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.

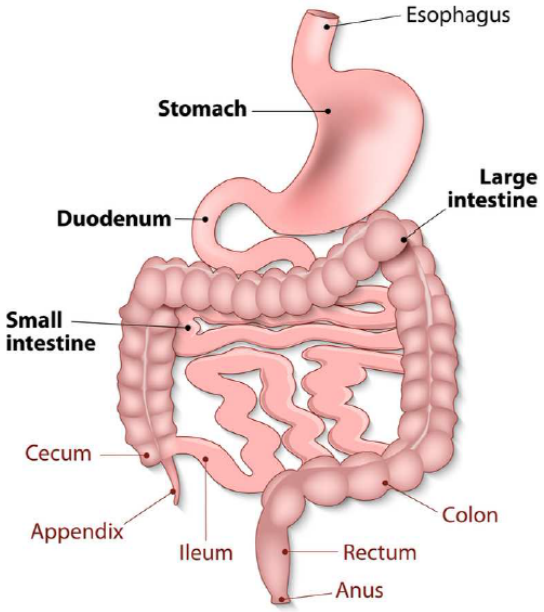

Figure 1: Schematic overview of the gastrointestinal tract. Source: shutterstock.com

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].

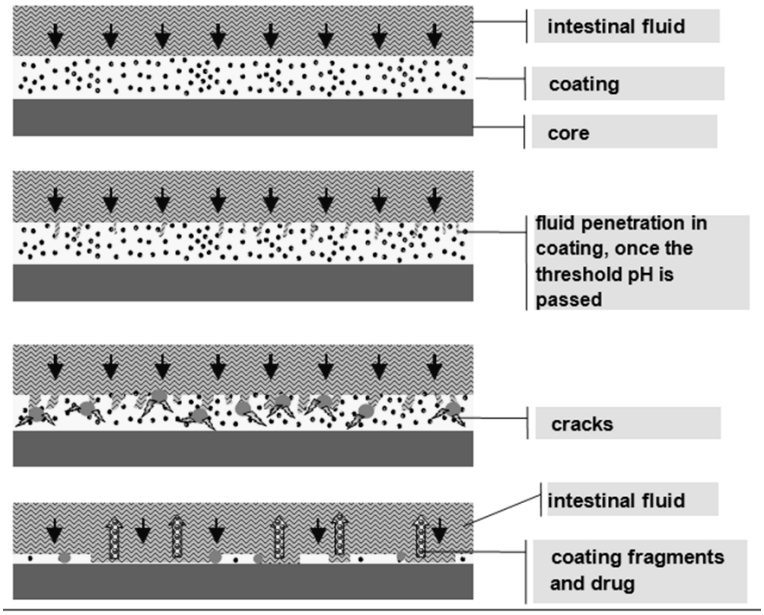

Figure 2: Overview of the mechanism and release from a ColoPulse dosage form

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.

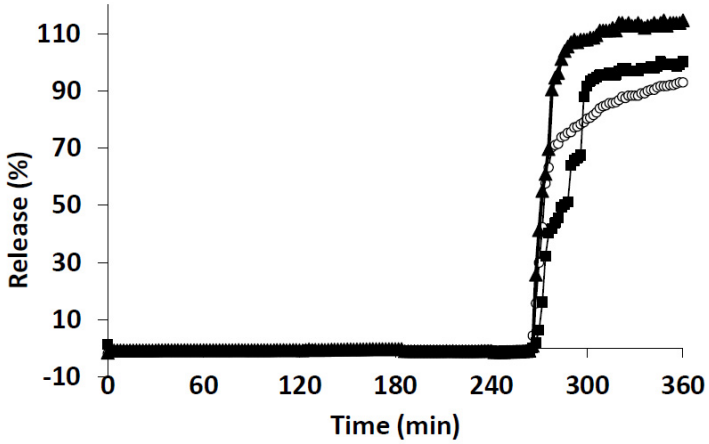

Figure 3: Typical release profile from a ColoPulse 25 mg caffeine tablet, coat thickness 15.3 mg/cm^2 (n = 3)

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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