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Infliximab formulation strategy for a stable ileo-colonic targeted oral dosage form intended for the topical treatment of inflammatory bowel disease

Bahez Gareb a,b,*, Max Beugeling b, Silke Posthumus b, Antonius T. Otten c, Gerard Dijkstra c, Jos G.W. Kosterink a,d, Henderik W. Frijlink b

a Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands
b Department of Pharmaceutical Technology and Biopharmacy, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, the Netherlands
c Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands
d Department of PharmacoTherapy, Epidemiology and -Economics, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, the Netherlands

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ABSTRACT

Research shows that topically active infliximab therapy may be efficacious in the treatment of inflammatory bowel disease (IBD) with expected fewer side effects related to systemic exposure. Oral administration of infliximab with site-specific delivery could enable such therapy. In the present study, infliximab was incorporated in a sugar glass matrix (IFX-I) for additional stability and compounding flexibility after which IFX-I was compounded to ileo-colonic-targeted tablets containing 5 mg infliximab (ColoPulse-IFX). Potential critical steps in the production process that may decrease the formulation stability were identified and investigated. Furthermore, the long-term stability of IFX-I (6 months) and ColoPulse-IFX (12 months) stored either at room temperature (25 °C ± 2 °C/60% RH ± 5% RH) or refrigerated (5 °C ± 3 °C) was investigated according to ICH guidelines. Size-exclusion chromatography, fluorescence spectroscopy, and ELISA analyses were used to investigate the infliximab stability, content, tertiary protein structure, and potency at t0, t3, t6, t9, and t12 months. The coating performance of ColoPulse-IFX was investigated in a gastrointestinal simulation system at t0 and t12 months. All the analyses showed that IFX-I and ColoPulse-IFX were stable, potent, and that the coating performance was maintained during the entire storage period at both storage conditions. Thus, IFX-I is a stable dry-powder formulation and ColoPulse-IFX is a promising oral dosage form for the topical treatment of ileo-colonic IBD. This formulation strategy may serve as a new platform for the development of oral peptide or protein formulations that are targeted to the ileo-colonic region in IBD.

1. Introduction

Ulcerative colitis (UC) and Crohn’s disease (CD) are chronic inflammatory bowel diseases (IBD) that affect the gastrointestinal tract (GIT). IBD is characterized by gut epithelial dysfunction, which results in an increased exposure of the gut wall to luminal antigens. Consequently, immune cells secrete proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), resulting in the disease symptoms and tissue damage [1–4]. The inflammation sites in the GIT are generally localized regions and the immunological environment and cytokine profile in these regions correlate with IBD type [3–7], disease activity [8–11], and relapse [12,13]. Ileo-colonic inflammation is observed in approximately 50% of the IBD patients [4,14]. Anti-TNF-α therapy in IBD is efficacious and resolves the disease symptoms that are associated with the elevated TNF-α levels. Currently all the anti-TNF-α therapies in the treatment of IBD are proteins such as monoclonal antibodies or soluble TNF-α receptors and are administered

* Corresponding author. Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands.
E-mail address: b.gareb01@umcg.nl (B. Gareb).

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systemically by subcutaneous injections or intravenous infusions [15–17]. These routes of administration are reported by IBD patients as undesired and score significantly less favorable compared to oral therapy [18,19].

Infliximab is a chimeric monoclonal antibody against TNF-α. Systemic infliximab exposure is associated with adverse events that may complicate the treatment of IBD. These adverse events include infusion reactions [20,21], the development of antinuclear antibodies (ANA) [22–25], psoriasisiform lesions [26], osteonecrosis of the jaw [27,28], an increased risk of opportunistic infections [29–31], developing lymphoma [32], and the induction of anti-drug antibodies (ADA) [33–35]. However, research shows that the anti-inflammatory effects of infliximab are correlated with the site-specific immunological effects in the GIT and that the tissue concentrations at these sites correlate with the clinical response rather than serum levels alone [36–38]. These data suggest that site-specific as opposed to systemic TNF-α inhibition in IBD may be an efficacious treatment option with expected fewer side effect [17]. Oral administration of infliximab that is targeted to the ileo-colonic region could enable such therapy for ileo-colonic IBD patients.

The ColoPulse coating technology is a formulation strategy that targets oral dosage to the ileo-colonic region in IBD. This coating consists of a pH sensitive polymer matrix (pH threshold of ≥ 7.0) in which a superdisintegrant, such as croscarmellose sodium, is incorporated in a non-percolating manner. During GI transit [39–41], the coating remains intact in the stomach (pH ~ 1.0–2.0) and jejunum (pH ~ 6.0–6.8). In the terminal ileum (pH ~ 7.5), however, the coating starts to disintegrate, which exposes the incorporated superdisintegrant to the luminal fluids. The rapid swelling of the superdisintegrant exerts a great force on the coating matrix that results in rapid coating disintegration, even if the exposure time of the coating to luminal fluids with a pH > 7.0 is short [42]. Drug release from the coated tablet core ensues after the coating is disintegrated, resulting in the ileo-colonic targeting of the formulation. In vitro studies [42–45] as well as five clinical trials in healthy subjects and CD patients [46–50] have shown that ColoPulse-coated oral dosage forms reproducibly target the ileo-colonic region.

Previously we developed a novel ileo-colonic targeted oral dosage form containing 5 mg IFX (ColoPulse-IFX) with the objective to treat ileo-colonic IBD topicaly [43]. The stresses associated with compounding proteins such as infliximab into a coated and ileo-colonic targeted tablet may be detrimental for the stability and potency of the formulation. Incorporating proteins in sugar glass matrices can increase the stability of protein formulations by means of preserving the native protein conformation in a rigid, amorphous sugar glass matrix [51,52]. Therefore, we incorporated infliximab in an insulin sugar glass matrix (IFX-I) for additional stability during the compounding process and the storage period. Subsequently, IFX-I was used for the production of ColoPulse-IFX.

For the clinical application of ColoPulse-IFX, the formulation should be produced on a large scale in a Good Manufacturing Practice (GMP) compounding facility. The stresses associated with the compounding process of larger batches of coated tablets are generally greater compared to the production process of smaller batches in a laboratory setting. The increased stresses may negatively impact the formulation stability and hinder the clinical feasibility and applicability. Furthermore, the long-term stability as well as the desired storage conditions of the formulation should be investigated. To date, no formulation strategy nor extensive long-term stability data of a feasible oral dosage form containing an antibody that is targeted to the distal GIT for the topical treatment of inflammation sites have been reported [17,53]. Together, obtaining these results should show whether the formulation strategy is feasible for the production of orally administered and ileo-colonic targeted infliximab therapy in IBD. Additionally, the formulation strategy data may give guidance in the development of novel peptide or protein formulations that are administered orally and targeted to the ileo-colonic region in IBD.

The objective of this study was to identify and investigate the critical steps in the formulation strategy of ColoPulse-IFX that may negatively impact the stability. In addition, the objective was to investigate the long-term stability of IFX-I (6 months) and ColoPulse-IFX (12 months) at room temperature or refrigerated according to ICH guidelines [54]. The objective of the IFX-I stability study was to investigate whether the dry-powder was stable during storage before further compounding whereas the objective of the ColoPulse-IFX stability study was to investigate whether this formulation is a feasible and stable oral dosage form for the treatment of IBD.

2. Materials and methods

2.1. Chemicals

The chemicals used for the present study were potassium dihydrogen phosphate, t alc, sodium chloride (S pruyt-Hillen, L ijsselstein, The Netherlands), microcrystalline cellulose (DMV Fonterra Excipients, F oxhol, The Netherlands), polyethylene glycol 6000 (PEG 6000, Fagron, Capelle aan den IJssel, The Netherlands), poly sorbate 20 (Sigma- Aldrich, St. Louis, MO, USA), infliximab (Remsima, C elltrion, Incheon, Korea), insulin (Frutarfit T EX!, Sensus, Roosendaal, The Netherlands), methacrylic acid–methyl methacrylate copolymer 1:2 (Eu dragit S100, Evonik, Essen, Germany), croscarmellose sodium (F MC, Brussels, Belgium), sodium stearyl fumarate (JRS Pharma, Ro senberg, Germany), acetone, hydrochloric acid 37%, sodium hydroxide, (VWR, Fontenay- sous-Bois, France), disodium hydrogen phosphate dihydrate, and sodium dihydrogen phosphate dihydrate (Merck, Darmstadt, Germany).

2.2. Design of the study

The graphical abstract of the present study shows a schematic overview of the study design. Compounding infliximab into coated tablets exposed the protein to great stresses. The identified major stresses of the compounding process were shear stress (mixing procedure), compaction force (tabletting procedure), and exposure to a water-organic solvent mixture (coating procedure). Therefore, the infliximab in commercially available Remsima vials was reconstituted with an insulin sugar solution and subsequently lyophilized. This yielded the infliximab sugar glass powder in which the infliximab was incorporated in a sugar glass matrix (IFX-I) for additional stability and compounding flexibility.

The stability of infliximab during the production process was investigated. First, it was investigated whether the shear stress associated with mixing a large amount of the dry-powder tablet mixture, which contained IFX-I and the tablet core excipients, had an effect on the stability of infliximab in the uncoated tablet core (Uncoated-IFX). Thereafter, the effects of the coating procedure on the infliximab stability of Uncoated-IFX was investigated by simulating several coating procedures. The stability data from these experiments give insight into the critical steps that should be controlled during the GMP upscaling of the production process.

Although the compaction force is also a critical process parameter, this variable was kept constant in the present study. Changing the compaction force alters the tablet geometry and porosity, which in turn changes the behavior of the tablet bed during the coating procedure and tablet core dissolution characteristics, respectively. Furthermore, an increase in compaction force might negatively impact the infliximab stability in the tablet core. The introduction of these variables were considered undesired since the formulation characteristics should comply with the set requirements (Supplementary Table 1) and these were met with the applied compaction force.

The present study also investigated the long-term stability of IFX-I and ColoPulse-IFX. IFX-I (6 months) and ColoPulse-IFX (12 months) were stored either at room temperature (25 °C ± 2 °C/60% RH ± 5% RH) or refrigerated (5 °C ± 3 °C) according to the ICH guidelines on
long-term stability studies [54]. The requirements of the stability study are given in Supplementary Table 1 and can be summarized as a content and potency of 90–110% with no apparent deviations compared to reference infliximab.

Analyses at t0, t3, t6, t9, and t12 months investigated the stability, content, fragmentation, tertiary protein structure, potency, and coating performance of IFX-I and ColoPulse-IFX with size-exclusion chromatography (SEC), fluorescence spectroscopy, ELISA, and the gastrointestinal simulation system (GISS), respectively (Table 1).

### 2.3. Lyophilization and production of IFX-I

The infliximab in commercially available Remsima vials (100 mg infliximab per vial) was reconstituted with 10 ml 5% (w/v) inulin dissolved in demineralized water. This infliximab solution was aliquoted, snap-frozen in liquid nitrogen, and lyophilized (Christ, Salm & Kipp, Breukelen, The Netherlands). The primary drying step of the lyophilization procedure was 24 h at −35°C and 0.220 mbar followed by the secondary drying step consisting of 30 h at 25°C and 0.050 mbar. The lyophilized cake (IFX-I) was sieved (0.4 mm) for further compounding.

### 2.4. Tablet core production

The components of the investigated formulations are given in Table 2. The Uncoated-IFX that was used to investigate the infliximab stability during the mixing process was produced by mixing the dry-powder mixture in a Turbula mixer (Bachofen, Basel, Switzerland) at 90 rpm for 10 min (rough mixing) or by mixing the powder manually (gentle mixing). The resulting powder mixture was tested before and after tabletting with the SEC method described in Section 2.9. The tablets that were used to investigate the long-term infliximab stability during the entire storage period as well as to investigate the stability during the coating process were produced by manually mixing the dry-powder tablet mixture until a homogenous mixture was obtained using a validated mixing process. For the GISS experiments (see Section 2.12), caffeine (25 mg) was added to the formulation (ColoPulse-IFX-caff). The experimental justification and explanation for this approach has been discussed in detail elsewhere [43].

All the tablet cores were produced by compacting the dry-powder mixture at 3 kN with a rate of 0.5 kN/s (Instron, Norwood, MA, USA).

### 2.5. Tablet coating

The ColoPulse coating suspension was prepared by mixing Eudragit S100/PEG 6000/croscarmellose sodium/talc in a ratio of 7/1/3/2 (w/v). The ColoPulse coating solvent mixture was acetone/water 97/3 (v/v). For the coating procedure, the coating suspension was continuously sprayed onto the tablets in a mini-rotating drum coater (Erweka, Heusenstamm, Germany) that was modified in-house in view of optimizing the ColoPulse coating process. To induce solvent mixture evaporation and coating film formation, a hot-air blower was aimed at the mini-rotating drum for mild heating [43]. The batch size (varied), pan load, pan speed, tablet bed temperature, gun-to-bed distance, and spray rate (varied with batch size) during the coating process were 17.5–75 g, 35 rpm, 30°C, 10 cm, 2.5–4.5 g/min, respectively. For the experiment described in section 2.6, a pan load of 17.5 g was chosen in view of reducing the generated acetone vapors during the stress test.

### 2.6. Infliximab stability during the coating process

To investigate the effects of the coating procedure on the infliximab stability, an acetone/water 97/3 (v/v) mixture (coating solvent) without the other ColoPulse coating constituents was sprayed onto the Uncoated-IFX tablets for 30 min in the exact same manner as described in Section 2.5, with (control) and without the hot-air blower (minor acetone stress; tablet bed temperature not regulated). Additionally, Uncoated-IFX tablet were exposed for 60 min to a saturated acetone vapor pressure in a closed glass container (major acetone stress).

### 2.7. Long-term stability study of IFX-I and ColoPulse-IFX

IFX-I, ColoPulse-IFX, and ColoPulse-IFX-caff (Table 1) were all stored in separate closed polypolyene containers in which a desiccant was present. One container of each formulation was stored at room temperature (25°C ± 2°C/60% RH ± 5% RH) and the other container of the same formulation was stored refrigerated (5°C ± 3°C) [54]. The storage period of IFX-I was 6 months and the experiments were conducted at t0, t3, and t6 months. The storage period of ColoPulse-IFX was 12 months and the experiments were conducted at t0, t3, t6, t9, and t12 months. The storage period of ColoPulse-IFX-caff was 12 months and the experiments were conducted at t0 and t12 months.

### 2.8. Sample preparation

Pulverized ColoPulse-IFX tablets or an amount of IFX-I corresponding to 5 mg infliximab was quantitatively transferred in 10 mL PBS pH 6.8 (150 mM) containing 0.05% (w/v) polysorbate 20. The resulting mixture was mixed for 10 min and filtered through a 0.45 μm filter. The filtrate was analyzed with SEC, fluorescence spectroscopy, and ELISA.

For the tablet crushing strength experiments, ColoPulse-IFX crushing strength was determined with a tablet hardness tester (Erweka, Heusenstamm, Germany).

### Table 2: The component of the investigated formulations.

<table>
<thead>
<tr>
<th>Components</th>
<th>IFX-I</th>
<th>ColoPulse-IFX</th>
<th>ColoPulse-IFX-caff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcrystalline cellulose (mg)</td>
<td>Ad 350</td>
<td>Ad 350</td>
<td>Ad 350</td>
</tr>
<tr>
<td>Silicon dioxide (mg)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Croscarmellose sodium (mg)</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Sodium stearyl fumarate (mg)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>–</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>ColoPulse (mg/cm²)</td>
<td>–</td>
<td>11–15</td>
<td>11–15</td>
</tr>
</tbody>
</table>

a. Amount varied since the content of infliximab per gram IFX-I differed from batch to batch. Range: 88–91 mg infliximab per gram IFX-I. Theoretical infliximab content was 90 mg infliximab per gram IFX-I.

b. Expressed as applied Eudragit S100 per cm² of tablet surface.

c. Fluorescence spectroscopy to investigate the tertiary protein structure.

d. ELISA to investigate the TNF-α-binding potency.

e. Gastrointestinal simulation system (GISS) to investigate the in vitro coating performance.
2.9. SEC analysis

Filtered samples were analyzed with SEC (Phenomenex, BioSep 5 μm SEC-x3000 290 Å, Torrance, CA, USA) for infliximab content and potentially formed fragments and/or soluble aggregates. The wavelength, injection volume, flow rate, column temperature, run time, and mobile phase were λ = 280 nm, 20 μL, 1.0 mL/min, 22 °C, 15 min, and PBS pH 6.8 (150 mM) containing 0.05% (w/v) polysorbate 20, respectively.

2.10. Fluorescence spectroscopy analysis

Fluorescence spectroscopy was applied to investigate the tertiary protein structure of infliximab [43,55]. Filtered samples were 10 × diluted in PBS pH 6.8 (150 mM) that contained 0.05% (w/v) polysorbate 20. The samples were analyzed in a fluorescence quartz cuvette (path length 10.00 mm). The content of the cuvette (sample) was magnetically stirred to prevent photobleaching and the intrinsic fluorescence of infliximab was analyzed (Photon Technology International, Inc., Birmingham, AL, USA). The excitation wavelength, slit width, sample temperature, and recorded spectrum range were λ = 295 nm, 2.5 nm, 20.0 °C, λ = 300–360 nm, respectively.

2.11. ELISA analysis

The TNF-α-binding potency of infliximab was analyzed with ELISA. A validated sandwich-type ELISA kit (MabTrack M2920, Sanquin, Amsterdam, The Netherlands) was used for the analysis [43]. The filtered samples were 500 × diluted in human serum. This solution was further diluted 200 × with the provided dilution buffer from the ELISA kit. The ELISA protocol of the manufacturer was followed [56]. This protocol consisted of three incubation steps, two wash steps, and the addition of a stop solution to stop the reaction. Subsequently, the OD450 nm was measured (BioTek, Winooski, VT, USA). During each analysis, quality control (QC) reference samples of 5 mg fresh infliximab stock were also analyzed. The infliximab potency was calculated with the supplied 6-point calibration curve and the analyzed QC values were taken as 100% potency.

2.12. Gastrointestinal simulation system

The coating performance of ColoPulse-IFX was investigated in the in vitro model GISS, which simulates GI transit. Briefly, the stomach (pH 1.2 for 2 h), jejunum (pH 6.8 for 2 h), ileum (pH 7.5 for 30 min), and colon (pH 6.0 for as long as needed) are simulated consecutively during each experiment. The GISS is described in detail elsewhere [57].

An USP dissolution apparatus II (Rotax, Basel, Switzerland) coupled with a UV-VIS spectrophotometer (Thermo Fisher, Madison, WI, USA) was used. The medium temperature, paddle speed, path length, and wavelength were 37 °C, 50 rpm, 10.00 mm, and λ = 274 nm, respectively. The medium constituents as well as the medium volumes were variable since during each experiment, buffers were added consecutively for the pH change to simulate the GI transit. The initial medium volume was 500 mL (stomach) and the final volume was 1000 mL (colon). During each experiment, the pH was measured to ensure the desired pH.

Caffeine (25 mg) was added to ColoPulse-IFX (ColoPulse-IFX-caff; Table 2) as a model drug to investigate the coating performance in the GISS with UV-VIS spectroscopy since the infliximab release could not be analyzed accurately due to the resulting low concentrations (0.25–5.0 μg/mL) in the release media. However, the infliximab release from ColoPulse-IFX correlates with the caffeine release from ColoPulse-IFX-caff and this has been described in detail elsewhere [43].

3. Results

3.1. Infliximab stability during compounding

The effects of the mixing procedure of the dry-powder tablet mixture were investigated. The powder was mixed either manually (gentle mixing) or in a Turbula mixer (rough mixing). The mixed powders were first analyzed for infliximab content with SEC, thereafter the powders were tableted, and the uncoated tablets (Uncoated-IFX) were again analyzed for infliximab content with SEC (Supplementary Table 2). When the dry-powder mixture was mixed manually, the average (n = 3) infliximab content of the powder and Uncoated-IFX was 99 ± 3% and 100 ± 1%, respectively. When the dry-powder mixture was exposed to greater shear stress during mixing in the Turbula mixer, a remarkable increase in the average infliximab content was observed (109 ± 3%) which decreased substantially (77 ± 4%) after the tableting procedure. No changes other than the changes in the area of the infliximab peak on the SEC chromatogram were observed.

The effects of the coating procedure were investigated by spraying (30 min) an acetone-water mixture (coating solvent) without the other ColoPulse coating constituents on Uncoated-IFX with (control) and without (minor acetone stress) using the hot-air blower that was utilized during our validated coating procedure. Additionally, Uncoated-IFX was exposed for 60 min to a saturated acetone vapor pressure in a closed glass container (major acetone stress). Fig. 1 shows the representative SEC chromatograms of the control, minor acetone-stressed, and major acetone-stressed Uncoated-IFX tablets. The SEC chromatogram of control Uncoated-IFX shows the infliximab peak at approximately 8.5 min with no apparent deviations compared to fresh infliximab stock (see Supplementary Figure 1 for the infliximab reference chromatogram). The SEC chromatogram of minor acetone-stressed Uncoated-IFX shows an additional peak at approximately 12 min (peak area 4% of total) whereas the area of the infliximab peak at 8.5 min decreased (peak area 96% of total). The SEC chromatogram of major acetone-stressed Uncoated-IFX shows the same additional peak at 12 min. However, the area of this additional peak is greater (peak area 19% of total) whereas the area of the infliximab peak at 8.5 min decreased accordingly (peak area 81% of total).

3.2. Appearance and mass of IFX-I and ColoPulse-IFX

No noticeable changes in the appearance of IFX-I and ColoPulse-IFX were tableted, and the uncoated tablets (Uncoated-IFX) were again analyzed for infliximab content with SEC ( Supplementary Table 2 ).
were observed during the entire storage period at the different storage conditions. Furthermore, no noticeable changes in the flowability of the IFX-I powder were observed. The average mass gained during the storage period was <1.0% for IFX-I and ColoPulse-IFX. These data suggest that no moisture was absorbed during the entire storage period at both storage conditions.

3.3. Tablet crushing strength of ColoPulse-IFX

Supplementary Table 3 shows the average (n = 3) tablet crushing strength of ColoPulse-IFX during the storage period at both storage conditions. At t0 months, the average (range) crushing strength was 382 (366-392) N. The tablet crushing strength complied with the set requirement of >200 N (Supplementary Table 1) during the entire storage period at both storage conditions. No remarkable changes in crushing strength were observed for ColoPulse-IFX stored at room temperature. However, a small increase in the tablet crushing strength was observed during the refrigerated storage period.

3.4. Infliximab content

SEC analysis was used to investigate the infliximab content as well as the potentially formed protein fragments and/or soluble aggregates during the long-term stability study (Table 3). At t0 months, the average (n = 3) infliximab content of IFX-I and ColoPulse-IFX was 100 ± 1% and 102 ± 5%, respectively. The infliximab content of IFX-I as well as ColoPulse-IFX remained within the set limit of 90–110% on all the timepoints during the entire storage period at both conditions. No noticeable trends were observed regarding the content. Furthermore, no additional peaks or changes were observed on the SEC chromatograms of IFX-I or ColoPulse-IFX during the analyses on all timepoints.

Supplementary Figures 1 and 2 show the representative SEC chromatograms of IFX-I and ColoPulse-IFX on the last timepoints, respectively. The SEC chromatograms of ColoPulse-IFX showed an additional peak at approximately 12.5 min during the analyses on all timepoints. This peak had a different shape and was less sharply defined compared to the additional peak that was observed in Fig. 1. Moreover, the presence of this additional peak had no effect on the area of the infliximab peak at 8.5 min, which remained ±100%. Earlier investigations showed that this peak was the result of the pH sensitive polymer Eudragit S100 that is present in the ColoPulse coating [43]. This observation was consistent with the SEC chromatogram of a ColoPulse-IFX placebo tablet that contained the same components as ColoPulse-IFX without the added infliximab (Supplementary Figure 2, chromatogram 1, black line).

These data show that no degradation or decrease in the infliximab content of IFX-I and ColoPulse-IFX was observed during the entire storage period at the two different storage conditions as analyzed with SEC.

3.5. Infliximab tertiary protein structure

Fluorescence spectroscopy was used to investigate the tertiary protein structure of infliximab during the long-term stability study [43, 55]. Fig. 2 shows the average fluorescence spectra of IFX-I and ColoPulse-IFX. Fig. 2 also shows the average spectra of fresh infliximab stock for reference and infliximab stressed for 1 h at 60 °C as an indication of the expected changes in the spectrum that may result from stress-induced alterations. In view of clarity, the spectra of IFX-I at t3 months and the spectra of ColoPulse-IFX at t3 and t9 months are not shown. However, these spectra were similar to all the depicted IFX-I and ColoPulse-IFX spectra, respectively. Fig. 2 shows no apparent deviations of the IFX-I spectra at t0 and t6 months compared to the infliximab reference spectrum. Furthermore, no apparent deviations of the ColoPulse-IFX spectra at t0, t6, and t12 months compared to the infliximab reference spectrum were observed. However, the spectrum of infliximab stressed for 1 h at 60 °C shows a remarkable increase in the fluorescence intensity. This was likely the result of the denaturation of the native tertiary protein structure of infliximab [43]. These data show no apparent alterations in the native infliximab tertiary protein structure of IFX-I and ColoPulse-IFX that resulted from the storage period at the two different storage conditions.

3.6. Infliximab TNF-α-binding potency

ELISA analysis was used to investigate the TNF-α-binding potency of infliximab during the long-term stability study (Table 3). The average infliximab potency of IFX-I and ColoPulse-IFX at t0 months was 97 ± 6% ± 5%, respectively. The infliximab content of IFX-I as well as ColoPulse-IFX remained within the set limit of 90–110% on all the timepoints during the entire storage period at both conditions. No noticeable trends were observed regarding the content. Furthermore, no additional peaks or changes were observed on the SEC chromatograms of IFX-I or ColoPulse-IFX during the analyses on all timepoints.

Supplementary Figures 1 and 2 show the representative SEC chromatograms of IFX-I and ColoPulse-IFX on the last timepoints, respectively. The SEC chromatograms of ColoPulse-IFX showed an additional peak at approximately 12.5 min during the analyses on all timepoints. This peak had a different shape and was less sharply defined compared to the additional peak that was observed in Fig. 1. Moreover, the presence of this additional peak had no effect on the area of the infliximab peak at 8.5 min, which remained ±100%. Earlier investigations showed that this peak was the result of the pH sensitive polymer Eudragit S100 that is present in the ColoPulse coating [43]. This observation was consistent with the SEC chromatogram of a ColoPulse-IFX placebo tablet that contained the same components as ColoPulse-IFX without the added infliximab (Supplementary Figure 2, chromatogram 1, black line).

These data show that no degradation or decrease in the infliximab content of IFX-I and ColoPulse-IFX was observed during the entire storage period at the two different storage conditions as analyzed with SEC.

Table 3

The average ± s.d. (n = 3) infliximab content (SEC analysis) and potency (ELISA analysis) of IFX-I and ColoPulse-IFX stored either at room temperature (Room) or refrigerated (Refrig). The results are expressed as % infliximab compared to 5 mg fresh infliximab stock. N.A.: not applicable (see also Table 1).

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>IFX (%)</th>
<th>SEC</th>
<th>ColoPulse-IFX (%)</th>
<th>SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA</td>
<td></td>
<td>ELISA</td>
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<tr>
<td></td>
<td>Room</td>
<td>Refrig</td>
<td>Room</td>
<td>Refrig</td>
</tr>
<tr>
<td>t0 month</td>
<td>97 ± 6</td>
<td>100 ± 1</td>
<td>98 ± 2</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>t3 month</td>
<td>105 ± 4</td>
<td>101 ± 5</td>
<td>103 ± 1</td>
<td>104 ± 1</td>
</tr>
<tr>
<td>t6 month</td>
<td>102 ± 6</td>
<td>102 ± 4</td>
<td>106 ± 1</td>
<td>107 ± 1</td>
</tr>
<tr>
<td>t9 month</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>t12 month</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>
and 98 ± 2%, respectively. The infliximab potency of IFX-I as well as ColoPulse-IFX remained within the set limit of 90–110% on all the timepoints during the entire storage period at both conditions. No noticeable trends were observed regarding the potency.

3.7. In vitro coating performance

The GISS was used to investigate the coating performance of ColoPulse-IFX during the long-term stability study. Caffeine was added to ColoPulse-IFX (ColoPulse-IFX-caff, Table 2; Section 2.12.) as a model drug to investigate the initial moment of coating disintegration. Fig. 3 shows the average release profiles of ColoPulse-IFX-caff at t0 and t12 months. The initial moment of coating disintegration in the GISS at 10 months was at t270 min, which corresponds to the simulated ileum. The initial moment of coating disintegration in the GISS at t12 months was at t250 and t260 min when stored refrigerated or at room temperature, respectively. Both these timepoints correspond with the simulated ileum. Furthermore, after the coating disintegrated at the targeted site, caffeine release from the tablet core was fast and complete. Therefore, these data show that the coating performance as well as drug release characteristics of the formulation was maintained during the entire storage period at both storage conditions.

4. Discussion

The stresses associated with compounding infliximab into ileo-colonic targeted tablets can be detrimental for the formulation stability. The investigation of the identified process steps of the formulation strategy showed that the mixing and coating procedures are critical steps that may negatively impact the formulation stability. Controlling these steps resulted in a stable IFX-I dry-powder formulation for the production of ColoPulse-IFX, which is an ileo-colonic targeted tablet containing 5 mg infliximab intended for the topical treatment of IBD. The results obtained with SEC, fluorescence spectroscopy, and ELISA showed that the infliximab in IFX-I and ColoPulse-IFX was stable and remained potent up to 6 months and 12 months, respectively, when either stored at room temperature or refrigerated. Furthermore, the coating performance and the drug release characteristics of ColoPulse-IFX were maintained during the entire storage period at both storage conditions. Therefore, ColoPulse-IFX is a stable and promising oral treatment option for ileo-colonic IBD. To the best of our knowledge, this study reports for the first time [17,53] a stable oral dosage form that contains a highly efficacious, well studied, and well tolerated monoclonal antibody [58] that is targeted to the distal GIT in IBD with the objective to inhibit TNF-α topically.

The infliximab was incorporated in a sugar glass matrix to obtain additional stability. In general, the stabilizing effects of sugar glass matrices can be attributed to several mechanisms of which the preservation of the native protein structure by the rigid, amorphous matrix is thought to be the main mechanism [51,52,59]. Therefore, the preservation of the native infliximab structure in the inulin sugar glass matrix may contribute to the increased stability by limiting the molecular mobility and protein degradation kinetics during the compounding process and the subsequent storage period.

The increased stability is desired since the stresses associated with the compounding process and subsequent storage may be detrimental for the protein structure. For instance, the mixing of dry-powders in a mixer imposes shear stress on the protein formulation. Furthermore, the compaction forces that are needed for tableting may result in alterations of the protein structure. Additionally, a coating procedure in a rotating coating drum in which a coating mixture of water and organic solvent is continuously sprayed onto the tablets exposes the protein to mechanical stress as well as the water-organic solvent, which may denature the protein. Proteins may also adsorb to insoluble tablet excipients during compounding and/or storage, which may result in incomplete drug release from the formulation after a given storage period.

The investigated critical steps of the formulation strategy showed that the mixing procedure of the dry-powder had an effect on the infliximab content. The results (Supplementary Table 2) of the mixing experiments showed that gently mixing the dry-powder and subsequently tableting the powder did not alter the infliximab content of either the dry-powder itself (99 ± 3%) or the tableted powder mixture (100 ± 1%). However, an apparent increase in the infliximab content (109 ± 3%) of the roughly mixed dry-powder was observed whereas a decrease in the infliximab content (77 ± 4%) of the tablets produced with the same powder mixture was observed. We hypothesize that the increase in shear stress by the rough mixing procedure unfolds the infliximab in such a manner that the aromatic amino acid residues that absorb UV light at 280 nm during the SEC analysis (e.g. tryptophan and tyrosine) are less shielded in an aqueous solution and therefore the

![Fig. 3. The average (n = 3) ColoPulse-IFX-caff release profile in the GISS. Bars depict standard deviations. No efforts were made to simulate the entire colonic transit (6-12 h) since the objective of this experiment was to investigate the initial site of coating disintegration in the simulated GIT.](image-url)
assessing the mixing mode should be considered as a critical process parameter to be evaluated during the tableting process. The tablet formulation used in the present study was a GMP-imab formulation intended for the production of tablets under GMP. Low shear mixers were used to mix the dry-powder ingredients, and the mixing-speed balance as well as the mixing process were evaluated. A suitable mixing process might be proposed to investigate whether the apparent increase in content is indeed caused by infliximab aggregates. Further investigations of the dry-powder mixture showed that using only fluorescence spectroscopy was not sufficient to distinguish between an actual small increase in the infliximab content (109 ± 3% versus 100%) and any detrimental alterations in the three-dimensional structure since both phenomena resulted in an increase in the infliximab fluorescence intensity (Fig. 2). Therefore, several different protein analysis methods should be combined for further investigations. For future research, circular dichroism spectroscopy, liquid chromatography–mass spectrometry, and/or ELISA could be used to investigate whether the apparent increase in content is the result of conformational changes and whether this corresponds with a change in potency.

The coating procedure of Colopulse-IFX had an effect on the infliximab content. An increased exposure to acetone vapor resulted in infliximab fragmentation. Acetone, and organic solvents in general, can denature proteins [62]. In addition, peptide fragmentation through aldimine formation between the ketone group of acetone and the amide group of peptides and antibodies has been reported [63,64]. It may be that exposure to acetone vapor results in both phenomena of which fragmentation and denaturation was observed as a decrease in the infliximab chromatogram peak area, whereas only the fragmentation was seen as an additional peak with a retention time of approximately 12 min (Fig. 1). Hence, a balanced coating procedure that exposes the formulation to a minimum of acetone vapor is desired. On the one hand, the coating procedure should be fast so that the coating time is limited. However, a faster coating time corresponds with an increased amount of coating mixture sprayed per unit time onto the tablets.

This notion also applies to the investigated mixing procedure. On the one hand, the mixing process should be fast so that the amount of time that the formulation is exposed to shear stress is kept to a minimum. On the other hand, the mixing should not be too fast so that the imposed shear stress does not have detrimental effects on the infliximab structure and/or stability. The dry-powder mixtures in the present study were mixed manually to simulate a gentle mixing procedure. However, during the large-scale manufacturing process of the formulation, the mixing process is not a feasible option. A suitable mixing process might be a procedure with a low impeller speed or, alternatively, a procedure with a low mixing time. The determined mixing-speed balance as well as the mixing mode should be considered as a critical process parameter to be controlled during further development.

The formulation stability studies showed that IFX-I remained stable up to 6 months at both storage conditions. A suitable dry-powder infliximab formulation intended for the production of tablets under GMP is necessary. After the production of IFX-I, the necessary quality control tests should be performed to ensure that the raw material for the production of the tablets complies with the set requirements. In a GMP facility, these tests may take up several days to weeks after which the raw material is released for further compounding. Additionally, not all tableting facilities might be equipped with a validated freeze-dryer, which necessitates the lyophilization of infliximab at another facility. An IFX-I storage period of at least 6 months at either room temperature or refrigerated adds to the compounding flexibility of Colopulse-IFX.

The protein analyses of Colopulse-IFX showed that this formulation was stable up to 12 months at both storage conditions. In view of patient convenience, storage at room temperature is desired. However, this storage condition may be associated with lower protein stability. Alternatively, the formulation could be stored refrigerated in view of an increased shelf life. The main disadvantages of refrigerated storage is the necessity of a refrigerator and the relatively humid conditions that may result in water penetration into the formulation. Water can act as a plasticizer in sugar glass or polymer matrices, and therefore, can lower the glass transition temperature (Tg) [61]. The Tg designates the temperature at which the sugar glass or polymer matrix transitions from a rigid glassy state to a more mobile rubbery state. The increased mobility of the sugar glass matrix (i.e. the matrix of IFX-I contained into Colopulse-IFX) may be detrimental for the infliximab stability and conformation.

The mass data of IFX-I and Colopulse-IFX did not show an increase over time (<1.0%). This suggests that no substantial amount of water penetrated into both formulations stored at both storage conditions. However, a small increase in the Colopulse-IFX tablet crushing strength (Supplementary Table 3) was observed during the refrigerated storage period, which did not affect the coating performance of the formulation (Fig. 3). This increase in tablet crushing strength may be explained by the uptake of small amounts (<1.0%) of water by the Colopulse coating polymer matrix, which consists of the pH-sensitive polymer Eudragit S100. Penetrated water into the film coating may act as a plasticizer and lowers the Tg of the polymer matrix, which may result in a more mobile, rubbery state at room temperature; the temperature at which the tablet crushing strength experiments were conducted. A greater force is then needed to break the less rigid and more flexible polymer. However, due to the small sample size (n = 3), data from only two timepoints (t6 and t12 months), and no further efforts made to investigate this phenomenon, this explanation remains speculative.

Since water uptake by both IFX-I and Colopulse-IFX was minimal, we hypothesize that the desiccant present in the closed polypropylene containers was sufficient to protect IFX-I from moisture absorption since insulin is relatively hygroscopic [65,66]. We further hypothesize that the film coating layer of Colopulse-IFX protects the entire tablet core from moisture penetration whereas the tablet excipients (cellulose, croscarmellose sodium) in the tablet core may act as an additional protective barrier. It is assumed that small amounts of moisture is absorbed by these excipients (~80% of the tablet core mass) rather than the IFX-I that is present in Colopulse-IFX. Given the presented results, it is advisable to store IFX-I and Colopulse-IFX at room temperature in colder climates, but refrigerated storage is advisable in warmer climates.

Research shows that the clinical efficacy of infliximab is correlated with the site-specific anti-inflammatory effects in the tissues of the GIT as opposed to drug blood levels, whereas the adverse events that may complicate IBD treatment are associated with the systemic exposure to infliximab. These findings suggest that site-specific TNF-α inhibition in IBD may be efficacious and has fewer expected side effects [17]. Moreover, in vitro studies on the stability of infliximab in simulated colonic conditions are encouraging. Infliximab recoveries of 75% and 40% are reported after 1 h and 2 h simulations, respectively [67,68]. These observations are corroborated by clinical studies on the in vivo stability of antibodies in the GIT. For instance, fecal recoveries of up to 50% of active antibodies are reported after oral administration of non-formulated doses that transit through the entire GIT. An increased recovery was observed when an enteric-coated antibody formulation was orally administered. Interestingly enough, no systemic antibody absorption and exposure was reported in these studies [69,70].
Research on topically administered macromolecules such as therapeutic antibodies and nucleotides in IBD animal models shows that these compounds penetrate into the inflamed regions of the GIT and exert a pharmacological effect. These studies show that on average the penetration into the inflamed regions is greater compared to the unaffected sites of the GIT as a result of the enhanced permeability of the inflamed tissues. These observations are consistent with the results from IBD clinical trials that investigated orally administered and topically active anti-TNF-α proteins or antibodies. The results of these trials show that topically active anti-TNF-α therapy is feasible and associated with a favorable clinical response. Moreover, these drugs penetrate into the inflamed tissues of the GIT whereas no systemic exposure is reported (reviewed in Ref. [17]). Taken together, we hypothesize that a substantial fraction of orally administered antibodies that are targeted to the ileo-colonic region remain intact and topically active, whereas no systemic exposure is expected.

ColoPulse-IFX is intended for the daily oral treatment of ileo-colonic IBD with topically active infliximab and the rationale of the 5 mg dose is discussed elsewhere [43]. The objective of this approach is to maximize the site-specific infliximab concentration in the GIT while minimizing the systemic exposure. In addition, daily topical infliximab therapy continuously exposes the GI tissues to relatively high infliximab concentrations. We hypothesize that this treatment approach may be efficacious and may eliminate the adverse events associated with the systemic exposure to infliximab. Additionally, this treatment approach may also be applied for existing (e.g. ustekinumab or vedolizumab) or new peptide or protein drugs to treat ileo-colonic IBD. The formulation strategy of IFX-I and ColoPulse-IFX may serve as a platform for the development of novel, stable, orally administered, and ileo-colonic targeted peptide or protein formulations. The data on the stability of the formulation during the compounding process may give guidance on the production steps that should be considered critical and should be controlled during the development of these novel formulations.

The strengths of this study were the different analyses that investigated the stability of infliximab during the compounding process as well as the storage period. However, several limitations remain. First, in the present study we did not investigate the degradation products that were observed in Section 3.1. Future research should elucidate the mechanism behind the degradation as well as the identity of these compounds. Second, it is currently unclear whether ileo-colonic-targeted infliximab is efficacious in the treatment of IBD. The data from the present study together with earlier published data on site-specific TNF-α inhibition in IBD [17] indicates that the efficacy and safety of ColoPulse-IFX can be investigated in a clinical trial. This trial should be designed so that the efficacy of ColoPulse-IFX can be compared to systemically administered infliximab. The results from this trial should determine whether ColoPulse-IFX is a valuable addition to the treatment options of ileo-colonic IBD.

5. Conclusion

Compounding infliximab into ileo-colonic targeted tablets with the objective to treat ileo-colonic IBD with topically active infliximab is feasible. Several protein analysis methods showed that IFX-I and ColoPulse-IFX were stable up to 6 months and 12 months, respectively, stored either at room temperature or refrigerated. The results showed no degradation, fragmentation, aggregation, or loss of potency of IFX-I and ColoPulse-IFX during the entire storage period. Therefore, ColoPulse-IFX is a promising oral dosage form for the treatment of ileo-colonic IBD. This formulation strategy may serve as a development platform for orally administered and colon-targeted peptides or proteins. Further research is needed to investigate the clinical efficacy, pharmacokinetics, and safety of ColoPulse-IFX.

Credit authorship contribution statement

Bahez Gareb: Conceptualization, methodology, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization, project administration.

Max Beugeling: Conceptualization, methodology, formal analysis, data curation, writing—original draft, writing—review and editing, visualization, project administration.

Silke Posthumus: Methodology, investigation.

Antonius T. Otten: Writing—review and editing.

Gerard Dijkstra: Conceptualization, writing—review and editing, supervision.

Jos G.W. Kosterink: Conceptualization, resources, writing—review and editing, supervision.

Henderik W. Frijlink: Conceptualization, resources, writing—review and editing, supervision.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2021.102552.

References


