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.