12 h post dose, in all four cases.

The cumulative percentage non-fermented ^{13}C- and $^{15}N_2$-urea expressed as percentage of the dose recovered (PDR) per collected urine volume is shown for each subject in figure 3. The cumulative PDR of ^{13}C and ^{15}N in urine after 24 h is shown in table 2. The cumulative PDR of ^{13}C in urine from the coated colon targeted capsule (median 11.9%, range 7.4-25.9%) was in all collections lower ($p < 0.05$) than the cumulative PDR of ^{15}N (median 81.6%, range 76.6-86.8%) in the same collection and the cumulative PDR of ^{13}C from the uncoated capsule for the same subject (median 73.1%, range 64.0-77.9%).

The median cumulative PDR at t = 24 h of ^{15}N from the uncoated capsules in experiment 1 and 2 (73.7% versus 81.6%) showed 7.9% absolute difference.

The median cumulative PDR at t = 24 h of ^{15}N and ^{13}C from the uncoated capsule in experiment 1 (73.7% versus 73.1%) showed 0.6% absolute difference.

The median interindividual variation in cumulative PDR (3.4%) was in the same range as the median interlabel variation (2.9%). Median interday variation in cumulative PDR of ^{15}N was 9.7%. Calculations of median variations were performed by combining the data from uncoated ^{13}C and ^{15}N-urea because we considered kinetics of both isotopes as equal after review of the results.

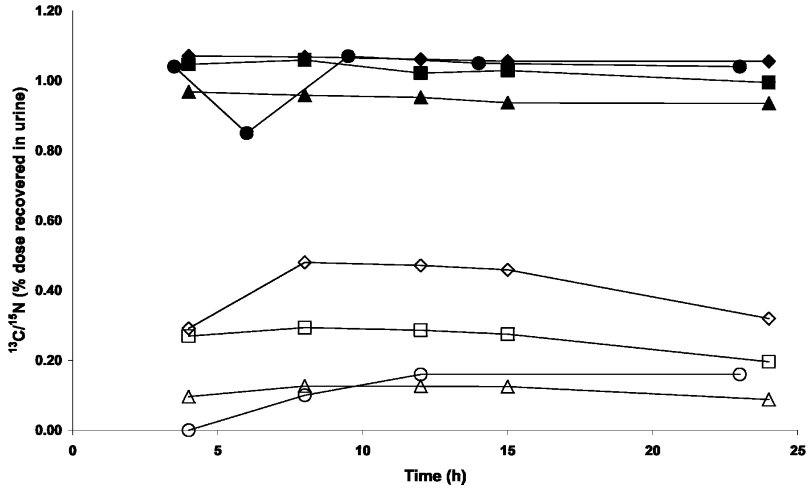
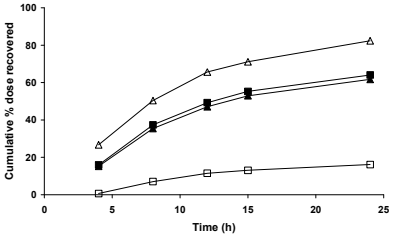
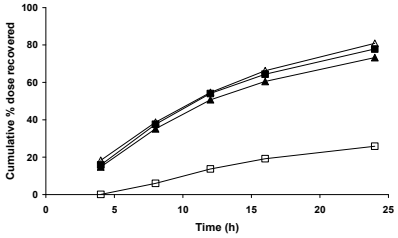


Figure 2: Ratio $^{13}C/^{15}N$ (corrected for values at t = 0) as percentage of the dose recovered in urine after intake of an uncoated ^{13}C-urea capsule (closed symbols) and a coated ^{13}C-urea capsule (open symbols). Similar symbols reflect the same subject. The time point is the time at which urine collection was finished.



3A: subject 1



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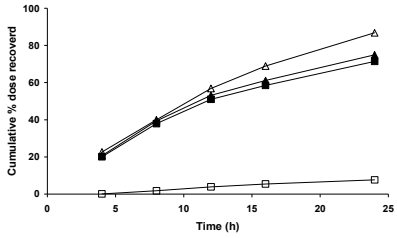

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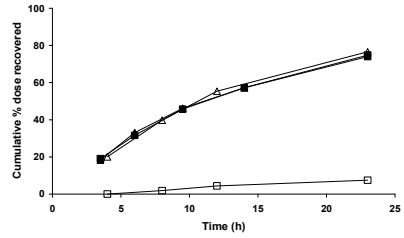

3D: subject 4

Figure 3: Cumulative percentage non-fermented ^{13}C- and $^{15}N_2$-urea recovered from urine as a function of time.
■ ^{13}C uncoated capsule (day 1); ▲ ^{15}N uncoated capsule (day 1)
□ ^{13}C coated modified-release capsule (day 2); Δ ^{15}N uncoated capsule (day 2)

Table 2: Cumulative PDR of ^{13}C and ^{15}N in urine at t = 24 h

	Uncoated capsules			**Coated capsule**
Subject	**^{15}N (exp 1)**	**^{15}N (exp 2)**	**^{13}C (exp 1)**	**^{13}C (exp 2)**
1	61.8	82.3	64.0	16.1
2	73.3	80.9	77.9	25.9
3	74.9	86.8	71.4	7.6
4	74.0	76.6	74.8	7.4
Median	73.7	81.6	73.1	11.9
Mean	71.0	81.6	72.0	14.3
SD	6.2	4.2	5.9	8.7
CV	8.7	5.2	8.2	61.2

Median inter-individual variation (uncoated ^{13}C+^{15}N)	3.4%
Median interday variation (^{15}N)	9.7%
Median interlabel variation (exp 1)	2.9%

3.2.2. Breath-data

In figure 4 the breath ^{13}C exhalation data are shown, expressed as the cumulative PDR versus time curves over a time period of 15 h after intake of the coated capsule. All 4 subjects exhibited a significant exhalation of ^{13}C in breath varying from 54.5 to 81.5%. These percentages represent the fermented fraction of ^{13}C-urea. The curves also indicate a lag time of > 3 h. Figure 5 shows the fermented (breath) and non-fermented (urine) fractions of ^{13}C-urea recovered 15 h after intake of a coated capsule. Total recovery was large (> 77%), whereas the non-fermented fraction was limited (< 32%).

3.3. Modeling

When $F_{non\text{-}fermented}$ calculated from a single urine-sample taken ≥ 12 h post dose was compared to $F_{non\text{-}fermented}$ calculated from the 24 h urine collection the absolute difference had a mean value of 8.6% (95% CI 5.5-11.7%, p = 0.068). The relationship between the $^{13}C/^{15}N$-ratios could be described by equation (1) obtained from the regression line.

Eq. 1: $(^{13}C/^{15}N)_{24h\text{-}collection} = (^{13}C/^{15}N)_{single\text{-}collection} / 1{,}51$ ($R^2 = 0.9977$)

Using this equation the mean difference between the calculated $F_{non\text{-}fermented}$ from a single sample ≥ 12 h post dose and the 24 h urine collection was 0.1% (95% CI -0.3 to 0.5%, p = 0.67).

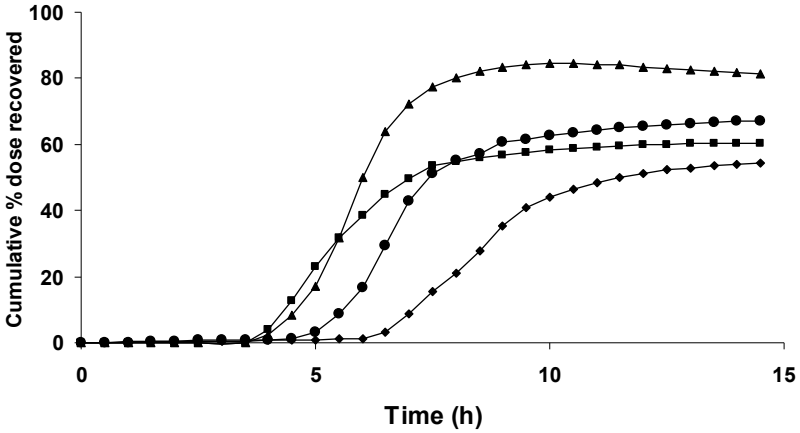

Figure 4: Cumulative percentage recovery of ^{13}C in breath as percentage of the administered dose ^{13}C-urea (corrected for CO_2-retention) in all four subjects

4. Discussion

4.1. *Kinetics of* ^{13}C and $^{15}N_2$-urea

The method of sample preparation and IRMS analysis proved to be reliable as shown by the spiking results. The accuracy was high with a recovery over 95% and variation was low with a precision under 4%. Furthermore the $^{13}C/^{15}N$-ratio was linear in a range of 0 to 1.0 ($r^2 = 0.9999$).

A prerequisite for the successful application of the dual stable isotope approach to determine local bioavailability is comparable kinetics of ^{13}C and $^{15}N_2$-urea. The so-called "isotope effect" [11,12] is the sum of differences in metabolism and physical properties (such as polarity, lipophilicity, protein binding) between the two different labeled compounds. Since urea is the end product of the nitrogen metabolism and therefore undergoes limited recycling, the kinetic isotope effect differentiating between ^{13}C and $^{15}N_2$-urea is expected to be absent. This hypothesis was tested in the first experiment. Two uncoated capsules containing 100 mg ^{13}C-urea and 50 mg $^{15}N_2$-urea respectively were taken simultaneously. Release of labelled urea will occur in the stomach and absorption of intact molecules into the systemic circulation will be fast. The mean of the $^{13}C/^{15}N$-ratio in urine for uncoated capsules shortly after administration was 1.01 ± 0.06. This reflects the equimolar concentration in the urea distribution volume (UDV), pointing to comparable dissolution in the stomach, absorption, distribution and renal excretion. The interlabel variation appeared to be very limited as shown by the median recoveries from ^{15}N and ^{13}C in experiment 1 (73.7% versus 73.1%). This confirms the aforementioned hypothesis that both urea-isotopes have comparable kinetics and that the isotope effect is absent.

The cumulative PDR at t = 24 h for ^{13}C or ^{15}N from uncoated capsules was high (61.8 - 86.8%) as one expects for a small, water-soluble molecule, which is readily absorbed from the intestine. The amount not recovered can partly be explained by the so-called urea salvage. Intact absorbed urea diffuses from the systemic circulation back into the intestine where it may either be fermented (colon) or excreted via the feces. In earlier work we found this fraction to be 7.5% at 12 h after oral administration [10]. The major part of the non-recovered isotope label is probably still present in the urea distribution volume and will gradually be excreted in the urine afterwards, as the elimination half-life of urea is 7 h [12]. This hypothesis is supported by two observations. First, the cumulative PDR-time curves in urine did not reach a plateau level within 24 h (figure 3). Second, fermentation was finished within 15 h (figure 4).

Since the "isotope effect" is absent for ^{13}C- and $^{15}N_2$-urea and the urea salvage is limited after release of urea in the stomach, $^{15}N_2$-urea excretion in urine collected over a certain time interval can serve as an internal standard correcting for day-to-day variation in urea kinetics. The fraction of non-fermented ^{13}C-urea after delivery in the colon may be quantified relative to this standard.

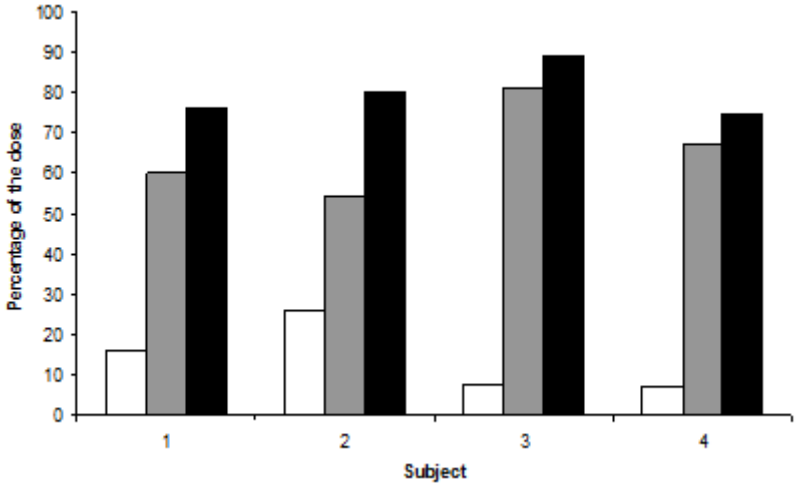

Figure 5: Fractions fermented and non-fermented ^{13}C-urea after intake of a coated modified-release capsule; □: $F_{non\text{-}fermented}$ ▒: $F_{fermented}$ ■: total recovery of ^{13}C

4.2. Dual-label stable isotope strategy

The second experiment aimed to give information on the release of ^{13}C-urea in the ileocolonic intestinal segment (urease-rich region). In an earlier proof-of-concept clinical study we showed that urea is fermented in the colon, which leads to reduced availability of intact ^{13}C-urea in blood and urine from a ColoPulse-capsule [9].

4.2.1. Urine and breath data

After concomitant administration of $^{15}N_2$-urea in an uncoated and ^{13}C-urea in a ColoPulse-capsule, the $^{13}C/^{15}N$-ratio in urine became constant about 12 h after intake (figure 2). The ratio was much smaller than 1 in all subjects, which is explained by limited absorption of intact ^{13}C-urea from the ColoPulse-capsules in comparison to uncoated capsules (11.9% versus 73.1%). The fraction non-fermented of ^{13}C- and $^{15}N_2$-urea expressed as PDR per collected urine volume for each subject (figure 3) also illustrates the comparable kinetics of ^{13}C- and

$^{15}N_2$-urea and the difference in segment of release of the uncoated and the ColoPulse-capsule.

The release profile of the ColoPulse-capsules obtained from the $^{13}CO_2$ response in breath (fig. 4) is comparable to the one we found in the single dose, two-period crossover study [11]. Median values found for the fermented fraction were 63.5% in this study versus 69.2% earlier. For the non-fermented fraction this was 14.8% (urine) versus 16.0% (blood). These results support our earlier finding that combining breath and urine data yields results comparable to combining breath and blood data.

The value of the $^{13}C/^{15}N$-ratio in urine 12 h after intake becomes constant. This is explained by the release characteristics of the ColoPulse-capsule, which starts releasing its contents when the ileocolonic region is reached about 3 h after intake (figure 4).

The orocecal transit time (OCTT) is highly variable in healthy subjects and is dependent on the time interval between intake of the capsules and intake of food. The range of 3-5 h as observed for the lag time in the healthy volunteers in this study is in agreement with published OCTT data. Urease activity is related to the presence of bacterial load, which is normally absent in the mid small intestine, low in the distal small intestine and high in the colon. An exception is when bacterial overgrowth is present in the small intestine. However, this is not expected to occur in healthy subjects and therefore the subjects were not screened for this pathology. Furthermore no non-invasive, absolute diagnostic test is available for this purpose.

As might be expected, at 12 h all ^{13}C-urea has been released in the intestines and is absorbed or fermented or encapsulated in viscous feces. The curves of figure 4 indicate a lag time of > 3 h. The combination of the delayed and the high ^{13}C response in breath proves that the capsule released its content in the urease-rich ileocolonic region.

The median cumulative PDR of ^{15}N released from the uncoated capsules in experiment 1 and 2 (73.7% versus 81.6%) showed 7.9% absolute difference indicating day-to-day variation in urea kinetics. The median interday variation (9.7%) appeared to be larger than the interlabel (2.9%) and interindividual variation (3.4%), supporting the proposal to apply $^{15}N_2$-urea released in the stomach as an internal standard to correct for day-to-day variations.

4.3. Single urine sample

When $F_{non\text{-}fermented}$ calculated from a single urine-sample taken ≥ 12 h post dose was compared to $F_{non\text{-}fermented}$ calculated from the 24 h urine collection the absolute difference in $F_{non\text{-}fermented}$ had a mean value of 8.6% ($p = 0.07$). This difference is caused by the excretion of ^{15}N-label during the first 4 h post dose when the coated ^{13}C-urea capsule still did not release its content.

We tried to find a reliable mathematical relationship between $^{13}C/^{15}N$-ratio in a single collection and in a 24 h-collection, taking into account the earlier start of ^{15}N-label excretion. This algorithm is to be used in future studies to be able to calculate the F non-fermented from a single urine sample. For each subject the mean $^{13}C/^{15}N$-ratio of the single urine collections ≥ 12 h post dose was plotted against the $^{13}C/^{15}N$-ratio in the 24 h urine collection. The obtained linear correlation coefficient was 0.9977 indicating a very strong relationship. This was confirmed by calculation of the $F_{non\text{-}fermented}$ both from a single sample (≥ 12 h post dose) and the 24 h urine collection. No difference could be detected between the $F_{non\text{-}fermented}$ obtained between these methods. The mean difference between these outcomes was only 0.1%, ($p = 0.67$) showing the validity of the model as used in this study.

The strong relationship between the $^{13}C/^{15}N$-ratio in a single urine sample collected ≥ 12 h post dose and that in the 24 h urine collection implies that the non-fermented fraction, needed to evaluate bioavailability of the content of a ColoPulse-capsule can be determined by analyzing the $^{13}C/^{15}N$-ratio in any urine sample obtained between 12 and 24 h after administration.

Table 3: Sample size calculations for a local bioavailability study of a colon drug delivery system applying non-invasive stable isotope technology ($\alpha = 0.05$, $\beta = 0.2$)

Reference	Detectable difference	Population variance	Required group size
Schellekens et al, 2010 [10]	20%	CV = 49%	36
	10%		144
This paper	20%	CV = 18%	5
	10%		21

4.4. Increase of study power by one-day design

Heck et al. [13] reported already in 1979 that the application of stable isotope technology in bioavailability studies permits smaller group sizes by increased study power via elimination of day-to-day variation which is unavoidable in a two-day study design. To further evaluate the ColoPulse-technology, we

calculated the difference in required group size when applying a one- or two day study design by equation (2):

Eq. 2: $$z_\beta = \frac{\delta}{\sqrt{\frac{2\sigma^2}{n}}} - z_{\alpha/2}$$

We established a level of significance of 95% ($\alpha = 0.05$, corresponding $z_{\alpha/2} = 1.96$) and a power of 80% ($\beta = 0.2$, corresponding $z_\beta = 0.84$). We choose to be able to detect a difference (δ) in local bioavailability of 10 or 20%. The population variance (σ) was estimated based on bioavailability data of our two-day [10] and one-day studies. As is shown in table 3, the group size (n) is smallest in the one-day study design and decreases more than proportional with a decrease in population variance.

Together with the elimination of blood samples, the reduction of breath samples by performing a study in one day and the absence of day-to-day variation in urea kinetics add additional advantages to the earlier proposed study design using coated and uncoated capsules containing ^{13}C-urea on different days [11]. We will further investigate this approach in a clinical study to evaluate the release profile and local bioavailability of colon-specific tablets in both healthy subjects and patients with Crohn's disease.

Another part in clinical development of a new drug delivery system or drug product is determination of bioequivalence when comparable devices or products are already available. The dual-label stable isotope strategy was not intended for studies with active substances, because drug specific characteristics cannot be tested. However the principle can be used for testing bioequivalence of colon-specific drug delivery devices. In that case the study has to be performed on two different days, but all the other mentioned advantages of this approach are still applicable including the absence of day to day variation. The single challenge is the availability of a comparator drug delivery device containing ^{13}C-urea.

5. Conclusion

Application of a dual-label stable isotope strategy of $^{15}N_2$- and ^{13}C-urea is suitable for the evaluation of bioavailability of colon-specific drug delivery systems. Since both isotopes can be taken at the same time, day-to-day variation in urea kinetics is eliminated and study power is increased.

Compared with the conventional two-period study design, our approach reduces clinical study costs by a decrease in study run through time (one period instead of two) and in sample-load by omitting blood-samples, reducing breath samples by 50% and only taking one urine sample. With this feasibility study we showed that combination of breath and a single urine sample provides sufficient information to assess ColoPulse-capsules in vivo without radioactive exposure in a non-invasive, low-cost study design.

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CHAPTER 6

Gastrointestinal pH and transit time profiling in healthy volunteers using the IntelliCap system confirms ileo-colonic release of ColoPulse tablets

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Abstract

Introduction

ColoPulse tablets are an innovative development in the field of oral dosage forms characterized by a distal ileum and colon-specific release. Previous studies in humans showed release in the ileo-colonic region, but the relationship between gastrointestinal pH and release was not experimentally proven *in vivo.* This information will complete the *in vivo* release-profile of ColoPulse tablets.

Materials and Methods

Release from ColoPulse tablets was studied in 16 healthy volunteers using the dual label isotope strategy. To determine gastrointestinal pH profiles and transit times the IntelliCap system was used. A ColoPulse tablet containing ^{13}C-urea and an uncoated, immediate release tablet containing $^{15}N_2$-urea were taken simultaneously followed by a standardized breakfast after three hours. Five minutes after intake of the tablets the IntelliCap capsule was swallowed and pH was measured until excretion in the feces. Breath and urine samples were collected for isotope analysis.

Results

Full analysis could be performed in 12 subjects. Median bioavailability of ^{13}C-urea was 82% (95% CI 74 - 94%, range 61 - 114%). The median lag time (5% release of ^{13}C) was 5:42 h (95% CI 5:18 - 6:18 h, range 2:36 - 6:36 h,) There was no statistically significant difference between lag time based on isotope signal and colon arrival time (CAT) based on pH (median 5:42 vs 5:31 h p = 0.903). In all subjects an intestinal pH value of 7.0 was reached before release of ^{13}C from the ColoPulse tablet occurred.

Discussion and conclusions

From the combined data from the IntelliCap system and the ^{13}C-isotope signal it can be concluded that release from a ColoPulse tablet *in vivo* is not related to transit times but occurs in the ileo-colonic region after pH 7.0 is reached. This supports our earlier findings and confirms that the ColoPulse system is a promising delivery system for targeting the distal ileum and colon.

1. Introduction

Distal ileum and Colon-specific delivery of medicines is clinically relevant because their efficacy can be improved, side effects can be reduced and the bioavailability of drugs that are metabolized or poorly absorbed in the higher parts of the small intestine can be enhanced. This offers interesting perspectives for the treatment of for instance inflammatory bowel diseases with peptides [1].

In the literature different strategies for colon-specific delivery have been described. They include pH-responsive systems, time-based systems and systems triggered by the colon flora, as well as combinations of such systems [1,2]. The recently developed ColoPulse technology is a promising pH-responsive system being able to specifically deliver the active substance to the ileo-colonic region. Because of the non-percolating incorporation of a super-disintegrant in the coating, by which a more reliable and pulsatile release is achieved, it differs from other pH-responsive systems. The dissolution of this coating is triggered by the physiologically occurring increase in pH from 5.5 in the upper small intestine to 7.5 in the ileo-colonic region [3].

Until now bioavailability from ColoPulse dosage forms has been studied in healthy volunteers and in patients with Crohn's disease using stable isotopes of urea [4,5,6]. All studies showed a mean total bioavailability of ^{13}C-urea of > 76%, but the relationship between gastrointestinal pH and release from a ColoPulse dosage form has so far not been proven experimentally in vivo. To obtain more insight in the behaviour and functioning of ColoPulse tablets and capsules in vivo, studies on the gastrointestinal pH with the concomitant administration of a ColoPulse tablet are justified.

In the literature different devices for gastrointestinal pH measurement have been described [7]. They can be divided in two subgroups: the "static" devices and the "freely moving" devices. With representatives of the first group only the oesophageal, intra-gastric or peri-mucosal colonic pH can be measured. Their main disadvantage is the inability to measure the pH along the entire length of the gastrointestinal tract. The second group mainly comprises pH sensitive wireless radiotelemetry capsules (RTC). After administration of a RTC the subject is able to perform normal daily activities. Emitted radio signals are detected by a recorder mostly worn around the waist. If the device functions properly, measuring ends when the RTC is excreted with the feces. Disadvantages of the RTCs used so far are the frequent loss of signals, batteries running out of power before excretion, large pH-drift (up to approximately 1 unit) and difficulties in determining the exact location of the capsule in the gastrointestinal tract. In table 1 a summary of the available literature on freely

Table 1: Summary of available literature on gastrointestinal pH measurement →

Study	Device	Subjects	Battery life	pH Sampling interval	Position detection
Watson et al, 1972 [8]	Radiotelemetry Capsule	2 healthy subjects 7 patients with miscellaneous gastrointestinal disorders	10 days	60 min	Abdominal x-ray
Evans et al, 1988 [9]	Radiotelemetry capsule (Remote control systems Ltd, UK)	72 healthy subjects	-	12 seconds	"locator" to detect highest signal intensity
Fallingborg et al, 1989 [10]	Radiotelemetry Capsule (Remote control systems Ltd, UK)	39 healthy subjects	-	15 – 120 min (not between 11 pm and 8 am)	Fluoroscopy
Raimundo A et al 1992 [11]	Radiotelemetry Capsule	7 patients with acute colitis 6 patients with ulcerative colitis in remission	-	-	Based on pH
Fallingborg et al, 1993 [12]	Radiotelemetry Capsule	7 patients with ulcerative colitis	-	30 min (not between 11 pm and 8 am)	Fluoroscopy
Sasaki et al, 1997 [13]	Radiotelemetry Capsule (Remote control systems Ltd, UK)	4 healthy subjects 4 patients with Crohn's disease	1	1 second	Based on pH, x-ray, contrast colonogram and a radio-directional probe
Press et al, 1998 [14]	Radiotelemetry Capsule (7036, Oakfield instruments, UK)	12 healthy subjects 11 patients with ulcerative colitis 15 patients with Crohn's disease	-	-	"locator" to detect highest signal intensity
Fallingborg et al, 1998 [15]	Radiotelemetry Capsule (remote control systems Ltd, UK)	13 healthy subjects 9 patients with Crohn's disease	-	10-15 min	Fluoroscopy
Ewe et al, 1999 [16]	Radiotelemetry Capsule (7036, Oakfield instruments, UK)	15 healthy subjects 15 patients with Crohn's Disease 5 patients with ulcerative colitis	24 h	6 seconds	Metal detector
Maqbool et al, 2009 [17]	SmartPill, (Buffalo, NY, USA)	10 healthy subjects	5 days	5 seconds, after 24 h 20 seconds	Based on pH
Rubin et al, 2009 [18]	Smartpill, (Buffalo, NY, USA)	10 patients with active ulcerative colitis	-	-	Based on pH, motility and temperature
Lalezari et al, 2012 [19]	SmartPill, (Buffalo, NY, USA)	10 healthy subjects 9 patients with IBS	5 days	5 seconds	Based on pH
Schaar et al, 2013 [20] / Koziolek et al, 2014 [21]	(IntelliCap Medimetrics Eindhoven, NL)	2x 10 healthy volunteers	> 48 h	10 seconds	Study 1: based on pH and temperature. Study 2: also based on 99MTc

[a]- = no information in publication

with freely moving devices in healthy volunteers and/or inflammatory bowel diseases

Study	Food intake during study	Data loss	pH drift device during study	Total transit time	Remarks
Watson et al, 1972 [8]	Device intake after breakfast. No restrictions in food and beverages	-[a]	0.1 unit	-	
Evans et al, 1988 [9]	Device intake after overnight fasting. breakfast after leaving the stomach. No restrictions in food and beverages	In 14 subjects > 75% loss in the small intestine	pH 4: < 0.6 unit pH 9.2 < 1.0 unit	Mean: 23.3 h	Measurement up to 48 h. Median signal loss 20.4%. 2 subjects > 1.0 unit pH drift
Fallingborg et al, 1989 [10]	Device intake after overnight fasting; breakfast after leaving the stomach. Food and beverages according to the protocol	-	< 0.9 unit	9-129 h	
Raimundo et al, 1992 [11]	-	-	-	-	
Fallingborg et al, 1993 [12]	Device intake after overnight fasting; breakfast after leaving the stomach. No restrictions in food and beverages	-	< 0.4 unit	8 - 123 h	Measurement max 39h
Sasaki et al, 1997 [13]	Device intake after overnight fasting; breakfast after leaving the stomach. Food according to the protocol	-	< 0.5unit	-	
Press et al, 1998 [14]	Device intake after overnight fasting; breakfast after leaving the stomach. No restrictions in food and beverages	In 4 subjects > 75% loss in 24 h	< 0.5 unit	-	Measurement in the colon was marked as unpredictable. 4 subjects had to repeat the study
Fallingborg et al, 1998 [15]	Device intake after > 8h fasting; breakfast after leaving the stomach	-	< 0.4 unit	-	Difference in small intestine transit time between resected patients and healthy volunteers
Ewe et al, 1999 [16]	Device intake after > 8h fasting; breakfast after leaving the stomach	6 subjects excluded, several reasons	-	Median 24-31 h	In 1 subject > 2 weeks retention of RTC
Maqbool et al, 2009 [17]	2000 kcal diet, 30% fat. Device intake after breakfast	-	-	-	
Rubin et al, 2009 [18]	-	-	-	Median 24.6 h	No complications with the device
Lalezari et al, 2012 [19]	Device intake after > 8h fasting; breakfast after leaving the stomach	-	-	-	
Schaar et al, 2013 [20] / Koziolek et al, 2014 [21]	Device intake with water after overnight fasting. Food 4 h after device intake	Mean 3.5% (one subject 13%)	-	Average 30:34 h	Two publications, same studies

moving devices studied in inflammatory bowel diseases and/or healthy volunteers is presented [8-21].

Recently a medical device for the *in vivo* measurement of pH and temperature in the gastrointestinal tract was developed by Medimetrics (Eindhoven, The Netherlands): the IntelliCap system [20,21]. Furthermore the system can be used for electronically controlled drug delivery in defined sections of the gastrointestinal tract to quantify regional drug absorption [22]. It differs from the so far available freely moving RTCs by more accurate and more frequent measurements, minimal signal loss, a built-in drug reservoir and improved battery power (> 72 h) to ensure reliable and complete data acquisition. By combining diagnostic functionalities and the capability to generate adjustable controlled drug release profiles, the IntelliCap system can play a promising role in pharmaceutical drug profiling and formulation development.

In this paper we describe a study performed in healthy volunteers in which we studied the relationship between gastrointestinal pH obtained with the IntelliCap system as well as the release from a ColoPulse tablet to prove that release from a ColoPulse tablet indeed does occur in the ileo-colonic region and after a pH value of ≥ 7.0 is reached

2. Materials and methods

2.1. Subjects

Sixteen healthy volunteers (10 male, 6 female, age 18-65) were initially included in this study (table 2). Participant recruitment started January 2011 and ended April 2011. Written informed consent was obtained from all participants. They had no history of gastrointestinal diseases or gastrointestinal surgery. None of the subjects used antibiotics or drugs influencing the gastrointestinal transit time for at least three months prior to the start of the study. A possible *Helicobacter pylori* infection was excluded with a ^{13}C-urea breath test (INFAI, Köln, Germany).

Table 2: Demographics of included subjects (healthy volunteers)

	Median (range)
Sex (male/female)	10/6
Age (year)	27.5 (19-63)
Weight (kg)	77.0 (54.5-121.4)

A flowchart summarizing recruitment and analysis is shown in Fig. 1.

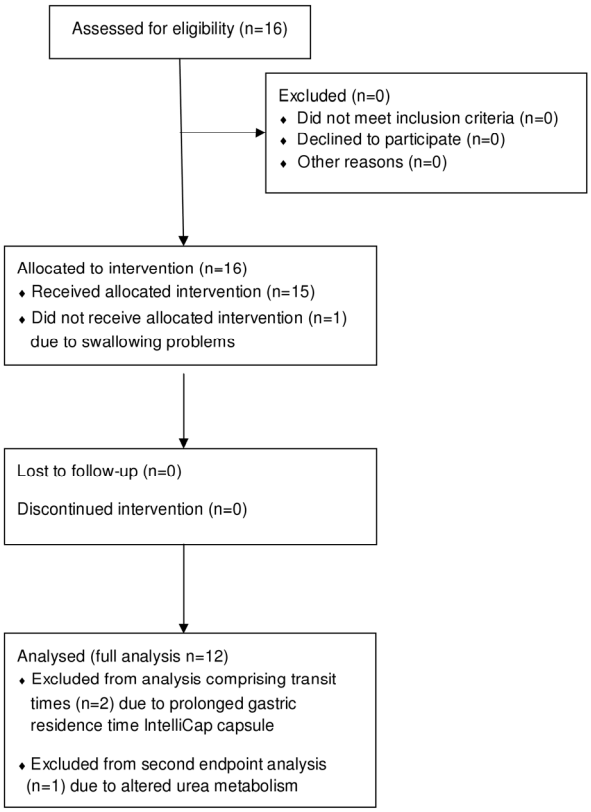

Figure 1: Flowchart.

2.2. Study-design

This bioavailability study was performed as an open-label, non-randomized, single arm clinical trial and was part of a study described by Maurer et al [6]. The design was based on a previous feasibility study in which two stable isotopes of urea are administered simultaneously [23]. Subjects were fasted from 8 p.m. the day before the test day. Only water, apple juice (until 11 p.m.) and unsweetened tea without sugar were allowed. On the test day an uncoated tablet containing 50 mg $^{15}N_2$-urea and a ColoPulse tablet containing 50 mg ^{13}C-urea were taken simultaneously at around 8 a.m. with 100 mL apple juice. Five minutes thereafter the IntelliCap capsule was swallowed with another 100 mL apple juice. A standardized breakfast was taken three hours after the intake of the tablets. The meal consisted of a standardized double sandwich and 200 mL

unsweetened tea. Approximately 6 and 10 hours after tablet intake, respectively lunch and dinner were taken. There were no food-restrictions for lunch and dinner except foods rich in ^{13}C, like corn products, cane sugar and pineapple. During the test day, (that ended at 8 a.m. the next morning) water, apple juice and tea were the only beverages allowed.

Sampling, administration of the tablets and IntelliCap capsule took place in a controlled facility until 5 p.m. Thereafter subjects went home were they continued sampling of breath and urine according to the study protocol. All necessary information was recorded in a diary. A summary of the study design is shown in table 3.

Table 3: Study schedule, activities are marked with an X (T0 is 8 a.m.)

Time (h)	-12	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	24
Arrival, start fasting	X																	
Intake tablets		X																
Intake IntelliCap		X																
Meal					X			X				X						
Urine Sample		X				X			X		X			X			X	X
Breath sample		X	X	X	X	XX	XX	X	XX	XX	XX	XX	XX	X	X	X	X	
Return home											X							

2.3. Ethics statement

The study was approved by the ethical committee of the University Medical Center Groningen (ref 2009.188 / EudraCT 2009-01347121) and was performed according to the principles of the Declaration of Helsinki. The study has been registered in the ISRCTN register (ISRCTN18301880). This was not done before the start of recruitment because at that time this was not required by internal procedures and the ethical committee that approved this study.

2.4. IntelliCap system

The IntelliCap system was supplied by Medimetrics (Eindhoven, The Netherlands) and consisted of a capsule and a portable unit. The size of the IntelliCap capsule was 27 x 11 mm. A complete description including illustrations of the IntelliCap system can be found in the literature [20]. The drug reservoir was filled with normal saline solution which had no function in this study and was not expelled from the IntelliCap capsule during the experiments.

Data were measured until excretion of the IntelliCap capsule or until the battery ran out of power. The excretion of the IntelliCap capsule from the body had to be confirmed by collecting the device from the stool. If the IntelliCap capsule was not retrieved within 96 hours it was probably missed and its absence from

the body was confirmed with an abdominal X-ray. No follow up was required after retrieving the IntelliCap capsule or confirmation of its absence by X-ray.

2.5. Analysis of pH profiles

The pH profiles were analysed for gastrointestinal landmarks (ingestion, pylorus, ileocecal valve and excretion) using the following criteria:

- Ingestion: rapid and sustained rise in temperature from room to body temperature and a rapid drop of > 3 pH units
- Pylorus: rapid and sustained rise of at least 3 pH units
- Ileocecal valve (cecum): first and rapid drop of > 0.8 pH units at least 1 h after the pylorus to pH ≤ 6.5
- Excretion: rapid and sustained drop in temperature from body to room temperature

Gastrointestinal residence and transit times were derived from the identification of gastrointestinal landmarks and are defined as follows:

- Gastric residence time (GRT): elapsed time between ingestion and pylorus
- Small intestine transit time (SBTT): elapsed time between pylorus and ileocecal valve
- Colonic arrival time (CAT): elapsed time between ingestion and ileocecal valve
- Colonic transit time (CTT): elapsed time between ileocecal valve and excretion
- Whole gut transit time (WGTT): elapsed time between ingestion and excretion

2.6. Chemicals, isotopes and coated tablets

All substances were of pharmacopoeial grade (Ph. Eur. or USP) and were obtained via a certified wholesaler as described before [6]. The stable isotopes ^{13}C-urea and $^{15}N_2$-urea (AP 99%) were obtained from an FDA-controlled facility (Isotec, USA). Tablet cores containing 50 mg ^{13}C- or 50 mg $^{15}N_2$-urea and 25 mg caffeine were compounded in the Department of Hospital and Clinical Pharmacy of the University Medical Center Groningen and analysed according to the European Pharmacopoeia 7th edition

A ColoPulse coating of 13-17 mg/cm^2 was applied on the tablets containing ^{13}C-urea .The coating was composed of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol:talc in a ratio of 7:1:3:2 (w/w). The solvent was an acetone-water 97:3 mixture (w/w). Coating thickness was determined and expressed as the amount

of Eudragit S100 applied per cm^2. Caffeine was added to the ^{13}C-urea tablet cores for quality control purposes and was used as a marker substance for the *in vitro* determination of the release characteristics lag- and pulse time in the *in vitro* dissolution test. Caffeine was also added to the $^{15}N_2$-urea tablet cores to obtain comparable tablet cores, with no particular function in this tablet. All tablets, coated and uncoated, met established pharmaceutical quality control criteria [6].

2.7. Urea-kinetics

To study the bioavailability from a ColoPulse tablet in the ileo-colonic region the difference in kinetics and fate between ^{13}C-urea and $^{15}N_2$-urea was used. An overview of the relevant kinetic steps can be found in Maurer et al [23,24]. Release of ^{13}C-urea in the ileo-colonic region (urease-rich) from a ColoPulse tablet leads to *in situ* fermentation of ^{13}C-urea into $^{13}CO_2$ which is subsequently exhaled in breath. The delivery of the isotope in the colon can therefore be established by measuring the $^{13}CO_2$ response in breath. Unfermented urea (i.e. release in the small intestine, urease-poor) can be measured as the amount of ^{13}C-urea in urine. The second stable isotope of urea, $^{15}N_2$-urea, in an uncoated tablet functions as a reference and reflects 100% release in a urease-poor region. Release of $^{15}N_2$-urea in the small intestine from an uncoated capsule leads to recovery of $^{15}N_2$-urea in urine. Bioavailability can be described by the difference between kinetics of ^{13}C- and $^{15}N_2$-urea [23,24].

2.8. Sample collections and analysis

Breath samples were collected every 0.5-1 h up to 15 h after intake of the tablets (table 2) and were analysed as described before [23]. Briefly, $^{13}C/^{12}C$ isotope ratios in the CO_2 of breath samples were analysed by using a validated breath ^{13}C-analyser (Thermo Fisher Scientific, Bremen, Germany) based on isotope ratio mass spectrometry (IRMS).

Urine samples were collected during 24 h after intake of the tablets at prescribed intervals (table 2) in 500 or 1000 mL containers containing an aliquot of 6M HCl. Urine volumes were recorded and 20 mL samples were stored at -80˚C until analysis. Concentrations of total ^{15}N and ^{13}C were determined as described before using an elemental analyzer interfaced with IRMS [23].

2.9. Calculations

The Percentage of the administered Dose Recovered (PDR) of ^{13}C and ^{15}N in each urine sample, the ratio of the PDRs ^{13}C versus ^{15}N (the $^{13}C/^{15}N$-ratio), the fermented ($F_{fermented}$) and not-fermented ($F_{not\text{-}fermented}$) fraction of ^{13}C urea were calculated as described before [23,24]. In short:

- $F_{fermented}$ was calculated as the cumulative (c)PDR of ^{13}C in breath over a 15 h time period
- $F_{not\text{-}fermented}$ = cPDR ^{13}C / cPDR ^{15}N in a 24 h urine collection
- Bioavailability = $F_{fermented} + F_{not\text{-}fermented}$
- The lag time was derived from the cPDR of ^{13}C in breath and was defined as de time between administration of the tablets and the time the cPDR reached the value of 5% of cPDR at t = 15 h

All data were corrected for baseline-concentrations of ^{13}C and ^{15}N in breath and /or urine. Furthermore, breath data were corrected for CO_2-retention as described before [4].

2.10. Statistical procedures

This study was performed as a bioavailability study. Based on previous data on transit times of a ColoPulse tablet a sample size of 10 patients is needed to detect a clinically relevant difference of 15% between lag-time based on isotope-signal and colon arrival time based on pH with 80% power and a significance level of $\alpha = 0.05$ (two sided). Because this study was part of another study [6] requiring a higher sample size and anticipating some drop-out 16 subjects were included.

The results were evaluated by descriptive statistics with SPSS version 22. Normal distribution of the data was investigated with the Shapiro-Wilk test. The center was characterized by the mean and standard deviation (pH-data) or the median and corresponding bootstrap based 95% confidence intervals (95% CI). The dispersion was characterized by the coefficient of variation (CV) and range because not all data were normally distributed. A (parametric) paired-samples t-test (two tailed, $\alpha = 0.05$) was used to compare the results within groups when data were normally distributed for both variables. A (non-parametric) Wilcoxon signed rank test was performed to compare the results when at least one of the variables was not normally distributed. Differences were considered significant when $p < 0.05$.

2.11. Endpoints

The endpoint was to investigate the relationship between the gastrointestinal pH-profile obtained with the IntelliCap system and release of ^{13}C-urea from a ColoPulse tablet and to confirm that release occurs in the ileo-colonic region after pH 7.0 has been reached.

3. Results

The results of 15 out of 16 healthy volunteers initially included in the study were evaluated (Fig. 1). One volunteer could not swallow the IntelliCap capsule and was therefore excluded without replacement. Two other subjects (6 and 15) appeared to have a prolonged gastric residence time and the IntelliCap capsule was still in the stomach when breakfast and the following meals were taken. Because their gastric residence time was respectively 17 and 22 h data of these subjects were excluded from any analysis comprising transit times. Their lag time based on the isotope signal was within the normal range. Subject 5 was also excluded from analysis comprising lag time and bioavailability, because of a probably altered urea metabolism. This was concluded from the fact that the cPDR ^{13}C in breath was < 6.5% after 15 h combined with a cDPR of unfermented ^{13}C in urine of 70% at t = 24 h. The pH profile of this subject was normal with a GRT of 0:15 h and a SBTT of 3.15 h. The coating functioned well, because the appearance of ^{13}C in the urine sample could be seen in the sample collected between t = 4 and 7 h and not earlier. This means that ^{13}C-urea in stead of being fermented was absorbed into the bloodstream when it was released at the ileo-colonic region. The data from the remaining 12 subjects were available for all analyses.

IntelliCap capsules could be recovered from the feces within 72 hours after intake in 13 out of 15 subjects. For two subjects the temperature data indicated that the IntelliCap capsule had left the body, but the subjects failed to retrieve it from the feces. Absence from the body was confirmed with an abdominal X-ray. No adverse events potentially related to the IntelliCap system were observed during the study.

In three subjects the portable unit ran out of power after circa 60 hours. This did not influence the data collection necessary for the endpoint analysis because in all subjects the IntelliCap capsule already passed the cecum. However, for these subjects time of excretion and whole gut transit time (WGTT) could not be determined.

In three other subjects the communication between the capsule and portable unit was interrupted varying from 4 to 12 h because the subjects did not wear the portable unit close enough to the body or did not wear it. This also did not influence the data collection for endpoint analysis because all interruptions occurred more than 24 h after intake, when the IntelliCap capsule already had passed the cecum. For one of these subjects excretion, CTT and WGTT could not be determined because excretion occurred during the period of interrupted communication. No other loss of data was encountered in the study.

All gastrointestinal pH profiles recorded with the IntelliCap system were analysed according to the mentioned methods. A summary of the gastrointestinal transit times is shown in Fig. 2 and a representative example of a pH and temperature profile is shown in Fig. 3. The residence in the stomach, passage of the pylorus, course of pH in the small intestine and the ileocecal valve (cecum) are all clearly visible in this figure. From Fig. 2 it is obvious that there are large inter-individual differences in transit times. For example, the colon arrival time (CAT) differs from 3:25-8:20 h (median 5:31 h, 95% CI 4:51 – 5:48 h, CV 26%) and the whole gut transit time for the IntelliCap capsule was 10:01-59:39 h (median 27:08 h, 95% CI 22:49 – 59:11 h, CV 52%). Gastric residence time varied between 0:15 and 3:14 h (median 1:30 h, 95% CI 1:05 – 2:08 h, CV 59%). The median difference between the time when pH 7.0 was reached and the CAT was 2.26 h and in most subjects pH remained > 7.0 until the cecum was reached. A summary of measured pH values in the stomach, small intestine and colon is shown in Fig. 4.

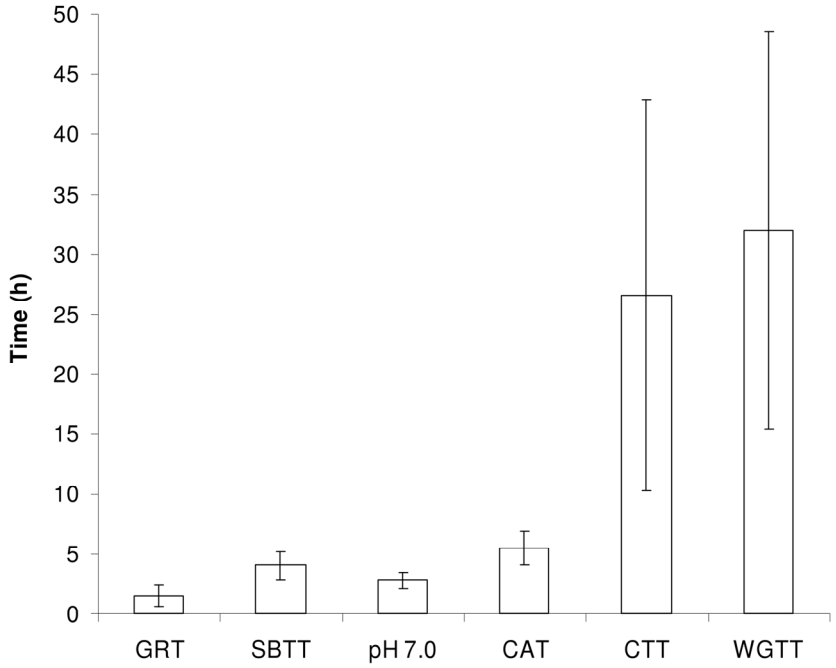

Figure 2: Mean (gastrointestinal residence and transit times determined with the IntelliCap system. Data are presented mean and standard deviation of 13 evaluable subjects (for CTT and WGTT n = 9).

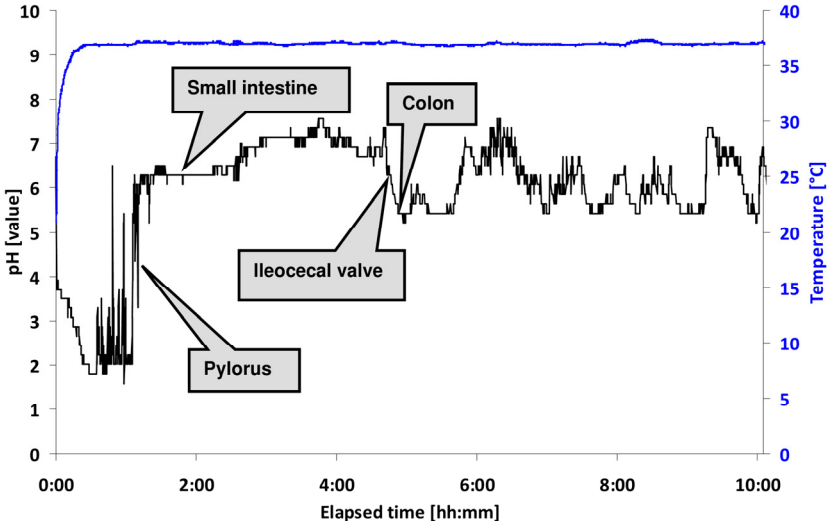

Figure 3: Example of pH-profile of the first 10 hours after intake of the IntelliCap capsule (subject 14)

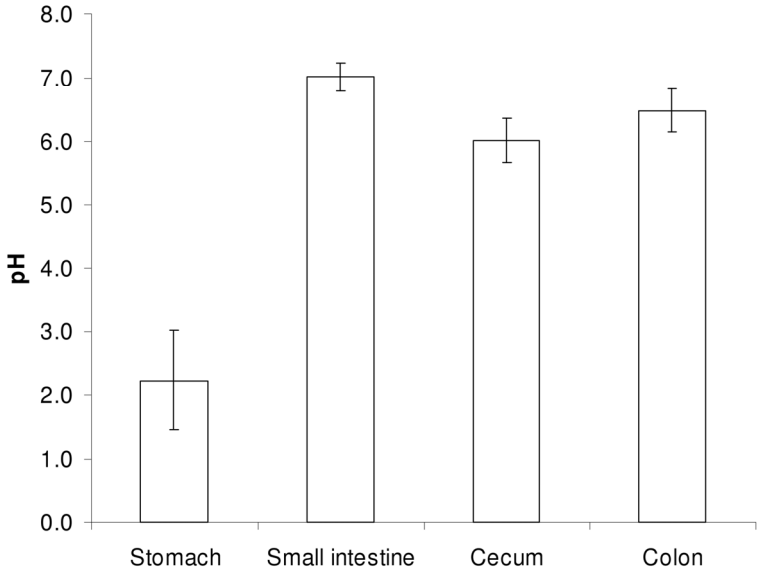

Figure 4: Summary of pH in the stomach, small intestine and colon as measured with the IntelliCap system. Data are presented as mean and standard deviation of 15 evaluable subjects.

Lag time and bioavailability of ^{13}C-urea from a ColoPulse tablet were calculated as described. The median lag time was 5:42 h (95% CI 5:18 – 6:18 h, range 2:36 - 6:36 h, CV 18%) and median bioavailability was 82% (95% CI 74 - 94%) range 61 - 114%, CV 10%). More detailed results can be found in S1 summary and in Maurer et al [6].

There was no statistically significant difference between CAT based on pH-data (IntelliCap) and the lag time of the ColoPulse tablet based on the stable isotope signal of ^{13}C-urea in breath (median 5:31 vs 5:42 h, p = 0.903, parametric test). A representative example is shown in Fig. 5. Information about all subjects can be found in Fig. 6.

In all subjects a pH value of 7.0 was reached before release of ^{13}C from the ColoPulse tablet occurred, as measured in exhaled breath. There was a statistically significant difference between the time when pH 7.0 was reached and the lag time (185 vs 342 minutes, p = 0.002, non-parametric test).

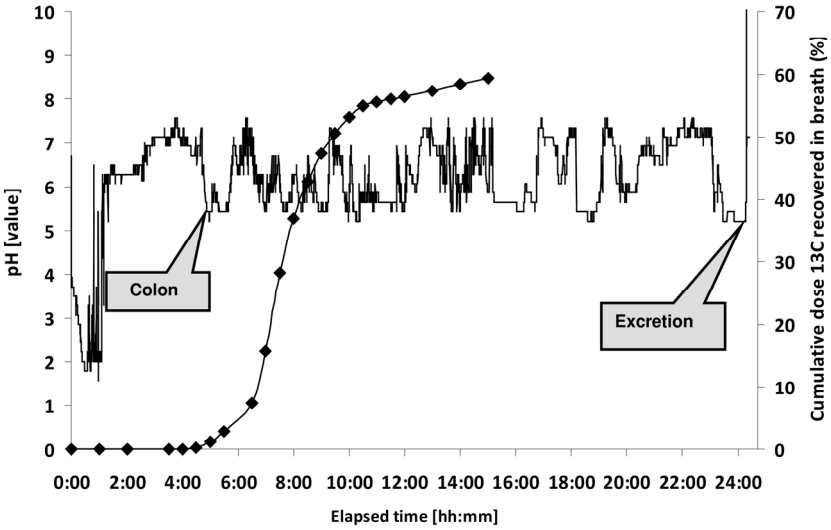

Figure 5: Colon arrival time based on pH (-) corresponds with release of ^{13}C (♦) (subject 14). See also Fig. 4 for the first 10 hours.

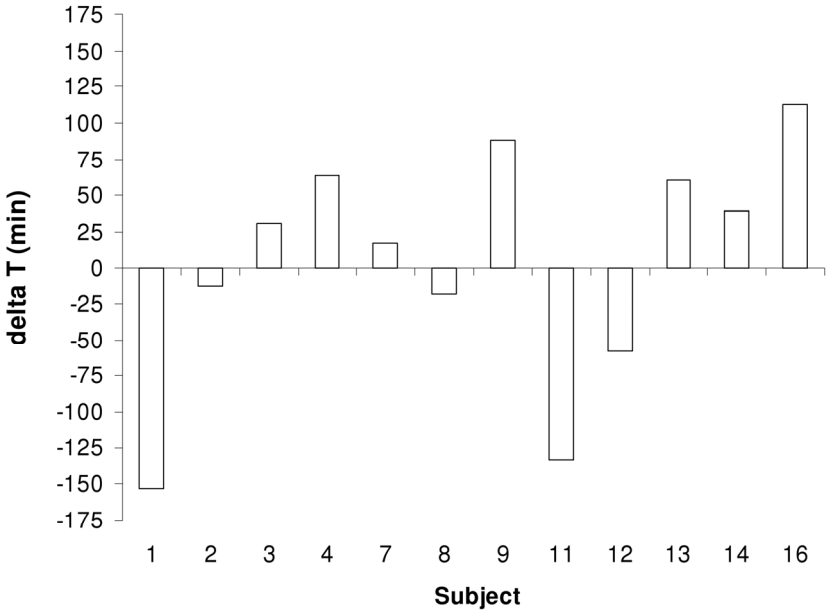

Figure 6: Difference (delta T) between lag-time (based on isotope signal) and CAT (based on pH) for each subject (n = 12).

4. Discussion

This is the first study in humans combining release from ColoPulse tablets and *in vivo* gastrointestinal pH measurements using the IntelliCap system. There was no difference between CAT (pH-data) and lag time (isotope data) found in this study which delivers proof that release of ColoPulse tablets occurs in the ileo-colonic region. Furthermore the results show that release from the ColoPulse tablet does not occur before an intestinal pH value of 7.0 is reached.

Several studies have been published with radiotelemetry capsules (RTC). Through the years the functioning of RTCs improved considerably. The first RTCs were used in the 1970's and had logging intervals from 5 to 120 minutes to save battery capacity [8,10]. Furthermore high data loss (75%) has been described [9]. Thereafter RTCs with shorter logging intervals up to 5 seconds became available and battery life improved considerably [16,17,19]. However in some studies data loss was still described mostly attributed to the angle between the RTC and the antenna [16]. In the current study with the IntelliCap system data loss was observed in three subjects out of 15. This didn't occur in the first 8 hours after intake of the capsule, when data were also sent to the control center,

but only when the subjects were at home and did not keep their receiver close enough to the body. Therefore this event did not influence the outcome of our study, because the IntelliCap capsule already passed the cecum at the time data loss occurred. On the other hand, this shows that an overnight stay in a controlled facility is preferable when longer pH profiling is needed.

The IntelliCap system was able to record complete gastrointestinal pH and temperature profiles as well as derived transit-times from intake to excretion. The observed gastrointestinal transit times for the small intestine and the colon are within the range of earlier published data of healthy volunteers, only the median GRT appeared to be increased [10,25]. Published data collected with RTC and dosage forms labeled with gamma emitting radionuclides mention an average GRT of about ≤ 1 h in fasted, healthy volunteers while we observed an average gastric residence time of 1:30 h in the evaluable subjects. A likely explanation is the intake of the tablets with apple juice, since it is known that food increases the GRT of pharmaceutical dosage forms [25]. Due to its caloric content apple juice apparently also has a delaying effect. However in this study, the apple juice was given to get the same study design as previous studies with ColoPulse formulations. This was done to be able to compare the functioning of ColoPulse tablets used in this study with capsules which were used in previous studies. In the future ColoPulse formulations can also be administered with water.

All subjects showed an elevated gastric pH (pH 3-4) immediately after administration probably due to administration with apple juice, which decreased to a more acidic level of around pH 1.6 in about 30 minutes. Because in our study the determination of the location of the IntelliCap capsule was only based on pH, pH values of the different segments of the small intestine and colon could not be determined. The median pH values of the stomach, small intestines and colon as observed in the majority of subjects are consistent with published data from fasted, healthy volunteers [7].

No difference was found between the colon arrival time based on the ^{13}C-isotope signal (lag time of ColoPulse tablets) and pH-measurements (from IntelliCap system). This proves the site-specific release of the active substance from the ColoPulse tablets in the ileo-colonic region. Simultaneous migration of the ColoPulse tablet and the IntelliCap capsule after leaving the stomach is supported by the literature. According to Davis et al [25] no difference in intestinal transit times was seen between solid dosage forms with the same size of ColoPulse tablets and the IntelliCap capsule. Gastric emptying of large single unit systems however, was highly influenced by the presence of food in the stomach. Even a light breakfast delayed emptying in some subjects. This may

explain the increased stomach residence time of the IntelliCap capsule in two subjects as seen in the current study. In these subjects colon arrival time and bioavailability based on isotope signal were within the normal range. However the relatively large IntelliCap capsule was retained in the stomach for respectively 17 and 21 h, probably because they returned to their "normal" meal intakes when the IntelliCap capsule was still in the stomach. After pylorus passage of these two IntelliCap capsules intestinal transit times were comparable to those of the other subjects.

In this study we observed no relation between the lag-time of a ColoPulse tablet and the time when pH 7.0 was reached or the CAT. However, in none of the subjects release from the ColoPulse tablet occurred before pH 7.0 was reached.

The difference between the time when pH 7.0 was reached and the CAT was approximately 2.5 hours, supporting the fact that release occurs in the distal ileum and colon. Remarkably, this difference in time is relatively large and differs from parameters used in vitro dissolution tests that were performed with the ColoPulse tablets in the gastrointestinal simulation system (GISS) [26]. We use this in vitro test for quality control of ColoPulse tablets and normally dissolution of the coating and subsequent release occurs within 30 minutes after raising pH from 6.8 to 7.5. However, the volumes of intestinal fluid in vivo differ from the volumes used in the GISS. According to Schiller et al [27] the fluid volume of the small intestine has a maximum of 319 mL while the volume in this stage of the GISS is as high as 940 mL. Furthermore the fluid is not distributed homogenously along the small intestine in vivo with water pockets and "dry" segments randomly scattered. This contributes to the relatively slow dissolution of the Eudragit-S coating and is probably the cause of the relatively large difference between the time point at which pH 7 was reached and the CAT. The clinical relevance of this phenomenon seems to be limited because median bioavailability was 82%.

5. Conclusion

Based on the combined data from the IntelliCap system and the urea-isotope signal from a ColoPulse tablet as obtained in this study in healthy volunteers it can be concluded that release from ColoPulse tablets indeed occurs in the distal ileum and colon and after pH 7.0 is reached. This supports our earlier observations and confirms that the ColoPulse system is a promising delivery system for site-specific delivery and local therapy in inflammatory bowel diseases present in the distal ileum and colon.

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CHAPTER 7

ColoPulse tablets perform comparably in healthy volunteers and crohn's patients and show no influence of food and time of food intake on bioavailability

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Abstract

ColoPulse tablets are an innovative development in the field of oral drug delivery and are characterized by a colon-specific release. Until now ColoPulse dosage forms (only capsules) have been studied in healthy volunteers having a standardized breakfast three hours after administration but not in specific patient groups and not with a shorter interval between administration and breakfast. Information on bioavailability and release characteristics of ColoPulse tablets in Crohn's patients and the influence of food and time of food intake is a prerequisite to properly design future clinical studies with active substances in these patients.

In the current cross-over study bioavailability and drug release characteristics of ColoPulse tablets were compared in healthy volunteers and in Crohn's patients in remission. Furthermore the influence of food and time of food intake on the *in vivo* drug release behaviour of ColoPulse tablets was investigated.

In this study the dual label isotope strategy was used which means that a ColoPulse tablet containing ^{13}C-urea and an uncoated, immediate release tablet containing $^{15}N_2$-urea were taken simultaneously. Breath and urine samples were collected during the test day for isotope analysis. The appearance of the stable isotopes in breath and/or urine provides information on the site of release from the dosage form, release characteristics and bioavailability.

Both tablets were administered on two different days in a cross-over design: the first day with a breakfast (non-standardized) one hour after administration and the second day with a standardized breakfast three hours after administration of the tablets. There was no difference in instructions for administration between both days.

Results of 16 healthy volunteers and 14 Crohn's patients were evaluated. At least 86% (51 out of 59) of all ColoPulse tablets administered in this study released their contents at the desired intestinal region. There was no significant difference in bioavailability between healthy volunteers and Crohn's patients on both days (day 1 75.8% vs 90.2%, $p = 0.070$ and day 2 83.4% vs 91.4%, $p = 0.265$). There was also no significant influence of food and time of food intake on bioavailability in healthy volunteers (75.8% and 83.4%, $p = 0.077$) and in Crohn's patients (90.2% and 91.4%, $p = 0.618$) when day 1 and day 2 were compared. Release characteristics did not significantly differ between healthy volunteers and Crohn's patients. However, food and

time of food intake had some, clinically non-relevant, influence on the release characteristics within both groups which is in line with the fact that food affects gastro-intestinal transit times.

This study shows that ColoPulse tablets enable the site-specific delivery of drugs or other compounds (e.g. diagnostics) deep in the ileo-colonic region of the intestine of Crohn's patients in a comparable amount and rate as in healthy volunteers. Food and time of food intake had no relevant influence on bioavailability. In conclusion ColoPulse delivery systems are promising and deserve further research for local therapy with immunosuppressive drugs in Crohn's patients in the near future.

1. Introduction

Oral dosage forms with a site-specific drug release in the colon are of interest because they have the potential to improve the drugs efficacy or to minimize side effects of both locally or systemically acting drugs especially in the case of systemic administration. This may have advantages in the treatment of for instance inflammatory bowel diseases. Furthermore, colon-specific dosage forms may be used to improve the bioavailability of drugs that are poorly absorbed in the higher parts of the small intestine or are metabolized in the upper intestinal tract, such as peptide-based drugs [1].

In the literature different strategies for colon-targeting have been described. They include pH-responsive systems, time-based systems and systems triggered by the colon flora, as well as combinations of such systems [1,2]. The ColoPulse technology is a typical example of a pH-responsive system that delivers a drug to the ileo-colonic region. Release from the coated ColoPulse system is triggered by a physiologically occurring variation in the gastrointestinal pH in the terminal ileum and occurs at pH > 7.0. It differs from other pH responsive systems because of the non-percolating incorporation of a disintegrant in the coating, yielding a highly reliable and pulsatile release pattern in the targeted region [3].

In two previous studies [4,5] we determined the bioavailability and release profile from a ColoPulse capsule in healthy volunteers using the stable isotope ^{13}C-urea. Release of ^{13}C-urea in the ileocolonic intestinal region (urease-rich) from a ColoPulse capsule leads to *in situ* fermentation of ^{13}C-urea into $^{13}CO_2$ which is subsequently exhaled in breath. The delivery of the isotope in the colon was established by measuring the $^{13}CO_2$ response in breath. The release characteristics could also be derived from the breath ^{13}C-measurements and correlated well with the release characteristics derived from blood. Unfermented urea (i.e. release in the small intestine, urease-poor) was measured in blood and urine. Bioavailability was defined as the sum of the recovery of fermented ^{13}C-urea in breath and the amount of unfermented urea in blood or urine and ranged from 93 to 99%. A strong correlation ($r = 0.943$) was found between blood and urine kinetics, indicating that non-invasive urine sampling could replace blood sampling. In these studies the release of ^{13}C-urea from a ColoPulse capsule was compared with the release from an uncoated, immediate release capsule containing ^{13}C-urea administered on a second test day as a reference.

We recently improved the study design by using an uncoated capsule with a second stable isotope of urea, $^{15}N_2$-urea, as a reference on the same day in stead of a ^{13}C-urea uncoated capsule on a different day [6]. This simplifies the study

design, reduces costs and eliminates day-to-day variation in urea metabolism. Release of $^{15}N_2$-urea in the small intestine from an uncoated capsule leads to recovery of $^{15}N_2$-urea in urine. Bioavailability can be described by the difference between kinetics of ^{13}C- and $^{15}N_2$-urea (figure 1, a modified version of this figure was published before [6]). The difference in kinetics of these urea isotopes reflects release characteristics, can be used to determine the gastro-intestinal region of release and can be helpful in determination of the cause of a failure in release from a ColoPulse dosage form.

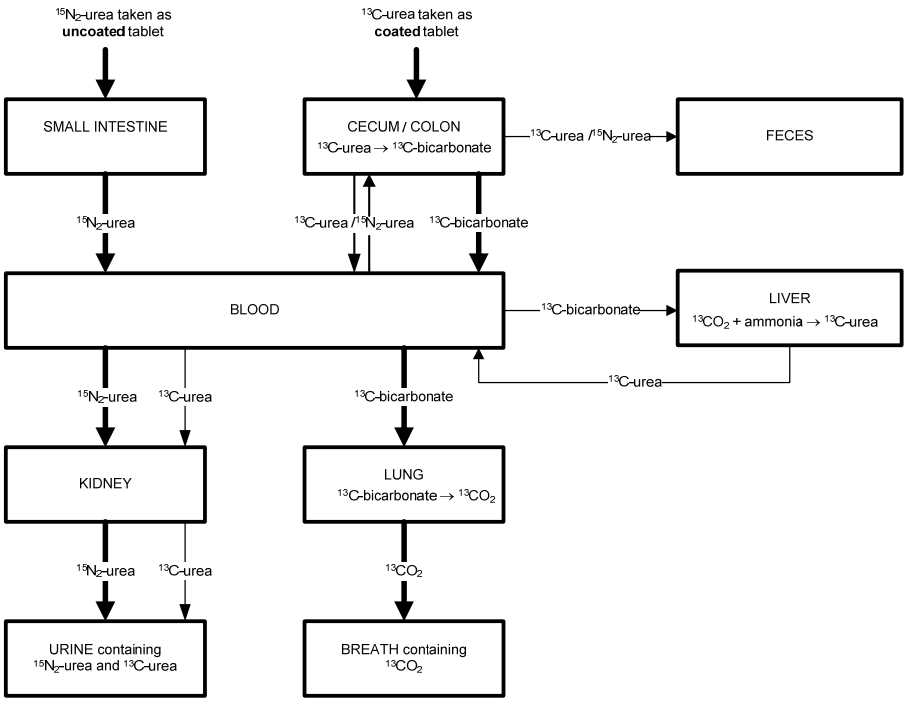


Figure 1: Absorption, metabolism and elimination of ^{13}C-urea and $^{15}N_2$-urea. The weight of the arrow symbolizes the importance of the kinetic step. $^{15}N_2$-urea administered as an uncoated tablet is mainly excreted in urine. ^{13}C-urea administered as coated tablet is converted to ^{13}C-bicarbionate by bacterial fermentation in the colon followed by absorption in blood and exhalation as $^{13}CO_2$ in breath.

Reliable delivery in the targeted intestinal region of the contents from ColoPulse coated capsules has been shown in 18 healthy volunteers [4-6]. Driven by this success we are currently developing ColoPulse tablet formulations for colon-specific delivery and subsequent local therapy of the intestinal mucosa with

immunosuppressive drugs, like TNF-α inhibitors, for Crohn's patients. Currently these types of drugs are only available as parenteral formulations. This requires, depending on the type of drug, parenteral self-administration or frequent visits to the out-patient clinic for intravenous administration. Parenteral administration of immunosuppressive drugs always harbors the risk of infusion reactions, i.e for Infliximab in 10 - 40% of the cases [7]. Furthermore parenteral administration can lead to unwanted systemic side effects.

We believe that administration of such drugs in a ColoPulse tablet may overcome the mentioned problems. However, before we can test this hypothesis and studies with an active substance can be carried out in a specific patient group, it is necessary to determine the performance of the ColoPulse system in this group of patients and to compare it with the performance in healthy volunteers. This is especially of importance, since it is so far unknown whether the conditions of the diseased intestine might affect the performance of the ColoPulse system.

In this paper we describe a cross-over study in healthy volunteers and in Crohn's patients in remission with the aim to determine drug release characteristics in terms of lag- and pulse time and bioequivalence (marker substance bioavailability) of ColoPulse tablets between both groups. Furthermore, the influence of food and time of food intake on the *in vivo* drug release behaviour of ColoPulse tablets is investigated both in healthy volunteers and in Crohn's patients, a prerequisite to properly design future clinical studies with immunosuppressive drugs.

2. Material and methods

2.1. Study design

The bioavailability study design was based on a previous feasibility study in which two stable isotopes of urea are administered simultaneously [6]. The current study was performed on two different test days with at least one week wash-out between both test days. On the respective test days, further designated as 'day 1' and 'day 2', an uncoated tablet containing 50 mg $^{15}N_2$-urea and a ColoPulse tablet containing 50 mg ^{13}C-urea were taken simultaneously at around 8 a.m. On day 1 a non-standardized breakfast chosen by the subjects was taken one hour later, on day 2 a standardized breakfast three hours later, this to investigate the influence of food and the time of food intake on bioavailability. Food and time of food intake were varied simultaneously to mimic realistic conditions for further clinical studies in which subjects have to take the tablets

every day for a period of several weeks. We decided to investigate only the administration schedules which we planned to use in future clinical studies.

During the experiments the subjects' food and liquid intake were standardized as described before [6] except for the breakfast on day 1. This breakfast was chosen by the subjects and varied largely between the subjects; from two crackers with marmalade to six slices of bread with various types of filling. This represents the variety of breakfasts among different people on a normal day. The breakfast of day 2 consisted of a standardized double sandwich and 200 ml (unsweetened) tea. The subjects were fasted from 8 p.m. the day before both test days. Only water, apple juice (until 11 p.m.) and tea without sugar were allowed. On the test day the tablets were swallowed with 200 ml of apple juice. Approximately 5 and 10 hours after tablet intake, lunch and dinner were taken. There were no food-restrictions for lunch and dinner except foods enriched in ^{13}C, like corn products, cane sugar and pineapple. During the test day, (that ended at 8 a.m. the next morning) water, apple juice and tea without sugar were the only drinks allowed.

The study was approved by the ethical committee of the University Medical Center Groningen (EudraCT 2009-01347121) and the study was performed according to the principles of the Declaration of Helsinki.

2.2. Subjects

16 healthy volunteers (10 male, 6 female, age 19-63) and 16 Crohn's patients (7 male, 9 female, age 22 - 64) in remission (Harvey Bradshaw ≤ 3, no intestinal stenosis, no ileocecal resection or other intestinal surgery) were initially included in the study. The healthy volunteers had neither a history of gastrointestinal diseases (ulcerative colitis, Crohn's disease, spastic colon, colon cancer, ileus, stoma, stomach- and/or gastrointestinal infection) nor of gastrointestinal surgery. None of the subjects used antibiotics or drugs influencing the gastrointestinal transit time for at least three months prior to the start of the study. A possible *Helicobacter pylori* infection was excluded with a ^{13}C-urea breath test (INFAI, Germany).

2.3. Chemicals, drug substances and drug products

Polyethylene glycol 6000, acetone, caffeine, colloidal anhydrous silica, sodium stearyl fumarate, talc (BUFA, The Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany), were obtained via a certified wholesaler (Spruyt-Hillen, The Netherlands). Sucrose was obtained from VWR (the

Netherlands). Inulin 4.0 kD was obtained from Sensus (the Netherlands) and water for injections was obtained from Fresenius Kabi (Germany). All ingredients were of pharmacopoeial grade (Ph Eur or USP). The stable isotope labelled ^{13}C-urea and $^{15}N_2$-urea (AP 99%) was obtained from a FDA-controlled facility (Isotec, USA). Tablet cores containing 50 mg ^{13}C- or 50 mg $^{15}N_2$-urea and 25 mg caffeine were compounded in the Department of Hospital and Clinical Pharmacy of the University Medical Center Groningen and analysed according to the European Pharmacopoeia 7th edition (table 1). A coating was applied on the tablets containing ^{13}C-urea using the ColoPulse technology. The coating of ColoPulse tablets was composed of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol:talc in a ratio of 7:1:3:2 (w/w). The solvent was an acetone/water 97:3 mixture (w/w). Coating thickness was determined and expressed as the amount of Eudragit S100 applied per cm^2 [3]. Caffeine was added to the ^{13}C-urea tablet cores for quality control purposes and was used as a marker substance for the *in vitro* determination of the release characteristics lag- and pulse time in the *in vitro* dissolution test. Caffeine was also added to the $^{15}N_2$-urea tablet cores to obtain comparable tablet cores, with no particular function in this tablet. All tablets, coated and uncoated, met established quality control criteria (table 1).

Table 1: Quality control data of 50 mg $^{15}N_2$-urea tablets and ColoPulse 50 mg ^{13}C-urea tablets

Parameter	**Specification**	**Result $^{15}N_2$-urea (uncoated)**	**Result ^{13}C-urea (coated)**
Crushing strength (cores)	150-200 N	160 N	175 N
Friability (cores)	< 1% (Ph Eur)	0.03%	0.03%
Disintegration (cores)	< 15 min (Ph Eur)	1.5 min	3.5 min
Uniformity of dosage units (urea, n = 10)	AV < 15 (Ph Eur)	14.8	13.1
Uniformity of dosage units (caffeine, n = 10)	AV < 15 (Ph Eur)	11.1	11.6
Content (urea, n = 10)	90-110%	104.3%	106.3%
Coat thickness (n = 20)	13-17 mg/cm^2	n.a.[a]	15.1 mg/cm^2
Bursts or cracks in coating (n = 6)	none	n.a.	none
Lag time (n = 6)	> 240 min	n.a.	244 min
Pulse time (n = 6)	< 60 min	n.a.	21 min
Release at t360 min (n = 6)	> 80%	n.a.	106.3%

[a]n.a. = not applicable

The pulsatile release properties are reflected by the lag time ($t_{5\%\ release}$) and the pulse time. The lag time is the time point at which the tablets start to release the

active substance and was defined as the time at which 5% of 25 mg caffeine was released. The pulse time reflects the pulsatile release characteristics and was defined as the period between the lag time ($t_{5\%\ release}$) and $t_{70\%\ release}$. These parameters were established in a modified dissolution test with a total duration of 360 minutes in which the pH was varied in time to simulate the different stages of the gastrointestinal tract as described before [8].

2.4. Sample collections and analysis

Breath samples were collected every 0.5-1 h up to 15 h after intake of the tablets and were analyzed as described before [4]. Briefly, $^{13}C/^{12}C$ isotope ratios in the CO_2 of breath samples were analyzed by using a validated breath ^{13}C-analyser (Thermo Fisher Scientific, Bremen, Germany) based on isotope ratio mass spectrometry (IRMS).

Urine samples were collected during 24 h after intake of the tablets at prescribed intervals in 500 or 1000 ml containers containing an aliquot of 6M HCl. Urine volumes were recorded and 20 ml samples were stored at -80˚C until analysis. Concentrations of total N and C were determined based on element analysis. Urine aliquots of 25 µl were combusted in an elemental analyzer SL^{TM} (SerCon, Crewe, United Kingdom) using chromium(VI)-oxide at 900˚C to N_2, NO_x, H_2O and CO_2. NO_x was subsequently reduced to nitrogen gas over copper at 600˚C. Thereafter, the ^{13}C and ^{15}N enrichments were measured online by IRMS (Tracer mass 20-20^{TM}, SerCon, Crewe, UK).

2.5. Calculations

The Percentage of the administered Dose Recovered (PDR) of ^{13}C and ^{15}N in each urine sample, the ratio of the PDRs from ^{13}C versus ^{15}N-ratio (the $^{13}C/^{15}N$-ratio), the fermented ($F_{fermented}$) and not-fermented ($F_{not\text{-}fermented}$) fraction of ^{13}C urea were calculated as described before [4]. In short, the fermented fraction was calculated as the cumulative (c)PDR of ^{13}C in breath over a 15 h time period. The not-fermented fraction was calculated as the ratio of the cPDR ^{13}C and ^{15}N in a 24 h urine collection. Bioavailability was expressed as the sum of $F_{fermented}$ and $F_{not\text{-}fermented}$.

The lag time was derived from the cPDR of ^{13}C in breath and was defined as de time between administration of the tablets and the time the cPDR reached the value of 5% of cPDR at t = 15 h. The pulse time, reflecting the *in vivo* pulsatile characteristics, was calculated as the difference between $cPDR_{70\%}$ (70% of cPDR at t = 15 h) and $cPDR_{5\%}$.

When the cPDR in breath was < 10% at t = 15 h, the results of the corresponding test day for that subject were excluded from further analysis because in that case

the observed small amount of ^{13}C is probably caused by diffusion of ^{13}C-urea from the blood to the colon. This was observed in a previous study [5] in which it was found that on average 7.5% of the administered dose of ^{13}C-urea was recovered in breath 12 h after administration.

All data were corrected for baseline-concentrations of ^{13}C and ^{15}N in breath and /or urine. Furthermore, breath data were corrected for CO_2-retention as described before [4].

2.6. Bioequivalence

For bioequivalence the 90% confidence interval of the ratio of the population means for bioavailability should lie within an acceptance interval of 0.80 - 1.25 [9]. Lag- and pulse time were used to characterize the system.

2.7. Statistical procedures

The results were evaluated by descriptive statistics with SPSS version 18.0. Normal distribution of the data was confirmed with the ShapiroWilk test. The center was characterized by the mean and the dispersion by the standard deviation (SD). A paired-samples t-test and an independent samples t-test (both two tailed, $\alpha = 0.05$) were used to compare the results within and between groups, respectively. The 90% confidence interval of the ratio of the population means for bioavailability was calculated to evaluate bioequivalence.

2.8. Endpoints

The first endpoint of the study was to determine bioequivalence and drug release characteristics of ColoPulse tablets in healthy volunteers and Crohn's patients. A second endpoint was to investigate the influence of food and time of food intake on the *in vivo* drug (marker substance) release from a ColoPulse tablet.

3. Results

The results of 16 healthy volunteers and 14 of the 16 Crohn's patients initially included were evaluated. One patient was withdrawn just before the start of the study because of a urinary tract infection treated with antibiotics which was one of the exclusion criteria and one patient withdrew permission just before start and could not be replaced in time. One patient (subject 27) completed only the first day and the results of this day were used for further analysis.

Based on a first evaluation of the results from the 30 remaining subjects the data of eight test days (~14%) were excluded from further analysis for several reasons. In one healthy volunteer (subject 5) and in one patient (subject 25) (both on day 1) the coating appeared to be defect as the release pattern in urine

of the coated tablet ^{13}C-urea tablet was equal to that of the uncoated $^{15}N_2$-urea tablet. One patient (subject 29) appeared unable to ferment urea and the data of both test days were therefore excluded from further analysis. Finally, two healthy volunteers (subject 8 and 5, respectively day 1 and 2) and two Crohn's patients (subject 23 and 24, day 1) had a release of ^{13}C in breath < 10.0% being a reason for exclusion as explained in the methods section. For these subjects only the mentioned test day was excluded. The results of the other test day (if applicable) were still used in the analysis. In one healthy volunteer (subject 3, day 1) no lag- and pulse time could be calculated due to the absence of a sigmoid release profile, however bioavailability was available for further analysis. This test day was therefore not excluded.

3.1. Bioavailability

Bioavailability ($F_{fermented}+F_{not\text{-}fermented}$) was used to determine bioequivalence between healthy volunteers and Crohn's patients and to determine the influence of food and time of administration on bioavailability. The results clearly indicate release in or nearby the ileo-colonic region (cPDR ^{13}C in breath > 10%, lag time > 4 h) in 91% and 89% of the tablets administered to healthy volunteers and Crohn's patients, respectively. The mean course of the exhalation of ^{13}C in breath in healthy volunteers and in Crohn's patients on both days is shown in figures 2a and b. The lag time in Crohn's patients on day 2 in figure 1b is < 4 h. This is due to the results of 2 subjects (18 and 25).

The mean course of the excretion of ^{13}C and ^{15}N in urine (used to determine $F_{not\text{-}fermented}$) in healthy volunteers and in Crohn's patients on both days is shown in figure 3a and b. On all test days $F_{fermented}$ was 3 - 4 times higher than $F_{not\text{-}fermented}$. There was no difference in excretion of ^{13}C in urine between both days and this was similar for ^{15}N.

3.2. Bioequivalence and influence of food and time of food intake

Bioavailability, lag- and pulse time were evaluated to determine whether there is any difference between healthy volunteers and Crohn's patients in the *in vivo* drug release characteristics of the ColoPulse tablet and whether there is an effect of food and time of food intake on release and bioavailability. A summary of the results is shown in table 2A - C. More detailed results are presented in figures 4A and B. There was no significant difference in mean bioavailability between healthy volunteers and patients who took a non-standardized breakfast after 1 h (75.8 vs 90.2%, p = 0.070) neither was there a difference between both groups taking the standardized breakfast after 3 h (83.4 vs 91.4%, p = 0.265). The 90% confidence intervals (CI) around the ratio of the means were 0.72 - 0.99

(breakfast after 1 h) and 0.80 - 1.04 (breakfast after 3 h), respectively. The lower CI of day 1 is just outside 0.80 - 1.25.

To evaluate the influence of food and time of food intake the results for day 1 and day 2 were compared within both groups. There were no significant differences within the group of healthy volunteers ($p = 0.077$, 90% CI ratio 0.87 - 0.99) and within the group of Crohn's patients ($p = 0.618$, 90% CI 0.89 - 1.19).

The release pattern of a ColoPulse tablet is characterized by the parameters lag- and pulse time. There was no significant difference in mean lag time between volunteers and patients who took breakfast after 1 h (278 min vs 332 min, $p = 0.111$) as well as breakfast after 3 h (340 min vs 309 min, $p = 0.333$). The pulse time was not significantly different between both groups either. With breakfast at 1 h after administration of the tablets the pulse time was 224 minutes in healthy volunteers vs 274 minutes in Crohn's patients ($p = 0.343$). With breakfast 3 h after administration the pulse time was 218 and 225 minutes, respectively ($p = 0.825$).

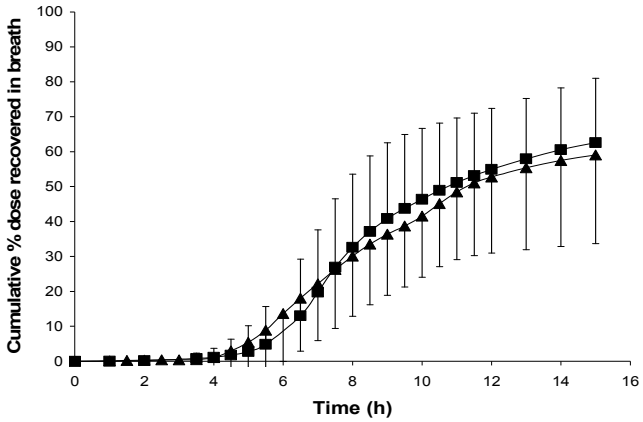
There was an effect of food and time of food intake on both release characteristics within both groups. There was a significant difference for the lag time within the group of healthy volunteers ($p = 0.021$) and for the pulse time within the group of Crohn's patients ($p = 0.015$) when the results of day 1 and day 2 were compared. However food and time of food intake had no significant influence on the lag time for Crohn's patients ($p = 0.780$) and the pulse time in healthy volunteers ($p = 0.917$).

Table 2: Summary of parameter results in healthy volunteers and Crohn's patients. Data are means and (SD) for 14 (day 1) + 15 (day 2) healthy volunteers and 10 (day 1) + 12 (day 2) Crohn's patients
Day 1: breakfast (non-standardized) after 1 h
Day 2: standardized breakfast after 3 h

Parameter	Day	Healthy volunteers	Crohn's patients	P-value[a]	90% CI of ratio
A: Bioavailability					
Bioavailability	1	75.8% (20.1)	90.2% (15.4)	0.070	0.72-0.99
Bioavailability	2	83.4% (14.1)	91.4% (22.2)	0.265	0.80-1.04
P-value[b]	n.a.[c]	0.077	0.618	n.a.	n.a.
90% CI of ratio	n.a.	0.87-0.99	0.89-1.19	n.a.	n.a.
B: Lag time					
Lag time	1	278 min[d] (81)	332 min (72)	0.111	
Lag time	2	340 min (57)	309 min (97)	0.333	
P-value[b]	n.a.[c]	0.021[e]	0.780	n.a.	
C: Pulse time					
Pulse time	1	224 min[d] (135)	274 min (103)	0.343	
Pulse time	2	218 min (94)	225 min (61)	0.825	
P-value[b]	n.a.[c]	0.917	0.015[e]	n.a.	

[a] determined with an independent samples t-test
[b] determined with a paired samples t-test
[c] n.a. = not applicable
[d] results of 13 healthy volunteers
[e] significant difference between day 1 and day 2

A: Healthy volunteers



B: Crohn's patients

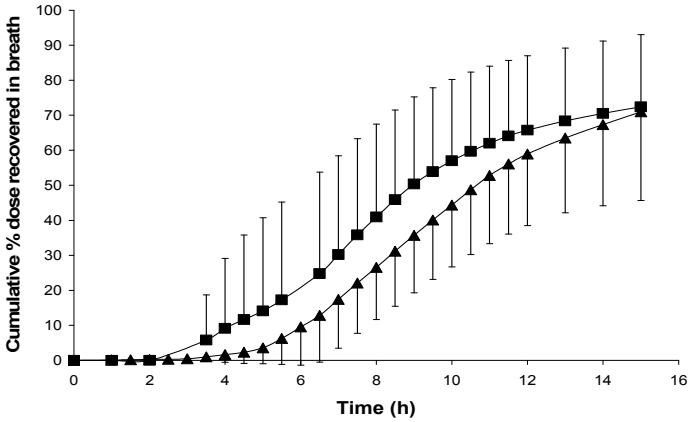
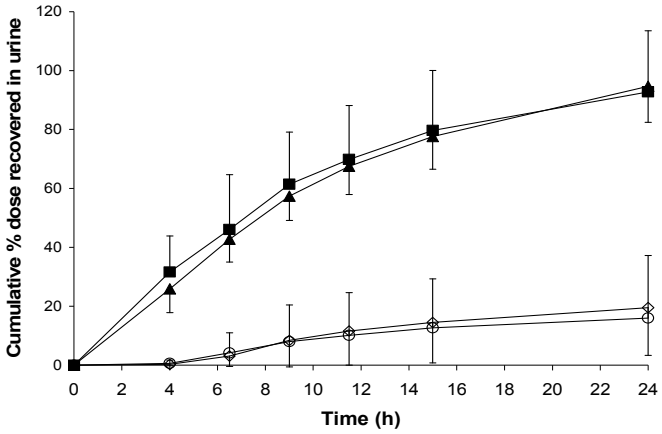


Figure 2: Appearance of ^{13}C in breath in healthy volunteers (mean of 16 subjects) and in Crohn's patients (mean of 14 (day 1) and 13 (day 2) subjects). The error bars represent the standard deviation.

▲ day 1: breakfast (non-standardized) after 1 h

■ day 2: standardized breakfast after 3 h

A: Healthy volunteers



B: Crohn's patients

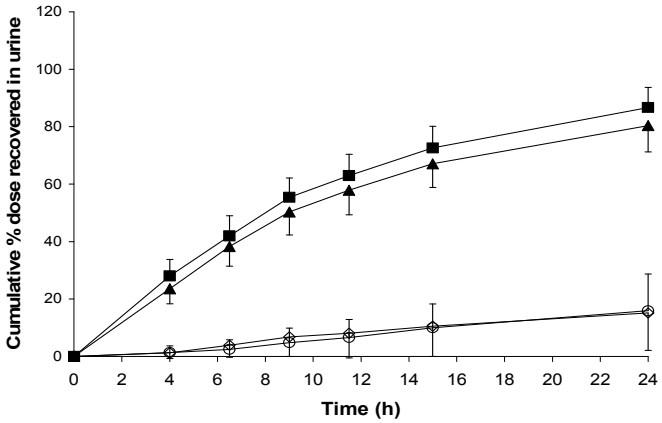


Figure 3: Appearance of ^{13}C and ^{15}N in urine in healthy volunteers (mean of 16 subjects) and Crohn's patients (mean of 14 (day 1) and 13 (day 2) subjects). The error bars represent the standard deviation.

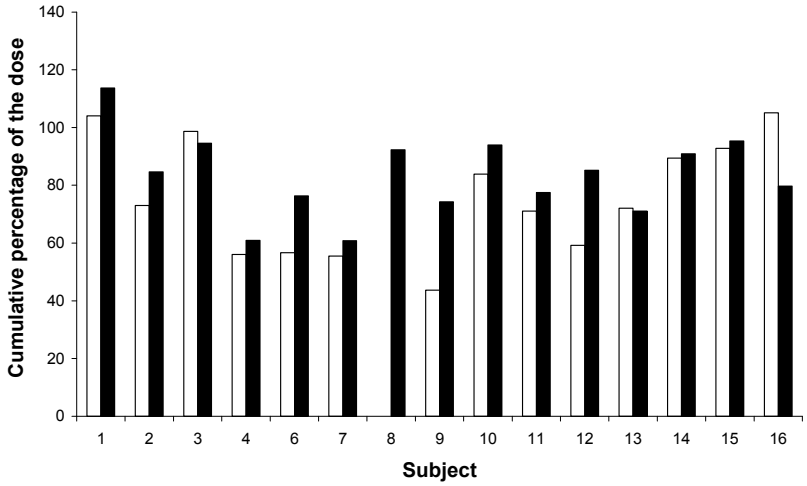
○ ^{13}C day 1: breakfast (non-standardized) after 1 h

◊ ^{13}C day 2: standardized breakfast after 3 h

■ ^{15}N day 1: breakfast (non-standardized) after 1 h

▲ ^{15}N day 2: standardized breakfast after 3 h

A: Healthy volunteers (subject 5 was excluded for both test days)



B: Crohns's patients (subject 29 was excluded for both test days)

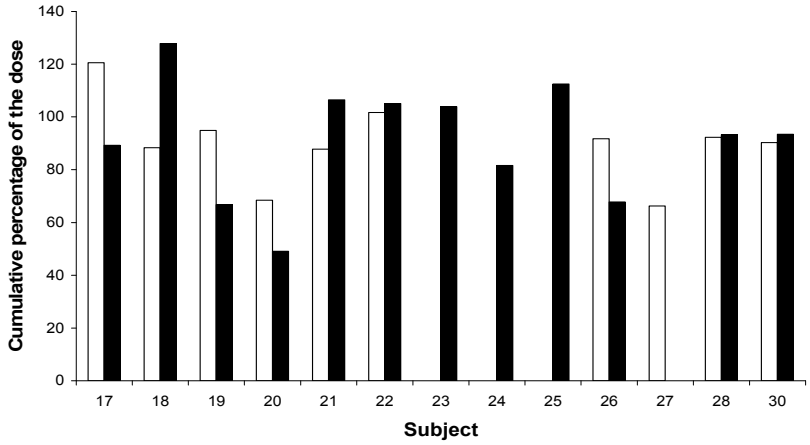


Figure 4: Bioavailability in healthy volunteers and Crohn's patients

□ day 1 : breakfast (non-standardized) after 1 h

■ day 2 : standardized breakfast after 3 h

4. Discussion

This is the first study in which the ColoPulse technology is tested in Crohn's patients. From the results obtained it can be concluded that the ColoPulse tablet delivers its content effectively in the ileo-colonic region of the intestine in both healthy volunteers and Crohn's patients. This study delivers clear proof that the behaviour of a ColoPulse tablet in Crohn's patients and in healthy volunteers is comparable. Furthermore, only small differences in bioavailability were found when the results from a non-standardized breakfast 1 h after intake of the tablets mimicking real life conditions were compared with a standardized breakfast after 3 h (both in healthy volunteers as well as in patients). Despite the fact that the amount and type of food taken by the subjects on the first day differed substantially, the mean bioavailability ranged between and 75.8 and 91.4% and criteria for bioequivalence (as used by the EMA [9]) were met in three out of four 90% confidence intervals of the ratio of population means for bioavailability.

At least 86% (51 out of 59) of all tablets administered in this study released the marker substance at the desired site of delivery. As described above, the results of eight test days out of 59 were excluded from further analysis due to low or altered bioavailability. On four of these test days $F_{fermented}$ was below 10%. This phenomenon was also observed in a previous study in healthy volunteers [4]. In that study two subjects out of 12 showed a low $F_{fermented}$ (4.5 and 7.5%).

There are several possible reasons for a low or altered bioavailability: no or unusual bacterial flora in the colon, too early release in a region with low urease activity (terminal ileum), a coating defect and incomplete release from the tablet or insufficient mass transport in the lumen due to a viscous fecal mass. The dual-label stable-isotope technique offers the possibility to discriminate between these possible causes of low or altered bioavailability and the results obtained from these eight patients can all be explained adequately.

In one Crohn's patient (subject 29) $F_{fermented}$ was relatively low on both test days (3.7 and 11.1%). Urinary excretion was around 75% at t = 24 h. However, appearance of ^{13}C in urine was $\geq$ 4 h delayed compared to ^{15}N. This probably means that the content was released in the right region but that no fermentation occurred. Because this happened on both days it is most likely that this subject had an unusual low urease activity in the ileocolonic region. Therefore we considered this patient not suitable for evaluation of the ColoPulse technology using ^{13}C-urea. One healthy volunteer (subject 5, day 2) showed a similar profile, low $F_{fermented}$ and delayed excretion of ^{13}C in urine compared to ^{15}N.

Total bioavailability was > 78%. The most likely explanation again is that the content of the tablets was released in a urease-poor region nearby the colon, most likely the terminal ileum.

For subjects 5 (day 1) and 25 the urinary excretion profile of ^{13}C from a ColoPulse tablet was equal to that of ^{15}N from an uncoated tablet. This is a clear indication of a coating defect with early release of the contents. Finally in subjects 8, 23 and 24 the content of the tablets was released incompletely, maybe due to the fact that pH 7.0 was not reached or that the tablet became trapped in the viscous fecal mass with no further systemic absorption. In this group excretion of ^{13}C in urine was delayed compared to ^{15}N, but total bioavailability was also relatively low indicating an incomplete release of the marker substance from the tablets.

The Eudragit S polymer used for the ColoPulse coating has a dissolution threshold at pH = 7.0. It is known from the literature that the intraluminal pH in healthy volunteers gradually changes from about 6.6 to 7.5 (from jejunum to terminal ileum). A pH above 7 is encountered in a short intestinal region were the ColoPulse tablet resides only 0.5 to 1 h [10]. It is not known whether the gastrointestinal pH-profile of Crohn's patients differs from that of healthy volunteers. In the literature there is only scarce information available on the intestinal pH profiles in Crohn's patients. One study describes that the median pH value in healthy controls compared to that of Crohn's patients was significantly lower in the stomach (1.55 vs 2.4) but not in the terminal ileum, cecum and right colon [11]. In another study it was found that there was no difference in intraluminal pH of the gastrointestinal tract between controls and patients with active Crohn's disease [12]. Based on this, we assumed for our study that the pH in the intestinal lumen of Crohn's patients would not differ compared to that of healthy volunteers. Therefore we expected no difference in the performance of the ColoPulse system between healthy volunteers and Crohn's patients (meaning bioequivalence). The results from this study support our hypothesis.

In the development of new oral dosage forms, especially controlled release formulations, and their applications, food-interaction studies are of great importance [13]. The previous studies with the ColoPulse technology in healthy volunteers were done with a standardized breakfast 3 h after intake of a ColoPulse coated capsule. This schedule is not feasible in daily (clinical) practice. From the perspective of the patient, non-restricted food and beverage intake are preferred. However, it cannot be excluded that effects on the drug release profile will occur due to a temporary high rise (pH > 7.0) in gastric pH caused by the intake of certain food and fluids. We considered the intake of a

ColoPulse coated tablet 1 h before breakfast in this stage of development an acceptable and feasible option for patients.

The used study design with almost no restrictions in food and drinks differs from commonly conducted food-interaction studies. This was done to come as close as possible to a setting with daily use of medication. The results show that even in this setting, there was no difference in bioavailability and in the location of drug release within the groups of healthy volunteers and Crohn's patients when a non-standardized breakfast 1 h after intake of the tablets was compared with a standardized breakfast after 3 h. This is an important precondition for the further development of ColoPulse tablets to be used in this particular patient group.

This design gives only information for active substances administered once daily. If other dosage regimens are used in future studies the absence of food influence has to be verified for the particular conditions applied. Furthermore, the influence of food on the bioavailability of a specific drug substance has to be investigated using the applicable EMA guidelines [9,14].

The drug release characteristics of the ColoPulse system were assessed by the lag- and pulse time. Although there appeared to be no significant differences when the results of healthy volunteers and Crohn's patients were compared, differences were found within both groups when food effects were studied. In healthy volunteers the lag time was significantly shorter with administration 1 h before breakfast compared to 3 h before breakfast (278 vs 340 minutes). This could be explained by the principle that after a period of fasting a subsequent meal activates the gastrointestinal motility. This causes the dosage form to pass the ileo-cecal junction. The two hours difference in breakfast after intake of the tablets could explain the shorter lag time with breakfast after 1 h. The fact that there is no difference in bioavailability between both days supports this [15]. In the literature an altered gastrointestinal motility was observed in Crohn's patients compared to healthy controls which can be an explanation of the fact that the interval between administration and breakfast does not have an effect on the lag time in Crohn's patients [16]. However, there is no literature available about the influence of a subsequent meal on gastrointestinal motility.

In Crohn's patients the pulse time was longer with administration of the tablet 1 h before breakfast compared to 3 h before breakfast (274 versus 225 minutes). The pulse time is influenced by the rate of disintegration of the tablet coating and core, dissolution, metabolism and kinetics of ^{13}C-urea. However the observed difference cannot be explained by one of these aspects. The clinical relevance of this difference seems limited, especially for chronic therapy and the type of medications Crohn's patients' use. This is supported by the fact that the

difference in bioavailability within both groups was not found to be significant and to be within the 90% confidence intervals for bioequivalence. Furthermore, it should be kept in mind that the residence time in the first part of the colon is several hours. This makes a difference of approximately 1 hour in lag- and pulse time relatively short and less meaningful. In particular the lag time is mainly determined by the small intestinal transit time. This transit time is determined solely by the variability of the intestinal motility of the subject.

Compared to previous studies in healthy volunteers with ColoPulse capsules, the ColoPulse tablets exhibited longer pulse times (median around 200 minutes) than the capsules (median 99 minutes) [5]. However, a large range of 276 minutes was noticed in the study with capsules. A possible explanation could be the fact that the tablet cores are relatively dense compared to the contents of a capsule. The dissolution process when little fluid is available takes probably more time for the tablets. The clinical relevance of this phenomenon seems to be negligible since the pulse time is less than the residence time in the proximal colon and the active substance will arrive at the desired site of delivery.

5. Conclusions

This clinical cross-over study in healthy volunteers and Crohn's patients shows that the ColoPulse technology enables the site-specific delivery of drugs or other compounds (e.g. diagnostics) deep in the ileo-colonic region of the intestine of Crohn's patients with a mean bioavailability of around 90%. Bioavailability was similar in healthy volunteers and in Crohn's patients and met in most tests the criteria for bioequivalence. Food had no relevant effect on bioavailability and drug release characteristics of the ColoPulse system. We conclude that the ColoPulse system is a delivery system that deserves further research for its application in local therapy with immunosuppressive drugs in Crohn's patients in the near future.

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CHAPTER 8

General discussion and future perspectives

In previous research at our institute the ColoPulse concept was developed and a proof of concept study was performed in healthy volunteers. With this thesis we aimed to perform the next steps in the pharmaceutical and clinical development of ColoPulse dosage forms with a special focus on inflammatory bowel disease. We studied the *in vivo* release from ColoPulse tablets with an optimized study design, compared to the earlier mentioned proof of principle study, in healthy volunteers and in patients with Crohn's disease. The results obtained in this thesis have brought us a step forward into the direction of developing an oral dosage form of infliximab or another active protein/monoclonal antibody, that offers clinical advantages compared to the current parenteral treatment with infliximab in inflammatory bowel disease.

1. Formulation of ColoPulse tablets

In chapter 2 we obtained insight in several critical process parameters influencing release from a ColoPulse tablet using a quality by design approach. This systematic approach can be used to optimize pharmaceutical preparations and to improve the control over and the quality of the production process [1]. With the current composition, containing a hydroxypropylmethylcellulose (HPMC) interlayer between the tablet and the ColoPulse coating, substances with a neutral to weakly acidic or basic pH can be formulated in a ColoPulse tablet. For these substances we managed to create a platform technology so that it is now known which process parameters will result in a tablet with an adequate release profile. This will make future development of ColoPulse tablets more efficient. Studies confirming the physical and chemical stability of the active substance are still necessary however. From chapter 2 it also appeared that more research has to be done towards the formulation of active substances with acidic and basic pKa (pKa < 3 and > 11) into a ColoPulse tablet. Possible solutions as increasing the thickness of the HPMC interlayer, increasing the amount of filler material, lowering the drug load or titrate the core with acidic or basic substances remain to be investigated. This has to be done preferably in two separate studies, for acidic and basic substances, and using a quality by design approach. Combined, this will result in a complete profile of the formulation possibilities of a ColoPulse tablet.

2. Infliximab: formulation of ColoPulse tablets and kinetics

Monoclonal antibodies against tumor necrosis factor alpha (TNF alpha) such as infliximab are very effective in the treatment of inflammatory bowel diseases [2]. Infliximab was the first anti-TNF alpha antibody and is until now administered intravenously. Small-scale studies describe the successful local

administration of infliximab by injection in patients with Crohn's disease [3-5]. Several disadvantages related to parenteral therapy have been reported however. In chapter 3 we showed that infliximab could be successfully formulated in a ColoPulse tablet by incorporating this antibody into a sugar glass of the flexible oligosaccharide inulin. Tablets stored at 25ºC in a closed vial still displayed a mean biological activity of 83% 16 months after production compared to a fresh infliximab solution [6]. With this study we showed that the formulation and production of 5 mg ColoPulse infliximab is technically feasible, but some improvements remain necessary to be able to perform the next crucial step: a clinical study. A relatively simple improvement will be to reduce the storage temperature to 2-8ºC. By storage at a lower storage temperature, the difference with the glass transition temperature will be increased with approximately 20ºC which favors the stability of infliximab [7]. Lowering the storage temperature in combination with a reduction of the relative humidity in the container will possibly be even more effective, because water uptake during storage acts as a plasticizer. This lowers the glass transition temperature resulting in destabilization [8]. Furthermore, the stability indication profile has to be extended with methods of analysis to gain more knowledge about the mechanism of degradation of infliximab upon storage. Examples of these methods are LC-MS (intact mass and subunits), peptide mapping, FT-IR and glycosylation assays [9,10]. A different sugar glass formulation based on another sugar or a combination of sugars may also be an approach to prevent degradation, because size and molecular flexibility of sugars relate to their protein stabilizing ability [8].

Because oral therapy with ColoPulse infliximab tablets is still a scenario of the future and because these tablets are only intended for disease located in the ileo-colonic region, it remains necessary to work on strategies to optimize intravenous infliximab therapy. Therapeutic drug monitoring (TDM) of infliximab is being described in the literature for several years already and it is known that the development of anti-drug antibodies and low serum concentrations are associated with poor clinical outcomes [11]. On the other hand no stopping rules for anti-TNF-alpha therapy are available for patients with inflammatory bowel disease in remission. There is evidence that TDM can play a role in the decision making of dosing and stopping anti-TNF therapy, because remission with low infliximab trough levels at the time of discontinuation is predictive of sustained remission after terminating infliximab treatment [12]. However, TDM of infliximab is not already commonly applied everywhere in daily clinical practice. In Chapter 4 we have developed a pharmacokinetic model for infliximab in patients with inflammatory bowel disease to be able to use TDM in predicting serum trough levels and in performing dose optimization in

this population [13]. In patients without anti-infliximab antibodies trough level dosing based on longer intervals can reduce hospital visits and subsequently reduce costs. Simulations based on our model showed that dosing every 12 weeks instead of every 8 weeks still resulted in adequate serum trough levels in our population. Although the outcomes of our retrospective study are promising, unfortunately disease activity parameters as endoscopy of fecal calprotectin were not available for most patients. Therefore the results should be confirmed in a prospective study. Currently, the analysis of monoclonal antibodies is less common in hospital pharmacies compared to bioanalysis of the small drug molecules, but this is expected to change in the near future as more data and analytical methods will become available. This change will contribute to a more cost-effective use of this effective, though expensive, therapy.

3. *In vivo* studying of ColoPulse dosage forms

Based on the characteristics of an Eudragit-S coating, release from a ColoPulse dosage form occurs after pH 7.0 has been reached. This correlates *in vivo* with the ileo-colonic region and is supported by the results of two studies by Schellekens et al. [15,16]. In these studies release of ^{13}C-urea in the cecum and colon (urease-rich segment) from a ColoPulse capsule was compared to release of ^{13}C-urea from an uncoated capsule at a different day. When ^{13}C-urea is released in the colon this leads to fermentation of ^{13}C-urea by bacterial urease in $^{13}CO_2$ which is absorbed in the bloodstream and subsequently exhaled in breath. ^{13}C-urea from an uncoated capsule is released in the stomach or the small intestine (urease-poor segment), is directly absorbed in the blood stream and subsequently excreted into urine. It was shown that subtraction of the results from a coated and an uncoated capsule containing ^{13}C-urea with administration on two different days could estimate the release characteristics from a ColoPulse capsule and that release occurred in the ileo-colonic region.

The feasibility of using two different stable isotopes of urea to determine the release profile from a ColoPulse dosage form is presented in chapter 5. The study comprises the results of an optimized strategy characterized by simultaneous administration of ^{13}C-urea in a coated capsule and $^{15}N_2$-urea in an uncoated capsule [14]. This design was an improvement of the earlier published design in the proof-of-concept study by Schellekens et al. on the use of ^{13}C-urea [15]. By using two different stable isotopes of urea on a single day, day-to-day variation in urea kinetics is eliminated and the study power is increased. Compared with a conventional two-period study design, this approach reduces clinical study costs, because run through time and sample load are reduced by approximately 50%. Moreover, the sampling is completely non-invasive by replacing blood-samples for a single urine sample or only 24 h collection of

urine. Our new approach resulted in a non-invasive (only breath and urine are necessary) and one-day study design. This design was subsequently used in the clinical studies described in chapter 6 and 7.

In Chapter 6 a study confirming release from a ColoPulse tablet in the ileo-colonic region in healthy volunteers is described. In this study we used a new medical device, the IntelliCap system®. One of the functionalities of the IntelliCap system® is continuous measurement of the pH in the gastro-intestinal tract [17]. The obtained results supported our previous results based on urea-kinetics and confirmed that release from a ColoPulse tablet occurs in the ileo-colonic region and after pH 7.0 has been reached. Ideally this study using the Intellicap system® should also be performed in patients with inflammatory bowel disease to make the picture complete. Unfortunately this was impossible in the planned study period, but several small studies comparing pH in patients with inflammatory bowel disease and healthy volunteers have been published. They showed that there is little to no relevant difference in gastrointestinal pH between both groups. Generally, it was seen that the gastric pH is slightly elevated in the patient group compared to healthy volunteers and that patients with ulcerative colitis had slightly higher pH values in the terminal ileum, the caecum and the right colon compared to patients with Crohn's disease. There was no difference in patients with active disease and patients in remission [18,19]. In patients with ileocecal resections, cecal pH was somewhat higher and the time at pH above 7.0 was a little shorter compared to controls [20]. After release from the dosage form (local) bioavailability of drug substances depends also on transit times. A reduction in transit time will decrease the exposure of the target organ to the drug substance. On the other hand an increase in transit time, will also increase the possibility of local inactivation for substances sensitive to enzymatic or environmental degradation. In the literature it has been described that regional transit times are prolonged in severe ulcerative colitis [21] and prolonged to normal in patients with Crohn's disease [22]. Based on the information above it was concluded that the ColoPulse concept will probably function properly in inflammatory bowel diseases, not only in patients in remission but also in patients with active disease or (limited) ileocecal resections. Therefore no additional research on the ColoPulse concept is necessary. However, clinical studies to investigate bioavailability en effectiveness are necessary for each drug substance to be formulated in a ColoPulse dosage form.

4. Influence of food and disease on release from a ColoPulse tablet

One of the remaining issues to be addressed before a clinical study with ColoPulse tablets containing active substances can be performed in patients with Crohn's disease, was to investigate the performance of ColoPulse tablets in this patient group and to study the influence of food intake on release characteristics. In chapter 7 we present the results of a cross-over study in healthy volunteers and patients with Crohn's disease in remission [23]. Using a practical approach, we studied the influence of food and time of food intake on release from a ColoPulse tablet. Bioavailability was similar in healthy volunteers and in Crohn's patients. Food had no clinically relevant influence on release parameters in both groups.

This is the starting point for our future research. Now we have developed ColoPulse infliximab tablets and showed that the ColoPulse concept works in patients with Crohn's disease, we can move forward in preparing a clinical study to investigate whether or not our hypothesis that local delivery of infliximab is non-inferior to parenteral therapy will be true. Depending on the results, the research can potentially be expanded with more active substances and more sub-groups of patients with inflammatory bowel disease.

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Summary

ColoPulse oral dosage forms exhibit a site-specific release in the lower gastrointestinal tract which may have certain advantages compared to non-controlled drug release. They have the potential to improve efficacy and to minimize side effects of locally acting drugs. Moreover, oral administration is considered more patient friendly than many other routes of administration (such as the parenteral route). In the literature different approaches for local, colonic delivery are described. They include pH-responsive systems, time-based systems, pressure-based systems, systems triggered by the colon flora as well as combinations of these. The ColoPulse technology is a representative of a pH responsive system. It differs from other modified release systems because of the non-percolating incorporation of a super-disintegrant in the coating by which a reliable and pulsatile release at the desired site in the gastrointestinal tract is achieved. Release is triggered by the physiologically occurring variation of the pH of the gastro-intestinal tract and starts when pH levels over 7.0 are reached. This corresponds *in vivo* with the ileo-colonic region. In the literature colon-specific oral delivery is considered a promising alternative for the current parenteral administration of macromolecular and peptide drugs due to the relatively neutral pH of the ileo-colonic region combined with the relatively low proteolytic activity of the colon compared to the small intestine.

One of the aimed patient populations that could profit from the ColoPulse technology are patients with inflammatory bowel disease. Currently one of the cornerstones in their treatment is parenteral administration of the monoclonal antibody infliximab. The main concerns in systemic exposure of infliximab are the development of anti-drug-antibodies which in turn are associated with a shorter duration of response and an increased risk of infusion reactions. To overcome this problem and the drawbacks with respect to parenteral administration, the development of an oral dosage form of infliximab may become a new strategy in the treatment of inflammatory bowel disease. Moreover, oral administration is a lesser burden to the patient than administration via an infusion.

In this thesis several studies are described, all aimed to improve the treatment of patients with chronic inflammatory bowel disease. We focused on pharmaceutical aspects regarding the formulation of ColoPulse tablets and especially ColoPulse infliximab tablets. We furthermore explored the pharmacokinetics of infliximab in an outpatient setting. This was combined with the collection of more insight and knowledge about the *in vivo* behavior of ColoPulse tablets in healthy volunteers and in Crohn's patients.

From earlier work it was already known that strong acidic and alkaline substances tend to influence the release profile of an active substance from a

ColoPulse dosage form. This was however not investigated systematically. Knowledge about factors that may affect the drug release behavior of the coating will be helpful in the selection of active substances to be formulated in a ColoPulse tablet. In chapter 2 a quality by design approach is presented, directed to obtain more insight into the effect of several critical process parameters on the release from a ColoPulse tablet. The critical process parameters included the pKa of active substance, the coating thickness, the exposure time to pH 6.8 and type of solvent used to prepare the coating suspension. Further the effect of a 2.7 mg/cm^2 hydroxypropylmethylcellulose (HPMC) seal coating between the tablet core and the ColoPulse coating applied to prevent the effect of strongly acidic or alkaline material on drug release was investigated. Release parameters lag time, pulse time and total release were determined using an abbreviated GISS dissolution test. From the results it could be concluded that acetone is the preferred coating solution. The application of a 2.7 mg/cm^2 HPMC coating between tablet core and ColoPulse coating did not act as the desired barrier to prevent any effect of the drug compound in the core on the coating. Neutral to weakly acidic and alkaline drug substances appeared to be excellent candidates for formulation in a ColoPulse tablet. In contrast, substances with low (< 3) and high pKa (> 11) were less or not suitable to use into the current ColoPulse formulation. The suitability of a ColoPulse coating for substances with pKa between 3 and 6 remains to be investigated in more detail. With the results of this study future development of ColoPulse tablets can be performed more efficiently.

Chapter 3 describes the development of an infliximab containing ColoPulse tablet. Formulation aspects are described, a stability indicating profile was composed and a stability study was performed under three different storage conditions during a period of 16 months. Infliximab tablets were compounded from the commercially available product Remicade®. To protect infliximab against degradation it was formulated into a sugar glass based on the oligosaccharide inulin before the tablets were produced. Tablets were stored under three conditions that differed in temperature, relative humidity or packaging. Under all storage conditions tablets showed no loss of content, potency or change in structure up to 4 months after production. After 16 months storage at ICH climate zone I conditions (25ºC, 60% relative humidity, closed vial), tablets still displayed a mean biological activity of 83% compared to a freshly made infliximab solution. Tablets in an open vial were less stable when stored at 40ºC, 75% relative humidity (73% and 12% biological activity remaining after 16 months respectively). We concluded that formulation of ColoPulse infliximab tablets is technically feasible. For use in a clinical study some optimization has to be performed including the packaging of the tablets.

Because ColoPulse infliximab tablets are only intended for patients with disease located in the ileo-colonic region, an important clinical focus will still be on intravenous therapy for patients with disease located elsewhere in the gastrointestinal tract. Currently infliximab is administered at fixed doses and intervals, but optimization is desirable because costs are high and adverse events or anti-infliximab anti-bodies can develop. The objective of the retrospective study described in chapter 4 was to develop a pharmacokinetic model for infliximab in patients with inflammatory bowel disease that can be used for dose optimization and to predict serum trough levels. Simulations with the developed model showed that dosing every 12 weeks instead of every 8 weeks could be considered in the treatment of patients with inflammatory bowel diseases without anti-infliximab-antibodies. Predicted trough levels were above 3.0 mg/L in a majority of the female patients (81% at 12 week intervals versus 92% at 8 week intervals). For male patients this was 56% versus 67% respectively. Considering the high percentage of patients in remission with trough levels $\leq$ 3.0 mg/L dose intensification or modification should always be combined with clinical and/or endoscopic disease activity parameters. Dosing every 12 weeks instead of every 8 weeks will reduce concomitant costs related to infliximab treatment and will reduce hospital visits with one third. We concluded that the developed pharmacokinetic model may be used to optimize therapeutic drug monitoring of infliximab but it needs to be confirmed in a prospective trial using calprotectin or endoscopy as disease activity parameters.

In chapter 5 we describe a feasibility study on the simultaneous administration of ^{13}C-urea and $^{15}N_2$-urea to study bioavailability and release from colon-specific drug delivery systems in one single day. In earlier studies only one stable isotope (^{13}C-urea) was used as a marker substance to determine bioavailability and release in a single dose, two-period crossover design. In the current study either an uncoated or a ColoPulse-capsule containing ^{13}C-urea combined with an uncoated capsule containing $^{15}N_2$-urea were taken by four healthy volunteers. When ^{13}C-urea is delivered in the colon, the ^{13}C-label is detected in breath as $^{13}CO_2$, due to intraluminal fermentation. Upon delivery in proximal parts of the intestine, ^{13}C-urea and $^{15}N_2$-urea will be absorbed unfermented and appear unaltered in the urine. The recoveries of ^{13}C and ^{15}N from uncoated capsules showed a ratio of 1.01 ± 0.06 during the first 24 h after administration. The ^{13}C/^{15}N-ratio after intake of a ColoPulse-capsule containing ^{13}C-urea showed considerable interindividual variation, but was constant between 12 and 24 h after intake. The cumulative percentage of the dose recovered (PDR) of ^{13}C in urine was in all cases much lower than the cumulative PDR of ^{15}N in the same collection. After 24 h for ^{13}C a median cumulative PDR of 11.9% was found for the ColoPulse-capsule versus 73.1% for uncoated

capsules. The $^{13}C/^{15}N$-ratio in a single urine sample at $t \geq 12$ h post dose could be used to predict the cumulative PDR of ^{13}C after 24 h. The ColoPulse-capsule showed a delayed sigmoid release-pattern with a lag time of > 3 h as derived from the time course of $^{13}CO_2$ in breath. We concluded that this one day, non-invasive study design based on the dual-label isotope strategy is suitable for the evaluation of bioavailability of colon-specific drug delivery systems. This approach also reduces study costs by a reduction in study run through time and sample load.

As described before an environment with pH over 7.0 triggers release from a ColoPulse dosage form. Release in the ileo-colonic region was proved based on the *in situ* fermentation of ^{13}C-urea by bacterial urease, which is only present in that region. However, release was never real-time correlated to gastrointestinal pH. In chapter 6 we describe a prospective study in healthy volunteers in which we studied the *in vivo* relationship between gastrointestinal pH and release from a ColoPulse tablet. The *in vivo* pH as measured with the IntelliCap system® was compared to the isotope signal from a ColoPulse tablet. There was no statistically significant difference between colon arrival time based on pH and lag time based on isotope signal (5:31 vs 5:42 h, $p = 0.903$). We concluded from the combined data that *in vivo* release from a ColoPulse tablet is not related to transit time, but is related to the gastro-intestinal site.

Until now ColoPulse dosage forms have only been studied in healthy volunteers and not yet in specific patient groups. Furthermore the interval between administration of a ColoPulse dosage form and breakfast / food has been continued to three hours, which is not feasible in daily life. In chapter 7 we describe a crossover study in health volunteers and patients with Crohn's disease in remission to compare release parameters and bioavailability from a ColoPulse tablet. Furthermore the influence of food and time of food intake was studied. In this study we used the dual-label isotope strategy as described in chapter 5. On the first day tablets were administered followed by a breakfast (non-standardized) after one hour. On the second day a breakfast (standardized) was consumed three hours after administration of the tablets. There was no significant difference in bioavailability between healthy volunteers and Crohn's patients on both days. No significant influence of food and time of food intake was found in healthy volunteers (75.8% vs 83.4%, $p = 0.077$) and Crohn's patients (90.2% vs 91.4%, $p = 0.618$). Food and time of food intake had some minor, clinically non-relevant, influence on the release characteristics within both groups, which is in line with the fact that food affects gastrointestinal transit times.

With the research described in this thesis we aimed to perform the next steps in the pharmaceutical and clinical development of ColoPulse dosage forms with a special focus on its use in inflammatory bowel disease. We studied the *in vivo* release from ColoPulse tablets with an optimized study-design in healthy volunteers and in patients with Crohn's disease. The results from the research described in this thesis have brought us a step forward into the direction of developing an oral dosage form of infliximab that offers potential clinical advantages compared to the current parenteral treatment of inflammatory bowel disease. From this point on we can move forward and start the preparations for a clinical study to investigate whether or not our hypothesis that local delivery of infliximab is non-inferior to parenteral therapy will be true.

Samenvatting

ColoPulse toedieningsvormen zijn tabletten of capsules die omhuld zijn met een extra coating-laag die gevoelig is voor de zuurgraad (pH) van het maag-darmkanaal. Ze zijn ontworpen om op een gecontroleerde manier geneesmiddelen in verderop gelegen delen van de darmen af te geven. Door de speciale coating blijven ze heel in de maag en in het eerste deel van het maag-darmkanaal, omdat de pH op deze plaatsen relatief laag is. De pH neemt vervolgens toe tot 7,5 op de plaats waar de dunne darm overgaat in de dikke darm (het colon) en gaat daarna weer omlaag. De ColoPulse coating is zo ontworpen dat deze opengaat bij een pH vanaf 7,0 waarna vervolgens het geneesmiddel in de buurt van het colon vrijkomt. Een desintegratiemiddel in de coating zorgt ervoor dat het geneesmiddel sneller vrijkomt dan normaal.

De meeste tabletten bevatten geen coating en vallen al in de maag uit elkaar. Het geneesmiddel wordt dan direct in het bloed wordt opgenomen en bereikt het laatste deel van de darmen nauwelijks. Als het laatste deel van de darm toch bereikt moet worden, gebeurt dit meestal door middel van een infuus met daarin het geneesmiddel. Een deel van het geneesmiddel komt dan via de bloedbaan alsnog op de juiste plek terecht. Door ColoPulse tabletten te gebruiken, zouden mogelijk ziektes waarvoor nu alleen behandeling met een infuus beschikbaar is ook met tabletten behandeld kunnen worden. Hierdoor komt er meer werkzame stof op de juiste plek en zou de kans op bijwerkingen op andere plaatsen af kunnen nemen. Daarnaast zijn tabletten over het algemeen prettiger voor de patiënt dan een infuus.

Patiënten met chronische darmontstekingen zoals de ziekte van Crohn en colitis ulcerosa zijn een groep waarvoor ColoPulse tabletten interessant zouden kunnen zijn. Hun behandeling kan uit diverse geneesmiddelen bestaan, maar een deel van hen krijgt een infuus met daarin het antilichaam infliximab. Infliximab remt de werking van het eiwit TNF-alfa dat een rol kan spelen bij ontstekingen en wordt iedere acht weken toegediend. Hierdoor worden de ontstekingen meestal rustiger. Aan de behandeling met infliximab kunnen ook nadelen zitten. Zo kunnen er infusie-reacties optreden en kan het lichaam antistoffen tegen infliximab maken, waardoor infliximab minder goed werkt. Daarnaast moet de patiënt voor deze behandeling steeds naar het ziekenhuis. Het zou dus een verbetering zijn als infliximab als tablet toegediend zou kunnen worden en de werkzame stof direct op de plek zou komen waar het nodig is, zodat het immuunsysteem minder makkelijk antistoffen tegen infliximab zal maken.

In dit proefschrift worden verschillende onderzoeken beschreven. Deze onderzoeken zijn er uiteindelijk allemaal op gericht om de behandeling van patiënten met chronische darmontstekingen te verbeteren, maar de weg er naar toe is lang. In dit proefschrift worden voornamelijk de farmaceutische aspecten

die van belang zijn voor het ontwikkelen en het testen van ColoPulse tabletten beschreven. Daarnaast ligt de focus op één specifiek geneesmiddel, het antilichaam infliximab.

Hoofdstuk 2 beschrijft een onderzoek waarin de invloed van verschillende factoren op het afgiftepatroon van een ColoPulse tablet systematisch wordt onderzocht. Hierdoor kan het ontwikkelen van ColoPulse tabletten in de toekomst efficiënter verlopen. Uit eerder onderzoek was al bekend dat er van zure stoffen slechts weinig vrijkomt wanneer zij worden verwerkt in ColoPulse tabletten. Mogelijk zijn er nog meer factoren die invloed hebben op de afgifte uit een ColoPulse tablet. Daarom is een aantal factoren op systematische wijze volgens een "quality by design" aanpak onderzocht: pKa van de werkzame stof, de dikte van de coating, tijd blootstelling aan pH 6,8 en type coatvloeistof. Daarnaast is het effect van een 2,7 mg/cm2 hydroxypropylmethylcellulose (HPMC) coating tussen de tabletkern en de ColoPulse coating bestudeerd. Om het afgifteprofiel vast te kunnen stellen, is naar een aantal uitkomsten gekeken. Hiervoor is een verkorte versie van het gastro-intestinaal simuleringssysteem (GISS) gebruikt. Dit is een laboratoriumopstelling waarin zuurgraad en passagetijden van het maag-darmkanaal in vier fases nagebootst worden. Op basis van de resultaten bleek aceton de voorkeur te hebben boven ethanol als coatvloeistof. De extra HPMC coating bleek niet het gewenst effect te hebben. Verder werd geconcludeerd dat zwak zure, neutrale en zwak basische stoffen het meest geschikt zijn om te verwerken in een ColoPulse tablet en dat er nog meer onderzoek nodig is naar stoffen met een pKa van 3-6.

In hoofdstuk 3 wordt de ontwikkeling van een ColoPulse infliximab tablet beschreven. Deze tabletten zijn gemaakt vanuit het poeder voor infusie Remicade® . Voordat de tabletten werden geslagen is infliximab gestabiliseerd door infliximab samen met inuline te vriesdrogen zodat er een suikerglas ontstond. Vervolgens is de houdbaarheid van deze tabletten gedurende 16 maanden bij drie verschillende bewaarcondities onderzocht. Omdat infliximab een complex eiwit is, zijn er meer stabiliteitstesten nodig dan gebruikelijk bij de ontwikkeling van een tablet. Na 4 maanden was er geen verschil in gehalte, biologische activiteit of structuur tussen de verschillende bewaarcondities. Na 16 maanden bewaren bij 25ºC, 60% relatieve luchtvochtigheid in een gesloten flacon was de biologische activiteit van de tablet 83% ten opzichte van een infliximab standaard. Tabletten die bewaard werden in een open flacon waren minder stabiel evenals tabletten die bewaard werden bij 40ºC, 75% relatieve luchtvochtigheid in een open flacon (respectievelijk 73% en 12% biologische activiteit na 16 maanden). Geconcludeerd werd dat de het ontwikkelen van ColoPulse infliximab tabletten technisch haalbaar is. Wel is er nog optimalisatie van bewaarcondities en verpakkingsmateriaal nodig.

In hoofdstuk 4 wordt een farmacokinetiek studie met infliximab beschreven. Deze studie is uitgevoerd omdat ColoPulse infliximab tabletten bedoeld zijn voor patiënten met chronische darmontstekingen in het colon. Bij ziekte op andere plaatsen zal de intraveneuze behandeling nog steeds noodzakelijk zijn. In de praktijk wordt infliximab in vaste doseringen en vaste intervallen toegediend. Omdat infliximab een duur geneesmiddel is, is het wenselijk dat het zo optimaal mogelijk gebruikt wordt. Daarnaast kunnen patiënten antilichamen tegen infliximab ontwikkelen, waardoor het minder goed werkt. In dit onderzoek is daarom gekeken of het mogelijk is om een model te ontwikkelen op basis van gemeten bloedspiegels van 42 patiënten waarmee de dosis en het doseer-interval van infliximab geoptimaliseerd kunnen worden en waarmee daarnaast dalspiegels die gerelateerd zijn aan respons voorspeld kunnen worden. Over het algemeen wordt aangehouden dat de dalspiegel minimaal 3,0 mg/L moet zijn. Op basis van het ontwikkelde model kan geconcludeerd worden dat het voor patiënten zonder antilichamen tegen infliximab mogelijk kan zijn om het infuus iedere twaalf weken te krijgen in plaats van iedere acht weken. De voorspelde dalspiegels waren bij het grootste deel van de vrouwelijke patiënten > 3,0 mg/L, namelijk 81% bij een interval van 12 weken en 92% bij een interval van 8 weken. Voor mannen was dit 56% versus 67%. Omdat bij een deel van de patiënten geen ziekteactiviteit aanwezig was, maar wel dalspiegels < 3,0 mg/L, is het advies om een daadwerkelijk aanpassing van dosering of doseerinterval altijd op basis van ziekteactiviteit uit te voeren. Het ontwikkelde model laat zien dat het mogelijk is om doseeradvies op basis van bloedspiegels te geven, maar het moet worden bevestigd in een prospectieve studie op basis van de gangbare ziekteactiviteit parameters calprotectine of door middel van een endoscopie.

In hoofdstuk 5 wordt een klinisch onderzoek beschreven naar de haalbaarheid van een nieuwe methode om met behulp van twee stabiele isotopen van ureum in één dag het afgifte profiel van colon-specifieke toedieningsvormen te kunnen bepalen. In eerdere studies werd alleen 13C-ureum gebruikt en werd afgifteprofiel van een gecoate en een ongecoate ColoPulse capsule op twee verschillende dagen vergeleken. Door 15N2-ureum in een ongecoate capsule tegelijk in te nemen met 13C-ureum in een ColoPulse capsule kan deze studie op één dag worden uitgevoerd, waardoor kosten en belasting van de proefpersoon omlaag gaan en de betrouwbaarheid wordt vergroot. Dit werkt als volgt: als 13C-ureum in het colon vrijkomt, wordt het door bacteriën die alleen daar aanwezig zijn gefermenteerd, opgenomen in bloed en vervolgens als 13CO2 uitgeademd. 13C-ureum wat eerder in het maagdarmkanaal vrijkomt, wordt niet gefermenteerd, opgenomen in het bloed en vervolgens uitgescheiden via de urine. Dit laatste geldt voor 15N2-ureum in een ongecoate capsule. In deze studie werden de haalbaarheid van deze methode getest in vier gezonde

vrijwilligers. De ratio 13C/15N verschilde initieel tussen de proefpersonen maar bleef constant tussen 12 en 24 uur na inname van de capsules. De hoeveelheid 13C die werd teruggevonden in urine was in alle gevallen veel lager dan de hoeveelheid 15N (11,9% versus 73,1%). De ratio 13C/15N vanaf twaalf uur na inname van de capsules kon gebruikt worden om de vrijgekomen hoeveelheid op t = 24 uur te voorspellen. Door combinatie van ademmonsters en één urinemonster kan in principe voldoende informatie worden verkregen om orale colon-specifieke toedieningsvormen te evalueren met daarbij een reductie van kosten en monsters van tenminste 50%.

In hoofdstuk 6 wordt een studie beschreven waarin meer onderzoek gedaan is naar het verband tussen afgifte van een ColoPulse tablet en de pH van het maag-darmkanaal van een proefpersoon. Afgifte vanuit een ColoPulse tablet start wanneer een pH van 7,0 is bereikt. Dit is in eerdere studies, waaronder de studie zoals beschreven in hoofdstuk 5, onderzocht met behulp van stabiele isotopen. Door deze gegevens te koppelen aan data over de pH, kan een nog completer beeld van het functioneren van ColoPulse tabletten worden verkregen. De pH van het maag-darmkanaal kan worden gemeten door middel van een speciale capsule, het IntelliCap systeem®. Deze capsule meet na inslikken iedere 10 seconden de pH. De data worden vervolgens doorgezonden naar een ontvanger. In deze studie namen in totaal 12 proefpersonen gelijktijdig een ColoPulse tablet met 13C-ureum een IntelliCap® capsule in. Vervolgens werden de 13C-ureum data vergeleken met de pH data. Hieruit bleek dat het tijdstip van de afgifte vanuit de ColoPulse tablet (5:31 uur na inname) gelijk was aan het tijdstip waarop de IntelliCap® het colon bereikte (5:42 uur, p=0.903). Op basis van deze gecombineerde data kan geconcludeerd worden dat de afgifte vanuit een ColoPulse tablet daadwerkelijk plaatsvindt in het colon.

Mogelijk is de afgifte vanuit een ColoPulse tablet anders bij patiënten met chronische darmontstekingen dan bij gezonde vrijwilligers. Tot nu toe is het afgiftepatroon van ColoPulse tabletten en capsules alleen maar onderzocht in gezonde vrijwilligers. Verder was de tijd tussen inname van een ColoPulse tablet en een ontbijt altijd tenminste drie uur. Dit is niet praktisch in het dagelijks leven. In hoofdstuk 7 worden de resultaten van een studie in gezonde vrijwilligers en patiënten beschreven. In deze studie werd gebruik gemaakt van ColoPulse tabletten met 13C-ureum en ongecoate tabletten met 15N2-ureum zoals beschreven in hoofdstuk 5. Op dag 1 werden de ColoPulse tabletten ingenomen en een uur daarna kregen de proefpersonen een zelf gekozen ontbijt. Op dag 2 kregen de proefpersonen 3 uur na inname van de tabletten een gestandaardiseerd ontbijt net als in vorige onderzoeken. Er werd geen verschil gevonden tussen gezonde vrijwilligers en patiënten met de ziekte van Crohn wanneer gekeken werd naar de hoeveelheid vrijgekomen 13C-ureum. Er was

geen verschil in hoeveelheid vrijgekomen ureum tussen gezonde vrijwilligers en patiënten met de ziekte van Crohn. Verder was er geen verschil in hoeveelheid vrijgekomen ureum bij een zelf gekozen ontbijt na 1 uur en een gestandaardiseerd ontbijt na 3 uur. Dit gold zowel voor gezonde vrijwilligers (75,5% versus 83,4%, p=0,077) als voor patiënten met de ziekte van Crohn (90,2% vs 91,4%, p=0,618). Wel was er een aantal, klinisch niet-relevante, verschillen in afgifte tussen beide groepen, die vooral gerelateerd zijn aan het feit dat voedsel de passagetijd van het maag-darmkanaal beïnvloedt.

In dit proefschrift zijn verschillende onderzoeken beschreven die allemaal een bijdrage leveren aan de farmaceutische en klinische ontwikkeling van ColoPulse tabletten met daarbij een focus op inflammatoire darmziekten. De resultaten van deze onderzoeken hebben de ontwikkeling van een infliximab tablet als alternatief voor parenterale toediening een stap dichterbij gebracht. Vanaf dit punt is nu gestart met de voorbereidingen voor een klinische studie om te onderzoeken of de veronderstelling dat infliximab tabletten in ieder geval even goed werken als een infuus inderdaad klopt.

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About the author

Curriculum vitae

Marina Maurer was born on January 18 1979 in Kampen (Overijssel, the Netherlands). She studied pharmacy at the University of Groningen (1997-2003) and graduated cum laude. She specialised in analytical chemistry and developed an interest in hospital pharmacy. In 2001 and 2002 she worked in short term jobs during summer in the laboratory of the hospital pharmacy of the University Medical Centre Groningen (UMCG).

After graduation Marina started working as a pharmacist in the Scheeperziekenhuis Emmen, the Netherlands. In 2004 she was appointed at the University Medical Centre Groningen, where she worked as a clinical trial pharmacist combined with pharmaceutical patient care.

From 2007 to 2010 she was trained as a hospital pharmacist under supervision of professor Jos Kosterink. As part of this training she specialized in pharmaceutical compounding. The research part of this training, conducted in collaboration with Dr. Reinout Schellekens, formed the starting point of the PhD project on further development and application of the ColoPulse technology. In January 2011 she was registered as a hospital pharmacist and started working at the compounding department of the UMCG, in charge of individual preparations.

Marina spends her spare time playing the violin in several orchestras and a string quartet. She is married with Albertjan Tollenaar and mother of Rosan (2012) and Vygo (2013). Together they love to travel and explore new destinations.

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