UNIVERSITÀ DEGLI STUDI DI MILANO

Facoltà di Farmacia

Dipartimento di Scienze Farmaceutiche "Pietro Pratesi"

Dottorato di Ricerca in Chimica del Farmaco (XXIII Ciclo)
CHIM/09

DEVELOPMENT OF AN ORAL MULTIPLE-UNIT DOSAGE FORM CONTAINING A HIGH LOAD OF 5-AMINOSALICYLIC ACID

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ANNO ACCADEMICO 2009/2010

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Preface

The work in this dissertation was undertaken in the Dipartimento di Scienze Farmaceutiche "P. Pratesi" at the University of Milan, between November 2007 and December 2010. It is the original work of the author, except where specifically acknowledged in the text. The part of the work presented in Chapter 1 has been carried out in collaboration with the Department of Chemical Engineering and Biotechnology at the University of Cambridge.

No part of the dissertation has been submitted for a degree to any other University.

This dissertation is approximately 30,000 words in length, including appendices, references, tables and equations. It contains precisely 31 figures and 16 tables.

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INTRODUCTION

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a spectrum of chronic, episodic, inflammatory conditions of the gastrointestinal (GI) tract. IBD is conventionally divided into two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC). Both UC and CD can affect individuals of any age but they are mostly diagnosed in patients aged between 15 and 30 years. In the Western world, the number of new cases of IBD diagnosed each year (incidence rate) has been estimated to be about seven per 100,000 (Ghosh *et al.,* 2000), with over 200 people per 100,000 living with UC at any given time. Both UC and CD cause significant gastrointestinal symptoms, including abdominal pain and blood diarrhoea. IBD also is associated with a spectrum of extraintestinal manifestation, including liver and biliary disease, osteoporosis, eye inflammation, arthritis, ankylosing spondylitis, schlerosing cholangitis, pyoderma gangrenosum and erythema nodosum.

Pathogenesis

While CD and UC share a number of gastrointestinal and extraintestinal manifestations and can respond to similar array of drugs, emerging evidences suggest that they result from fundamentally distinct, pathogenic mechanisms (Bouma et Strober, 2003). Crohn's disease may irregularly affect the entire GI tract, even though most commonly the small intestine and the area adjacent to the ileocecal valve. The inflammation in CD is not necessarily confluent and its transmural nature may lead to fibrosis and strictures or, alternatively, to fistulas formation. Conversely, UC is characterised by confluent mucosal inflammation of the colon starting at the anal verge and extending proximally for a variable extent (*e.g.* colitis, left-side colitis, pancolitis). From a histological point of view, the transmural lesions in

Chron's disease exhibit marked infiltration of lymphocytes and macrophages, granuloma formation and submucosal fibrosis, whereas the superficial lesions in ulcerative colitis have lymphocytic and neutrophilic infiltrates. Within the diseased bowel in Crohn's disease, the cytokine profile includes increased levels of interleukin-12 (IL-12), interferon-γ, and tumor necrosis factor-α (TNF-α), findings characteristic of T-helper 1 (TH1)–mediated inflammatory processes. In contrast, the inflammatory response in ulcerative colitis resembles more closely that mediated by the TH2 pathway. In Figure 1, a summary of proposed pathogenic events of IBD and potential sites of therapeutic intervention are shown.

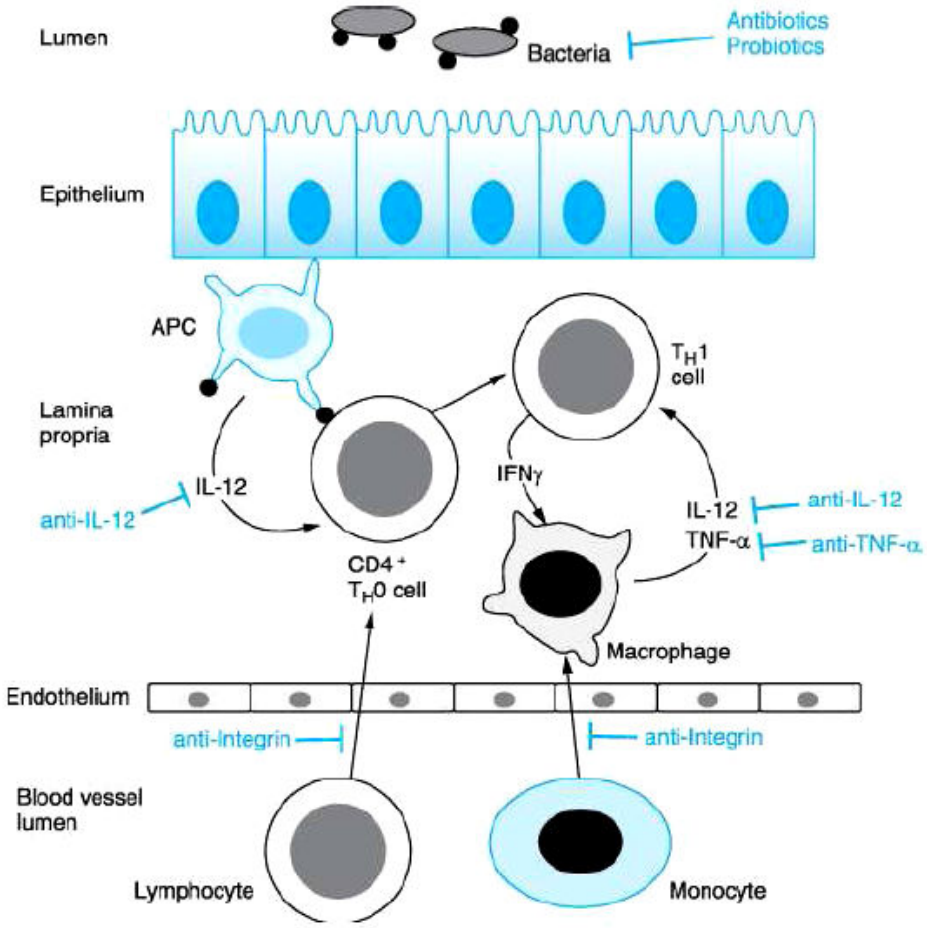

Figure 1: Proposed pathogenesis of inflammatory bowel disease and target sites for pharmacological intervention. Adapted from Sellin *et al.,* 2006.

The interactions among bacterial antigens in the intestinal lumen and immune cells in the intestinal wall is represented. If the endothelial barrier is impaired, bacterial antigens (dark circles) can gain access to antigen-presenting cells (APCs) in the *lamina propria*. APCs present, consequently, the antigen(s) to CD4+ lymphocytes and also secrete IL-12, thereby inducing the differentiation of TH1 cells in Crohn's disease (or type 2 helper T cells in ulcerative colitis). The TH1 cells produce a characteristic array of lymphokines, including IFN-α, which in turn activates macrophages. Macrophages positively regulate TH1 cells by secreting additional IL-12 and TNF- α. In addition to general immunosuppressants that affect multiple sites of inflammation (*e.g.* glucocorticoids, thioguanine derivatives, methotrexate, and cyclosporine), more specific sites for therapeutic intervention involve the intestinal bacteria (antibiotics and probiotics) and therapy directed at TNF-α.

Aetiology

The aetiology of IBD appears multifactorial: an underlying immune dysregulation coupled with an intolerance to gut flora seems fundamental to the pathogenesis that, in some cases, are associated with genetic mutations or are initiated by environmental factors. An inappropriate and excessive response to dietary triggers, unidentified infectious agents or the normal colonic bacterial population (Kovvali and Das, 2005) by an inadequately regulated mucosal immune system are thought to play a major pathophysiological role in the chronic inflammation and manifestation of symptoms of UC and CD. Various environmental and host factors (*e.g.* genetic-, epithelial-, immune and non-immune) are involved in the pathogenesis of IBD, even though the exact mechanism by which the intestinal mucosa loses tolerance to its bacterial neighbors remains elusive. In addition to environmental factors, genetic

predisposition, stronger in CD than in CD, also seems nowadays to play a key role in the pathology. The genetic aspect of research is quite focused on numerous chromosomal loci. Important insights into pathogenesis also have emerged from genetic analyses of CD.

The role of host genetic regulation of the innate immune response in the pathogenesis of CD has been brought to sharp focus by the identification of a gene called nucleotide-binding oligomerization domain-2 (NOD2/CARD15). NOD2/CARD15 is an intracellular element responsible for the indirect recognition of bacterial peptidoglycan through the binding of muramyl dipeptide, thereby playing an important role in the natural immunity to bacterial pathogens (Hugot *et al.,* 2001). Consistent with this model, other bacterial antigens have been identified. Dalwadi *et al.* (2001) and Lodes *et al.* (2004), identified pseudomonal protein I2 and a flagellin protein, respectively, as dominant superantigens that induce the TH1 response in Crohn's disease. Thus, these converging experimental approaches are generating new insights into the pathogenesis of Crohn's disease that soon may translate into novel therapeutic approaches to IBD.

Pharmacotherapy

Medical therapy for IBD is still problematic due to its multifactorial, polygenic nature. A part from a total proctocolectomy for UC, there is no cure for IBD. Because no unique abnormality has been identified, current therapy for IBD seeks to dampen the generalized inflammatory response; however, no agent can reliably accomplish this, and the response of an individual patient to a given medicine may be limited and unpredictable. Based on this variable response, clinical trials generally

employ standardized quantitative assessments of efficacy that take into account both clinical and laboratory parameters (*e.g.,* the Crohn's Disease Activity Index).

Current treatments are aimed at reducing the symptom burden, controlling acute exacerbations of the disease and maintaining the disease quiescence or treating specific complications, such as fistulas. Choice of therapy depends largely on the severity of the disease, and may also be influenced by such factors as disease location, side effects, as well as costs. Conventional steroids, such as glucocorticoids, remain the treatment of choice for moderate to severe flares, but they are neither effective at preventing post-operative relapse (Bergman *et al.,* 1976) nor appropriate for long-term use because of side effects and their inability to maintain remission. Conventional steroids are thereby reserved for patients who have failed more first-line therapy. Non-systemic steroids, such as Budesonide have low systemic side effects owing to a high (80-90 %) first-pass metabolism (Kane *et al.,* 2002) and are effective as first-line agent for ileal and/or right colonic and perianal disease although maintenance benefits remain to be proven. Immunomodulators, such as 6-Mercaptopurine (6-MP) and its prodrug azathioprine (AZA), are preferred for long-term management, but they require several weeks to achieve their therapeutic effect have therefore a limited role in the acute setting. Cyclosporine and Tacrolimus, macrolide antibiotics, are used primarily to prevent allograft rejection in the transplant setting and can be effective for severe or refractory UC.

A more thorough appreciation of the intricacies of the inflammatory response and improved biotechnology have led to the development of biological agents that can target single steps in the immune cascade. For example Adalimumab (D2E7,

Humira®; Abbott Laboratories, Chicago, IL) is a subcutaneously administered recombinant of human IgG_1 monoclonal antibody that binds with high specificity and affinity to human TNF-α and consists of human-derived heavy and light chain variable regions and human IgG_1 constant region. Analogously, Certoluximab pegol (UCB; Smyrna, GA) is a monoclonal humanized anti TNF-α antibody Fab' fragment linked chemically to polyethylene glycol (PEG). Anti-TNF agents have been effective for patients with moderate to severe UC and CD, independent of concomitant medications.

However, potential side effects, costs and immunogenicity remain critical issues relating to current and future biological agents.

Drug delivery to the appropriate site(s) along the gastrointestinal tract also has been a major challenge, and second-generation devices have been developed to improve pharmacokinetic profiles, thus increasing efficacy and decreasing side-effects.

5-ASA (mesalamine)-based therapy

Mechanism of action and pharmacological properties

The current standard of care for mild-to-moderate UC and CD, with demonstrated efficacy and safety, involves 5-aminosalicylic acid (5-ASA, mesalamine) (Sutherland *et al.,* 2003). The parent compound of 5-ASA is sulfasalazine (SP), which consists of a 5-ASA molecule linked, by an azo-bond, to a sulfapyridine moiety. Sulfasalazine was developed originally as therapy for rheumatoid arthritis, but clinical trials serendipitously demonstrated a beneficial effect on the gastrointestinal symptoms of subjects with concomitant ulcerative colitis. SP was for many years the standard

therapy after pivotal introduction of remission and maintenance trials in the 1960 (Baron *et al.,* 1962). However, up to one-third of patients were intolerant of sulfasalazine with the most common adverse effects being nausea, dyspepsia, malaise or headaches. Less common effects were dyscrasias, male infertility and skin rashes. These adverse events were demonstrated to be arisen to the sulfapyridine moiety after it was recognised, in 1977, that the therapeutic effect of SP is due to the release of the 5-ASA moiety into the lumen by the action of colonic azo-reductase in the distal ileum and colon (Azad *et al.,* 1977). The diazo linkage in sulfasalazine prevents absorption in stomach and small intestine of both sulfapyridine and 5-ASA, that would be otherwise absorbed in the upper gastrointestinal tract when given individually. The therapeutic moiety (5-ASA) is therefore liberated after cleavage of the diazo bond by colonic bacteria (Figure 2).

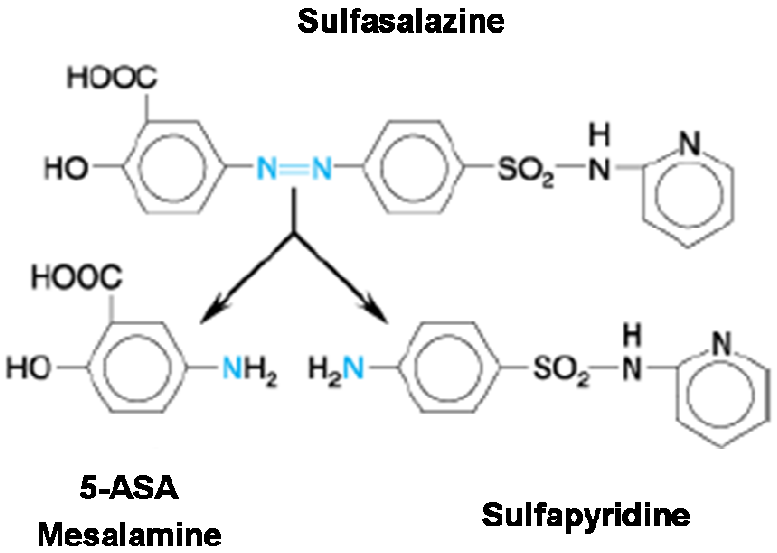

Figure 2: Structures of sulfasalazine and related agents. Blue N atoms indicate the diazo linkage undergoing cleavage by colonic azo-reductase, to generate the active moiety. Adapted from Sellin *et al.,* 2006.

In-vitro 5-ASA shares many of the pharmacological properties of the non-steroidal anti-inflammatory drugs (NSAIDs), including inhibition of cyclooxigenase, lipoxygenase, B-cells, and several key inflammatory cytokines. However, the therapeutic effect of 5-ASA does not appear to be related to cyclooxygenase inhibition; indeed, traditional NSAIDs actually may exacerbate IBD (Desreumax *et al.,* 2006).

A number of different but not mutually exclusive mechanisms have been proposed, including modulation of prostaglandin metabolism (Ligumsky *et al.,* 1981), inhibition of intestinal epithelial cell injury and apoptosis induced by oxidative stress (De Allegri *et al.,* 1990), inhibition of colonic production of chemo-attractant leukotrienes (Lauritsen *et al.,* 1996), increase in the intestinal epithelial cell heat shock protein response (Burress *et al.,* 1997) and inhibition of the activity of the nuclear factor-kappa B (NF-jB) pathway (Klotz *et al.,* 2005). However, the key to an integrated understanding of the mechanism of action was the realization that 5-ASA can activate selective proxisome proliferators-activated receptor ligand-γ (PPAR- γ), a nuclear receptor that controls cell proliferation and apoptosis (Desreumax *et al.,* 2006). The anti-inflammatory actions of 5-ASA produce effects similar to activation of PPAR- γ, *e.g.* modulation of inflammatory cytokine production, leading to decreased transcriptional activity of NF-jB, and reduced synthesis of prostaglandins and leukotrienes (Kaiser *et al.,* 1999). Activation of PPAR- γ also has anti-tumourigenic effects which manifest as anti-proliferative activities, pro-differentiation activities, pro-apoptotic activities, inhibition of the formation of aberrant crypts foci and inhibition of the development of colon tumours. PPAR-γ molecules are expressed at particularly high levels in the epithelium of the colon and

adipose tissue, at least an order of magnitude higher than in the small intestine (Figure 3).

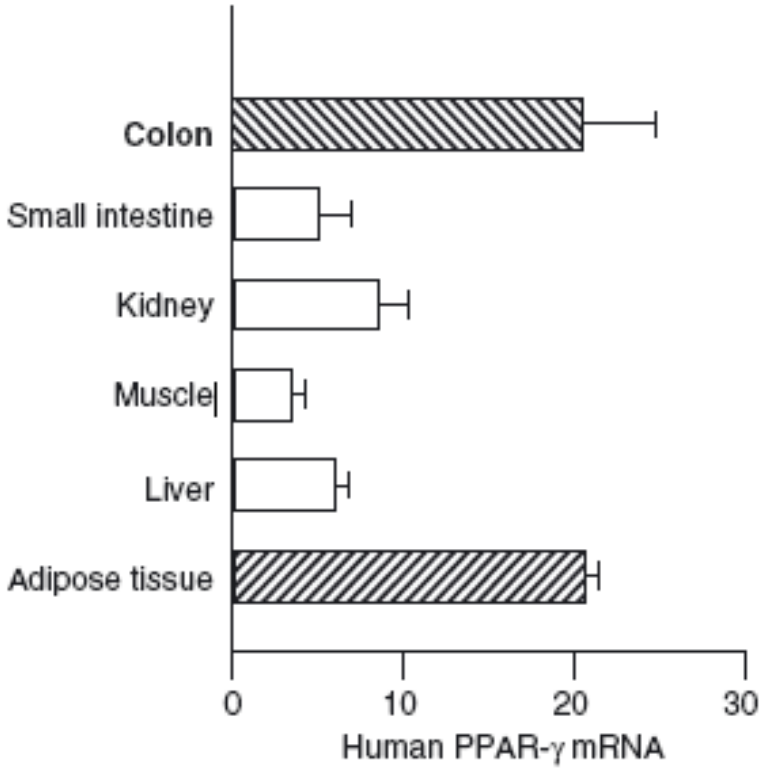

Figure 3: Expression of peroxisome proliferator-activated receptors (PPAR)-γ. Adapted from Desreumaux *et al.,* 2002.

Rousseaux *et al.* (2005) demonstrated that 5-ASA is able to bind PPAR-γ, to induce its translocation from the cytosol of epithelial cells to the nucleus, to promote a PPAR-γ conformational change, and to recruit a co-activator named DRIP, concluding that PPARγ is an essential receptor mediating the common 5-ASA activities in IBD.

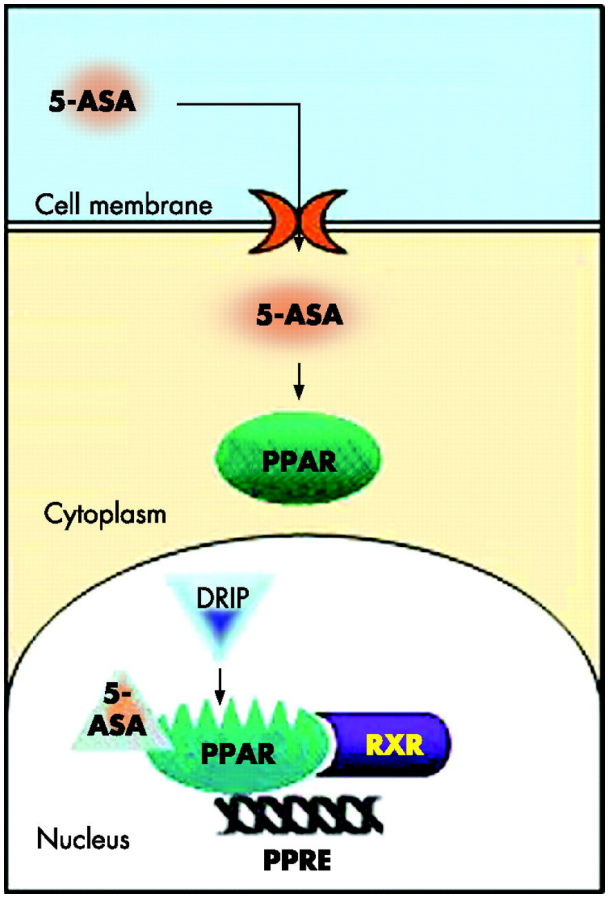

Figure 4: Molecular mechanisms of peroxisome proliferator activated receptor γ (PPARγ) activation by 5-aminosalicylic acid (5-ASA). Adpated fro Dubuquoy *et al.,* 2006.

After local administration, 5-ASA crosses the cell membrane of the epithelial cell through a transporter and binds to PPARγ in the cytoplasm (Figure 4). 5-ASA then induces its nuclear translocation, promotes a PPARγ conformational change, and recruits the coactivator DRIP, leading to formation of a heterodimer between PPARγ and retinoid X receptor (RXR) and activation of the PPARγ response elements (PPRE).

5-ASA delivery strategies and limitations of current formulations

The action of 5-ASA is demonstrated to be predominantly topical at the site of inflammation (Qureshi *et al.,* 2005). A number of rectal and oral formulations have therefore been proposed, to achieve drug delivery to the target gastrointestinal tissue. Even though rectal preparations (*e.g.* suppositories, enemas and foams) are effective in the treatment of distal colitis, they are associated with several adverse events, including burning sensations as well as leakage, retention and bloating problems. Moreover, patients often dislike rectal formulations because of difficulty with this mode of administration and problems with discomfort (Segars *et al.,* 1992). In addition, rectal preparations allow only the distal colon and rectum to be reached, being therefore ineffective in the treatment of proximal colitis and pancolitis (ulcerative colitis that involves the entire large intestine). When the inflammation spreads above the left flexure and during periods of remission, oral delivery of 5-ASA is thereby needed.

However, oral administration of 5-ASA presents a challenge as the majority of the drug, when administered as a conventional dosage form, is rapidly absorbed in the small intestine, leaving little or no 5-ASA to achieve the inflamed mucosa and perform its therapeutic activity. In fact, when administered orally, 5-ASA is nearly completely systemically absorbed from the proximal small intestine and then extensively metabolised, in intestinal epithelial cells and the liver, to N-acetyl-5-ASA.

In order to prevent unwanted systemic absorption, thus enhancing the clinic outcome and tolerability of the treatment, a number of strategies have been proposed. These

strategies include the use of conventional dosage forms, based on prodrugs, and Drug Delivery Systems (DDS) based on delayed- or prolonged-release formulations.

A number of products have been initially developed based on 5-ASA prodrugs intended for activation by colonic bacteria.

As discussed already, the parent compound is sulfasalazine, that consists of a 5-ASA molecule azo-bonded to a sulfapyridine moiety. Sulfasalazine largely resists gastric breakdown and systemic absorption and, when in the colon, undergoes cleavage of the sulphapyridine-5-ASA bond due to bacterial azoreductase. Sulfasalazine has been widely prescribed for the last four decades due to its effectiveness and low costs. However, it is associated with dose-dependent adverse events (*e.g.* head-ache, nausea and dyspepsia) related to the sulfapyridine moiety. Different prodrugs have therefore been proposed, *e.g.* Olsalazine (Dipentum; Celltech Pharmaceuticals, Inc., Rochester, NY, USA), which consists of two azo-bonded 5-ASA molecules, and Balsalazide (Colazal; Salix Pharmaceuticals, Inc., Morrisville, NC, USA), in which 5-ASA is linked to a benzoic acid (inert carrier) derivative.

Prodrugs are effective in the treatment of ulcerative colitis but, since they largely by-pass the small intestine, they do not have significant effect neither in small bowel Crohn's disease nor in pancolitis.

A second approach that has been made to maximise delivery of 5-ASA to the affected regions of the GI tract was the development of DDS, including delayed- or prolonged-release formulations. Most of the mesalamine-based products currently available on the market are delayed-release oral dosage forms. These involve the use of pH-dependent coating polymers (*e.g.* methacrylates-based polymers) that dissolve over a certain value of pH (generally 7, since it is thought to correspond to the pH

value of the human terminal ileum). The release performance of these delayed-release formulation therefore rely on the hypothetical progressive raise of pH along the GI tract. Unfortunately, the endoluminal pH of patients affected by CD and UC is highly variable and it is demonstrated to fluctuate of 2-3 units (Fallingborg *et al.,* 1993), so that the pharmacokinetic profile and therapeutic effect of such products can be compromised. Delayed-release formulations include Asacol® (Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA) and Salofalk® tablets (Axcan Pharma, Mont St Hilaire, QC, Canada).

One of the latest approach for 5-ASA delivery is based on a prolonged-release formulation, marketed as Pentasa® (Shire US, Inc. Wayne, PA, USA). Pentasa® comprises 5-ASA granules enclosed within an ethylcellulose membrane, which allows pH-independent release of the drug to occur. These ethylcellulose-coated granules gradually release the active drug beginning in the stomach and continuing throughout the jejunum, the ileum and the colon to rectum, allowing therefore the entire intestine to be "covered" (Figure 6). This is the reason why Pentasa® is often used "off label" to treat Crohn's disease in addition to its indicated use in UC (Cohen *et al.,* 2006). However, the main limitation of Pentasa® is that a considerable extent of 5-ASA is released during the multiparticulates stay in the stomach. This would result in a relatively high loss of mesalamine due to systemic absorption occurring in the duodenum, and subsequently to less drug reaching inflamed tissues lower in the small intestine and in the colon (Klotz *et al.,* 1985; Vree *et al.,* 2001).

In Figure 5, a scheme representing sites of release of different 5-ASA formulations is depicted.

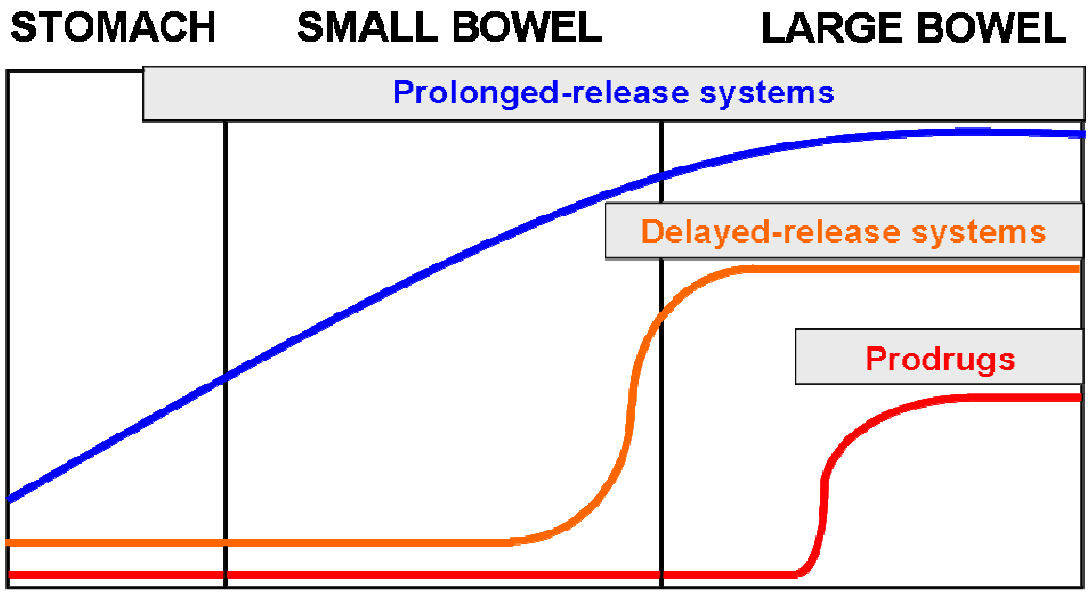

Figure 5: Theoretical release profiles from different 5-ASA oral dosage forms.

Oral dosage forms currently available on the market have several limitations. Firstly, prodrugs and delayed-release formulations are not equally effective in the treatment of both UC and CD as they may not deliver therapeutically effective doses of 5-ASA to the left side of the colon (a site affected in all patients with UC). Indeed, clinical studies have shown that mucosal 5-ASA concentrations using azo-bonded or delayed-release formulations are typically highest in the right-sided colon, whereas in the rectum the concentration of 5-ASA is much lower (Frieri *et al.,* 1999). Secondly, it has thus far been necessary to dose these formulations multiple times daily. In fact, recent clinical trials convincingly demonstrated that by increasing the standard dosage regimen of 5-ASA up to 4.8 g/day, both safety and efficacy and hence cost-effectiveness of therapy can be improved, with no increase in adverse events (Buckland and Bodger, 2008). This has been considered essential to ensure that therapeutically effective 5-ASA concentrations are maintained in the affected

areas, and indeed, formulations dosed in this way have been shown to be efficacious for the treatment of UC in clinical studies (Carter *et al.,* 2004).

Most of the oral formulations currently available on the market contain a relatively low drug dosage (generally 500 mg 5-ASA/unit), in particular for prodrugs whose 5-ASA content is always less than 300 mg/unit. Multiple daily administrations and a high number of capsules/tablets (up to 16 per day) are therefore required to meet the recommended 5-ASA dosage regimen. The inconvenience of frequent daily dosing, together with the number of capsules/tablets (pill burden) have been identified as key factors in reducing patient acceptability with therapy in UC and CD, leading to reduced drug efficacy and thus poorer disease control, *i.e.* an increased number of UC flares (Kane, 2007).

The limitations of current oral and rectal formulations have driven the evolution of 5-ASA therapeutics to ensure patient compliance as well as improve delivery of active drug to all affected areas of the colon. Recent developments include high-strength tablet formulations aiming to reduce daily administrations and improving compliance and innovative formulations (multiple-units) offering more convenient dosing in addition to improved drug delivery to the whole colon. A novel, high strength (1.2 g 5-ASA/tablet) formulation, SPD476 marketed as Lialda^{TM} in the US and Mezavant ^{TM} in the EU (otherwise known as MMX mesalamine; Shire Pharmaceuticals Inc., Wayne, PA, under license from Giuliani SpA, Milan, Italy) has been recently approved by the US Food and Drug Administration for the induction of remission in patients with active mild-to-moderate ulcerative colitis. MMX comprises a tablet, coated with a gastro-resistant pH-dependent polymer film that breaks down at pH ≥ 7, containing 1.2 g mesalamine embedded in a matrix, which is

claimed to disintegrate slowly over 24 h. Even though MMX mesalamine allows the pill burden to be reduced, thanks to the high dose of 5-ASA, it still relies on a pH-dependent approach.

Lately, 5-ASA multiple unit formulations, have been studied with the aim of providing less frequent dosing in an easy-to-swallow formulation (Raedler *et al.,* 2003). Multiple unit drug delivery systems are becoming more widespread. Provided as individual sachets containing a single dose, these formulations utilize granules to effect a delayed and sustained release of 5-ASA with similar delivery properties and systemic exposure to tablets (Lichtenstein *et al.,* 2007). Salofalk® 500 mg and 1 g (Axcan Pharma and Falk Pharma, Freiburg, Germany) and Pentasa® 1 g (Ferring A/S) are currently available in some European markets. Phase III trials are continuing in the US and a 1.5 g, once daily encapsulated mesalamine micropellet formulation (Salofalk Granustix®) is also under clinical development (Salix Pharmaceuticals Inc., Morrisville, NC, USA). Several clinical studies have been performed to assess and compare the efficacy of mesalamine granules *vs* conventional tablets in healthy volunteers. In one study of 14 healthy male volunteers, mesalamine containing granules (500 mg) released 5-ASA in the same target region (ileo-ciecal) and in a comparable timeframe to mesalamine tablets (Salofalk, 500 mg; Dr Falk Pharma GmbH, Freiburg, Germany) under fasting conditions (Brunner *et al.,* 2003). Pharmacokinetic analyses showed that the plasma under the curve vales were significantly lower for the granules than for the tablet formulation, suggesting a prolonged release of 5-ASA. The efficacy of mesalamine granules [1.5 d per sachet, taken twice daily (3g/day)] and tablets [2 x 500 mg tablets, three times daily] in patients with mild-to-moderate UC (n ¼ 362) have been

compared in a phase II, double-blind, active-controlled study (Raedler *et al.,* 2004). The study was designed to show non-inferiority of the multiparticulate formulation to tablets in terms of clinical remission within 8 weeks. Clinical remission was achieved by 67% of patients who received the multiparticulate formulation compared with 62.9% of patients who received the tablet formulation, supporting the non-inferiority of the granule formulation (OR 1.199; 95% CI: 0.758– 1.897). The effects of mesalamine prolonged-release granules [one packet four times daily or two packets twice daily (4 g/day), Pentasa® Sachet] has also been compared with prolonged-release mesalamine tablets [2 x 500 mg four times daily (4 g/day), Pentasa] in 227 patients with mild-to-moderate UC. Once again, the study was designed to confirm the non-inferiority of the multiparticulte formulation compared with the standard tablet.

Even though current mesalamine-based products have proven their efficacy and safety in the treatment of IBD, both patient acceptability and the clinical outcome might be improved.

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AIM OF THE PROJECT

Inflammatory bowel disease (IBD) is a group of chronic, inflammatory conditions of the gastrointestinal (GI) tract. IBD is conventionally divided into two major subtypes, *i.e.* Crohn's disease (CD) and ulcerative colitis (UC). As the name suggests, UC mainly affect the colonic region, even though it may extend proximally (*e.g.* colitis, left-side colitis and pancolitis). Conversely, the small intestine and/or the ileocolonic area are involved in CD.

The first-line drug therapy for mild-to-moderate acute exacerbations of UC and, occasionally of CD, is based on the use of 5-aminosalicylic acid (5-ASA, mesalamine).

Since the anti-inflammatory action of 5-ASA is thought to be predominantly topical at the site of inflammation, formulation strategies able to deliver the API to the targeted areas of the GI tract are needed. Current oral approaches are based on the use of prodrugs (selectively activated by colonic bacteria) and drug delivery systems (DDS) that enable a delayed- or prolonged-release of the drug.

Most of the mesalamine-based products, currently available on the market, are associated with a number of limitations. Both prodrugs and delayed-release systems are, in fact, not equally effective in the treatment of UC and CD, as most of the therapeutically effective dose of 5-ASA is delivered to the distal areas of the large intestine, thus leaving the proximal small intestine (which is, as stated, an area commonly affected by CD) potentially untreated. Moreover, delayed-release formulations rely on the hypothetical progressive raise of pH along the GI tract. This might represent an important limitation, considered the high variability of this physiological parameter, in particular in subjects suffering of IBD (Haddish-Berhane *et al.,* 2006).

On the other hand, prolonged-release formulations should allow a slow and pH-independent release of 5-ASA throughout the entire intestine to occur, thus potentially meet the overall IBD needs. However, a considerable extent of 5-ASA is released by these formulations, already in the stomach. This would result in a relatively high loss of mesalamine, due to considerable systemic absorption occurring in the duodenum, and subsequently to less drug reaching inflamed tissues lower in the small intestine and/or in the large bowel.

An other important limitation, common to most of the mesalamine-based preparations currently available on the market, is the drug dose (500 mg 5-ASA/unit), relatively low. If considering the complex IBD dosing regimen of 2.4-4.8 g/day, multiple daily administration are therefore required, thus reducing patient acceptability and potentially impairing the therapy.

On the basis of the above premises, the aim of the research project has been the development of a multiple-unit oral dosage form, containing a high dose (> 1,000 mg) of 5-ASA, in order to achieve a once/twice-a-day administration, improving compliance. Moreover, this formulation is intended to prevent/minimise gastric breakdown and allow a slow and pH-independent release of 5-ASA, throughout the entire intestine, to occur. Multiparticulates, based on high density pellets, have been sought as promising alternatives for the administration of 5-ASA, both for their biopharmaceutical (*e.g.* more even and predictable distribution and transit in the GI tract) and technological (*e.g.* high drug loading and bulk density) advantages.

The overall project has been structured into three stages:

1) Development of a multiple-unit drug containing core, based on highly loaded 5-ASA pellets [chapter 1].
2) Assessment of the scale-up ability of the promising highly-loaded multiple-unit formulation [chapter 2].
3) Tailoring of drug release accordingly to specific IBD therapeutic needs, and proposal of a high strength multiple-unit drug delivery system (DDS) [chapter 3].

[Chapter 1] Firstly, the feasibility of producing a multiparticulate core based on highly-loaded (> 90wt%) 5-ASA pellets needed to be assessed. Extrusion-spheronisation (E-S) was investigated as the manufacturing technique, as it offers the potential to produce pellets with high drug load and density.

A ram extruder apparatus (capillary rheometer) was initially employed to investigate, systematically, the rheological behavior of highly-loaded 5-ASA pastes undergoing extrusion. In particular, the effect of the chemical (acidity) and physical (particle size and shape) characteristics of 5-ASA were studied. The influence of the E-S aid in the processing of highly-loaded 5-ASA pastes, was also assessed. The water retention ability, quantified by centrifuge testing and supported by ram extrusion, as well as the rheological performance of a standard microcrystalline cellulose (MCC) grade (*i.e.* Avicel PH101) and colloidal ones (*i.e.* Avicel RC591 and CL611) containing sodium carboxymethylcellulose were therefore investigated. Moreover, ram extrusion of solid-liquid paste formulations through multi-holed square-ended dies was used to assess the extrudability of different 5-ASA/MCC paste formulations. Extrudates were spheronised using a bench-top device (Caleva) and the quality of extrudates monitored via visual and automated shape analyses.

The research activity pertaining to the rheological studies and feasibility of processing highly-loaded 5-ASA paste formulations was performed at the "Department of Chemical Engineering and Biotechnology" of the University of Cambridge (UK) and it was item for publication (Di Pretoro *et al*., 2010)

[Chapter 2] Being our next objective, the realization of an industrial scalable multiple-unit dosage form, a feasibility study on the scale-up ability of both the milling and the E-S processes was performed. Firstly, in collaboration with a company specialised in milling processes (IMS Micronizzazioni S.p.A., Milano, Italy) we investigated the possibility of reproducing, by an industrial scale microniser, a batch of 5-ASA with similar physical characteristics to those obtained *via* a bench-scale apparatus (planetary ball-mill).

Afterwards we investigated the feasibility of transferring a promising highly-loaded 5-ASA multi-particulate formulation, previously identified in the ram extruder, to a pilot-scale equipment (basket extruder, Nica®). Considered the complexity and multifactoriality of the E-S process, a factorial approach, namely a mixed fractional factorial design (MFFD), aiming to identify both the process and formulation parameters that affect mostly the E-S process of highly-loaded 5-ASA paste formulations, was carried out. In particular, the effect of three formulation components (5-ASA:MCC ratio, water and an extra binder amounts) and two process parameters (extrusion speed and spheronisation time) on the process yield and on the final properties of the pellets (*i.e.* size, shape, mechanical resistance, bulk density and dissolution properties), was investigated. The independent variables and the relevant experimental space investigated were selected on the base of preliminary trials.

27 runs, including 3 replicates of the central point, were performed. An optimisation study, based on the desirability function approach, was finally undertaken to identify the combination of factors that would provide the optimal response values to be obtained.

[Chapter 3] The drug release of highly-loaded 5-ASA pellets was accordingly tailored to meet specific IBD therapeutic needs. To achieve modular sustained- and pH-independent release profiles, pellets were coated with a polyvinyl acetate polymeric dispersion, up to different theoretical weight gains. An outer, additional, acrylate polymeric layer was applied to avoid/minimise 5-ASA loss from pellets during the gastric residence time. Coating process was performed in an pilot-scale fluidized bed apparatus (Glatt® Process Technology GmbH, Binzen, Germany).
Once the optimal coating levels were identified, preliminary attempts to develop a final DDS in the dosage form of capsules, containing more than 1,000 mg API were also made.

The *in-vitro* drug release performance of the final DDS was finally tested and compared to that of two commercially available products, namely a high strength single-unit DDS (Lialda™ tablets, 1,200 mg 5-ASA) and a multiple-unit dosage form (Pentasa® capsules, 500 mg 5-ASA).

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Di Pretoro G., Zema L., Gazzaniga A., Rough S.L., Wilson D.I., 2010. Extrusion-spheronisation of highly-loaded 5-ASA multiparticulate dosage forms. Int. J. Pharm. 402, 153-164.

CHAPTER 1

EXTRUSION-SPHERONISATION OF HIGHLY-LOADED 5-ASA MULTIPARTICULATE DOSAGE FORMS

G. Di Pretoro, L. Zema, A. Gazzaniga, S.L. Rough, D.I. Wilson. Extrusion-spheronisation of highly-loaded 5-ASA multiparticulate dosage forms. Int. J. Pharm. 402 (2010) 153-164.

Abstract

The aim of the current work was to develop an extrusion-spheronisation (E-S) route to manufacture pellets with a high loading (≥ 90 wt%) of 5-aminosalicylic acid (5-ASA). Ram extrusion studies, supported by centrifuge testing, were employed to investigate the effect of the chemical (acidity) and physical (particle size and shape) characteristics of 5-ASA on the ability of microcrystalline cellulose (MCC)-based pastes to retain water when subjected to pressure. Liquid phase migration (LPM) within the paste during the extrusion, and hence variation in water content of extrudates and reproducibility of the final E-S product, was generally observed. The extent of LPM was found to be related to both the drug loading and its physical properties, most notably the particle shape (needle-like). A reduction in particle size, combined with a change in the shape of the 5-ASA particles, allowed LPM to be reduced considerably or eliminated. The performance of colloidal grades of MCC (Avicel RC591 and CL611) as alternative extrusion aids to the standard Avicel PH101 was also investigated: these proved to be superior aids for the highly-loaded 5-ASA pastes as their greater water retention capacity mitigated LPM. Combining these results yielded a route for manufacturing pellets with 5-ASA loading ≥ 90 wt%.

Keywords: 5-aminosalicylic acid; ram extrusion; liquid phase migration; microcrystalline cellulose; granulation

1. INTRODUCTION

5-ASA treatment requirements

Crohn's disease (CD) and ulcerative colitis (UC) are chronic, episodic inflammatory conditions of the gastro-intestinal tract, which primarily affect the ileum and the colon, respectively (Cohen, 2006). The first-line drug therapy for mild to moderate acute exacerbations of CD and UC is, at present, based on 5-aminosalicylic acid (5-ASA, mesalamine). Since the anti-inflammatory action of 5-ASA is thought to be predominantly topical at the site of inflammation, oral modified-release delivery systems (*e.g.* enteric coated products) are generally employed to maximise drug delivery to the affected regions while minimising systemic absorption. 5-ASA is generally administered at the dosage regimen of 2.4 g/day, even though recent clinical trials have convincingly demonstrated that by doubling the standard dose of mesalamine, both safety and efficacy and hence cost-effectiveness can be improved, with no increase in adverse events (Buckland and Bodger, 2008). However, most of the oral formulations currently available on the market are associated with a number of limitations, mainly a relatively low drug dosage (generally 500 mg 5-ASA/unit). Frequent daily dosing and a high number of tablets/capsules are therefore required for the treatment of CD and UC, at the expense of patient acceptability and adherence to therapy (Cervený *et al.*, 2007).

To improve compliance, a high strength solid formulation containing more than 1 g 5-ASA/unit would be of interest, allowing once/twice daily administrations to be achieved. Multi-particulate dosage forms, based on high density pellets, have been sought as promising alternatives for the administration of 5-ASA, both for their

biopharmaceutical (*e.g.* more even and predictable distribution and transportation in the gastro-intestinal tract) and technological (*e.g*. high drug loading) advantages (Bechgaard and Hagermann*,* 1978). Extrusion-spheronisation (E-S) was investigated as the manufacturing technique in the present work, as it offers the potential to produce highly-loaded pellets (Gazzaniga *et al.,* 1998). The E-S route has been used by several other workers to prepare granules containing 5-ASA where the focus has been on identifying extrusion aids (*e.g*. Goskonda *et al*. 1994; Chuong *et al*., 2008), as well as coating methods (Wei *et al*., 2010) to allow colonic delivery. The 5-ASA content of the pellets rarely exceeded 50 wt%. Both Hileman *et al*. (1993) and Rudolph *et al*. (2001) have reported pellets with high 5-ASA loadings, at 80 and 77.4 wt% d.b. levels, respectively approaching that desired for a high strength dosage form. Higher 5-ASA loadings, approaching the 90% sought in this study, have not been achieved, to the authors' knowledge.

Extrusion-spheronisation

Extrusion-spheronisation is a multi-stage mechanical process, in which a wet mass is forced through a die/screen and afterwards shaped into small spherical/near-spherical granules. The liquid phase (generally water) provides interparticle cohesion, lubricates particle contacts, promotes wall slip and can also bear some of the stress generated during the forming process. However, the pressure exerted on the liquid phase during extrusion can cause it to move faster with respect to the particulate network, giving rise to variation in the liquid content of the paste within the barrel and hence the extrudates. The redistribution of the liquid phase within the solid matrix is known as liquid phase migration (LPM) or 'dewatering' (Mascia *et al.*,

2006). The occurrence of LPM phenomena is affected by the amount and the viscosity of the liquid phase, solids packing and structure, along with process parameters (*e.g.* extrusion velocity and die/screen geometry) (Bains *et al.,* 1991).

The physical and chemical properties of the active pharmaceutical ingredient (API) are important in paste formulation: the solubility of the API in the liquid phase, its particle size characteristics and packing behaviour can affect the amount of liquid necessary to obtain a wet mass with the required 'plasticity' (Vervaet *et al*., 1995). Hagsten *et al.* (2008) identified particle size, specific surface area and packing behaviour to be key factors affecting the variation in processability (in terms of the amount of liquid required for extrusion) of 131 batches of 5-ASA processed by E-S.

Particle size and particle morphology also have a significant influence on the rheology of the wet powder masses and the occurrence of LPM. Fielden *et al.* (1992) investigated the influence of lactose size distribution on extrusion behaviour and pellet characteristics of lactose/water pastes. Increasing the particle size resulted in high extrusion pressures and promoted LPM. Particle packing and solids matrix strength are both controlled by particle shape and volume fraction. The porosity and pore size distribution in the wet mass, and thereby the permeability of (and likelihood of LPM within) the extrudate, are also determined by particle shape and volume fraction. The rheological behaviour of suspensions is also sensitive to particle shape, with the intrinsic viscosity of particles in the Krieger-Dougherty equation known to increase as the shape becomes less spherical (Krieger and Dougherty, 1959). 5-ASA crystals are normally needle-like: Kraeger *et al.* (2004)

studied the influence of size and shape of another needle-shaped API, paracetamol, on dry powder properties and pelletisation, but the impact of particle shape on extrusion-spheronisation has not, to our knowledge, been considered in depth.

E-S aids

Since the active ingredients do not generally have either lubricant or plasticising properties, an extrusion spheronisation aid, namely an excipient able to provide mechanical structure and rheological stability, is usually required. Microcrystalline cellulose (MCC) is a well established E-S aid whose interaction with water is primarily physical rather than chemical. MCC absorbs water and confers the appropriate degree of plasticity to the bulk, as well as controlling the movement of water through the wet powder mass during extrusion. Fielden *et al.* (1988) suggested, on the basis of thermal studies, that MCC could be described as a 'molecular sponge': it is capable of physically retaining a large quantity of water within its fibrous network, but also of allowing its removal to take place by evaporation and when subjected to pressure (*e.g.* spheronisation).

Not all APIs can be processed with the standard grade of MCC (*e.g.* Avicel PH101), particularly when high loadings (*i.e.* above 70 wt%) are required. The inability of Avicel PH101 either to allow the extrusion (Tomer *et al.,* 2001) or the spheronisation (Tomer *et al*., 2002) of highly-loaded formulations seems to be related to its limited water-retention capacity when subjected to pressure. Podczeck and Knight (2006) reported that the inability of Avicel PH101 to support E-S of pastes containing

80 wt% of a water-insoluble drug (ibuprofen) was associated with the migration of water within the formulation during the extrusion stage.

Modified MCC grades, co-processed with varying amounts of sodium carboxymethyl cellulose (NaCMC) are able to retain higher quantities of water than the standard Avicel PH101 and have therefore been proposed as alternative E-S aids for high solids loadings. These modified MCC grades (*e.g.* Avicel RC591 and CL611) were identified by several workers, *e.g.* O'Connor *et al*. (1984) and Hileman *et al.* (1993), as promising aids in the extrusion of high solids loading formulations, improving extrusion performance and surface quality of the extrudates formed. This behaviour was found to be related to their ability to restrict migration of water in the wet mass when subjected to pressure (Podczcek *et al.*, 2007). However, the improved quality of surface extrudates was always obtained at the expense of rigidity of the extrudates, which led to difficulties in spheronisation. In fact, good quality extrudate does not always imply its suitability for spheronisation, as demonstrated by Rough and Wilson (2005).

The aim of the current work was to assess the feasibility of an E-S route for the manufacture of pellets with high 5-ASA loading (*i.e.* > 80 wt%). Initial attempts to produce pellets in a radial screen extruder (Nica System, model E140), using Avicel PH101 as the E-S aid and water as the liquid binder, were undertaken. A screening design of experiments, aiming to identify the most critical process and formulation parameters for the obtainment of pellets with desirable properties (*e.g.* high drug content, low friability, high density and sphericity), was determined. However,

variability in extrudate water content coupled with a lack of reproducibility of pellet properties was generally observed, in such a way that the experimental design was invalidated. In particular, the optimal amount of water necessary to achieve both a plastic mass suitable for extrusion, and extrudates with appropriate characteristics for spheronisation, could not be identified. A likely hypothesis to explain the variability in extrudate water content could be that pastes undergo dewatering when subjected to high pressures, *i.e.* during the extrusion process. A systematic study was therefore performed in a ram extruder apparatus to investigate the influence of both solid phase components, namely the active ingredient (physical and chemical characteristics) and the E-S aid, on the rheological properties of highly-loaded 5-ASA pastes undergoing extrusion.

2. MATERIALS AND METHODS

2.1 Materials

5-aminosalicylic acid (5-ASA, mesalamine) (Erregierre S.p.A., Bergamo, Italy) was used both as received from the supplier (un-milled, labelled uM) and after micronisation. Calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$, labelled $CaSO_4$) (Fisher Scientific Inc., Pittsburgh, PA, USA) and a high density grade of 5-ASA (labelled 5-ASA HD) (Farchemia S.p.A, Treviglio, Italy) were chosen as reference materials to investigate the influence of prolate (needle-like) particles on the extrusion process. The standard Avicel PH101 grade of microcrystalline cellulose (MCC) (IMCD S.p.A, San Donato Milanese, Italy) was used as the primary extrusion-spheronisation aid. Two modified grades of MCC co-processed with sodium carboxymethyl cellulose (NaCMC), namely Avicel RC591 (12 wt% NaCMC) and Avicel CL611 (15 wt% NaCMC), were evaluated as alternatives to Avicel PH101. Reverse osmosis water was used as the binder for paste preparation.

2.2 Methods

2.2.1 Micronisation

Two batches of micronised 5-ASA were obtained by milling uM in different equipment. The BM batch was prepared in a planetary ball mill (Pulverisette 7, Fritsch, Germany); the grinding process was performed in a 45 mL stainless steel grinding bowl containing fourteen stainless steel balls (20 mm diameter), for 12 h at 250 rpm. A standard Teflon seal was positioned between the lid of the grinding bowl and the bowl itself. The powder-to-ball weight ratio was 1/35. The JM batch was kindly provided by IMS Micronizzazioni S.p.A (Milano, Italy). The batch was

produced by air-jet milling (Chrispro® Jet-Mill, Micro-Macinazione S.A., Molinazzo di Monteggio, Switzerland). The mill had a spiral chamber of 300 mm diameter. The milling gas was air, injected through 8 nozzles at 8 bar. The starting material was charged (700-750 g min^{-1}) through a venturi feeder, using a pressure of 8 bar.

2.2.2 Powder characterisation

2.2.2.1 Powder X-Ray Diffraction

Powder X-ray diffraction (PXRD) was performed on a Philips PW1050/25 diffractometer (Philips Analytical Inc., Natick, MA, USA) using Cu-K_α radiation (λ = 1.54 Å) generated with 30 kV and 15 mA. Samples were packed in the same aluminum sample holder to promote reproducibility of conditions, taking care also to minimize preferred orientation effects. The operating conditions were as follows: scan speed 0.5° 2θ min^{-1}; step size 0.02° 2θ; range $5 \leq 2\theta \leq 35°$.

2.2.2.2 Thermal analysis

Differential scanning calorimetry (DSC) was performed on a DSC2010 calorimeter (TA Instruments, New Castle, DE, USA), between ambient and 350 °C, under nitrogen purging at 70 mL min^{-1}. DSC runs were performed on 2-3 mg samples in non-hermetically sealed aluminum pans at a scanning rate of 10 K min^{-1}. Data reported are the average of at least 3 determinations.

2.2.2.3 Solid, bulk and tapped densities

The solid density of the three batches of 5-ASA (uM, JM and BM) was evaluated using a Micromeritics AccuPyc 1330 helium pycnometer (Norcross, GA, USA). The gas pressure was set to be 0.087 psia with a purging time of ~ 15 min. Samples of 0.3-0.5 g were analysed in a controlled environment (26.1 °C, relative humidity

50%). All the analyses were conducted in triplicate. For the bulk and tapped densities, a sample mass of about 50 g was poured into a 250 mL glass cylinder mounted on an automated tap density tester (Stampfvolumeter STAV 2003, Engelsmann A.G., Ludwigshafen, Germany). The volume of powder was determined after zero and 1,250 taps, and the bulk and tapped densities were calculated as the ratio of the mass to the corresponding volumes, respectively. The results presented are the mean of three replicates.

2.2.2.4 BET surface measurements

Surface area determination was performed by means of a SA3100 Surface Area Analyzer (Beckman Coulter, UK) according to the BET method using N_2 as adsorbate gas (USP 32 *Physical Test. Specific Surface Area. Volumetric Method*). Prior to surface area measurements, the samples (approximately 2 g) were degassed at 90 °C under vacuum (0.4 Pa) for 1 h to remove physically adsorbed gases (water vapour). The measurement was carried out in triplicate.

2.2.2.5 Particle size and shape

Particle size characterisation was performed using a laser light scattering and an automated particle imaging apparatus. The laser scattering device was a Coulter LS 230 (Beckman Coulter Inc., Fullerton, CA, USA) equipped with variable speed, microvolume and dry powder modules. Lorentz-Mie theory (diffraction-diffusion) was used as the optical model. The refractive indices of 5-ASA and $CaSO_4$ were 1.691 and 1.521, respectively. Isopropyl alcohol (refractive index 1.377) and pure sunflower oil (refractive index 1.473) were used as suspending agents for 5-ASA and $CaSO_4$, respectively. Powder samples were sonicated prior to analysis to break up

potential aggregates. Data collected are reported in terms of volume-based relative size distribution.

The particle imaging device (Morphologi® G3, Malvern Instruments Ltd, Worcestershire, UK) was able to determine both particle size and shape. The machine featured a Nikon CFI 60 Brightfield/Darkfield optical system and a 1/1.8" global shutter progressive scan CCD camera (5 megapixels). Powder samples (3 μm^3) were dispersed using the integrated sample dispersion unit, via an instantaneous pulse of compressed air (range: 0.8-2.5 bar depending on the particle size of the powders). Image analyses were performed at 20× and 50× magnification, according to a standard operating procedure. Particle dimensions were quantified in terms of volume-based size distributions. Particle shape was quantified in terms of circularity (calculated as $4\pi A/p^2$, where A is the projected area and p the projected perimeter of the particle) and aspect ratio (defined as minor axis/major axis). At least 60,000 particles were analysed. Two-dimensional micrographs were also collected.

2.2.3 Paste preparation

Dry powders (200-300 g) were placed in a planetary mixer (Kenwood KM 200, Southampton, UK) and different amounts of liquid binder slowly poured onto the powder surface. Granulation was performed at a constant speed of 25 rpm for 10 min. The process was occasionally interrupted to scrape off the material adhering to the wall of the bowl and the mixing blade. Mixtures of 5-ASA batches and MCC were dry blended for 5 min prior to liquid addition. Pastes were stored for 24 h in airtight containers prior to extrusion to allow the water to equilibrate throughout the

mixture. All experiments were performed at room temperature in an air-conditioned laboratory (21 ± 3 °C, humidity 35 ± 5%).

2.2.4 Centrifuge testing

The centrifuge used was an MSE minor 's' (Meadowrose Scientific Ltd, Oxfordshire, UK) operating over the range of 500–4600 rpm. Paste samples were filled into cylindrical copper containers (22 mm internal diameter (i.d.), 85 mm height), featuring a punctured base (several holes, i.d. 2 mm), to a sample height of 50 mm. A filter paper (1 μm pore size) was placed at the bottom of the container to prevent loss of solid through the holes during centrifugation. The container was located in a stainless steel holder attached at the end of a shaft rotating around the centrifuge axis. Samples were weighed before and after centrifugation performed at constant rotational speed for 60 and 120 min; trials were performed at different rotational speeds. All the experiments were carried out at room temperature (21 ± 1 °C) and environmental relative humidity (45 ± 5%) to reduce the effects of water evaporation.

The centrifugal pressure, P_c, was calculated by the following relationship (Hassler and Brunner, 1945):

$$P_c = 0.5\rho\omega^2\left(r_{out}^2 - r_{in}^2\right) \quad (1)$$

where ρ is the liquid phase density, ω the angular velocity, and r_{in} (75 mm) and r_{out} (85 mm) are the radial distances from the centrifuge axis to the inner and outer faces of the sample, respectively. Final water content (and drainage) was quantified in

terms of the water/ MCC mass ratio, calculated as the mass of liquid retained by the sample after being centrifuged and the amount of MCC present in the sample.

2.2.5 Extrusion-spheronisation

Extrusion testing was performed in a computer-controlled ram extruder (Zwick/Roell, Zwick Testing Machines Limited, Leominster, UK). The apparatus consisted of a 25 mm i.d. barrel, a ram attached to the cross-head of the strain frame and a load cell that measured the applied force and the ram displacement, as described by Rough *et al.* (2000). Various concentric cylindrical square-entry dies with different die land lengths (L) and diameters (D) were used. All surfaces in contact with the paste were stainless steel except the piston, which featured a Teflon seal. The barrel was filled with approximately 80 g of paste; to ensure uniform compaction a pre-load (range: 1-2 kN) was applied. Pastes were extruded at a constant ram velocity (V_{ram}) in the 0.1-10 mm s^{-1} range. Extrudates were spheronised in a 120 mm diameter cross-hatch plate spheroniser (Caleva, Sturminster Newton, Dorset, UK), at a constant speed of 1,600 rpm. Pellets were dried in a vacuum oven at 40 °C for 24 hours.

3. RESULTS AND DISCUSSION

3.1 Ram extrusion study of Avicel PH101-based 5-ASA pastes

Extrusion pressure (P_{ex})-ram displacement profiles for Avicel PH101-based pastes containing increasing amounts of uM were compared at different experimental conditions (die length and diameter, ram velocity and paste water content). A steady increase in P_{ex} during the extrusion was generally observed for all the formulations tested. The phenomenon was more evident at lower extrusion velocities (data not shown). LPM is often manifested as a divergence from the expected steady-state P_{ex} value, and it was most noticeable with single-holed capillary dies having a high *L/D* ratio and/or at relatively low ram (and hence extrusion) velocities. The effect of die geometry and extrusion velocity on LPM has been discussed elsewhere (*e.g.* Rough *et al.,* 2002). In order to mitigate the effects of LPM caused by relatively low ram velocities, a square-entry multi-holed die (6 notionally identical holes, D = 1 mm, L = 4 mm, evenly spaced in a ring, radial distance from the die centre 7.5 mm) was employed for the majority of the tests, so that relatively low extrusion velocities could be attained with correspondingly high ram velocities (the ram velocity being directly proportional to the number of die holes multiplied by the extrusion velocity).

The paste water content was chosen to be just sufficient to give a plastic mass and suitable paste rheological properties for extrusion. The water/Avicel PH101 ratio was varied systematically to evaluate the influence of moisture content on the E-S process. The 1.2:1 water/Avicel PH101 ratio (by mass) was identified, by centrifugation testing (see section 3.4.1), as the lower liquid limit for a plastic mass.

An increase in the liquid content resulted in over-wet pastes, whereas a decrease yielded dry and stiff pastes that were unsuitable for extrusion.

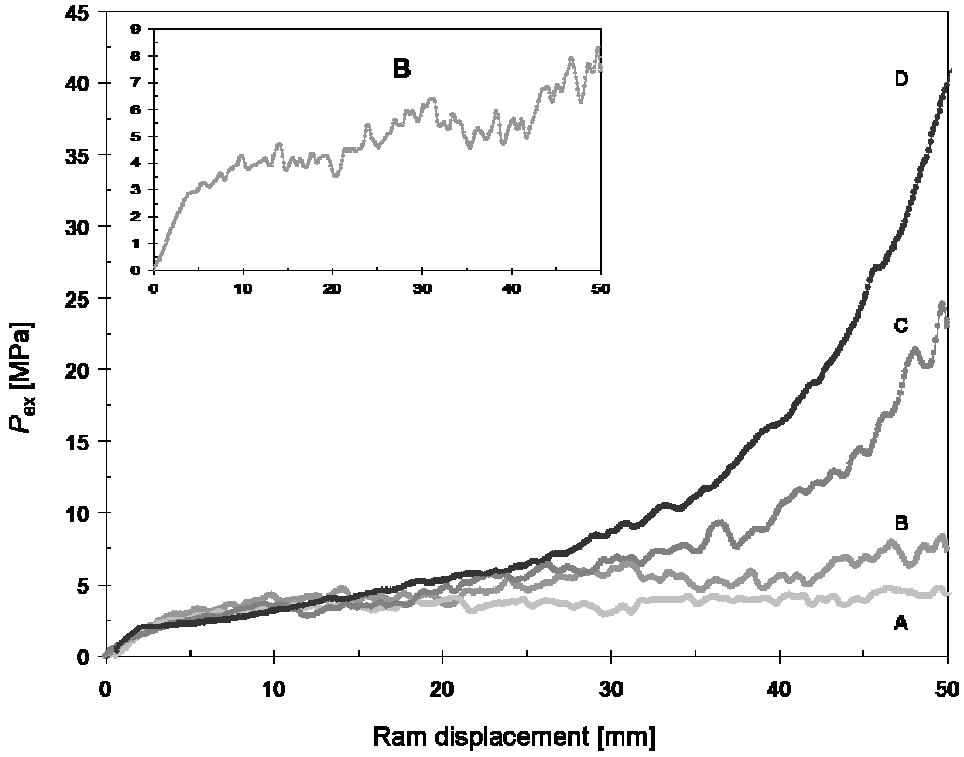


Figure 1.1: Extrusion profiles for Avicel PH101-based pastes containing (A) 10, (B) 20, (C) 30 and (D) 50 wt% uM at V_{ram}= 1 mm s^{-1}, 6-holed die (D = 1 mm, L = 4 mm). Water:Avicel PH101 ratio = 1.2:1. Inset shows a magnification of profile B, indicating how LPM occurred even at low drug loadings.

By way of example, Figure 1.1 shows the P_{ex}-ram displacement profiles of pastes containing uM at 10-50 wt% of the total solids, performed using the multi-holed die at a ram velocity of 1 mm s^{-1} (corresponding to an extrusion velocity of 104 mm s^{-1}). For a given ram displacement, P_{ex} increases as the uM content increases, indicating that LPM was related to the uM loading in the paste. For comparison, extrusion of a water/Avicel only paste under these conditions gave a steady P_{ex} value of ~ 2 MPa. Above 50 wt% 5-ASA, substantial dewatering of the paste was observed, resulting in P_{ex} approaching the mechanical limit of the apparatus. A systematic study of 5-ASA

loading with Avicel PH101 was therefore not possible as LPM effects dominated the extrusion behaviour.

The increase in LPM with 5-ASA loading could arise from either a physical or a chemical interaction of the API with Avicel PH101, thereby modifying the rheological properties of the final wet mass. 5-ASA is sparingly soluble in water and therefore is present as 'hard' solids within a 'soft' MCC matrix. At high drug loadings, 5-ASA could affect the ability of the Avicel PH101 to support extrusion. Alternately, as 5-ASA solution is acidic, the liquid holding capacity of the MCC could be modified.

The effect of the physical properties of 5-ASA, specifically the particle size and shape, and the potential influence of 5-ASA acidity on the behaviour of Avicel PH101-based pastes during extrusion were therefore investigated. In addition, the performance of two colloidal grades of MCC, namely Avicel RC591 and CL611, as E-S aids was compared to that of the standard Avicel PH101.

3.2 Influence of 5-ASA acidity

5-ASA is a zwitterionic drug, with pKa values of 6.0, 3.0 and 13.9 corresponding to the NH^{3+}, COOH and OH groups, respectively (Allgayer *et al.,* 1985). Since water was used as the liquid binder, 5-ASA could alter the pH of the mobile phase, thereby influencing the ability of the MCC to work as a molecular sponge. The liquid retention ability of Avicel PH101-based pastes was therefore assessed using RO water and saturated aqueous solutions of 5-ASA (pH = 4 ± 0.05, ~ 0.9 g L^{-1}) as the wetting/binding agent. Data were collected from centrifuge and ram extrusion testing, performed on different pastes prepared with 45-65 wt% liquid phase. The

results from these tests did not show any significant differences between water and 5-ASA saturated solution as binder, thereby indicating negligible influence of 5-ASA acidity on MCC liquid retention and flow behaviour.

3.3 Influence of 5-ASA physical properties

In order to assess the effect of 5-ASA physical properties on the extrusion behaviour of Avicel PH101-based pastes, three batches of 5-ASA, having different particle size and shape characteristics and hence different bulk properties, were investigated. The 5-ASA batches were:

(i) the material as received by the supplier, labelled uM;

(ii) the material in (*i*) after being subjected to jet milling, labelled JM;

(iii) the material in (*i*) after being subjected to ball milling, labelled BM.

Their particle size characteristics, measured by the laser scattering and automated microscope devices, are summarised in Table 1.1.

Table 1.1: Particle size data for 5-ASA batches.

		uM		JM		BM	
Particle size parameter / µm	**Volume basis**	**Laser scattering**	**Automated microscope**	**Laser scattering**	**Automated microscope**	**Laser scattering**	**Automated microscope**
	d_{10}	4.75	9.62	2.44	7.37	0.115	8.49
	d_{50}	41.4	20.3	5.58	13.0	1.58	28.5
	d_{90}	453	117	14.3	28.5	3.97	59.7

Both milling procedures reduced the particle dimensions, as indicated by the d_{10}, d_{50} and d_{90} values. Divergences between the two measuring techniques are evident: the laser scattering data indicate that the BM material has a smaller particle size and narrower size distribution than JM whereas the opposite trend is indicated by the

microscopy results. These differences were not surprising, as the devices employ different techniques and needle-like particle shapes further complicate the analysis. The micrographs in Figure 2.1 confirm the difference in size and shape between the different batches. The BM particles are smaller than those in the other batches, while the JM particles are still needle-like although with a smaller aspect ratio than uM.

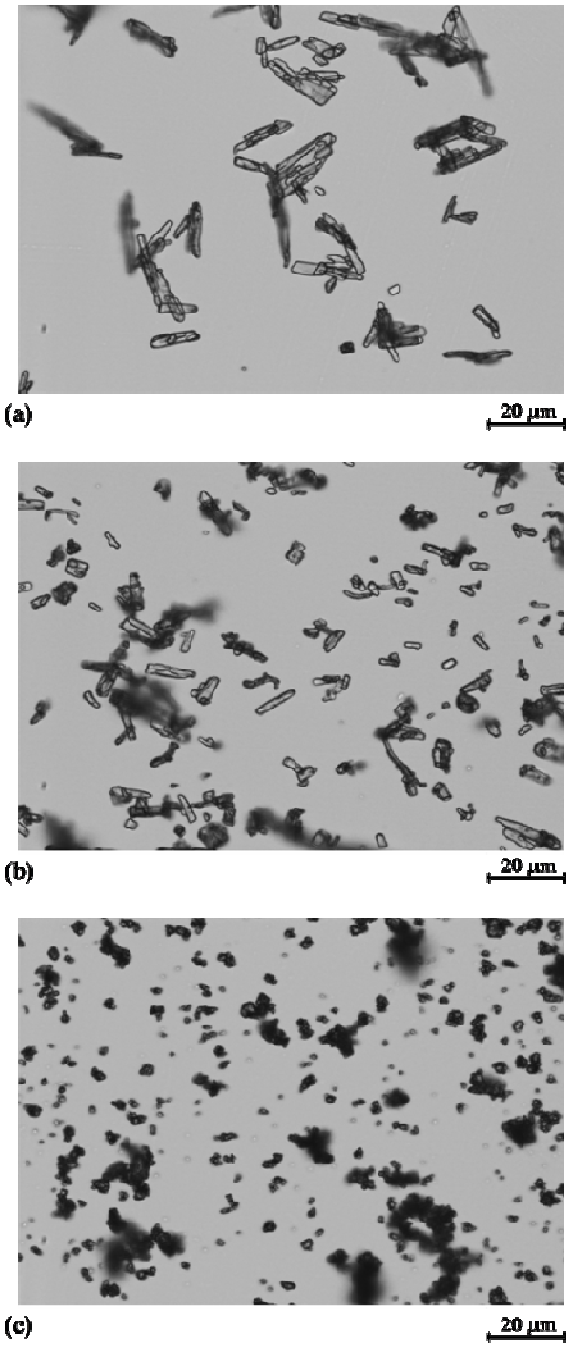


Figure 2.1: Micrographs of (a) uM, (b) JM and (c) BM batches of 5-ASA (magnification 50×).

The reliability of laser scattering for aspherical particles has been discussed by Latimer *et al.* (1978). In contrast, the microscope system captures 2-dimensional images of the particles and calculates particle size and shape parameters from these. Aggregates of BM particles are evident in Figure 2.1(c), which are likely due to uneven dispersion of the sample in the microscopy preparation step: these aggregates will skew the particle size analysis data away from the trends indicated by the micrographs and laser sizing results. The need for careful sample preparation is evident.

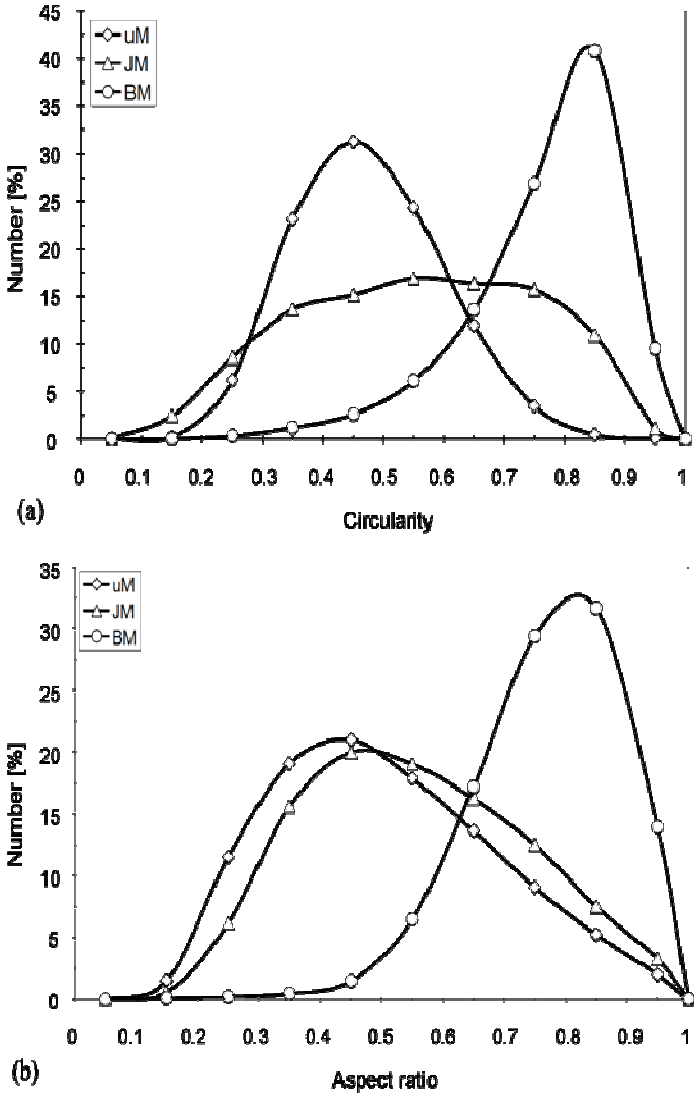


Figure 3.1: Shape parameters for uM, JM and BM batches of 5-ASA obtained from image analysis: (a) circularity and (b) aspect ratio.

The particle shape distribution data in Figure 3.1 quantify the differences evident in the 2-D micrographs in Figure 2.1. Other measures of shape could be readily calculated from the data sets: we chose to report circularity and aspect ratio as these capture the key results of milling. Ball milling increases the circularity of the 5-ASA from the broad distribution centred at 0.45 obtained for both uM and JM materials to a narrower distribution with a mean of 0.85. The aspect ratio distributions in Figure 3.1(b) reflect the above differences between uM and BM. Figure 3.1(a) also indicates that jet milling gives a noticeably broader distribution in circularity.

Table 2.1: Density values (± s.d.) and specific surface area for 5-ASA batches.

Batch	Density / g cm^{-3}			Specific surface area/ m^2 g^{-1}
	solid	bulk	tapped	
uM	1.530 ± 0.001	0.240 ± 0.002	0.410 ± 0.008	1.94 ± 0.025
JM	1.517 ± 0.001	0.245 ± 0.005	0.344 ± 0.004	2.65 ± 0.022
BM	1.515 ± 0.001	0.480 ± 0.002	0.670 ± 0.003	5.89 ± 0.043

Milling therefore changed the particle dimensions and shape. The impact on the powder handling properties is summarised in Table 2.1. The solid density values are very similar, as expected. In comparison with the uM values, the data indicate that the jet milling process did not modify either the bulk or the tapped densities of the 5-ASA powder considerably. The tapped density decreases slightly after jet milling, probably due to electrostatic attractive forces becoming stronger as the particle dimensions decrease, increasing particle aggregation and thereby leading to poorer packing ability. In contrast, the bulk and tapped density values for BM are about

50% and 60% higher, respectively, than the uM values. The differences in packing between JM and BM are consistent with the change in particle morphology and dimensions. The specific surface area (BET) results confirm the reduction in particle size upon milling. The trend supports the laser scattering sizing results (Table 1.1) in that the BM surface area is greater than that of the JM.

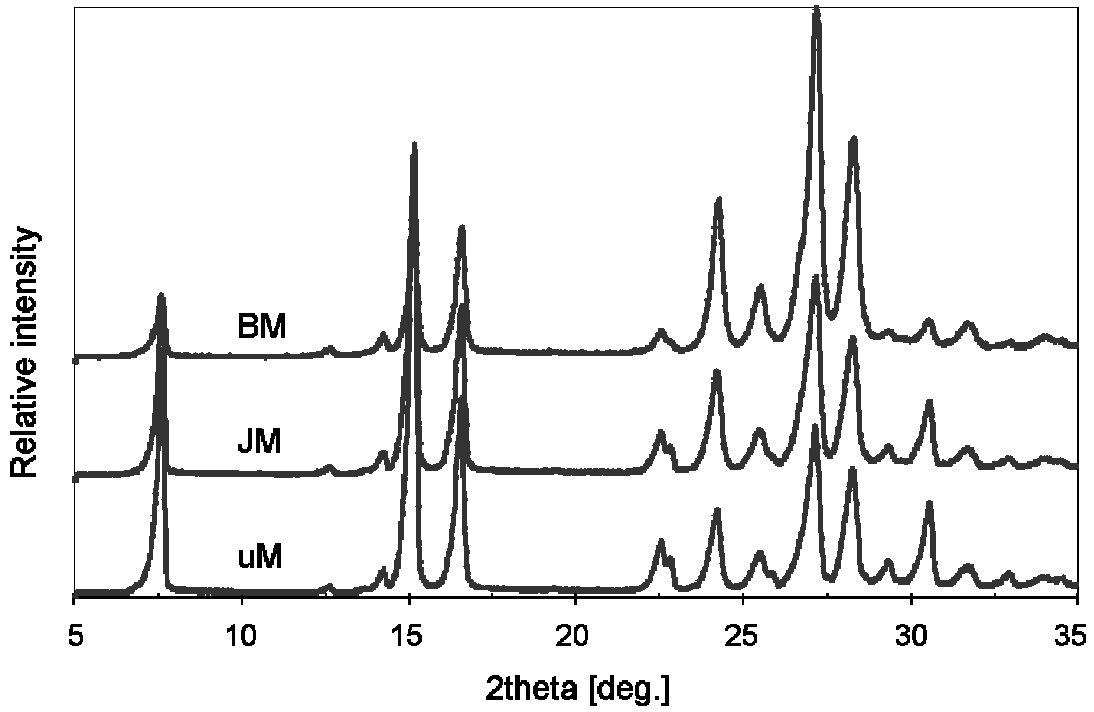


Figure 4.1: X-ray diffraction patterns for uM, JM and BM batches of 5-ASA. Profile baselines are shifted for clarity.

Modification of the solid state on milling was evaluated by PXRD and DSC. Figure 4.1 shows similar PXRD patterns for the three materials. The peak angular positions are in excellent agreement, indicating that milling did not affect the crystalline form of 5-ASA. Small differences in the peak relative intensities are attributed to preferred orientation effects; this is consistent with the differing morphology of the 5-ASA crystals, being predominantly prolate for the uM and the JM batches, and more pseudo-spherical for BM, as discussed above. Similarly, thermograms of all samples

showed a single endothermic melting event at approximately 280 °C with a comparable associated enthalpy (about 980 J g^{-1}).

3.3.1 Ram extrusion study

The effect of 5-ASA particle size and morphology on Avicel PH101 based pastes undergoing extrusion was investigated at varying experimental conditions of die length and diameter. Figure 5.1 presents some of the extrusion pressure-ram displacement profiles obtained for (a) 50 and (b) 90 wt% loaded 5-ASA pastes, performed at V_{ram} = 1 mm s^{-1} using a 6-holed square-entry die (D = 1 mm, L = 4 mm).

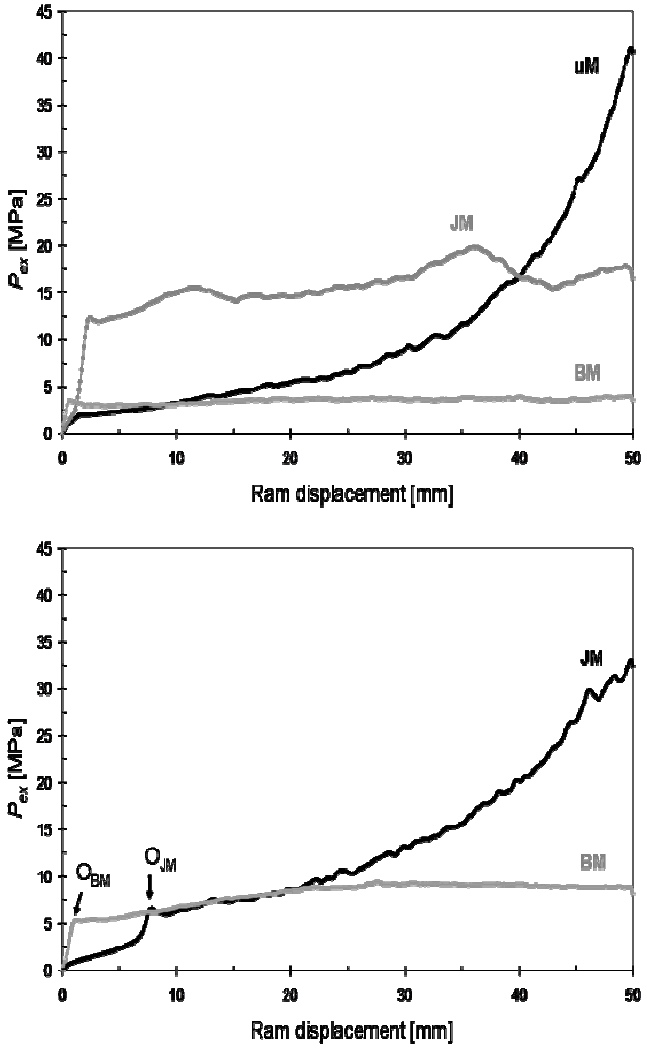


Figure 5.1: Extrusion profiles for Avicel PH101/5-ASA (uM, BM and JM) pastes at V_{ram}= 1 mm s^{-1}, 6-holed die (D = 1 mm, L = 4 mm). 5-ASA loading: (a) 50 and (b) 90 wt%. Points labelled O_{JM} and O_{BM} in (b) represent onset of flow for JM and BM pastes, respectively.

Figure 5.1(a) compares the ram extrusion profiles for 50 wt% loaded 5-ASA pastes. The 50 wt% loaded uM paste starts extruding at an initial P_{ex} value of ~ 2 MPa, but soon after the onset of the extrusion P_{ex} steadily increases, reaching a final value of ~41 MPa. This phenomenon is consistent with significant dewatering occurring during extrusion, leading to an over-wet initial mass, requiring a smaller stress to start flowing. Subsequent portions of paste being extruded are consequently drier, and higher pressures are hence required to maintain flow. As discussed in section 3.1, LPM increases with increasing drug loading; at 90 wt% loading, the uM paste was unable to be extruded since severe dewatering occurred, yielding a dry immovable plug in the barrel.

50 wt% loaded JM and BM pastes start extruding at slightly higher P_{ex} values than uM, namely 13 and 4 MPa, respectively; however, unlike uM, the extrusion pressure reaches a steady state instead of progressively increasing, indicating that dewatering is minimal. Hence, a decrease in 5-ASA particle size improves the flow characteristics of wet powder masses and allows LPM to be considerably reduced or eliminated. This is particularly important when the longest particle dimension approaches the size of the die diameter. However, differences between JM and BM data are observed; the extrusion profiles for BM pastes are smooth and continuous, unlike JM which shows more fluctuation. The more jagged appearance of the P_{ex} profiles for the JM paste could be due to the greater tendency of the material to stick-slip at the die wall. The differences between JM and BM are more pronounced when higher drug loadings (90 wt%, Figure 5.1(b)) are processed. The extrusion profile for the JM paste shows a compaction stage prior to the extrusion of the wet mass, most likely due to the different packing properties of the milled materials. The initial

extrusion pressure of 90 wt% JM paste (7 MPa) is almost half that of the corresponding 50 wt% loaded formulation (13 MPa), despite the increase in drug loading; this is once again consistent with liquid phase migration occurring during the extrusion, as is also evident from the steady increase in P_{ex} up to the final value of 33 MPa. In contrast, the BM paste shows a relatively steady flow state throughout the extrusion, after an initial extrusion pressure of ~ 6 MPa.

These results indicate that the particle size and shape of 5-ASA are critical parameters affecting the packing as well as the flow properties of the pastes. On the basis of the different behaviour of JM and BM pastes, we postulate that the shape of the 5-ASA particles, in addition to their size, is a key factor in the extrusion of highly-loaded pastes with Avicel PH101 as an extrusion aid at this length scale. In order to confirm this hypothesis, the behaviour of a reference material, having a similar particle morphology to that of 5-ASA, undergoing extrusion was investigated.

3.3.2 Influence of needle-like morphology of 5-ASA: control test

The dehydrate (gypsum) polymorph of calcium sulphate (labelled $CaSO_4$) was selected as a reference material: it is a sparingly soluble material with prolate shaped particles similar to those of 5-ASA. In order to assess the influence of particle shape of the active ingredient on Avicel PH101-based pastes undergoing extrusion, a commercial high density grade of 5-ASA (labelled HD) was also investigated. Particle size/shape characteristics and micrographs of $CaSO_4$ and HD are reported in Table 3.1 and Figure 6.1, respectively. Divergences between the laser scattering and the automated microscope as sizing methods were again evident. HD and $CaSO_4$ particles are prolate, even though their aspect ratios are different from that of uM crystals (Figure 3.1). The uM particles are more tapered whereas HD and $CaSO_4$ crystals are more coarse. Moreover, HD particles are slightly more acicular than $CaSO_4$ ones, as confirmed by the circularity data in Table 3.1.

Table 3.1: Particle size and shape values for $CaSO_4$ and 5-ASA HD.

		$CaSO_4$		5-ASAIID	
Particle size parameter / µm	Volume basis	Laser scattering	Automated microscope	Laser scattering	Automated microscope
	d_{10}	9.44	15.7	10.8	18.7
	d_{50}	30.0	54.4	64.2	37.1
	d_{90}	107	116	166	64.1
Particle shape parameter	Circularity (mean)	0.63		0.38	
	Aspect ratio (mean)	0.58		0.53	

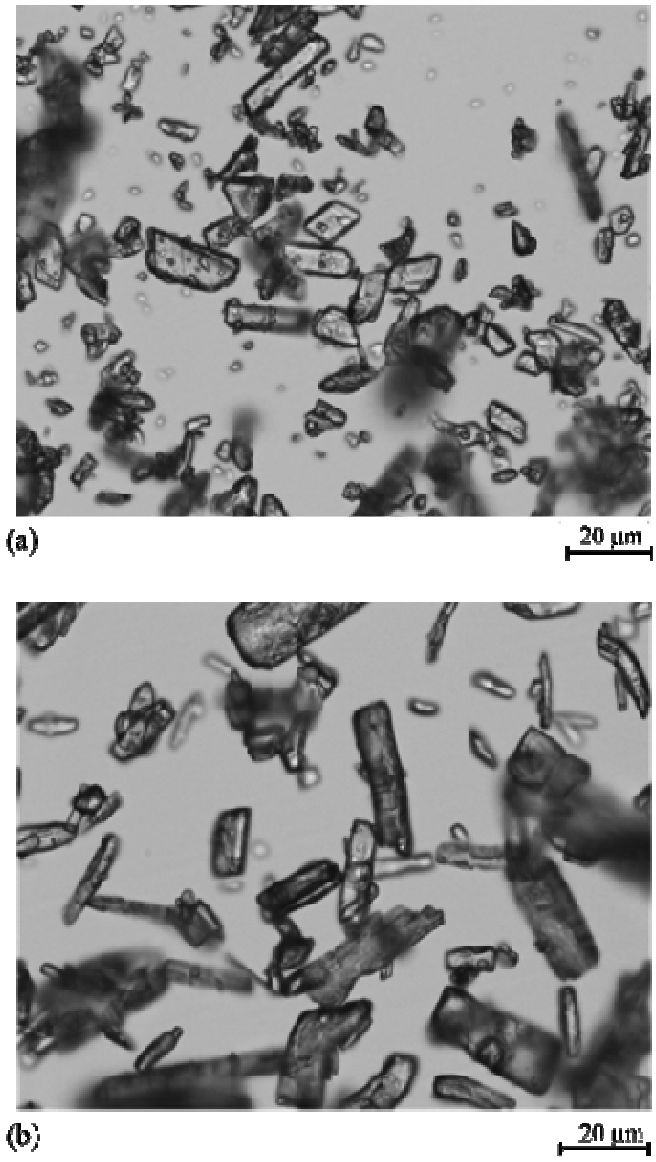


Figure 6.1: Micrographs of (a) $CaSO_4$ and (b) 5-ASA HD (magnification 50×).

A ram extrusion study, at varying conditions of die length and diameter and extrusion velocity, was performed on Avicel PH101-based pastes containing increasing amounts of active ingredient. Since $CaSO_4$ and 5-ASA differ in terms of their solid density values (2.32 and 1.53 g cm^{-3}, respectively), pastes were prepared on a volume rather than a weight basis. No sedimentation or segregation effects were observed.

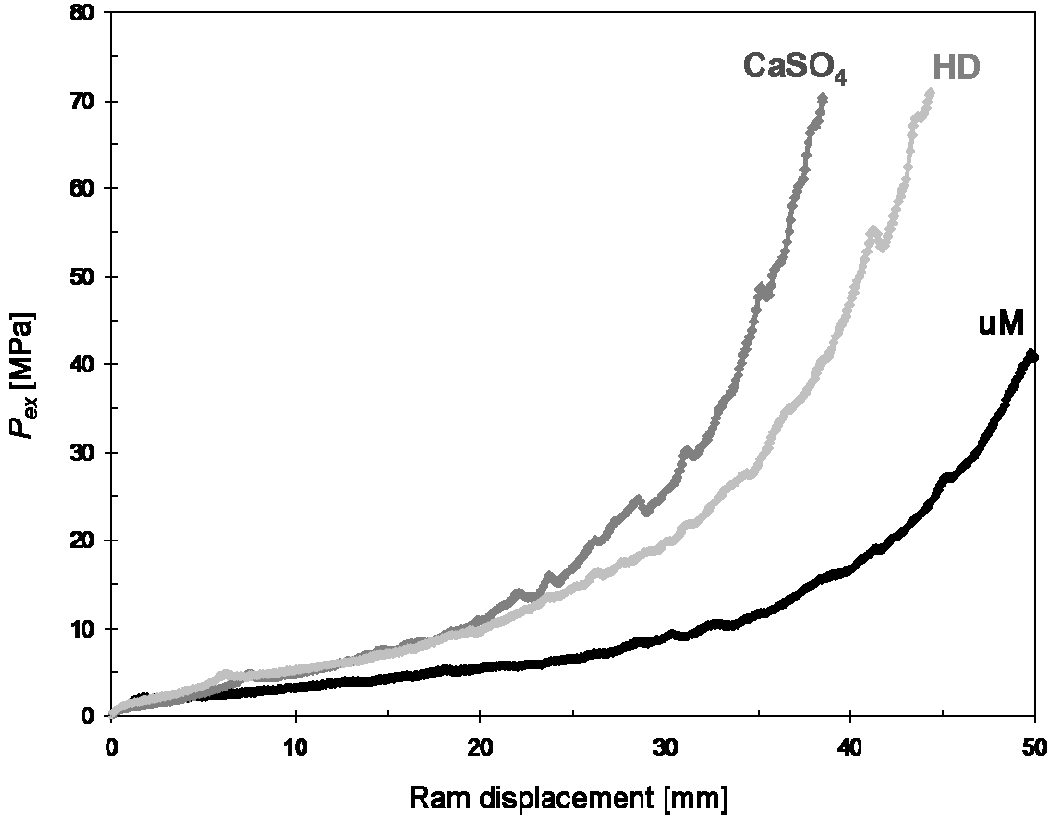


Figure 7.1: Extrusion profiles for Avicel PH101-based pastes containing 60 vol% of uM, 5-ASA HD or $CaSO_4$ at V_{ram} = 1 mm s^{-1}, 6-holed die (D = 1 mm, L = 4 mm).

Figure 7.1 presents the extrusion pressure-ram displacement profiles for 60 vol% active ingredient/Avicel PH101 pastes. In agreement with the particle shape characteristics, the HD and $CaSO_4$ pastes show a similar trend in the extrusion profile to that observed for uM, namely a steady increase in P_{ex} with ram displacement, indicating that dewatering of pastes is occurring during extrusion. Interestingly, LPM is more pronounced when the HD and $CaSO_4$ pastes are extruded, thus suggesting that particle shape, rather than particle size, is the critical parameter in the extrusion of Avicel PH101-based pastes. Both $CaSO_4$ and HD pastes yielded over-wet surface extrudates which could not be spheronised.

3.4 Influence of alternative MCC grades as E-S aids

The previous section demonstrated the influence of API shape on the extrusion of Avicel PH101/'hard API' pastes. The potential influence of the E-S aid was also investigated. The water retention ability, rheology and the performance as an E-S aid of Avicel PH101 for high 5-ASA loadings is now compared to that of two colloidal MCC grades containing NaCMC, namely Avicel RC591 and CL611.

3.4.1 Centrifuge testing

The water retention ability of the standard MCC and the colloidal grades was assessed by centrifugation, performed on pastes containing different amounts of water. Plots of water/MCC mass ratio *vs* centrifugal pressure are presented in Figure 8.1.

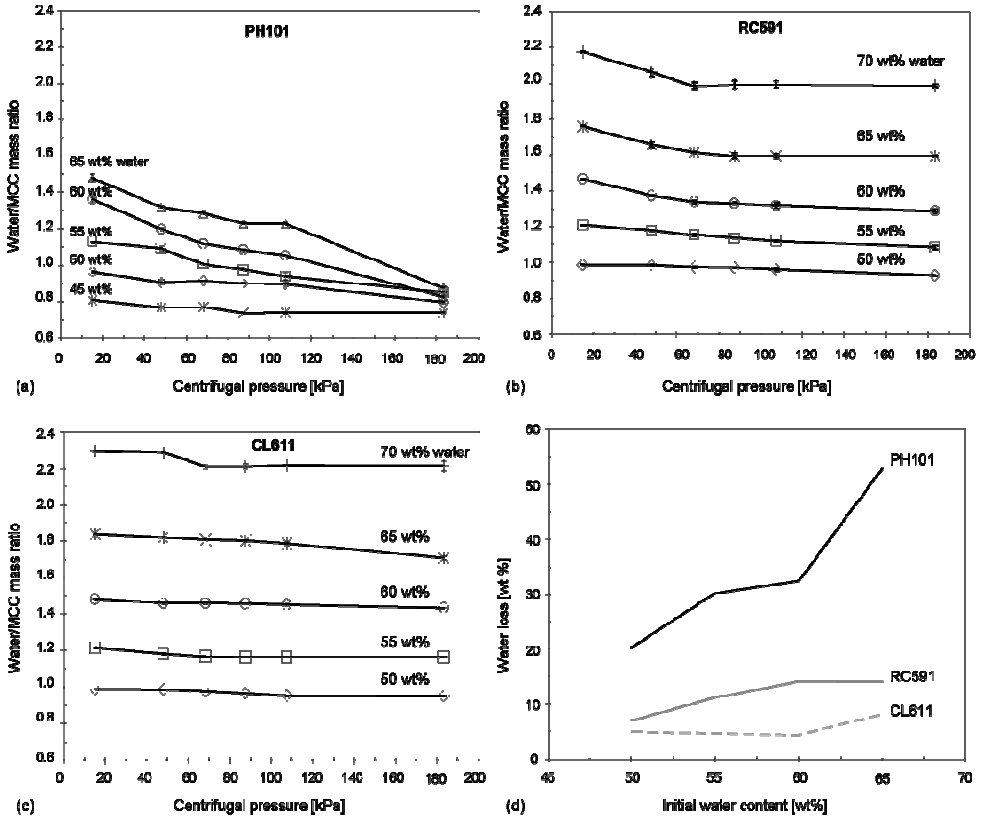


Figure 8.1: Water/MCC mass ratio vs centrifugal pressure profiles for Avicel (a) PH101, (b) RC591 and (c) CL611; (d) water loss profiles of MCC pastes at 180 kPa centrifugal pressure.

Figure 8.1(a) shows that Avicel PH101 is able to retain an amount of water almost equal to its own mass, even after the application of high centrifugal forces. However, for water contents above 50 wt%, the water-holding ability of Avicel PH101 steadily decreases with increasing centrifugal pressure. After being subjected to the highest centrifugal pressure value (180 kPa) for 60 min, all PH101 pastes reached a minimal water/MCC value of about 0.8. This suggests that the water/Avicel PH101 ratio of 1:1.2 might represent the maximum water quantity that the microcrystalline fibrils are able to retain within their internal structure. Once this saturation level is reached, additional water would be loosely associated in the free space between the cellulose particles and therefore readily lost during centrifugation. Extended centrifuge testing over 120 min (data not shown) gave similar results to those performed at 60 min. Figures 8.1(b) and (c) show that both the colloidal MCC grades are able to retain more than twice their own mass of water even when subjected to the highest centrifugal pressures. A plot of percentage water loss against initial paste water content for the three MCC grades (Figure 8.1(d)), at 180 kPa centrifugal pressure, also gives an indication of the water-retaining ability of the pastes. In order to validate the efficiency of the centrifuge testing, a control study using inert glass spheres (ballotini) having a particle size similar to that of MCC was performed (data not shown). As expected, the water added to the ballotini 'paste' was completely drained after the centrifuge run. The greater water-retaining ability of the colloidal MCC grades was further confirmed by ram extrusion testing.

3.4.2 Ram extrusion studies

3.4.2.1 MCC pastes

The rheological behaviour and the water retention ability of the standard PH101 grade of MCC was compared to that of the modified RC591 and CL611 grades. Ram extrusion testing was performed using a square-entry single-holed capillary die (D = 2 mm, L = 2 mm) over a ram displacement of 100 mm, on 50, 55, 60 and 65 wt% water/MCC pastes, at V_{ram} speeds of 0.1, 1 and 10 mm s^{-1}.

Table 4.1: Average P_{ex} values for MCC/water pastes at V_{ram}= 0.1, 1 and 10 mm s^{-1}, single-holed capillary die (D = 2 mm, L = 2 mm). Asterisked values indicate LPM: initial and final extrusion pressures are reported for a ram displacement of 100 mm. Coefficient of variation (CV, in %) given in brackets.

wt% water	V_{ram} / mm s^{-1}	P_{ex} / MPa (CV, in %)		
		PH101	**RC591**	**CL611**
50	0.1	2.31-11.2* (51.3)	7.75 (6.29)	2.76 (2.97)
	1	4.68-7.27* (14.3)	4.81 (2.32)	3.49 (2.11)
	10	7.11 (5.45)	7.76 (6.24)	5.19 (7.14)
55	0.1	0.797-2.86* (9.87)	2.30 (9.87)	1.47 (2.11)
	1	1.63-2.27* (15.9)	2.30 (9.87)	1.88 (1.73)
	10	2.86 (5.11)	3.67 (1.83)	2.90 (3.66)
60	0.1	0.532-0.925* (18.2)	0.532-0.922* (10.10)	0.717 (7.02)
	1	0.800-1.15* (13.4)	0.715 (7.86)	0.924 (2.75)
	10	1.25 (6.45)	1.77 (1.92)	1.49 (4.30)
65	0.1	0.076-0.213* (34.5)	0.323-0.567* (14.2)	0.369 (5.58)
	1	0.090-0.207* (20.1)	0.545 (5.09)	0.568 (1.36)
	10	0.155-0.315* (25.1)	0.797 (6.97)	0.919 (2.77)

Table 4.1 lists the average P_{ex} values for the formulations tested. For those formulations which exhibited LPM, initial and final P_{ex} values, marked by an asterisk, are reported. As expected, for a given MCC grade, the extrusion pressure increases with increasing V_{ram}, and decreases with increasing water content.

All PH101 pastes exhibited liquid phase migration at the lowest (0.1 mm s^{-1}) and intermediate (1 mm s^{-1}) ram velocities, whereas at the highest V_{ram} (10 mm s^{-1}) LPM was observed only for the 65 wt% water/PH101 formulation. Avicel RC591 only showed some LPM at V_{ram}= 0.1 mm s^{-1}, while the intensity of dewatering for RC591 pastes (profiles not shown) was always less pronounced than that observed for the corresponding PH101 pastes. LPM was not observed with Avicel CL611, and smooth extrusion profiles were obtained at all combinations of V_{ram} and water content investigated. By way of example, Figure 9.1 presents P_{ex} profiles for 50 wt% water/MCC pastes performed at V_{ram} = 1 mm s^{-1}.

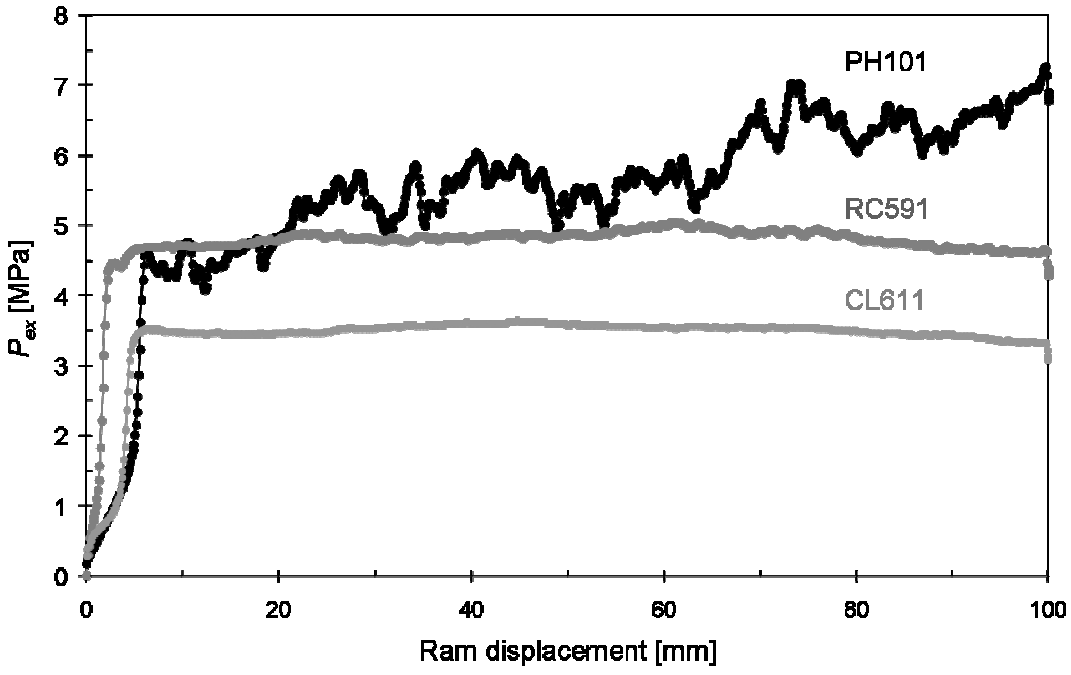
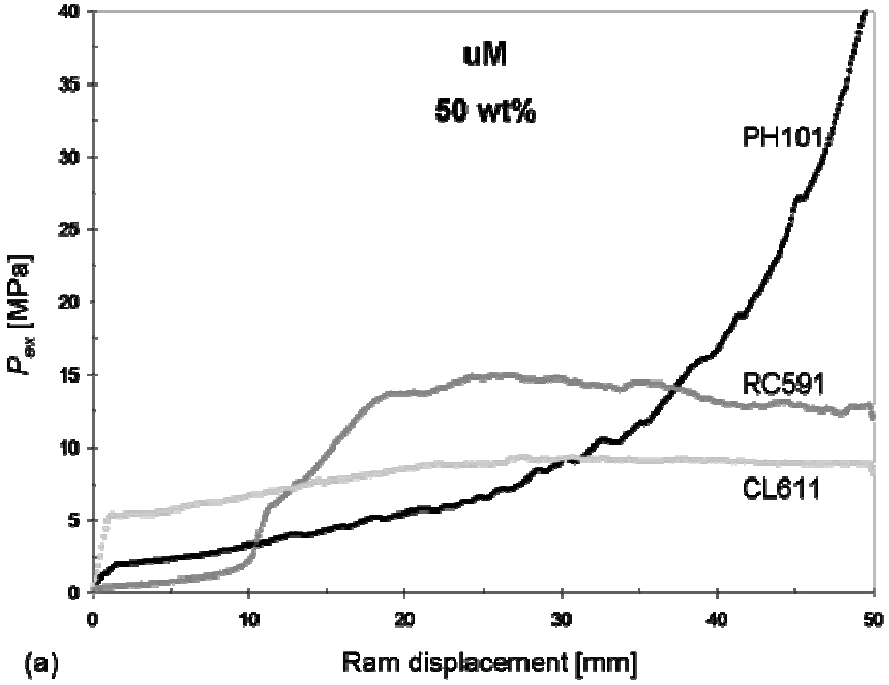


Figure 9.1: Extrusion profiles for 50 wt% water/MCC (Avicel PH101, RC591 and CL611) pastes at V_{ram} = 1 mm s^{-1}, single-holed capillary die (D = 2 mm, L = 2 mm).

The ram extrusion study, in agreement with the centrifuge results, demonstrates the improved ability of colloidal MCC grades to hinder water migration when subjected to pressure.

3.4.2.2 Colloidal MCC/5-ASA pastes

RC591 and CL611 grades of MCC were investigated as alternative E-S aids to Avicel PH101 in 5-ASA based pastes. The amount of water used for paste preparation was selected on the basis of the centrifuge testing. Since colloidal MCC grades have a greater water-retaining ability than the standard PH101, the amount of water necessary to obtain a plastic mass suitable for extrusion was greater. The water/colloidal MCC mass ratio selected was 1.5:1; both a wet mass and extrudates with appropriate characteristics of plasticity could be obtained with this amount of water.



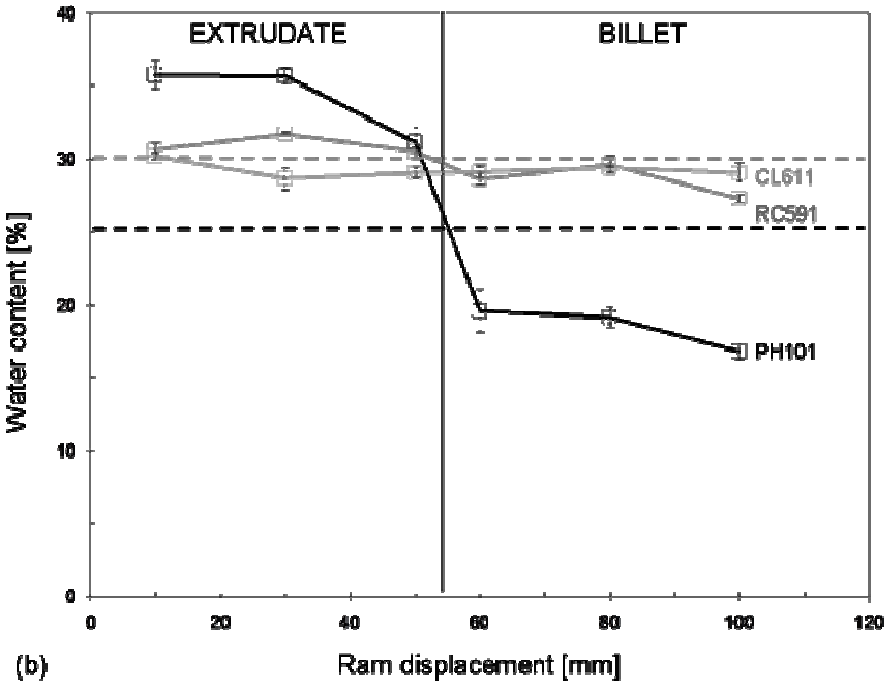


Figure 10.1: (a) Extrusion profiles for 50 wt% uM/MCC (Avicel PH101, RC591 and CL611) pastes at V_{ram} = 1 mm s^{-1}, 6-holed die (D = 1 mm, L = 4 mm) and (b) water content profile of extrudates throughout ram extrusion and the remaining paste billet. Horizontal lines in (b) indicate initial paste water content of colloidal MCCs (grey) and Avicel PH101 (black). Error bars indicate standard error (3 replicates).

Figure 10.1(a) shows an example of the P_{ex}-ram displacement profiles obtained for 50 wt% uM/MCC pastes. By replacing the standard MCC grade with the colloidal ones, LPM was reduced or eliminated. In contrast to the Avicel PH101 based paste, P_{ex} for the colloidal grades of MCC reached steady state shortly after extrusion started, indicating that little dewatering occurred. This was confirmed by monitoring the water content distribution of both the extrudate during ram extrusion and the remaining billet within the barrel (see Figure 10.1(b)). The Avicel PH101 based formulation showed a relatively large difference in extrudate water content from that of the initial paste. This is manifested as an over-wet extrudate and a relatively dry paste billet in the barrel. On the contrary, the water content of the extrudate and the billet for both the colloidal MCC grades is close to the initial value, thereby indicating that the water was strongly held within the MCC-NaCMC fibril network.

The extrudates obtained were subjected to spheronisation: both RC591 and CL611 based formulations yielded spherical granules, whereas PH101 failed, yielding over-wet extrudates that aggregated during spheronisation.

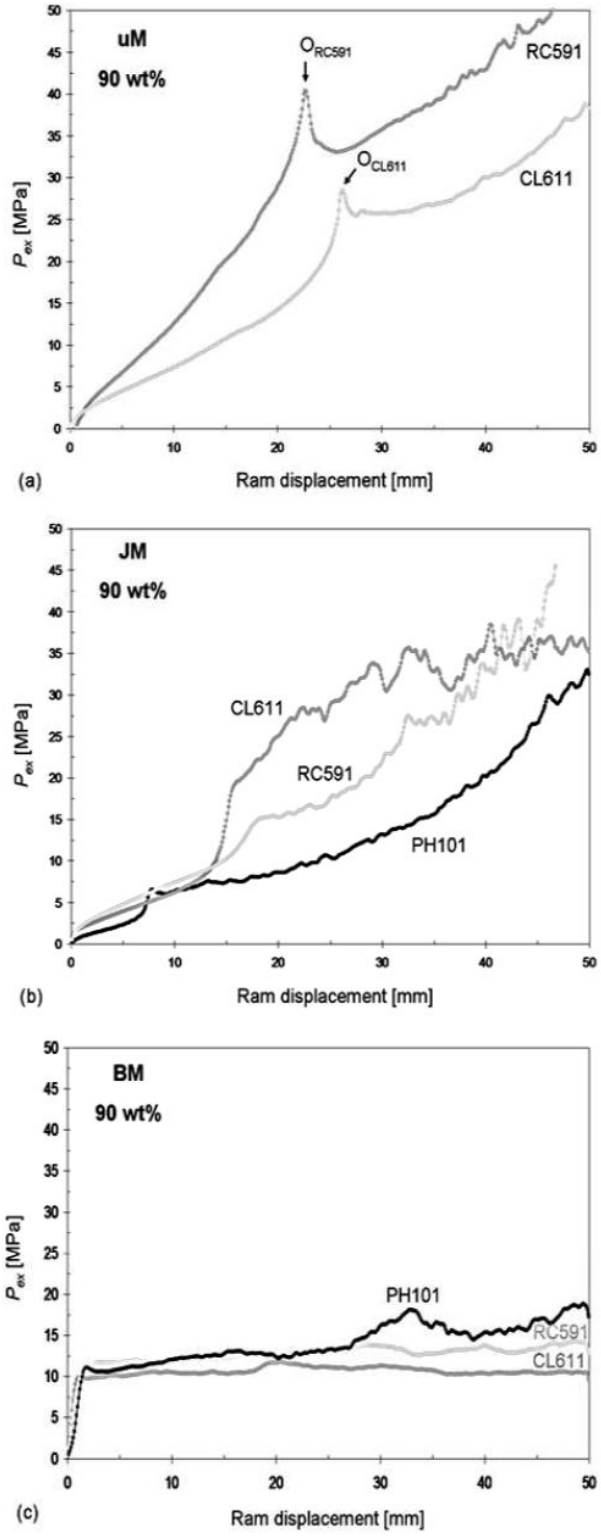


Figure 11.1: Extrusion profiles for MCC-based pastes containing 90 wt% of (a) uM, (b) JM and (c) BM at V_{ram} = 1 mm s^{-1}, 6-holed die (D = 1 mm, L = 4 mm). Points labelled O_{RC591}, O_{CL611} and O_{PH101} in (a) and (c) indicate the onset of extrusion for Avicel RC591, CL611 and PH101 pastes, respectively.

The behaviour of these colloidal MCC grades with high loadings of 5-ASA (*i.e.* 90 wt%) was also assessed. Figure 11.1 presents the extrusion profiles obtained for 90 wt% API loaded pastes with uM, JM and BM. Both the colloidal MCC grades allowed a 90 wt% uM loaded formulation to be extruded (Figure 11.1(a)), whereas Avicel PH101 failed (see section 3.3.1). However, both uM/RC591 and uM/CL611 started extruding at relatively high values of P_{ex} (41 and 28 MPa, respectively), after an extended compaction stage coupled with severe liquid phase migration throughout the extrusion. Analogously, JM pastes (Figure 11.1(b)) showed a similar extrusion trend, even though the overall P_{ex} values are slightly lower. Conversely, BM-based pastes (Figure 11.1(c)) gave steady extrusion profiles, following the onset of extrusion (occurring at 10 MPa for the CL611-based paste and at ~ 11 MPa for the PH101 and RC591 ones). The importance of particle shape, rather than particle size of 5-ASA in the extrusion process of MCC based pastes, is once again confirmed.

Previous workers, *e.g.* Harrison *et al.* (1985), demonstrated that steady-state flow during the extrusion process is necessary to obtain good quality extrudates (*i.e.* ones with a smooth surface); this was confirmed in the current study. However, good quality extrudate does not always imply its suitability for spheronisation. In fact BM/PH101 pastes yielded good quality (without surface defects) extrudates, but these were not able to give satisfactory spheres when subjected to spheronisation; the extrudates obtained did not have sufficient plasticity either to break up or to be rounded off within the spheroniser (Figure 12.1(a)). However, increasing the moisture content in the paste formulation, to enhance plasticity, led to over wet-surface extrudates and resulted in uncontrollable agglomeration. Conversely, both BM/RC591 and BM/CL611 pastes exhibited steady extrusion profiles and

furthermore yielded extrudates that were able to be broken up in the spheroniser and to form satisfactory spheres (Figure 12.1(b)).

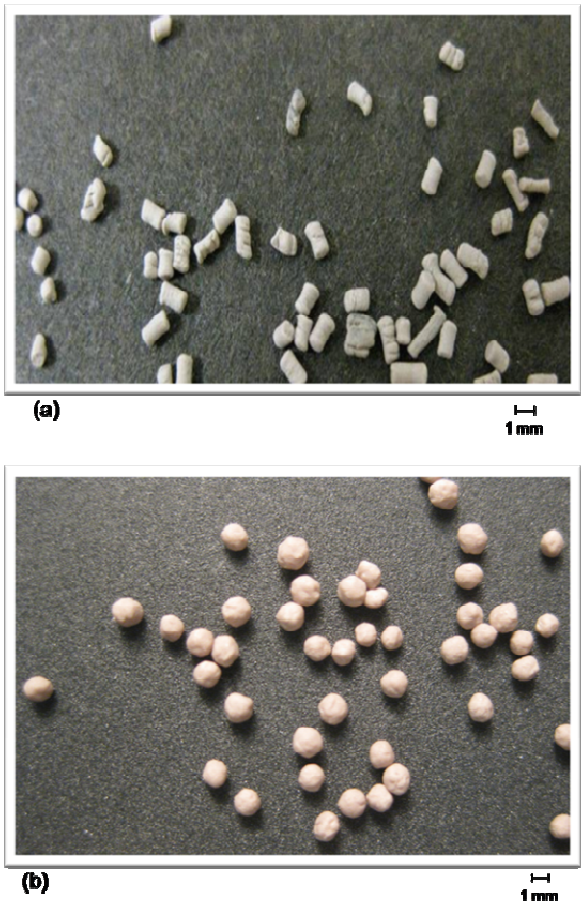


Figure 12.1: Micrographs of dried pellets containing 90 wt% BM and 10 wt% (a) Avicel PH101 and (b) CL611. Extrusion: 6-holed die (D = 1 mm, L = 4 mm); V_{ram} = 1 mm s^{-1}. Spheronisation: 10 min; 1600 rpm.

The overall results indicate that particle size and shape of the API (and thereby an appropriate process of milling) are important parameters to be taken into account when high drug loadings need to be processed by extrusion-spheronisation. Analogously, the E-S aid type used is also an important issue to be considered, as it can determine the feasibility of the spheronisation process and/or the characteristics of the final product. The influence of process parameters (*e.g.* spheronisation time and speed) on the properties of the final pellets will be investigated further.

4. CONCLUSIONS

The feasibility of an E-S route for the manufacture of highly-loaded 5-ASA multiparticulate dosage forms was assessed. The influence of the API chemical and physical properties on the rheological behaviour of MCC-based pastes undergoing extrusion was investigated. Two batches of micronised 5-ASA, with different particle size and shape characteristics, were prepared and their extrusion behaviour was compared to that of the starting material. The performance as E-S aids of the standard Avicel PH101 grade of MCC and of colloidal ones (*i.e.* Avicel RC591 and CL611), alongside high loadings of 5-ASA, was also evaluated. Centrifugation testing, supported by ram extrusion studies, was used to establish the water retention ability of paste materials and to determine the minimal water content required to obtain a wet mass with an appropriate degree of plasticity for extrusion and spheronisation.

Acute liquid phase migration (LPM), resulting in a non-uniform distribution of water within the extrudates, was generally observed for the 5-ASA/Avicel PH101 paste formulations. This was demonstrated to be dependent on both the drug load and the type of extrusion aid. Drug particle morphology (needle-like) was identified as the critical parameter, which was confirmed by extruding calcium sulphate/MCC pastes, where the sulphate polymorph was selected to match the 5-ASA shape. A reduction in particle size, combined with a change in particle morphology, allowed LPM to be considerably reduced or eliminated. The effect of pH on MCC behaviour was found to be negligible. Colloidal grades of MCC were identified to be promising alternatives to the standard PH101 in extrusion of high 5-ASA loadings, due to their ability to hinder water migration when subjected to pressure. Based on the results

obtained, a multiparticulate E-S formulation containing not less than 90 wt% 5-ASA could be developed by combining an accordingly micronised API and colloidal MCC grade.

Acknowledgements

The authors wish to thank IMS Micronizzazioni S.p.A (Milano, Italy) for providing the air-jet milled batch of 5-ASA. The microcrystalline cellulose was kindly provided by IMCD S.p.A (San Donato Milanese, Italy). The assistance of Mr Z. Saracevic (Department of Chemical Engineering and Biotechnology, University of Cambridge) is gratefully acknowledged.

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Nomenclature

A	projected area of particle (m^2)
d_{10}, d_{50}, d_{90}	particle size (m)
D	die land diameter (m)
L	die land length (m)
p	projected perimeter of particle (m)
P_c	centrifugal pressure (Pa)
P_{ex}	extrusion pressure (Pa)
r_{in}, r_{out}	radial distances (m)
t	time (s)
V_{ram}	ram velocity (m s^{-1})
ρ	liquid phase density (kg m^{-3})
ω	angular velocity (rad s^{-1})

Abbreviations

5-ASA	5-aminosalicylic acid
API	active pharmaceutical ingredient
B.E.T	Brunauer-Emmett-Teller
BM	ball-milled 5-ASA
$CaSO_4$	calcium sulphate dihydrate
CD	Crohn's disease
DSC	differential scanning calorimetry
E-S	extrusion-spheronisation
HD	high density grade of 5-ASA
JM	jet-milled 5-ASA
LPM	liquid phase migration
MCC	microcrystalline cellulose
NaCMC	sodium carboxymethyl cellulose
PXRD	powder X-ray diffraction
UC	ulcerative colitis
uM	un-milled 5-ASA

CHAPTER 2

A FACTORIAL APPROACH TO HIGHLY-LOADED 5-ASA PELLETS BY EXTRUSION-SPHERONISATION

G. Di Pretoro, L. Zema, L. Palugan, D.I. Wilson, S.L. Rough, A. Gazzaniga. A factorial approach to highly-loaded 5-ASA pellets by extrusion-spheronisation. Submitted for publication to Int. J. Pharm.

Abstract

The aim of the current work was to assess the feasibility of scaling-up, from a bench- to a pilot-scale extruder-spheroniser, a multiparticulate formulation containing a high load of 5-aminosalicylic acid (5-ASA). A preliminary study, aiming to compare the behaviour of 5-ASA-based pastes in a ram (bench-scale) and a basket (pilot-scale) apparatus was performed. Considered the complexity and multifactoriality of the E-S process, a factorial approach, namely a mixed fractional factorial design (MFFD) has been employed for the study. The influence of some process and formulation parameters on desired pellets characteristics, such as pellets size, size distribution, mechanical resistance, dissolution properties and process yield, was investigated. Moreover, an optimisation study, based on the desirability function approach, was performed to identify the combination of factors that would allow the optimal response values to be obtained. The possibility of scaling up a highly-5-ASA-loaded multiparticulate formulation from a bench to a pilot-scale apparatus was successfully demonstrated. The MFFD aided in the identification of the most affecting process and formulation variables. In addition, the optimisation study allowed the operative conditions for the obtainment of a multiple-unit dosage form, containing up to 95 wt% 5-ASA, in a pilot-scale equipment, to be identified.

Keywords: 5-aminosalicylic acid; extrusion-spheronisation; high drug loading; design of experiment; optimisation study.

1. INTRODUCTION

Inflammatory Bowel Disease (IBD) is a spectrum of chronic, episodic, inflammatory conditions of the gastrointestinal (GI) tract. IBD is conventionally divided into two major subtypes, *i.e.* Crohn's disease (CD) and ulcerative colitis (UC). UC mainly affect the colon starting at the anal verge, even though it may extend proximally (*e.g.* colitis, left-side colitis and pancolitis). At converse, CD generally involves the small intestine and the ileocolonic area, with a variable extent.

The current gold-standard molecule for the treatment of IBD (UC in particular) is 5-aminosalicylic acid (5-ASA, mesalamine). As mesalamine is believed to act locally from within the gut lumen, prodrugs activated by colonic bacteria and oral modified drug delivery systems (DDS) are widely used to maximise deliver of 5-ASA to the target tissues, while minimising systemic absorption.

Most of the mesalamine-based products, currently available on the market, are delayed-release DDS. They involve capsules and/or tablets coated with a polymeric film soluble over a certain value of pH (generally 7 since it is thought to be the pH value corresponding to the human terminal ileum). These delayed-release DDS might have important limitations. Firstly, pH is a highly-variable physiological parameter, in particular in those subjects suffering of IBD (Haddish-Berhane *et al.,* 2006). Moreover, it has been demonstrated that delayed-release DDS are not equally effective in the treatment of both UC and CD, as they may not deliver therapeutically effective doses of 5-ASA to the small intestine and to the left side of the colon (a site predominantly affected in all patients with UC). Indeed, clinical studies have shown that mucosal 5-ASA concentrations using these formulations are typically highest in

the right-sided colon, whereas in the rectum the concentration of 5-ASA is much lower (Frieri *et al.,* 1999).

Another important limitation of the mesalamine-based products currently available on the market, is the relatively low dose of 5-ASA (generally 500 mg/unit). If considering in fact the complex IBD dosing regimen of 2.4-4.8 g/day, multiple daily administrations are required, thus reducing compliance and possibly compromising the therapeutic efficacy.

Lately, new approaches to both simplify dose regimen and improve drug delivery to the affected areas of the GI tract have been investigated. These formulations are based on multiple-unit dosage forms (*e.g.* granules) opportunely coated to achieve a slow, and pH-independent, release of the active drug throughout the entire intestine. Multiparticulates may have several advantages over the standard, single-unit dosage forms, both from a biopharmaceutical (*e.g.* more even and predictable transportation in the GI tract) and a technological (*e.g.* high flexibility in tailoring the drug release) point of view.

In our research project, highly-5-ASA loaded pellets were therefore sought as the drug core of a multi-particulate DDS with improved efficacy and compliance (Di Pretoro *et al.,* 2010). Extrusion-spheronisation (E-S) was chosen as the manufacturing technique for pellet preparation since its claimed advantage to incorporate high levels of drug (Gazzaniga *et al.,* 1998; Tomer *et al.*, 2002).

E-S formulations represent a complex systems, whose rheological behaviour is not yet fully understood and determined by both a number of particle-particle interactions and liquid phase phenomena (Wilson and Rough, 2006). Particle size,

morphology, packing behaviour and other related properties of solid components may dictate the rheological characteristics of such materials, in particular when the API represents the major component, by volume, of the paste formulation.

In a previous work we studied, systematically, the rheological behavior of highly-loaded 5-ASA pastes, using a bench-scale ram extruder (capillary rheometer, Zwick). We investigated the effect of chemical (acidity) and physical (particle size and shape) characteristics of 5-ASA on the E-S process of highly-API-loaded pastes, as well as the performance as E-S aids of a standard and colloidal MCC grades.

Liquid phase migration (LPM), resulting in a non-uniform distribution of water within the extrudates and subsequent un-controlled agglomeration during spheronisation, was generally observed. The extent of LPM was demonstrated to be mainly dependent on the drug load and was identified as arising from the particle morphology (needle-like) of the API.

Based on the results obtained we were able, by combining an accordingly modified grade of 5-ASA and colloidal MCC types, to develop a promising formulation for the obtainment of a multi-particulate dosage form containing up to 90 wt% 5-ASA.

Being our next objective the realization of an industrial scalable multiple-unit dosage form, a feasibility study on the scale-up ability of both milling and E-S was performed. Firstly, in collaboration with a company specialised in milling processes (IMS Micronizzazioni S.p.A., Milano, Italy) we investigated the possibility of reproducing, by an industrial-scale microniser, a batch of 5-ASA with similar characteristics to those obtained *via* planetary ball-milling. Afterwards we investigated the feasibility of transferring the promising formulation, as identified in the ram extruder, to a pilot-scale apparatus (basket extruder, Nica® E-140).

E-S scale up is, in fact, a source of inconsistency between lab-scale results and industrial products. It is important to note that formulations which operate satisfactorily in one extruder type do not readily transfer to different machines (Newton *et al.,* 1994). By changing extruder type different E-S products, in terms of density, particle size and size distribution, indeed, may be obtained. This is the reason why, when aiming to transfer a formulation, intended for E-S, from a bench- to a pilot-scale apparatus it is recommended to investigate again the influence of both the process and the formulation parameters on the new equipment. However, because of the complex nature of the E-S process and the high number of factors involved, it is not always possible to investigate systematically the effect of every single parameter.

Experimental designs are therefore often employed, also in industrial scale-up, since they allow to evaluate the effect or "response" of many factors and levels with a reduced amount of trials and assist in estimating the main effects of each experimental factor on the product. In particular, factorial design type studies have proven useful in formulation development to understand the effect of variables and to control them to produce products having desired attributes (Zema *et al.,* 2008).

A mixed fractional factorial design, well suited for exploring both process and product variables and their interaction with each other, was therefore used in our study. A statistical approach was also employed to optimise final paste formulations, aiming to produce pellets containing the highest loading of 5-ASA possible.

2. MATERIALS AND METHODS

2.1 Materials

5-aminosalicylic acid (5-ASA) (Erregierre S.p.A., Bergamo, Italy) was used after micronisation. The colloidal Avicel® RC591 grade of microcrystalline cellulose (MCC) (IMCD S.p.A, San Donato Milanese, Italy) was used as the extrusion-spheronisation aid in the experimental design. Another colloidal MCC grade (*i.e.* Avicel® CL611), and a standard one (*i.e.* Avicel® PH101) were also evaluated in preliminary trials. The primary liquid binder was water, with polyvinilpirrolidone (PVP K30) (BASF, Mount Olive, NJ, USA) and a low viscosity grade of hydroxypropyl methylcellulose (HPMC, Methocel® E5) (Colorcon, Inc. Harleysville, PA, USA) used as extra binders.

2.2 Methods

2.2.1 Micronisation

The un-milled, commercial batch of 5-ASA was micronised in an air-jet mill apparatus (IMS Micronizzazioni S.p.A., Milano, Italy). The mill had a spiral chamber of 300 mm diameter. The milling gas was air. Due to secrecy agreements the operative conditions cannot be reported.

2.2.2 Particle characterisation

2.2.2.1 Particle size and shape measurements

Particle size characterisation was performed by laser light scattering (Coulter LS 230, Beckman Coulter Inc., Fullerton, CA, USA). Powder samples were sonicated

prior to analysis to break up potential aggregates. Data collected are reported in terms of volume-based relative size distribution.

Particle shape, quantified in terms of aspect ratio (defined as minor axis/major axis) was determined by particle imaging device (Morphologi® G3, Malvern Instruments Ltd, Worcestershire, UK). At least 60,000 particles were analysed. Two-dimensional micrographs were also collected.

2.2.2.2 Powder X-Ray Diffraction

A Philips PW1050/25 diffractometer (Philips Analytical Inc., Natick, MA, USA) was used for powder x-ray diffraction (PXRD) analyses. Cu-K_α radiation (λ = 1.54 Å), generated with 30 kV and 15 mA, was used as the radiation source. Samples were packed in the same aluminum sample holder to promote reproducibility of conditions, taking care also to minimize preferred orientation effects. The operating conditions were as follows: scan speed 0.5° 2θ min^{-1}; step size 0.02° 2θ; range $5 \leq 2\theta \leq 35°$.

2.2.2.3 BET surface measurements

Surface area determination was performed by means of a SA3100 Surface Area Analyzer (Beckman Coulter, UK). According to the BET method, N_2 was used as the adsorbate gas (USP 32 *Physical Test. Specific Surface Area. Volumetric Method*). Prior to surface area measurements, samples (approximately 2 g) were degassed at 90°C under vacuum (0.4 Pa) for 1 h to remove physically adsorbed gases (water vapour). The measurement was carried out in triplicate.

2.2.3 Pellets preparation

5-ASA and the E-S aid were dry blended in a Turbula® Shaker-Mixer T2F (Glen mills Inc., Clifton, NJ) for 10 minutes at 48 rpm. The powder mixture was transferred in a planetary mixer (Kenwood KM 010, Southampton, UK), assembling a K-shaped mixing arm, and the liquid binder slowly poured onto the bulk surface. The extra binder (PVP), when scheduled in the experimental design, was firstly dissolved in water and the resulting solution used for paste preparation. Granulation was performed at a constant speed of 250 rpm for 5 min. Pastes were stored for 24 h in airtight containers prior to extrusion to allow the water to equilibrate throughout the mixture. The batch size for each paste formulation was 1 Kg.

The wet masses were extruded in a radial basket apparatus (Nica® model E140) equipped with a multi-holed screen (i.d holes= 1 mm; screen thickness= 1.25 mm;) and the resulting extrudates were then spheronised in a 64 cm diameter radial-hatch friction plate spheroniser (Nica® S320). All surfaces in contact with the paste were stainless steel. Pellets were dried in a vacuum oven at 40 °C for 24 hours. All experiments were performed at room temperature in an air-conditioned laboratory (21°C ± 2°C, humidity 20% ± 2%).

2.2.4 Pellets characterization

2.2.4.1 Sieve analysis

The particle size distribution of pellets was estimated by sieve analysis (Octagon 2000, Endecotts Ltd., UK; 5 minutes, amplitude 4) of approximately 100 g samples. ASTM standard sieves were used in the 250-1,400 μm range. Geometric mean diameter (d_{geo}) and geometric standard deviation (σ_{geo}) were calculated from weight distribution of diameters. Process yield of the interesting size fraction (710-1,000

μm) was also calculated from the weight distribution. The product obtained, having a particle size less than 250 μm and above 1,400 μm were considered as the "fine" and the "coarse" fractions, respectively.

2.2.4.2 Bulk density

A sample weight (W) of about 50 g was poured into a 250 mL glass cylinder and the volume (V_0) was determined. The bulk density was calculated as $\rho_b = W/V_0$. The results presented are the mean of three replicates.

2.2.4.3 Mechanical resistance

Friability- Resistance of pellets to abrasion was evaluated through a procedure adapted from *Ph. Eur. 6th Ed*. "Friability of granules and spheroids-method B" (2.9.41). The oscillating apparatus was replaced with a horizontal oscillating mixer (Turbula®). About 10 g of pellets were placed in a 105 mL glass container along with 20 glass spheres (7 mm diameter) and shaken for 10 minutes at 200 rpm. Loss of powder (< 250 μm) was determined and friability was calculated as the percent ratio between the amount of powder < 250 μm and the initial sample weight.

Tensile strength- The mechanical characteristics of pellets were investigated using a texture analyzer (TA.HD*Plus*, Stable Micro Systems Ltd, Godalming, UK) after equilibrating the pellets at 20 °C and 20% relative humidity for at least 48 hours. The fracture force (F) of 50 pellets per batch at a loading rate of 0.1 mm/s was determined. To calculate the tensile strength (σ), the diameter (d) of each pellet in the crushing direction was considered (Equation 1).

$$\sigma = \frac{1.6 * F}{\pi * d^2} \quad (1)$$

Both the friability and tensile strength tests were performed on the 710-1,000 μm granulometric fraction; non-spherical pellets were avoided for the calculation of the tensile strength to minimise errors.

2.2.4.4 Pellets shape measurements

Photomicrographs of pellets from the selected 710-1,000 μm size fraction were taken using a digital camera (Canon, J), linked to a stereomicroscope (Panagor, D). Images of about 300 pellets of every batch at a suitable magnification (1 pixel=10 μm) were translated into binary images. The images were analyzed by image analysis software (ImageJ) and the aspect ratio was calculated.

2.2.4.5 Dissolution testing

Dissolutions were performed in a USP 29 paddle apparatus (Dissolution System 2100B, Distek, North Brunwick, NJ, USA) at the stirring rate of 100 rpm. Phosphate buffer (pH= 6.8 ± 0.05) (1,000 mL), thermostated at 37 ± 0.5 °C, was the dissolution medium. The sample amount used for analysis (1,000 mg) was adjusted to obtain sink conditions. Medium samples were automatically withdrawn at predetermined time points and spectrophotometrically analysed (Lambda 25, PerkinElmer, Monza, MI, I) at 230 nm. Each batch was analyzed in triplicates. The mean dissolution time (MDT) was calculated from the dissolution profile by Equation (2) (Costa *et al.,* 2001).

$$\text{MDT}= \frac{\sum_{j=1}^{n} \hat{t}_j \, \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad (2)$$

where j is the sample number, n is the number of dissolution sample times, $\hat{t}_j$ is the time at midpoint between t_j and t_{j-1} and ΔM_j is the additional amount of drug dissolved between t_j *and* t_{j-1}.

2.2.5 Experimental design and Process Optimization

A $3\times2^{4-1}$ mixed fractional factorial design was employed to investigate the effect of three formulation components (5-ASA:MCC ratio, PVP and water amount) and two process parameters (extrusion speed and spheronisation time) on the selected responses. In particular, the responses investigated were: pellets yield (710-1,000 μm), fine (< 250 μm) and coarse (> 1,400 μm) fractions, particle size (d_{geo}) and size distribution (σ_{geo}), particle shape (aspect ratio, AR), bulk density, mechanical resistance (friability and tensile strength) and dissolution properties (mean dissolution time, MDT). A total number of 27 experiments, including 3 replicates of the central point, to estimate the significance lack of fit tests, were performed. Statistical analysis was carried out using a software package (Statistica 7, StatSoft Inc., Tuls, OK).

The least-squares method was used to estimate coefficients of successive polynomials. Adjusted R^2 (R^2_{adj}) was calculated in order to evaluate the fitting of polynomials. F-statistic was used to identify statistically significant terms. Significant effects were evaluated by the analysis of variance at $p < 0.05$. Less significant regression coefficients were progressively eliminated to maximise the R^2_{adj} parameter. Moreover, an optimization step of both process and formulation parameters was performed by applying the desirability function approach (Derringer and Suich, 1980).

3. RESULTS AND DISCUSSION

3.1 Micronised 5-ASA preparation

The physical characteristics, such as particle size and shape, of the active ingredients might be critical parameters when aiming to process, by E-S, high drug loadings. In the particular case of 5-aminosalicylic acid (5-ASA), the particle morphology (needle-like) was identified as the key factor in affecting the quality of extrudates, when 5-ASA-based pastes were processed by ram extrusion. In fact, liquid phase migration (LPM), resulting in a non-uniform distribution of water within the extrudates and subsequent un-controlled agglomeration during spheronisation, was generally observed (Di Pretoro *et al.,* 2010).

In a previous work we described a viable way of producing, *via* a bench-scale planetary ball-mill, a batch of 5-ASA with the appropriate characteristics of particle size and shape for ram extrusion of high (*i.e.* up to 90 wt%) 5-ASA loadings. However, even though planetary ball-milling is a highly efficient technique for fine grinding processing it may have few drawbacks, including potential contamination from the grinding media (*e.g.* balls), un-wanted mechanical activation, wide particle size distribution and low reproducibility of the product achieved. In cases where dry grinding, or a very narrow particle size distribution for the product, is needed, jet mills are preferred (Tuunila and Nyström, 1998). The jet mill is a static machine which does not have any grinding media. The grinding effect is produced mostly by inter-particle collision. Jet-milling have therefore several advantages over the ball-milling technique, such as low product contamination, no heat build-up, narrow size distribution and, nearly-spherical uniform particle shape.

A systematic study was therefore performed in an industrial jet-mill apparatus (Chrispro® Jet-Mill, Micro-Macinazione S.A., Molinazzo di Monteggio, Switzerland), aiming to identify the operative conditions for the obtainment of a 5-ASA batch, scalable to large-size plants and reproducible, with similar physical characteristics to those obtained *via* planetary ball-milling. Table 1.2 lists the results inherent to particle size and shape measurements for ball- and jet- milled products. The batch size of 5-ASA processed by air-jet milling was about 50 Kg in comparison to the barely 30 g of material processed *via* the bench-scale apparatus.

Table 1.2: Particle size and shape data for ball- and jet-milled 5-ASA batches. Data inherent to ball milled 5-ASA are adapted from Di Pretoro *et al.,* 2010.

PARAMETER		Ball-milled batch	Jet-milled batch
PARTICLE SIZE (μm, volume basis)	d_{10}	0.115	0.278
	d_{50}	1.58	1.77
	d_{90}	3.97	4.11
ASPECT RATIO (mean ± S.D.)		0.80 ± 0.002	0.88± 0.004
SPECIFIC SURFACE AREA (m^2/g) (mean ± S.D.)		5.89 ± 0.043	5.76 ± 0.033

Both particle size and size distribution of ball- and jet-milled products are very similar, as evident from the d_{10}, d_{50} and d_{90} values and confirmed by the specific surface area measurements. In addition, jet milling process was demonstrated to be as efficient as the ball-milling in changing the morphology of 5-ASA crystals. As evident from 2-D micrographs (Figure 1.2) and quantified by particle shape parameter (aspect ratio), the un-milled 5-ASA crystals are in fact rounded off after both jet- and ball-milling process.

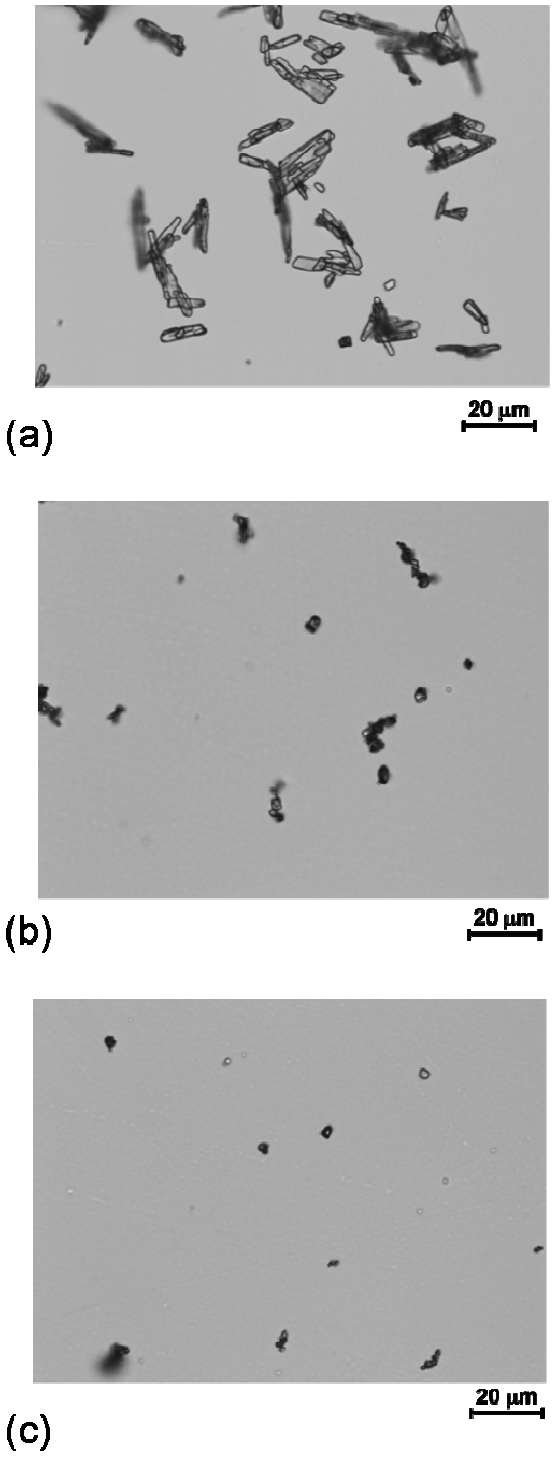

Figure 1.2: Micrographs of (a) un-, (b) ball- and (c) jet-milled batches of 5-ASA (magnification 50×).

Modification of the solid state on milling was also evaluated, by PXRD and DSC.

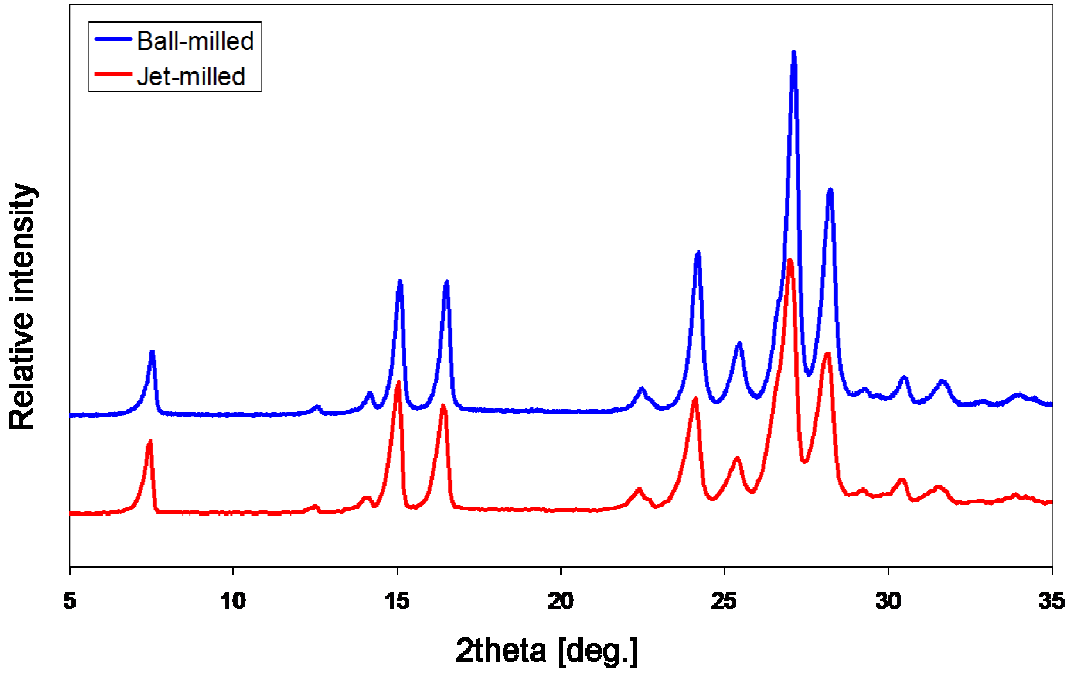

Figure 2.2: X-ray diffraction patterns for ball- and jet-milled batches of 5-ASA. Profile baselines are shifted for clarity.

Figure 2.2 shows similar PXRD patterns for the two materials. The peak angular positions are in excellent agreement, indicating that milling did not affect the crystalline form of 5-ASA. Similarly, thermograms of all samples showed a single endothermic melting event at approximately 280 °C with a comparable associated enthalpy (about 980 J g^{-1}).

An industrial batch (50 Kg) of 5-ASA, with similar characteristics in terms of particle size and shape to those obtained via a bench-scale planetary mill, was therefore achieved.

3.2 From bench-to-pilot scale extruder: preliminary settings

Extrusion scale up is often a source of inconsistency between lab-scale results and industrial products (Newton *et al.,* 1994). As mentioned, already, formulations which operate satisfactorily in one extruder type do not readily transfer to different

machines. Rheology of MCC-based pastes is strongly affected by the strain. Therefore, apparatus with different extrusion mechanisms and forces involved, may lead to products with different characteristics. For example, extruders based on a wiping action (*e.g.* screen extruders) provide low average shear rates followed by a rapid deformation, and hence the material is close to its yield stress during its processing. Radial extruders are therefore relatively insensitive to the solids particle size distribution in achieving good E-S products (Wilson and Rough, 2006). Conversely, in ram extruders low shear stresses are involved; this may lead to liquid phase migration phenomena, causing inconsistency in drug distribution within the dosage form as well as agglomeration of the pellets during E-S. Many approaches have been made to relate key deformation modes and rates in operation of lab- and pilot-scale extruders; Mascia (2009) demonstrated that paste properties vary significantly with strain rates and time scales of operation, which differ due to scaling between different extruders. By changing extruder type different E-S products, in terms of density, shape particle size and size distribution are therefore to be expected.

As yet it is not possible to predict the behaviour of paste formulations when processed in different apparatus nor it is possible to relate the rheological properties of pastes directly to the tendency to form good quality E-S products. Therefore, when aiming to scale up a formulation, which operate satisfactorily in a bench-scale extruder, to a pilot-scale apparatus it is strongly recommended to valuate again the influence of all the formulation and process parameters.

Basing on the above considerations, a set of extrusion trials was performed in a ram and a basket extruder apparatus, in order to compare the behaviour of the un-

milled and micronised (ball- and jet-milled) batches of 5-ASA alongside the standard and the colloidal grades of MCC. The quality of the extrusion products was determined by monitoring, when feasible, the attitude of the extrudates undergoing spheronisation (800 rpm, 5 min). In table 2.2, the outcome of the E-S trials, are summarised.

Table 2.2: Characteristics of E-S products obtained with ram and basket extruder apparatus, for the un-milled and micronised batches of 5-ASA (90 wt%) alongside three types of MCC.

5-ASA (90 wt%)	Avicel® grade	EXTRUDER TYPE	
		RAM	BASKET
Un-milled	PH-101	-	aggregates
	RC-591	dumbbells	dumbbells
	CL-611	dumbbells	dumbbells
Micronised	PH-101	rods	rods+dumbbels
	RC-591	spheres+dumbbels	spheres
	CL-611	spheres	spheres+dumbbels

Un-milled 5-ASA: as previously demonstrated, when a high loading (90 wt%) of un-milled 5-ASA was processed in ram extruder alongside Avicel PH101, acute liquid phase migration occurred, yielding an initial over-wet extrudate unsuitable for spheronisation and a dry immovable plug in the barrel that could not be extruded. This phenomenon is manly attributable to the high extrusion forces generated by the "pumping action" of ram extruder, but it is also arisen from the low water retention ability of Avicel PH101, as confirmed by previous studies (Podczeck *et al.,* 2007). In fact, by replacing the standard MCC grade with colloidal ones (*i.e.* Avicel RC591 and CL611), LPM was notably reduced, even though pellets obtained were far from being spherical (dumbbells shaped). Surprisingly, a similar outcome was achieved by processing the un-milled 5-ASA in the basket extruder, even though, contrary to

what observed in the ram apparatus, the un-milled 5-ASA/Avicel PH101 paste could be processed satisfactorily. This is most likely attributable to the ability of the wiping blades of basket apparatus to even out the pressure across the screen, which is in addition capable of flexing in response to the high stress involved.

Micronised 5-ASA: the micronised batch of 5-ASA produced by industrial jet-mill apparatus was firstly processed in the bench-scale ram extruder, to compare its performance with that of the ball-milled material. A substantial similarity between the two micronised products was observed. In fact, once again, by reducing the drug particle size and modifying the morphology of 5-ASA crystals, LPM was notably decreased or eliminated and a high 5-ASA loading (90 wt%) could be successfully extruded. Similarities between the ball and the jet-milled materials, other than for their chemo-physical properties but also for they behaviour undergoing ram extrusion, was therefore demonstrated.

The industrial jet-milled 5-ASA was afterwards tested in the pilot-scale basket extruder and its performance compared to that of the ram apparatus.

Both in ram and basket extruders, jet-milled 5-ASA/Avicel PH101 pastes yielded a very dense extrudate, that did not have sufficient plasticity either to break up or to be rounded off within the spheroniser. However, increasing the moisture content in the paste formulation, to enhance plasticity, led to over wet-surface extrudates and resulted in uncontrollable agglomeration. Conversely, by replacing Avicel PH101 with the colloidal ones, able to retain strongly water within their structure, plasticity was improved and good quality spheres where obtained. The best result, in ram extruder, was achieved by employing the MCC grade with the higher percentage in NaCMC, *i.e.* Avicel CL611. Conflicting results were obtained in the basket

apparatus. The best outcome (*i.e.* spherical pellets) was in fact achieved with Avicel RC591, whereas Avicel CL611 yielded both "spheres" and "rods".

The feasibility of processing high 5-ASA loadings in the basket extruder was assessed. Considered the complexity and the multifactoriality of the E-S process, an investigation of both formulation and process parameters, in the pilot-scale apparatus, was also performed. A factorial approach, namely a mixed fractional factorial design, was employed for this purpose.

3.3 Preliminary trials: setting the experimental space

Preliminary trials were performed to evaluate the most significant parameters and to establish their viable ranges for the experimental design (DOE). Since the colloidal Avicel RC591 grade of MCC yielded the most spherical pellets in basket extruder, it was selected as the E-S aid in the DOE.

The formulation variables selected were: (1) 5-ASA loading, quantified as 5-ASA:MCC ratio, (2) amount of water and (3) that of an extra binder (*i.e.* PVP). The process parameters were: extrusion speed and spheronisation time.

As the objective of the present work was to assess the feasibility of preparing, in a pilot-scale apparatus, pellets containing the highest loading of 5-ASA possible, the low (-1) and high (+1) levels for the 5-ASA:MCC ratio investigated, were set in the DOE at 90:10 and 95:5, respectively. These values corresponded, in formulation where no extra binder was added, to a 5-ASA loading of 90 and 95 wt%, respectively. When the extra binder was added in paste formulation, these values corresponded to an actual 5-ASA loading of 88.2 and 93.1 wt%, respectively.

Water is known to be a critical variable in the E-S process. In fact, it acts as a binder during wet massing, a lubricant during extrusion and a plasticiser during spheronisation (Hileman *et al.,* 1993). A careful choice of level values for this parameter is therefore necessary to obtain a wet mass with the appropriate degree of plasticity for extrusion and further spheronisation. An excess or, conversely, a lack of water added during paste formulation may result in severe over-wetted or dry masses, with consequent problems for extrusion and/or spheronisation.

Expectedly, the effective water amount was found to be strictly depended on the 5-ASA:MCC ratio. The optimal amount of water necessary to achieve a wet mass with a sufficient degree of plasticity, for each 5-ASA:MCC ratio, was therefore identified and set as the medium (0) level in the DOE. These values were then varied by ± 5 % to obtain the low (-1) and high (+1) levels for this variable.

The spheronisation time was also found to be dependent on the 5-ASA:MCC ratio. In fact, with the amount of MCC in the formulation decreasing, the extrudate strength decreased and shorter spheronisation times were required. Preliminary trials were performed to detect the useful "spheronisation time" range for each 5-ASA:MCC ratio. The behaviour of extrudates undergoing spheronisation was monitored by visual inspection and the minimal and maximal spheronisation time determined accordingly to the behaviour of such materials.

Since the amount of 5-ASA was increased up to 95 wt%, the influence of an extra binder on pellets characteristic was also considered. The performance of a low-viscosity grade of hydroxypropyl methylcellulose (HPMC, Methocel® E5) and that of a polyvinyl pyrrolidone (PVP K30) were preliminarily compared. PVP was chosen over the other since it provided the best binding properties combined with

minimal sticking of extrudates during spheronisation. To avoid an excessive decrease of 5-ASA loading in the final paste formulation, PVP amounts ranging from 0 to 5 wt% (dry mass), were considered. An amount of PVP equal to 2 wt% provided an acceptable balance between binding properties and minimal sticking; this amount was therefore set as the high level (+1) for the PVP amount in the experimental design, whereas no PVP was used when the low level (-1) was scheduled in the DOE.

The NICA® radial screen extruder used in our study has two independently variable drives: the feeder drive which conveys the material into the extrusion zone and the extruder drive which forces the material through the screen. By varying the extrusion speed may result in different extrusion pressures and thereby in different pellet densification. At converse, the involvement of the feeder drive in the extrusion process is minimal. This was therefore set as a constant in the DOE (90 rpm, which corresponds to a mean value between the minimal and the maximal speed of the apparatus). The influence of the extrusion drive was, instead, investigated in the DOE. The low and high levels for the extrusion speed variable were set at 34 and 140 rpm, respectively. These values corresponded to the lower and upper thresholds of the basket extruder. Along with the feeder speed, the spheroniser plate speed was also kept as a constant (800 rpm) in the DOE, in order to better investigate the influence of the other process parameters. Variables with their coded and actual values are summarised in Table 3.2.

Table 3.2: Coded and actual values for the factors used in the MFFD. Water and PVP amount depended on the 5-ASA:MCC ratio. Single (*) and double (**) asterisked values are relevant to those factors that depended on the 90:10 and 95:5 5-ASA:MCC ratio, respectively.

FACTOR	LEVEL		
	-1	**0**	**+1**
5-ASA:MCC ratio	90:10	-	95:5
Water amount (w/w%, dry mass)	36.1*	38.0*	39.9*
	33.3**	35.0**	36.8**
PVP amount (w/w%, dry mass)	0	-	2
Extrusion speed (rpm)	34	-	140
Spheronisation time (min)	5*	-	10*
	2**	-	5**

3.4 Statistical optimisation study

A $3\times2^{4-1}$ mixed fractional factorial design (MFFD) was employed to investigate the effects of the selected formulation (5-ASA:MCC ratio, water and PVP amounts) and operative (extrusion speed and spheronisation time) parameters on the process outcomes (yield and physico-technological properties of pellets). The MFFD responses defined were: particle size and size distribution (d_{geo} and σ_{geo}), particle shape (aspect ratio), process efficiency ($yield_{710\text{-}1,000\mu m}$, $fine_{(<250\mu m)}$ and $coarse_{(>1,400\mu m)}$ fractions), mechanical resistance (friability and tensile strength), bulk density and dissolution properties (mean dissolution time). Table 4.2 lists the experimental results relevant to the 27 trials performed.

All but one batch (trial 5) were successfully manufactured. The trial 5 failed to be processed as the amount of water added accordingly to the DOE was not sufficient to provide a wet powder mass with the appropriate consistency for extrusion. Brittle extrudates, yielding a powdery material when subjected to spheronisation, were therefore obtained.

Process yield of the desired size fraction (710-1,000 μm) was generally satisfactory (> 70%). Only in two cases (trials 22 and 23) uncontrolled agglomeration of extrudates occurred and an excess of coarse (> 1,400 μm) product was obtained.

Based on data reported in Table 4.2, the effect of factors (A, B, C, D and E) on the selected responses were calculated and statistically evaluated by the analysis of variance at $p < 0.05$. In Table 5.2 the regression and correlation coefficients as well as the ANOVA results for the MFFD are listed. The correlation between the selected factors and relevant responses was generally very good thus indicating the consistency of the approach (Table 5.2). The best fitting of data ($R^2_{adj} > 0.9$ and number of significant regression coefficients) was obtained for experimental data relevant to d_{geo} and $\sigma_{geo.}$

Table 4.2: $3\times2^{4-1}$ MFFD: factors and response values.

	Factor					Response									
	A	B	C	D	E										
Trial	5-ASA:MCC ratio	Water (wt%, dry mass)	PVP (wt%, dry mass)	Extrusion speed (rpm)	Spheronisation time (min)	d_{geo} (μm)	σ_{geo}	Aspect ratio	Density (g/mL)	Friability (%)	Tensile strenght (N)	MDT (min)	Yield (%)	Fine fraction (%)	Coarse fraction (%)
1	90:10	36.10	2	34	5	958.7	1.3	1.5	1.0	2.9	12.2	22.0	76.7	0.1	9.4
2	90:10	36.10	0	140	5	710.8	1.5	1.4	1.0	3.0	16.7	38.0	65.3	1.3	0.2
3	90:10	36.10	0	34	10	711.6	1.7	1.4	1.0	5.0	28.2	54.9	71.0	4.8	0.1
4	90:10	36.10	2	140	10	859.6	1.3	1.3	0.8	7.0	10.8	23.0	84.2	0.5	0.9
5	95:5	33.25	0	34	2	-	-	-	-	-	-	-	0.0	100.0	0.0
6	95:5	33.25	2	140	2	911.3	1.3	1.4	0.9	2.0	13.4	17.7	78.6	0.0	4.4
7	95:5	33.25	2	34	5	731.7	1.6	1.3	0.9	3.0	19.5	19.4	69.1	4.1	0.6
8	95:5	33.25	0	140	5	536.4	1.9	1.2	0.9	8.8	15.0	16.8	46.1	11.6	0.3
9	90:10	38.00	2	34	5	963.2	1.2	1.4	0.8	1.0	15.1	20.6	83.9	0.1	4.6
10	90:10	38.00	0	140	5	830.4	1.3	1.3	0.9	2.0	27.6	46.4	81.9	0.3	0.6
11	90:10	38.00	0	34	10	767.1	1.5	1.4	0.9	1.0	24.8	49.6	73.2	2.2	0.2
12	90:10	38.00	2	140	10	854.2	1.3	1.4	0.9	0.0	16.3	23.8	85.1	0.4	0.9
13	95:5	35.00	0	34	2	861.0	1.5	1.3	0.8	1.9	19.7	20.1	67.3	2.0	6.9
14	95:5	35.00	2	140	2	1257.4	1.2	1.4	0.8	1.0	14.5	15.3	27.6	0.0	61.5
15	95:5	35.00	2	34	5	1135.3	1.3	1.4	0.9	1.0	14.9	33.5	42.0	0.1	41.9
16	95:5	35.00	0	140	5	803.3	1.5	1.3	0.9	3.0	9.6	35.6	71.9	1.8	0.9
17	90:10	39.90	2	34	5	1107.6	1.2	1.6	0.9	2.0	23.8	23.9	54.7	0.0	29.1
18	90:10	39.90	0	140	5	911.3	1.2	1.4	0.9	1.0	19.7	43.0	85.0	0.0	1.9
19	90:10	39.90	0	34	10	902.1	1.3	1.5	0.9	1.0	20.1	48.9	84.9	0.3	1.4
20	90:10	39.90	2	140	10	1005.3	1.2	1.3	0.9	2.1	15.8	28.5	72.6	0.0	11.5
21	95:5	36.75	0	34	2	1019.3	1.3	1.3	1.0	1.0	8.4	38.8	62.0	0.0	22.5
22	95:5	36.75	2	140	2	1390.3	1.1	1.4	0.8	0.0	6.5	14.0	1.6	0.0	97.3
23	95:5	36.75	2	34	5	1366.6	1.1	1.4	0.8	0.0	7.2	24.0	5.5	0.0	89.4
24	95:5	36.75	0	140	5	963.5	1.3	1.3	0.8	2.9	3.2	42.2	78.3	0.1	6.0
25	92.5:7.5	36.50	1	87	5.5	1097.4	1.2	1.4	0.8	0.9	17.8	32.1	56.2	0.0	26.4
26	92.5:7.5	36.50	1	87	5.5	1113.5	1.2	1.3	0.8	1.0	17.8	33.5	52.4	0.0	30.3
27	92.5:7.5	36.50	1	87	5.5	1110.4	1.2	1.3	0.8	0.9	17.8	33.0	54.2	0.0	40.1

Table 5.2: Regression and correlation coefficients for the $3\times2^{4-1}$ MFFD. Asterisks indicate significant *p* values (< 0.05).

Polynomial term	Regression coefficient (*p* values, given in brackets)									
	d_{geo}	**σ_{geo}**	**Aspect ratio**	**Density**	**Friability**	**Tensile strenght**	**MDT**	**Yield**	**Fine fraction**	**Coarse fraction**
b_0	921.59* *(0,00000)*	1.3578* *(0,00000)*	1.3504* *(0,00000)*	0.87275* *(0,00000)*	1.3613* *(0,00342)*	17.820* *(0,00000)*	29.918* *(0,00000)*	63.911* *(0,00000)*	0.86177 (0,07691)	14.688* *(0,00196)*
b_A	39.770* *(0,00360)*	0.03224* *(0,00354)*	-0.03482* *(0,02271)*	-0.01474 *(0,10456)*	0.04366 *(0,85210)*	-3.3344* *(0,00025)*	-5.2980* *(0,00054)*	-12.639* *(0,00007)*	0.69656* *(0,02330)*	10.607* *(0,00053)*
b_B	167.86* *(0,00000)*	-0.16554* *(0,00000)*	0.02049 *(0,24624)*	-0.03165* *(0,00926)*	-1.6324* *(0,00006)*	-1.8909* *(0,03782)*	3.3270* *(0,04124)*	-6.9845* *(0,02561)*	-1.7951* *(0,00017)*	16.221* *(0,00010)*
b_C	123.50* *(0,00000)*	-0.11658* *(0,00000)*	0.02573 *(0,07953)*	-0.02068* *(0,02842)*	-0.52950* *(0,03792)*	-1.7523* *(0,01927)*	-7.7820* *(0,00001)*	-7.1031* *(0,00699)*	-1.0877* *(0,00159)*	13.619* *(0,00007)*
b_D	-2.1034 *(0,85619)*	-0.03029* *(0,00524)*	-0.03139* *(0,03684)*	-0.02226* *(0,01969)*	0.36134 *(0,14011)*	-1.8348* *(0,01523)*	-1.2448 *(0,31260)*	0.94170 *(0,67849)*	-0.18648 *(0,49992)*	-0.13988 *(0,95040)*
b_E	-35.198* *(0,00801)*	0.05340* *(0,00008)*	-0.02877* *(0,05293)*	0.00032 *(0,97058)*	0.52384* *(0,03972)*	-0.47141 *(0,48136)*	3.4302* *(0,01195)*	1.4202 *(0,53362)*	0.63693* *(0,03502)*	-2.8316 *(0,22411)*
b_B^2	-	0.02003 *(0,29173)*	0.03518 *(0,23572)*	0.02765 *(0,14160)*	1.5111* *(0,00731)*	-2.8554 *(0,05576)*	-	-	0.98896 *(0,10126)*	1.4786 *(0,75185)*
b_{AB}	82.158* *(0,00005)*	-0.07083* *(0,00005)*	-	-	-	-3.3321* *(0,00144)*	2.5278 *(0,10976)*	-6.9745* *(0,02579)*	-1.0038* *(0,01111)*	12.059* *(0,00109)*
b_{CB}	-	0.06544* *(0,00009)*	-	-0.01061 *(0,33310)*	-	1.5459 *(0,08062)*	-2.2816 *(0,14553)*	-14,794* *(0,00014)*	1.2170* *(0,00342)*	10.279* *(0,00328)*
b_{DA}	17.774 *(0,14111)*	-	0.02164 *(0,13406)*	-	-	-	-	-	-	2.2601 *(0,32592)*
b_{DB}	-	0.01857 *(0,11746)*	-	-	-	-	-	-	-	-
b_{DC}	-	-	-	-	-	-	-	-	-	-
b_{DE}	-47.237* *(0,00099)*	0.04281* *(0,00047)*	-	-	0.71283* *(0,00806)*	-1.8469* *(0,01470)*	-3.7985* *(0,00646)*	6.7623* *(0,00939)*	0.43591 *(0,12989)*	-9.2851* *(0,00143)*
b_{EB}	-	-0.04070* *(0,00336)*	0.01763 *(0,31536)*	-	-0.58763 *(0,06022)*	-1.5166 *(0,08580)*	-	3.3519 *(0,24808)*	-0.7648* *(0,04136)*	-2.9214 *(0,31084)*
Correlation coefficient										
R^2_{adj}	**0.93323**	**0.95979**	**0.48055**	**0.48707**	**0.74115**	**0.78769**	**0.48707**	**0.81323**	**0.76772**	**0.87065**

3.4.1 Pellet size and shape (d_{geo}, σ_{geo} and aspect ratio)

Narrow size distribution and spherical shape are amongst the claimed advantages of pellets prepared by extrusion-spheronisation. As widely described in literature, size distribution, dimension and shape of pellets are strictly related to both process and formulation parameters (Erkoboni, 2003). This was herein demonstrated; in fact all the formulation and process parameters investigated in the MFFD were identified to affect significantly both the size (d_{geo}) and the size distribution (σ_{geo}) of pellets.

Moreover, the ANOVA results shown in Table 5 indicate that the model proposed does have a very good fit for the data relevant to both d_{geo} and σ_{geo} ($R^2_{adj} > 0.9$).

Surprisingly, increasing the 5-ASA:MCC ratio in paste formulations, both d_{geo} and σ_{geo} increased. MCC, in addition to other process and formulation parameters, plays in fact a key role in the water retention capacity of pastes, when subjected to pressure (*e.g.* extrusion). Once the MCC saturation threshold has been reached, the addition of extra water may lead to liquid phase migration (LPM), where the pressure on the liquid causes it to flow relative to the solids, leading to non-uniform extrudate water composition (Rough *et al.,* 2002). Wet-surface extrudates (highly plastic and hence prone to agglomeration) and dry ones (contrariwise, not plastic enough to promote size enlargement) might be achieved. A batch of pellets constituted by both "aggregates" and "fines" may therefore be obtained and high σ_{geo} values are hence to be expected. This is the reason why paste formulations containing the highest 5-ASA loading (5-ASA:MCC = 95:5) were found to be particularly sensitive to the amount of water added. In fact, satisfactory pellets could be obtained only at the low (-1) and intermediate (0) water levels, whereas at the high level (+1) LPM occurred. This was confirmed by monitoring the moisture content of extrudates at differing points during extrusion. With water and PVP amount in paste formulation increasing, the size of pellets increased, whereas the size distribution was narrowed down (σ_{geo} decreased) (Fig. 3.2). Water and PVP, if exceeding a certain extent, might in fact enhance the plasticity/tackiness of the whole extrudate batch, thus promoting excessive size-enlargement or uncontrolled agglomeration of pellets. The whole batch of pellets obtained will therefore be characterised by over-size and/or aggregates with a size > 1,400μm, whereas σ_{geo} will be consequently low.

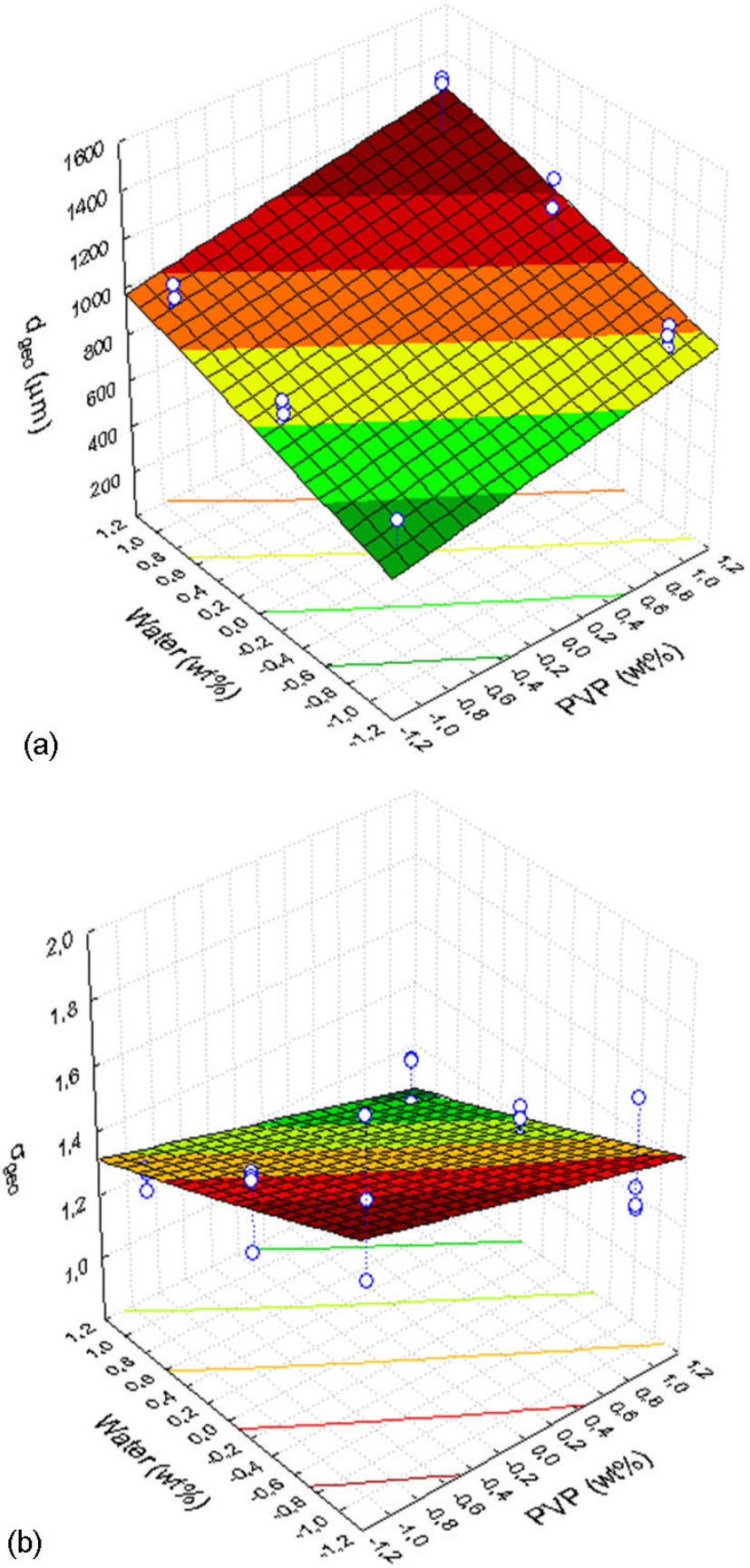

Figure 3.2: Effect of water and PVP amount on (a) d_{geo} and (b) $\sigma_{geo.}$

The spheronisation time has been recognised by several co-workers as an important factor related to pellet dimension (Thommes *et al.,* 2008). As expected, increasing the spheronisation time, the size of pellets decreased, whereas an increase in size distribution was observed. When undergoing spheronisation, the extrudates are in fact subjected to high mechanical stresses which may engender erosion phenomena and hence reduction of particle size and, conversely, increase of particle size distribution.

The shape along with the particle size is an important characteristic for pellets, in particular when considering to perform a coating step for the achievement of the desired drug release performance. The shape of pellets is commonly described by the aspect ratio (AR). Among the formulation parameters, 5-ASA:MCC ratio influenced statistically the AR of pellets (Figure 4.2). Surprisingly, the most spherical pellets were obtained with the highest 5-ASA:MCC ratio (95:5).

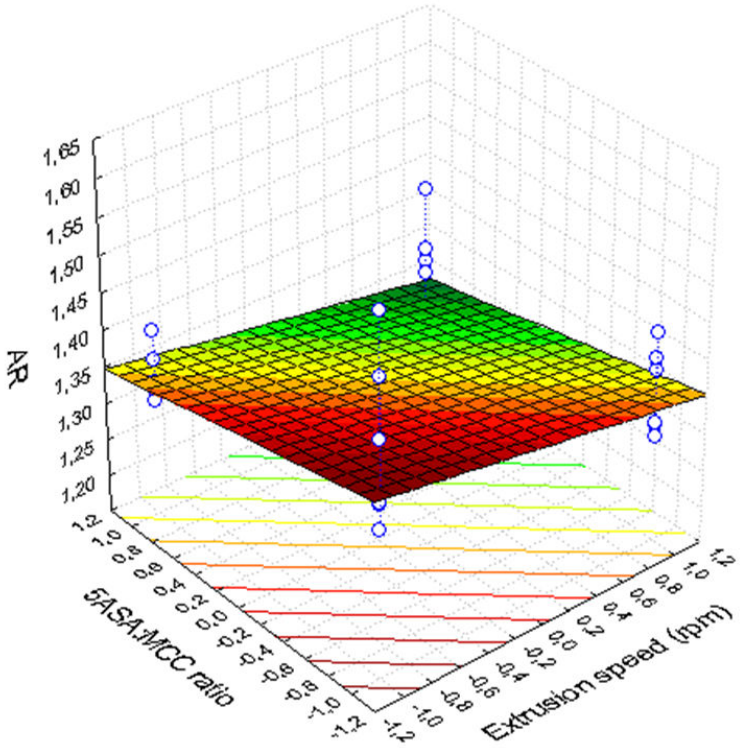

Figure 4.2: Effect of 5-ASA:MCC ratio and extrusion speed on AR.

Other than the 5-ASA loading, the process parameters also affected markedly the shape of pellets. AR, indeed, decreased with the extrusion speed and the spheronisation time increasing. A higher spheroniser speed transfers, in fact, more energy to the extrudate batch, thus allowing more frequent breaking in the first phase and plastic deformation (Thommes *et al.,* 2007). At higher extrusion velocities the extrudate surface presented in fact some superficial fractures (*e.g.* shark skin/cup-shaped). These breaks allowed extrudates to break up into shorter cylinders, more easily to deform when subjected to spheronisation. Conversely, at lower extrusion velocities the extrudate surface was smoother and longer cylinders were formed. These cup-shaped fractures were more evident when 95:5 5-ASA:MCC paste formulations were extruded, in particular at high extrusion velocities. That is thereby the most likely reason why 95:5 5-ASA:MCC ratio paste formulations yielded the most spherical pellets. Moreover, as already demonstrated by several workers, *e.g.* Rough *et al.* (2005), cup-shaped fractures might help the extrudate to be fragmented into reproducible segments thereby helping to achieve a more narrow size distribution, other than improving sphericity of pellets. This was again demonstrated; increasing the extrusion speed allowed in fact the pellet size distribution to be narrowed down ($< \sigma_{geo}$).

Apart from the 5-ASA:MCC ratio, neither the formulation parameters nor interaction terms influenced significantly the shape of pellets. Therefore, the model proposed did not fit the data inherent to AR very well ($R^2_{adj} < 0.5$). This suggests the hypothesis of a more complicated relationship between variables and responses, that could not be determined by the fractional factorial design applied.

3.4.2 Process efficiency: (yield$_{710\text{-}1,000\mu m}$, fine and coarse fractions)

Process yield is a key issue to be considered when aiming to industrial scale-up. In addition, the batch of pellets produced should be both reproducible and have a very narrow size distribution. This is particularly important when aiming at the development of a modified (*e.g.* controlled- or delayed-release) multiple unit dosage form. Small differences in the particle size might correspond, in fact, to huge differences in terms of specific surface area, and hence release performance. For this reason we selected, as the desired process yield, a narrow granulometric fraction, *i.e.* 710-1,000μm.

Process yied$_{710\text{-}1,000\mu m}$, fine$_{(<250\mu m)}$ and coarse$_{(>1,400\mu m)}$ fractions were significantly influenced by the formulation parameters. As already discussed in chapter 3.4.1, paste formulations containing a 5-ASA:MCC ratio equal to 95:5, were particularly sensitive to the amount of water added. Due to the low water retention capacity of pastes, LPM and hence non-uniform redistribution of liquid within the extrudates occurred, thus yielding both over-wet extrudates (and therefore over-size pellets) and dry ones (and hence a high percentage of fines). With the 5-ASA:MCC ratio increasing both the coarse and fine fraction therefore increased, whereas consequently the yied$_{710\text{-}1,000\mu m}$ decreased.

Moreover, with water and PVP increasing in paste formulation, a statistically significant increase of the coarse fraction, whereas a decrease of both fine fraction and process yield were observed (Figure 5.2).

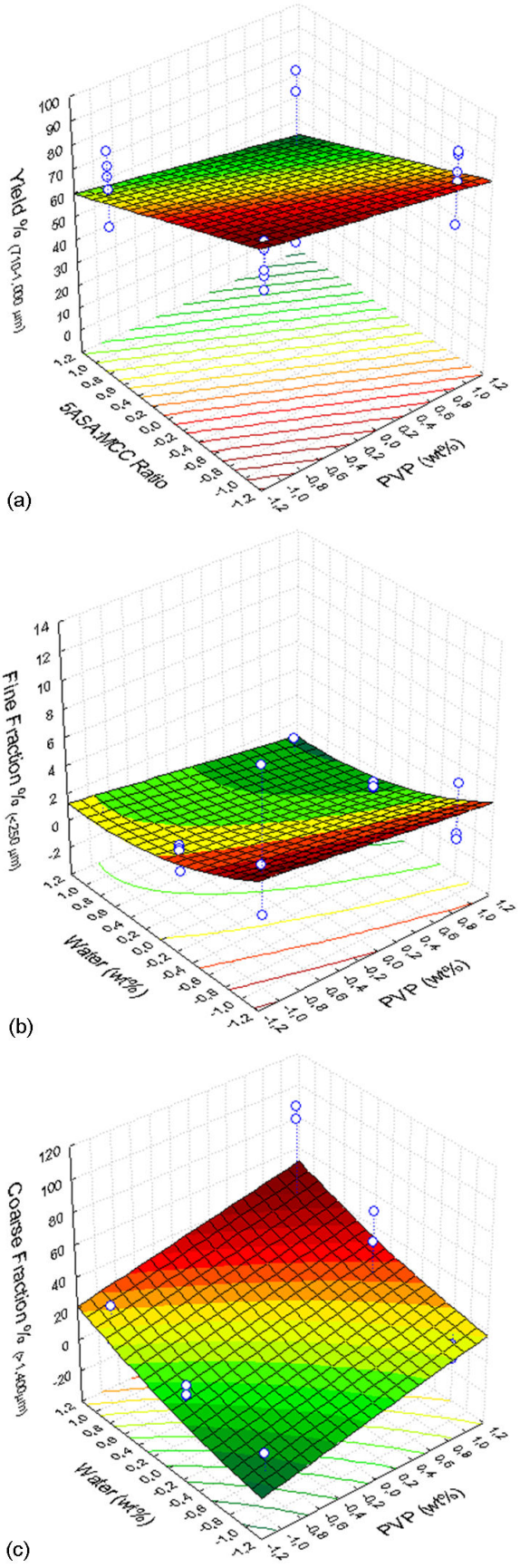

Figure 5.2: (a) Effect of 5-ASA:MCC ratio and PVP on process yield$_{710\text{-}1,000\mu m}$ and effect of water and PVP amounts on (b) fine and (c) coarse fractions.

As expected, the amount of fines was also influenced by the spheronisation time. When undergoing spheronisation, extrudates are in fact subjected to high mechanical stresses and subsequent possible erosion phenomena.

In order to optimise the yield of the desired size fraction, while minimising unwanted formation of fines and/or overgrowth pellets, it is necessary to consider carefully both the amount of water an PVP added in paste formulation and the residence time of extrudates within the spheroniser.

3.4.3 Mechanical properties: (friability and tensile strength)

A high mechanical resistance is an important property of pellets, in particular when considering of further process steps, like drying and coating.

Pellets friability was significantly influenced by the amount of water and PVP added in paste formulation. Expectedly, increasing these parameters the friability decreased. Both PVP and water promote, in fact, inter-particle binding and formation of a network of solid bridges (after solvent removal), thereby increasing the mechanical resistance of pellets. In point of fact, a deeper analysis of the quadratic term of water, suggests that the friability of pellets actually decreases when water is added within a certain experimental range, after which it starts increasing (Figure 6.2). Moreover, the impact of water on pellets friability is more pronounced at low levels for this parameter.

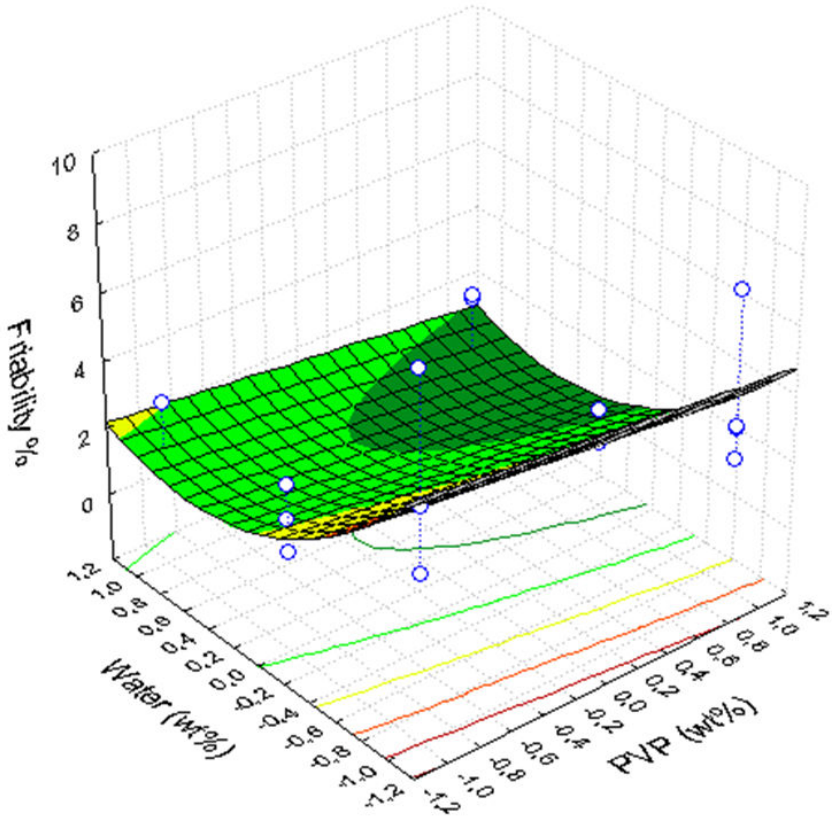

Figure 6.2: Effect of water and PVP amount on friability.

The spheronisation time also had a significant effect on pellets friability. Probably, the energy transferred to the extrudate batch increases, thus increasing the velocity of solvent removal from extrudates. The formation of liquid and solid (after drying) bridges is therefore less likely to happen thus decreasing the mechanical resistance of pellets.

Ts, unlike friability, was also influenced markedly by the 5-ASA:MCC ratio. With this parameter increasing, Ts decreased (Figure 7.2). MCC confers, in fact, strength to extrudate and to pellet matrix. Paste formulations containing the lowest amount of MCC yielded therefore spheres with lower resistance.

Surprisingly, Ts decreased with the PVP amount in paste formulation increasing. Water also have a negative influence on pellets Ts; in reality, the quadratic term of water indicates that Ts increases when water is added within a certain experimental range, whereas from this point onward it starts decreasing. Therefore, water seems to improve the mechanical resistance of pellets only into a certain extent, analogously to what observed in the granulation process.

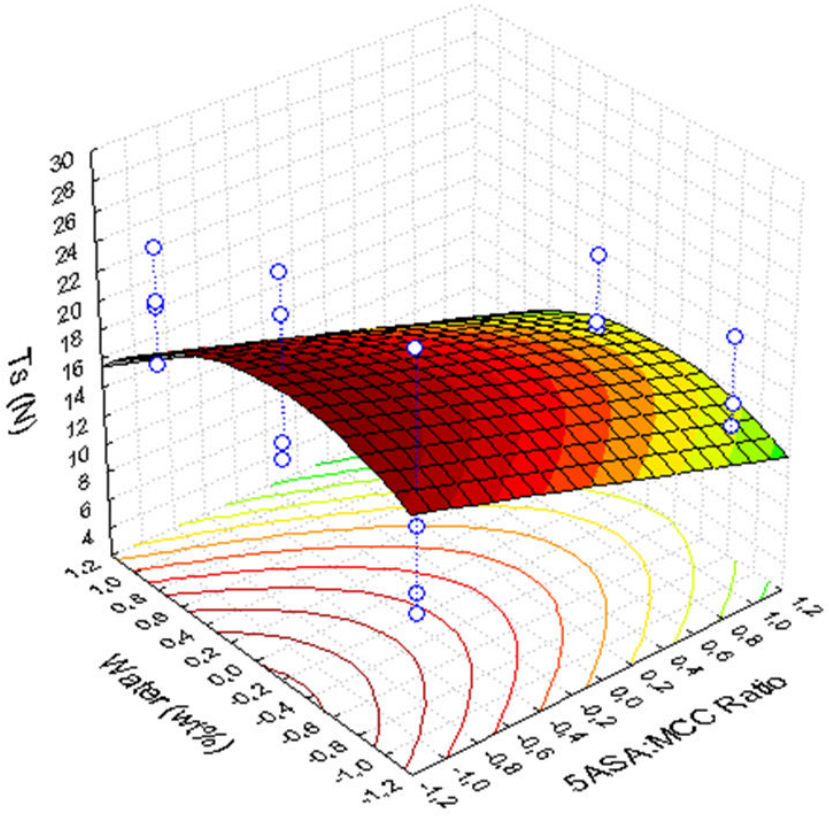

Figure 7.2: Effect of water and 5-ASA:MCC ratio on Ts.

The extrusion speed also has a statistically influence (negative) on the Ts of pellets. Lower extrusion velocities, by allowing a better packing of paste material, may in fact promote densification, thus increasing the mechanical strength of pellets.

3.4.4 Dissolution properties: (mean dissolution time)

With respect to the biopharmaceutical performance of pellets, the dissolution properties were evaluated by the mean dissolution time (MDT). Both formulation and process parameters, but the extrusion speed, had a significant influence on the drug dissolution rate.

Expectedly, MDT decreased with PVP in paste formulation increasing. PVP is in fact a water-soluble binder and thereby able to enhance the dissolution rate of drugs, in particular of sparingly soluble ones (Loftsson *et al.,* 1999). The 5-ASA:MCC ratio also affected remarkably the drug dissolution rate. With 5-ASA:MCC ratio increasing, the mechanical resistance decreases, while the contact surface area between the API and the solvent increases, thus likely increasing the dissolution rate (Figure 8.2).

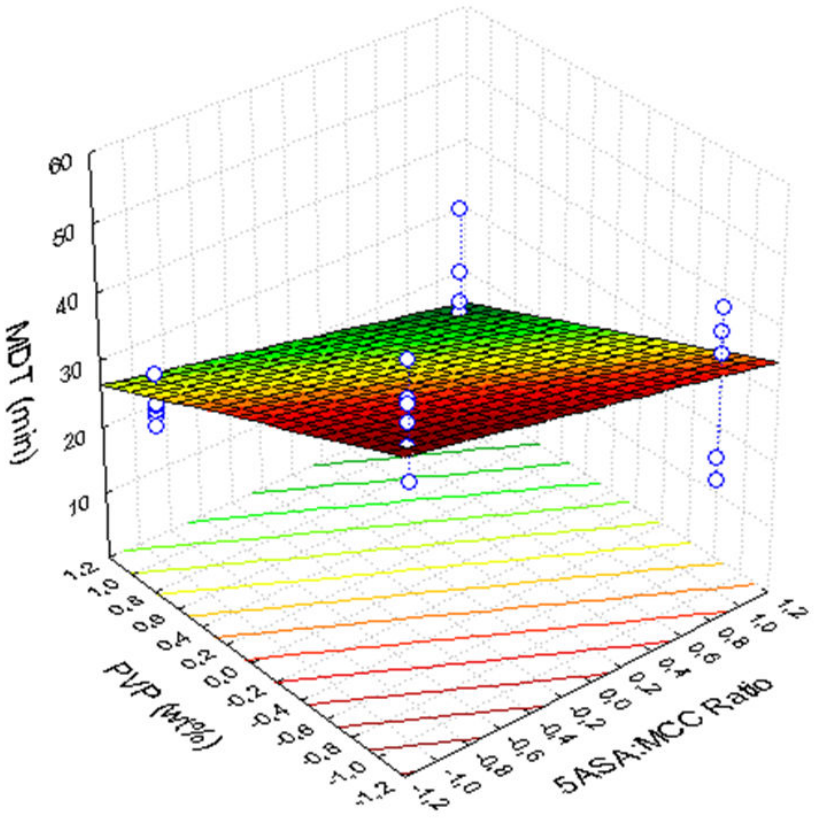

Figure 8.2: Effect of PVP and 5-ASA:MCC ratio on MDT.

Both the water amount and the spheronisation time had a significant, positive effect on the drug dissolution rate. This agrees with the reduced mechanical resistance of pellets with such parameters increasing. This is therefore likely related to the increased porosity of the pellets batch produced.

3.4.5 Pellets density

Other than the high drug loading, another important characteristic of pellets we sought in our study, in respect to the aim of producing a high strength final dosage form, was a high bulk density value.

The bulk density was found to be significantly affected by the amount of water and PVP, and by the extrusion speed parameters. As expected, with the extrusion speed increasing the bulk density decreased (Figure 9.2). In fact, lower extrusion velocities generally correspond to higher extrusion pressures, and hence greater densification of paste material.

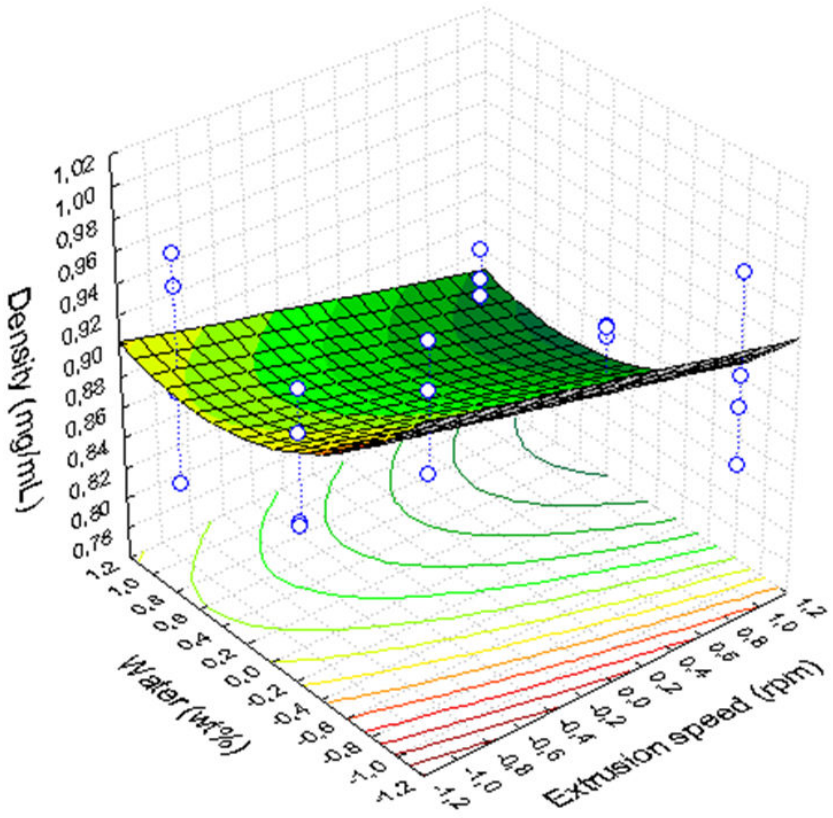

Figure 9.2: Effect of water and extrusion speed on bulk density.

Unexpectedly, with water and PVP amount in paste formulation increasing, pellets bulk density decreased. However, this may be attributed to a non uniform spherical shape of the pellets obtained when higher amount of either liquid or solid binders were used, since nearly spherical particles lead to higher bulk or tap density (L. Lachman, *et al.,*1986).

However, the effect of formulation and process parameters on the product bulk density should be confirmed as the predictability of the model proposed is not satisfactory ($R^2_{adj} < 0.5$).

3.5 Optimisation study

The goal of a design of experiments is to identify the combination of factors that would provide the optimal results to be obtained. With this aim, a desirability function approach was employed. Each response of the MFFD was therefore transformed into a dimensionless desirability scale (individual desirability, d_i). The individual desirability values were then combined using the geometric mean to give the overall desirability (D). For the calculation of D both Ts and MDT were excluded, since the results obtained for such parameters were already satisfactory in the whole experimental space.

The following optimal combination of factor levels was achieved: (1) 5-ASA:MCC ratio, 90:10; (2) water amount, 37.1 wt%; (3) PVP amount, 0 wt%; (4) extrusion speed, 5.1 rpm; (5) spheronisation time, 2 min.

As expected, the optimised formulation of pellets should contain the lowest amount of drug (5-ASA:MCC ratio = 90:10). However, since we demonstrated the possibility of producing satisfactory pellets also with the highest drug load, an additional optimisation study, was performed, setting the 5-ASA:MC ratio as a constant to 95:5. The new combination of factor levels achieved was: (1) water amount, 34.1 wt%; (2) PVP amount, 0 wt%; (3) extrusion speed, 45 rpm; (4) spheronisation time, 2 min. Three additional experiments, under these conditions, were performed to verify the predicted response and desirability values. Results are listed in Table 6.2.

Table 6.2: Predicted and experimental values for responses relevant to the trial performed under the optimal combination of factor values (water amount, 34.1 wt%; PVP amount, 0 wt%; extrusion speed, 34 rpm; spheronisation time, 2 min) with 5-ASA:MCC ratio = 95:5.

RESPONSE	VALUE		
	PREDICTED	EXPERIMENTAL	RELATIVE ERROR %
d_{geo} (μm)	826	861	**-4.25**
σ_{geo}	1.47	1.42	**3.40**
Aspect ratio	1.32	1.30	**1.52**
$Yield_{710\text{-}1,000\mu m}$ (%)	61.9	66.0	**-6.73**
Fine fraction $_{<250\mu m}$ (%)	2.95	2.30	**22.0**
Coarse fraction $_{>1,400\mu m}$ (%)	7.01	6.72	**4.14**
Friability (%)	2.08	1.61	**22.6**
Bulk density (g/mL)	0.900	0.901	**0.001**

*Relative error = [(predicted value-experimental value)/ predicted value]*100*

The values of the obtained responses and the calculated desirability of the new experiment were in good agreement with the predicted ones.

4. CONCLUSIONS

The feasibility of scaling-up a multiparticulate formulation, containing a high load of 5-ASA, from a bench- to a pilot scale apparatus was successfully demonstrated.

Firstly, the operative conditions for the production of an industrial batch of 5-ASA, with the appropriate characteristics of particle size and shape for extrusion of high API loadings, as previously identified in a bench-scale microniser, were found.

To provide a high level of process understanding and control capability, a mixed fractional factorial design (MFFD) was employed to investigate the effect of some process and formulation variables on desired pellets characteristics, such as size, size distribution, mechanical resistance, process yield and dissolution properties. Moreover, an optimisation study based on the desirability function approach was employed to identify the operative conditions that would allow the optimal response values to be identified.

The MFFD aided in investigating both process and formulation parameters that mostly affect the preparation of highly-loaded 5-ASA pellets in the pilot-scale apparatus. In addition, the statistical optimisation study allowed the operative conditions for the obtainment of pellets containing up to 95 wt% 5-ASA, and with good technological characteristics (e.g. mechanical resistance, size and shape), to be achieved

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CHAPTER 3

HIGH STRENGTH 5-ASA MULTIPLE-UNIT DRUG DELIVERY SYSTEM (DDS): *"PROPOSALS AND PROSPECTS"*

1. OBJECTIVE

The general aim of the research project has been the development of an oral drug delivery system (DDS) containing a high load (> 1,000 mg) of 5-aminosalicylic acid (5-ASA), in order to achieve a once/twice-a-day administration, thus improving compliance in inflammatory bowel disease (IBD).

Multi-particulates (pellets) has been chosen for the development of the DDS, in virtue of both their technological (*e.g.* high drug loading and density) and biopharmaceutical (*e.g.* flexibility in tailoring drug release and even and predictable distribution along the gastrointestinal tract) advantages.

As already and extensively discussed, 5-ASA is locally active and the location of the inflammation in IBD may vary and affect differing portions of the gastrointestinal (GI) tract, with predominance of the proximal intestine and ileocolonic region in the case of Crohn's disease (CD) and distal areas of the large bowel in that of ulcerative colitis (UC). In order to meet all the potential IBD needs, a formulation able to deliver 5-ASA throughout the entire intestine would be thereby desirable. Nevertheless, the possibility to target specific GI regions, thus treating selectively differing IBD conditions, is also an important challenge and it will be topic of a further investigation.

To obviate the high variability of pH along the GI tract, in particular in patients suffering of IBD, a drug release mechanism independent from pH should be seek. In addition, since 5-ASA is easily absorbed in the upper intestine (mainly in the

duodenum), drug release in the stomach should be avoided, thus maximising the therapeutic dose at the primary targets (lower in the small intestine and in the distal large bowel) while minimising risk of side effects.

In the previous stages of the research project we demonstrated the feasibility of an E-S route for the preparation of highly-loaded 5-ASA pellets in a bench-scale apparatus [chapter 1] as well as the possibility of transferring the E-S formulation to a pilot-scale equipment [chapter 2]. On the basis of the experimental observations we are now able to produce pellets with a high drug loading (*i.e.* 95 wt% 5-ASA) and good technological properties (*e.g.* bulk density and mechanical resistance).

The final stage of the research project was to evaluate the possibility of tailoring the 5-ASA release profile accordingly to the specific IBD therapeutic needs.

The first objective was the achievement of a prolonged, and pH-independent release of the API throughout the entire intestine. Therefore, 5-ASA pellets were coated with a polyvinyl acetate polymeric dispersion, to different theoretical weight gains. An additional enteric-coating polymeric layer was applied to avoid 5-ASA loss from pellets during the gastric residence time and allow drug release to start in the small intestine.

Preliminary attempts to develop a final dosage form were also made. In this respect, multiparticulates are highly flexible systems since they can be easily dispensed into various dosage forms. Pellets may in fact be compressed into tablets or alternatively filled into capsules or sachets.

Preliminary attempts to realise a final capsular dosage form were made. Capsules represent in fact a particular challenge when aiming to develop a high strength (> 1,000 mg) dosage form, since the limited volume available (max 1.38 mL, 000

size capsules). If a high strength formulation can be realised using capsules, it is therefore likely to be achieved with any of the other dosage forms above mentioned.

To evaluate the release performance of the DDS under development, two commercial reference products were selected: Lialda™, a single-unit high strength delayed-release preparation (1,200 mg 5-ASA, tablets) and Pentasa®, a multiparticulate prolonged-release formulation (500 mg 5-ASA, capsules).

2. MATERIALS AND METHODS

2.1 Materials

5-aminosalicylic acid (5-ASA, mesalamine), was purchased from Erregierre S.p.A., (Bergamo, Italy) and used after micronisation (IMS Micronizzazioni S.p.A., Milano, Italy). The colloidal Avicel® RC591 grade of microcrystalline cellulose (MCC), employed as extrusion-spheronisation aid for pellets preparation, was donated by IMCD S.p.A (San Donato Milanese, Italy). Kollicoat® grades SR 30D and MAE 30DP, used for pellets coating, were a gift from BASF (Mount Olive, NJ, USA). Propylene glycol, potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium chloride, sodium hydroxide and hydrochloric acid were all analytical grade and purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

Two commercially available mesalamine-based products, *i.e.* LIALDA™ (1,200 mg 5-ASA, tablets) (Shire Pharmaceuticals Inc., Wayne, PA, USA) and Pentasa® (500 mg 5-ASA, capsules) (Ferring Arzneimittel GmbH, Kiel), were purchased from local markets and used as reference preparations.

2.2 Methods

2.2.1 Pellets preparation

5-ASA (95 wt%) and Avicel® RC591 (5 wt%) were dry blended in a Turbula® Shaker-Mixer T2F (Glen mills Inc., Clifton, NJ) for 10 minutes at 48 rpm. The powder mixture was transferred in a planetary mixer (Kenwood KM 010, Southampton, UK), assembling a K-shaped mixing arm, and water slowly poured onto the bulk surface. The wet masses were extruded in a radial basket apparatus (Nica® model E140) equipped with a multi-holed screen (i.d holes= 1 mm; screen

thickness= 1.25 mm;) at the extrusion speed of 34 rpm. The resulting extrudates were spheronised in a 64 cm diameter radial-hatch friction plate spheroniser (Nica® S320) for 2 minutes at 800 rpm. Pellets were dried in a vacuum oven at 40 °C for 24 hours. All experiments were performed at room temperature in an air-conditioned laboratory (21°C ± 2°C, humidity 20% ± 2%). A detailed discussion inherent the choice of process and formulation parameters as well as the technological characterisation of pellets can be found in chapter 2.

2.2.2 Coating of 5-ASA pellets

5-ASA pellets of a size fraction 710-1,000 μm were selected for coating process. The pellet batch (300 g) was loaded into a fluidized bed apparatus, Glatt® GPCG-1 (Glatt Process Technology GmbH, Binzen, Germany), assembling a "bottom-spray" insert, and coating was performed accordingly to the different polymeric dispersions employed. The operative conditions are listed in Table 1.3.

- ***Kollicoat® SR 30D (KSR):*** the formulation of the polymeric dispersion used was: Kollicoat® SR 30 D, 50 wt%; 1,2 propylene glycol, 1.5 wt% (corresponding to 10 wt% on the dry polymer); water, 48.5 wt%. The plasticiser was firstly dissolved in water; KSR was then added and the resulting dispersion stirred for 30 min prior to film coating. 5-ASA pellets were coated with KSR up to theoretical weight gains of 2.5, 5, 7.5, and 10 %. The actual gains, determined by weighting pellets prior and after the coating process, were 1, 3, 5 and 7 w.g.%, respectively.

- Kollicoat® MAE30 DP (KMAE): to avoid gastric breakdown of 5-ASA, an outer layer of an enteric coating polymer was applied. The formulation of the KMAE polymeric dispersion used was: Kollicoat® MAE30 DP, 58.5 wt%; 1,2 propylene

glycol, 2.6 wt% (corresponding to 14.8 wt% on the dry polymer); water, 38.9 wt%. 5-ASA pellets, previously coated with KSR (5 w.g%) were further coated with KMAE up to theoretical weight gains of 5, 10 and 15 %. The actual gains determined were 3, 8 and 11 w.g.%, respectively.

Film-coating with KMAE was also performed on the un-coated 5-ASA pellets, up to theoretical weight gains of 5, 10, 15 and 20 %, corresponding to the actual values of 4, 8, 12 and 15, respectively.

Table 1.3: Process parameters for Kollicoat® SR 30D and MAE 30 DP.

PROCESS PARAMETER	Kollicoat® SR 30D	Kollicoat® MAE 30DP
Inlet temperature (°C)	70	60
Product temperature (°C)	40	50
Air flow (m^3/h)	100-120	80-100
Nozzle internal diameter (mm)	1.2	1.2
Spray pressure (bar)	1.8	1.5
Spray rate (g/min)	12	18
Curing	48h/ 40 °C	48h/ 40 °C

2.2.3 Drug release

The USP dissolution methods for mesalamine-based products are different depending on whether they are prolonged- (pH 7.5, 8 h) or a delayed-release (pH 1.4/2h; pH 6.0/1h; and pH 7.4/1.5 h) formulations. However, aiming to compare different products together, an alternative dissolution method proposed by Chuong *et al.,* (2008) has been adapted in the current study study. A 12-h dissolution testing, based on three different buffer stages to simulate pH conditions of gastric, small and large intestinal fluids, was carried out. The dissolution media and operative conditions are reported in Table 2.3.

Table 2.3: Dissolution media and testing times used in the test.

Buffer stage	Medium	pH value	Testing time (h)
I	HCl buffer	1.2	2
II	Phosphate buffer	6.0	2
III	Phosphate buffer	7.4	8

Dissolutions were performed in a USP 29 paddle apparatus (Dissolution System 2100B, Distek, North Brunwick, NJ, USA). The volume media was 1,000 mL (to keep sink conditions) at 37 ± 0.5 °C and a stirring rate of 100 rpm was employed. Medium samples were automatically withdrawn at predetermined time points and spectrophotometrically analysed (Lambda 25, PerkinElmer, Monza, MI, I) at λ= 303 for the acidic stage (Abs= 1.8384[API] + 0.0669; R^2=0.999) and λ= 330 nm for the buffer stages II and III (Abs= 0.0018[API] + 0.0648; R^2=0.999). Data reported are the average of 6 determinations.

The actual 5-ASA dose of un-coated and coated pellets was determined by dissolving an exactly weighted amount of pellets (~ 1,000 mg) in 1,000 mL pH 7.4 phosphate buffer. The solution obtained was then spectrophotometrically analysed at λ= 330 nm and the drug content thereby calculated by using the relevant calibration curve.

3. RESULTS AND DISCUSSION

5-ASA is a zwitterionic drug, with pKa values of 6.0, 3.0 and 13.9 corresponding to the NH^{3+}, COOH and OH groups, respectively (Allgayer *et al.,* 1985). The drug solubility is therefore pH-dependent. In Table 3.3 the solubility values of 5-ASA at pH 1.2, 6.0 and 7.4 are reported.

Table 3.3: Solubility values of 5-ASA, determined experimentally at pH 1.2, 6.0 and 7.4.

pH	Solubility (g/L)
1.2 (HCl buffer)	8.65
6.0 (phosphate buffer)	4.15
7.4 (phosphate buffer)	6.34

Being the API the major component by weight (95 wt%) of the multiparticulate formulation, the *in-vitro* dissolution performance of un-coated pellets is expected to be mainly influenced by the chemo-physical characteristics of the API. In Figure 1.3 the dissolution release performance of highly-loaded 5-ASA pellets at pH 1.2, 6.0 and 7.4 is shown.

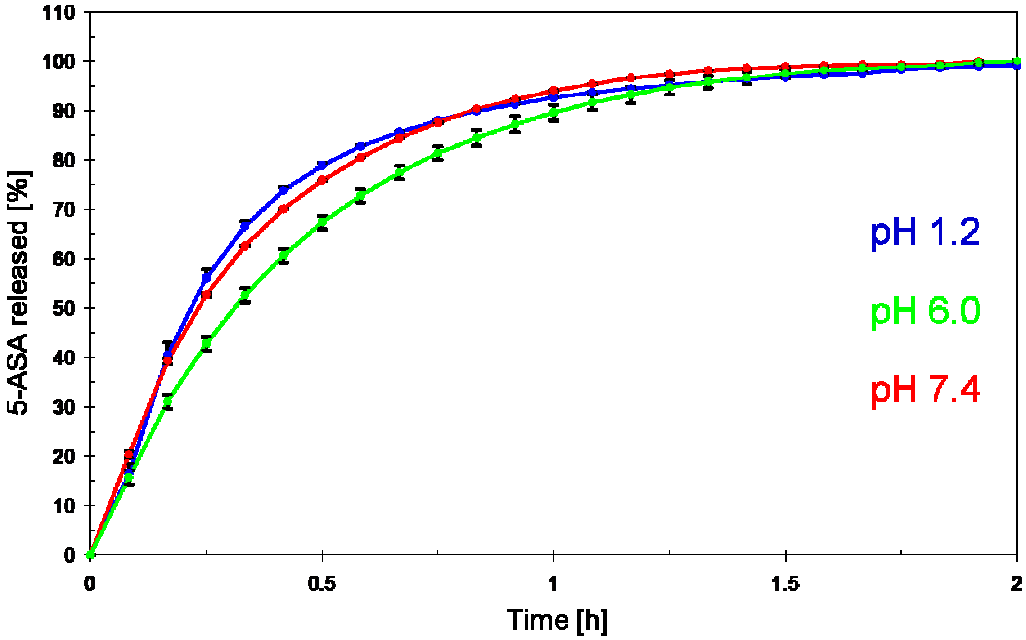

Figure 1.3: *In-vitro* mean (n=6) dissolution profiles of 5-ASA pellets (95 wt% 5-ASA, 5 wt% Avicel RC591) at pH 1.2, 6.0 and 7.4. Bars indicate standard deviation.

Expectedly, the chemo-physical characteristics of the API influenced, in part, the drug dissolution performance of highly-loaded 5-ASA pellets. About 80 % of 5-ASA is in fact dissolved within about 30 min at pH 1.2 and 7.4, whereas it takes about 45 min at pH 6.0.

However, the overall dissolution performance seems to be mostly affected by the technological characteristics of pellets. The dissolution profile, at each pH condition, is in fact slightly slackened. This is most likely arisen from the high density, in terms of high particles packing, of pellets prepared by extrusion-spheronisation (E-S), in particular if compared to granules and/or to pellets obtained by different techniques (*e.g.* drug layering). In addition, the presence of insoluble fillers such as the microcrystalline cellulose (MCC), used as the E-S aid, might contribute to a "matrix-like" release behaviour pellets.

3.1 Reference 5-ASA products: *a comparative study*

The aim of our work was to tailor the drug release accordingly to specific IBD therapeutic needs. Two commercial mesalamine-based products, namely a high strength single-unit (Lialda™) and a multiple-unit dosage forms (Pentasa®) were selected as reference formulations to our DDS.

MMX mesalamine (1,200 mg 5-ASA, tablets), marketed as Lialda™ in the US and Mezavant ™ in the EU (Shire Pharmaceuticals Inc., Wayne, PA, under license from Giuliani SpA, Milan, Italy), became after its introduction in the market in 2007 one of the most frequently prescribed products for the treatment of ulcerative colitis (UC). Another well-known mesalamine-based product is Pentasa®, a multiparticulate-based formulation available in capsule and tablet dosage forms (250 and 500 mg 5-ASA) and, most recently, in sachets containing up to 1,000 mg API. Pentasa®, in contrast to Lialda™ and to other delayed-release systems, allows a prolonged-release of 5-ASA throughout the entire intestine to occur. For this reason Pentasa® is, to date, the only formulation used "off-label" to treat Crohn's disease (CD) in addition to its indicated use in UC (Cohen R.D., 2006).

The *in-vitro* drug release performance of Lialda™ and Pentasa® was tested in a III stages pH conditions (1.2, 6.0 and 7.4) to simulate gastric, small and large intestinal fluids (Figure 2.3).

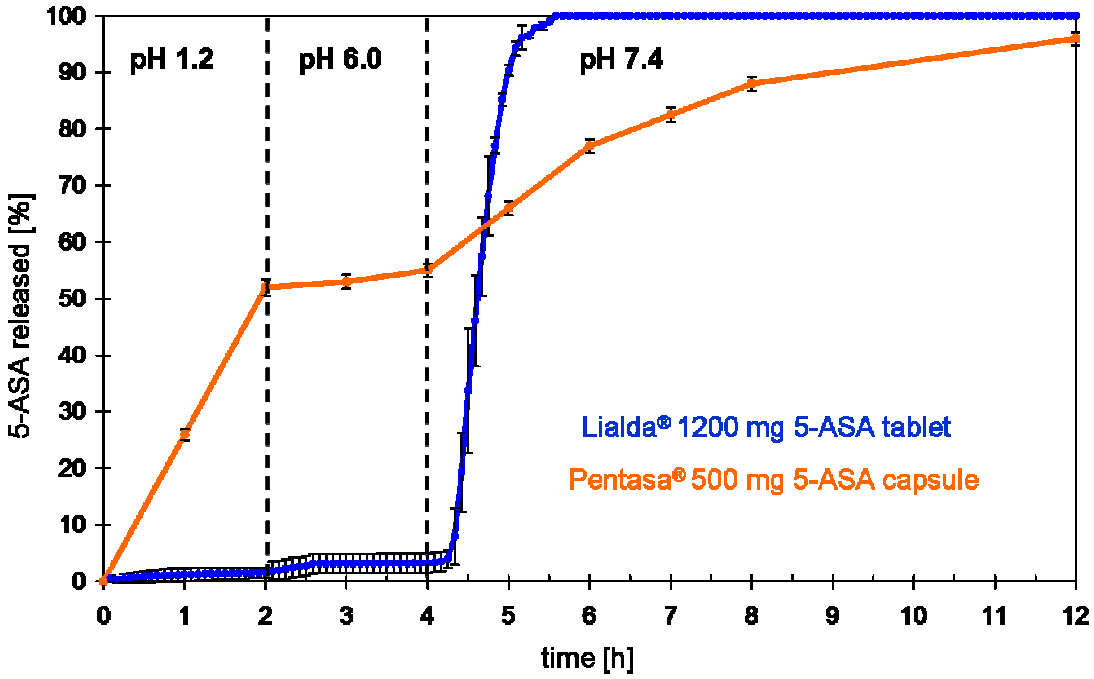

Figure 2.3: *In-vitro* mean (n=6) dissolution profiles of Lialda™ (1,200 mg 5-ASA, tablets) and Pentasa® (500 mg 5-ASA, capsules). Dissolution test performed at pH 1.2 (2h), 6.0 (2h) and 7.4 (8h). Bars indicate standard deviation.

As expected, no significant release of 5-ASA from Lialda™ tablets occurs under simulated gastric and small intestinal fluids condition, whereas almost the entire drug dose is released when the tablets approach a pH value > 7. Moreover a considerable variability of the time necessary for the polymeric film to dissolve and hence the tablet to start disintegrating, was observed (Figure 2.3). MMX technology consists in fact of a high strength mesalamine tablet coated with an acrylic-based resin (*i.e.* Eudragit® S), soluble at pH values exceeding 7. Such a pH-dependent mechanism may represent a limitation if considering the high variability of the above mentioned physiological parameter, in particular in subjects suffering of IBD. Moreover, the high drug dose is embedded in a single unit dosage form; this may represent a further limitation both from a safety (*e.g.* risk of dose dumping) and biopharmaceutical (*e.g.* distribution along the GI tract and drug release performance) point of view.

Based on the dissolution profiles obtained from Lialda™ tablets, the product appears to be more suitable for the treatment of distal IBD conditions, whereas it would not be expected to have any potential in the treatment of Crohn's diseases and/or in pathological manifestations where the inflammation spreads over the small intestine (*e.g.* proximal colitis or pancolitis). Lialda™, like most of the others delayed-release preparations, is indeed recommended for the treatment of distal IBD conditions, such as proctitis and distal UC.

On the other hand, Pentasa® is a multi-particulate DDS based on 5-ASA granules enclosed within an ethylcellulose film, that should allow a sustained, pH-independent release of the API to occur. In sooth, the release rate of 5-ASA from Pentasa® granules at pH 1.2 is about six times higher than that of simulated small and large intestinal fluids (pH 6.0 and 7.4, respectively). This unexpected behavior was confirmed by other co-authors (*e.g.* Schellekens *et al.,* 2007; Chuong *et al.,* 2008) and was also observed for Pentasa® tablet and sachet dosage forms (Rudolph *et al.,* 2001; Schellekens *et al.,* 2007).

A substantial amount (> 50%) of 5-ASA was released from Pentasa® granules within the 2-h stay at pH 1.2. Such a considerable extent of drug released in the stomach would result in a relatively high loss of mesalamine, due to systemic absorption occurring in the duodenum, and subsequently to less drug reaching inflamed tissues lower in the small intestine and in the colon (Klotz *et al.,* 1985; Vree *et al.,* 2001). Another limitation of Pentasa® is the relatively low drug loading (500 mg/unit), such to entail multiple daily administrations to meet the recommended drug dosage regimen (4.8 g/day 5-ASA).

However, Pentasa® is, to date, the only formulation that allows 5-ASA to be available within most of the GI tract, and that provides better release in the small intestine than other mesalamine-based preparations. For this reason Pentasa® is, as already discussed, the product of choice for the treatment of CD involving the small intestine.

3.2 Tailoring the drug release

Multiple unit systems offer the potential to easily modulate the drug release performance. Aiming to develop a final dosage form that would allow a slow, and pH-independent, release of the API throughout the entire intestine, 5-ASA pellets were coated with a polyvinyl acetate aqueous dispersion, *i.e.* Kollicoat® SR (KSR) up to theoretical weight gains of 2.5, 5, 7.5 and 10 %. The *in-vitro* dissolution profiles, performed at III buffer stages are shown in Figure 3.3.

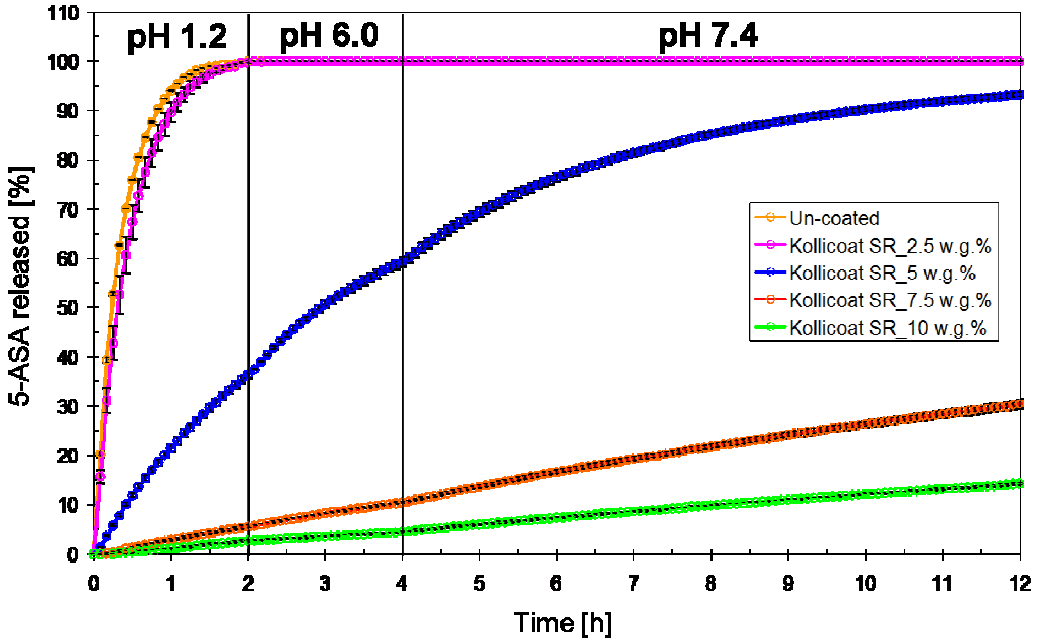

Figure 3.3: *In-vitro* mean (n=6) dissolution profiles of un-coated 5-ASA pellets (95 wt% 5-ASA, 5 wt% Avicel® RC591) and release profiles of pellets coated with Kollicoat® SR up to theoretical weight gains of 2.5, 5, 7.5 and 10 %. Test performed at pH 1.2 (2h), 6.0 (2h) and 7.4 (8h). Bars indicate standard deviation.

The entire amount of 5-ASA from un-coated pellets is dissolved within the 2-h at pH 1.2, as already shown in Figure 1.3.

The release profile of pellets coated with KSR to a theoretical weight gain of 2.5 % is similar to that of the un-coated units, thus indicating the inability of the polyvinyl acetate dispersion to form a continuous film when applied at such small amount. Increasing by little the KSR to a theoretical weight gain of 5 %, a prolonged- and relatively pH-independent, release over the 12-h dissolution testing was achieved.

Surprisingly, the release rate profile reached with such a small amount of KSR was already satisfactory to meet our needs, although the considerable amount (*i.e.* 36 %) of 5-ASA dissolved already at pH 1.2.

As expected, by further increasing the amount of KSR up to theoretical weight gains of 7.5 and 10 %, the drug release rate was considerably decreased. The extent of the drug release rate reduction was excessive, relatively to our objectives. At the end of the 12-h dissolution testing, in fact, only about 30 % 5-ASA was released from pellets coated with KSR to a weight gain of 7.5 %, whereas less than 15 % 5-ASA from those coated with KSR up to 10 w.g.%.

On the basis of the previous observations, pellets coated with KSR to a theoretical 5 w.g.%, were selected for further studies. In particular, to minimise unwanted systemic absorption while maximising the therapeutic efficacy, an outer layer of a methacrylic acid/ethyl acrylate copolymer (*i.e.* Kollicoat® MAE 30 DP) up to theoretical weight gains of 5, 10 and 15 %, was applied to 5-ASA/KSR (5 w.g.%) coated pellets. The *in-vitro* mean dissolution profiles of 5-ASA/KSR (5 w.g.%) pellets coated with differing amount of KMAE are shown in Figure 4.3.

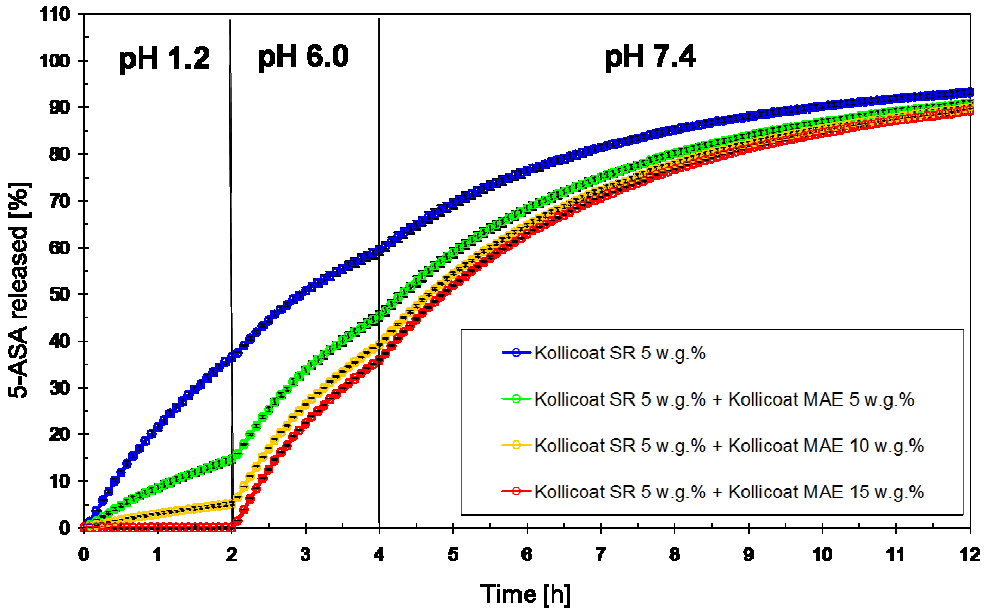

Figure 4.3: *In-vitro* mean (n=6) release profiles of 5-ASA pellets coated with Kollicoat® SR (theoretical weight gain 5%) and Kollicoat® MAE 30 DP up to theoretical weight gains of 5, 10 and 15 %. Test performed at pH 1.2 (2h), 6.0 (2h) and 7.4 (8h). Bars indicate standard deviation.

Kollicoat® MAE allowed a quite efficient *in-vitro* enteric protection to be achieved. In fact, an already small amount of KMAE (5 w.g.%) was able to reduce the amount of 5-ASA dissolved at pH 1.2 of about 22 %. By doubling the amount of KMAE (10 w.g.%) the drug amount dissolved was decreased of a further 9.5 %, whereas a complete enteric protection was reached by applying an amount of KMAE equal to 15 w.g.%.

Therefore, by combining a polyvinyl acetate (KSR) and an acrylate (KMAE) polymeric layers, to a theoretical weight gain of 5 and 15 %, respectively, the release profile of 5-ASA pellets was tailored accordingly to our objective.

This formulation is in fact able, *in-vitro*, to avoid completely 5-ASA release at pH 1.2. About 35 % of 5-ASA is released within the 2-h stay at pH 6.0, whereas the remaining dose of 5-ASA is then released at pH 7.4.

3.3 Final dosage form: *proposals and prospects*

Multiparticulates are highly flexible systems, other than for the possibility they offer to easily modulate the drug release, also because they can be readily dispensed into various dosage forms. Pellets may in fact be compressed into tablets or alternatively filled into capsules or sachets.

Regardless from the dosage form, an important characteristic of pellets to be considered when aiming to develop a high strength formulation is, along with the drug content, their bulk density value.

In table 4.3, the bulk density values and theoretical 5-ASA contents of the highly-loaded pellets, prior and after the film coating, are reported.

Table 4.3: Bulk density values and theoretical 5-ASA contents of un-coated, KSR (5 w.g.%) and KSR/KMAE (5/15 w.g.%) coated pellets.

Pellets	5-ASA theoretical content (%)	Bulk density (g/mL)
Un-coated	95.0	0.86 ± 0.002
KSR 5 w.g. %	90.4	0.90 ± 0.002
KSR 5 w.g. % + KMAE 15 w.g. %	78.7	0.91 ± 0.004

One of the main advantages of the extrusion-spheronisation technique is the possibility to produce pellets with a high drug loading and high density. This was herein demonstrated. In fact, the bulk density of the un-coated pellets is relatively high, in particular if compared to that of pellets prepared by other techniques and/or to granules.

Surprisingly, the application of a polymeric film increased (about 4 %) the bulk density of pellets. This may be attributable to an increased sphericity and surface smoothness, and hence to an improved flowability of the coated multiple units.

As previously stated, a good bulk density value, along with a high drug loading, are important requirements when aiming to develop a high strength dosage form.

Our multiparticulate system is able to meet both these requirements; a high strength drug delivery system (DDS), containing more than 1,000 mg 5-ASA is therefore likely to be achieved with any of the oral dosage forms proposed (*i.e.* capsules, tablets or sachets).

A "worse case" scenario, namely the development of a capsule dosage form, was preliminary considered for the multiple-unit DDS design. The largest capsules available for human use are, in fact, the 000 size, which have an overall capsule volume of 1.34 mL, corresponding to an actual available volume of approximately 1.18 mL.

At converse, the development of a high strength tablet dosage form would be relatively easier, as a consequence of bulk volume material reduction (and displacement of gaseous phase). A critical issue in this case would be, however, the protection of the functional coating when undergoing compression.

The simplest possible way to achieve a high drug loading would be the development of a sachet dosage form. Considering in fact that about 3,000 mg pellets can be filled into a sachet, that would correspond to an actual 5-ASA dose/unit > 2,000 mg.

Therefore, if a high strength preparation containing more than 1,000 mg 5-ASA can be realised using capsules, the feasibility with other dosage forms (*i.e.* tablets or sachets) is likely to be expected as well.

Gelatine 000 size capsules (Capsugel Ltd., Bornem, BE) were filled, manually, with 5-ASA pellets:

1) un-coated, labelled F0

2) coated with Kollicoat® SR (5 w.g.%, theoretical), labelled F1

3) coated with Kollicoat® SR (5 w.g% theoretical) and Kollicoat® MAE (15 w.g.% theoretical), labelled F2

The theoretical and actual 5-ASA contents was calculated on the basis of the net weight of capsules and the experimental content was also determined (Table 5.3).

Table 5.3: Net weight of un-coated, KSR (5 w.g.%) and KSR/KMAE (5/15 w.g.%) coated pellets in 000 size gelatine capsules (n=10). Theoretical 5-ASA content calculated as net of excipients used for pellet preparation and coating polymers. Experimental 5-ASA content determined at pH 7.4 phosphate buffer.

Formulation	Code	Pellets weight (mg ± s.d.)	5-ASA content (mg ± s.d.)		
			theoretical	**experimental**	Δ %*
Un-coated	F0	1199 ± 1.73	**1139 ± 0.981**	**1153 ± 1.12**	+ 1.18
KSR 5 w.g. %	F1	1237 ± 1.53	**1113 ± 1.15**	**1135 ± 2.47**	+ 1.95
KSR 5 w.g. % + KMAE 15 w.g. %	F2	1253 ± 3.78	**961.2 ± 1.22**	**1100 ± 3.04**	+ 12.6

*Δ%= (experimental - theoretical)/ experimental*100

The capsule filling with formulations was generally satisfactory. As expected from the bulk density values, listed in Table 4.3, the highest loadings were achieved with the KSR and KSR/KMAE coated pellets, thus confirming the ability of the film layer to increase the bulk density and improve the packing behavior of pellets.

The experimental 5-ASA content was always higher than the expectation, most likely because the coating weight gains were calculated on a theoretical basis.

The actual 5-ASA contents (%) of F0, F1 and F2 preparations were also higher than those expected, *i.e.* 96.2, 91.8 and 87.8 %, respectively.

The feasibility of preparing a high strength DDS, containing more than 1,000 mg 5-ASA, in a capsule dosage form was therefore demonstrated.

Prospects- To further increase the drug dose, alternative dosage forms will be evaluated. In particular, the possibility of realising tablets able to disintegrate promptly in the stomach, thus releasing the multiple-units will be assessed. Formulation strategies to protect the functionality of the polymeric layer (*i.e.* KSR and KMAE) undergoing compression will also be systematically investigated.

The *in-vitro* dissolution performance of our multiple-unit DDS, namely to F1 and F2 preparations, was compared to that of Pentasa® and Lialda™ (Figure 5.3). The 5-ASA release rate (mg/h) from each formulation was also determined, by dividing the amount of 5-ASA released at each phase by the relative residence time (Table 6.3).

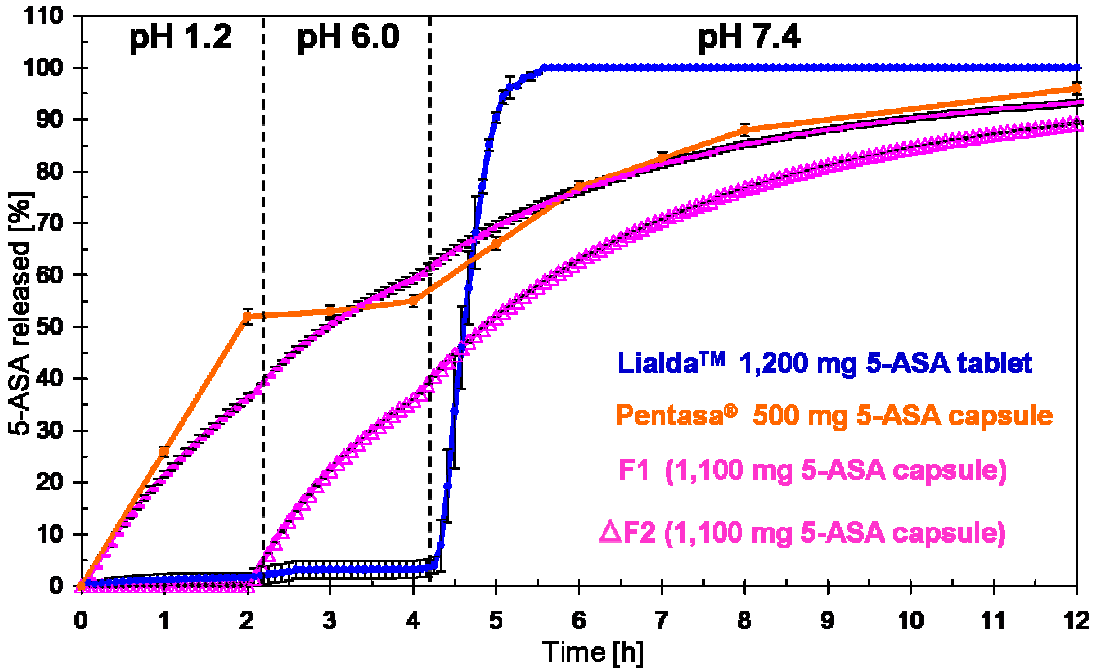

Figure 5.3: Comparative mean (n=6) dissolution profiles of Lialda™ (1,200 mg 5-ASA, tablets), Pentasa® (500 mg 5-ASA, capsules), F1 (1,100 mg 5-ASA, capsules) and F2 (1,100 mg 5-ASA, capsules). Test performed at pH 1.2 (2h), 6.0 (2h) and 7.4 (8h). Bars indicate standard deviation.

The overall dissolution profiles of F1 and Pentasa® seem to be quite similar. In reality, the release rate of the two preparation is comparable only at pH 7.4 (Table 6.3). Under simulated gastric conditions, the amount of 5-ASA released from F1 is in fact about 1.4 times lower than that of Pentasa® granules, whereas in the simulated small intestine it is about 8 times higher.

In addition, the release profile of F1 is notably less sensitive to pH changes than that of Pentasa® granules.

Table 6.3: Release rates of 5-ASA, during the III buffer stages, from Pentasa®, Lialda™, F1 and F2 (normalised to a dose of 500 mg).

	5-ASA release rate (mg/h per phase)		
	I	II	III
Lialda™	9.50	10.0	57.6
Pentasa®	130	7.50	26.6
F1	91.3	57.0	21.2
F2	0.10	89.8	33.8

As expected from the dissolution profiles (Figure 4.3), the 5-ASA release from Lialda™ tablets was minimal in both the simulated stomach as well as conditions, whereas nearly the entire drug dose is released within the simulated large intestinal fluids. This is the reason why Lialda™ is useful in the treatment of distal IBD manifestations. As for Pentasa®, the drug release rate under simulated gastric conditions was about 5 times higher than in the large bowel and even 17 times higher than in the simulated small intestine. Moreover, the amount of 5-ASA released within the 2-h stay in the simulated small intestine is potentially minimal if aiming to treat proximal IBD manifestations.

At converse, F1 preparation allows a considerable amount of 5-ASA to be released in the small intestine. An overall better release than that of the reference multiparticulate product was, therefore, achieved already without the application of an enteric coating polymer.

However, with the additional enteric protection (F2) the *in-vitro* performance was notably improved. In fact, 5-ASA loss under simulated gastric condition was avoided and a continuous drug release throughout the entire intestine was achieved.

As stated before, an optimal mesalamine-based preparation, to meet different IBD needs should have some important characteristics: 1) a high strength (> 1,000 mg) 5-ASA to achieve a once/twice daily administration, improving compliance; 2) prolonged-release mechanism to allow 5-ASA to be released throughout the entire intestine; 3) avoid/minimise drug release in the stomach to reduce risk of side-effects and maximise the therapeutic drug dose.

We were therefore able to satisfy all the above mentioned requirements.

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CONCLUSIONS

CONCLUSIONS

The aim of the research project was to develop an oral multiple-unit drug delivery system (DDS), containing a high dose of 5-aminosalicylic acid (5-ASA) for the treatment of inflammatory bowel disease (IBD). A DDS containing more than 1,000 mg 5-ASA was sought to reduce frequency of administration, improving compliance. Multiparticulates were selected in virtue of their biopharmaceutical advantages, such as reduced risk of dose-dumping and more even distribution along the GI tract. Accordingly to specific IBD therapeutic needs, the DDS was intended to prevent gastric breakdown and allow a slow and pH-independent release of 5-ASA throughout the small and the large intestines to occur.

The overall project has been divided into three stages:

4) Development of a multiple-unit drug containing core, based on highly loaded 5-ASA pellets [chapter 1].
5) Assessment of the scale-up ability of the promising highly-loaded multiple-unit formulation [chapter 2].
6) Tailoring of drug release accordingly to specific IBD therapeutic needs, and proposal of a high strength multiple-unit drug delivery system (DDS) [chapter 3].

1) Firstly, the feasibility of preparing pellets containing a high load of 5-ASA (*i.e.* > 90 wt%), by extrusion-spheronisation (E-S) technique, was assessed. The influence of the chemical (acidity) and physical (particle size and shape) characteristics of 5-ASA on the rheological behaviour of microcrystalline cellulose (MCC)-based pastes was systematically investigated using a bench-scale ram

extruder (capillary rheometer). Liquid phase migration (LPM) within the paste during the extrusion, and hence variation in water content of extrudates and reproducibility of the final E-S product, was generally observed. The extent of LPM was found to be related to both the drug loading and its physical properties. In particular, drug particle morphology (needle-like) was identified as the most critical parameter in the process. Therefore, a reduction in particle size, combined with a change in particle morphology, allowed LPM to be considerably reduced or eliminated. The effect of pH on MCC behaviour was, instead, found to be negligible.

The performance of colloidal grades of MCC (Avicel RC591 and CL611) as alternative extrusion aids to the standard Avicel PH101 was also investigated: Avicel RC591 and CL611 proved to be superior aids for the highly-loaded 5-ASA pastes due to their ability to hinder water migration when subjected to pressure.

Based on the results obtained, by combining an accordingly micronised API and colloidal MCC grade, a multiple-unit formulation containing not less than 90 wt% 5-ASA could be developed.

2) The possibility of scaling-up the promising multiple-unit formulation, identified in the bench-scale ram extruder, to a pilot-scale equipment (basket extruder) was afterwards assessed. Preliminary, the possibility of reproducing, by an industrial-scale microniser, a batch of 5-ASA with physical characteristics (*i.e.* particle size and shape) to those obtained *via* a bench-scale apparatus (planetary ball-mill), was considered.

To provide a high level of process understanding and control capability, a mixed fractional factorial design (MFFD) and a statistical optimisation approach were

employed in the study, to investigate the effect of some process and formulation variables on desired pellets characteristics (*e.g.* size and shape, size distribution, mechanical resistance, process yield and dissolution properties).

The MFFD aided in investigating both process (extrusion speed and spheronisation time) and formulation (5-ASA:MCC ratio, water and extra binder amounts) parameters that significantly affect the preparation of highly-loaded 5-ASA pellets in the pilot-scale equipment. Moreover, the statistical optimisation study allowed a combination of process and formulation parameters for the obtainment of pellets containing up to 95 wt% 5-ASA, and with good technological characteristics (*e.g.* mechanical resistance, size and shape), to be obtained.

3) Finally the feasibility of tailoring the drug release of highly-loaded 5-ASA pellets, accordingly to the specific IBD therapeutic needs, was undertaken. To achieve a modular sustained- and pH-independent release of 5-ASA, pellets were film-coated with a polyvinyl acetate polymeric dispersion up to different theoretical weight gains (w.g.%). A film thickness corresponding to a theoretical 5 w.g.%, was identified as being satisfactory to achieve a prolonged-release of 5-ASA within 12-h *in-vitro* dissolution testing, under simulated gastric (2-h), small (2-h) and large (8-h) intestinal fluid conditions. The *in-vitro* dissolution performance of the DDS was similar to that of a commercial reference multiparticulate product, *i.e.* Pentasa® (500 mg 5-ASA, capsules).

Moreover, the application of an outer acrylate-based layer to a theoretical weight gain of 15 % allowed a complete enteric protection, and hence potential reduction of side-effects and improvement of therapy, to be achieved.

By combining a polyvinyl acetate and an acrylate-based polymeric layers, a multiparticulate formulation could be tailored accordingly to our objectives.

First attempts to realise a high strength DDS in a capsule-dosage form, containing more than 1,000 mg 5-ASA, yielded satisfactory results. The multiple-unit preparation developed proved its superior *in-vitro* performance to that of the reference products tested.

FUTURE WORK

The areas identified in this work as requiring further work are:

i) To investigate the possibility of further increasing the 5-ASA dose/unit. Alternative dosage forms to capsules will therefore be considered. In particular, the feasibility of developing a high strength multiple-unit tablet dosage form, able to disintegrate in the stomach thus promptly releasing the multiparticulates, will be investigated. With this aim, technological strategies that would ease the tabletting process while protecting the functionality of polymeric layers (*e.g.* enteric- or sustained-release coatings) undergoing compression, will be evaluated.

ii) To investigate alternative formulation strategies for 5-ASA delivery to specific areas of the GI tract.

iii) To investigate the *in-vivo* release performance of the DDS.