Drug Development and Industrial Pharmacy, 2011; 37(9): 1100–1109

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.560156

informa
healthcare

REVIEW ARTICLE

Multiparticulate systems containing 5-aminosalicylic acid for the treatment of inflammatory bowel disease

Tereza Bautzová[1,2], Miloslava Rabišková[1], and Alf Lamprecht[2]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Czech Republic, and* [2]*Laboratory of Pharmaceutical Engineering, Faculty of Medicine and Pharmacy, University of Franche-Comté, Besançon Cedex, France*

Abstract

Background: In recent years, many achievements have been realized in the therapy of inflammatory bowel disease (IBD) although its etiology remains unknown. Thus IBD treatment is symptomatic and targets general inflammatory mechanisms. Oral formulations containing 5-aminosalicylic acid (5-ASA) have become the standard therapy for mild-to-moderate IBD.

Objective: This article is a review of recently published research dealing with new 5-ASA dosage forms. Thus promising candidates for IBD treatment evaluated *in vitro* are reported; systems tested *in vivo* in trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats are mentioned; and 5-ASA formulations used in clinical studies are presented. Moreover, all oral dosage forms containing 5-ASA or its prodrugs are reviewed; their characteristics and utilization in IBD treatment are discussed.

Conclusion: In several clinical studies, it has been shown that multiparticulates such as pellets offer more advantages as compared with single unit forms, that is, coated tablets. Prolonged presence close to the site of the action, improved drug bioavailability, and easier administration of large drug doses belong to the benefits of pellets.

Keywords: Controlled drug delivery, 5-aminosalicylic acid, pellets, tablets, commercialized products, promising candidates

Introduction

Inflammatory bowel disease (IBD) comprises two idiopathic inflammatory disorders of the intestinal tract: ulcerative colitis (UC) and Crohn's disease (CD)[1]. Clinical manifestations include bloody diarrhea, fecal urgency, tenesmus, mucosal ulcerations, weight loss, and generally feeling unwell[2]. This pathology is widespread in western countries and, although its exact etiology is poorly understood, individual genetic background, environmental signals such as stress, and immunological influences may all contribute to the disease process[3]. Probably, in hereditarily susceptible population, environmental factors such as water, food, and infection trigger excessive reaction of intestinal immunity. Their action may cause an inflammatory stimulation to the intestinal mucosa and damage it[4].

UC is a refractory, chronic, and nonspecific inflammatory disease of the rectal and colonic mucosa. Abdominal pain occurs in the lower left part. The colon wall is thinner compared with CD and shows continuous inflammation with no patches of healthy tissue in the diseased section[5,6]. Contrarily, CD can involve any part of the gastrointestinal (GI) tract from the mouth to the anus, but most frequently involves the distal small bowel and colon[7]. CD patients commonly experience pain in the lower right abdomen. Bleeding from the rectum is much less common as in the patients with UC[8]. The inflammation may occur in patches in one or more organs in the digestive system. A diseased section of colon may appear between two healthy sections[9]. The colon wall may be thickened and, because of the intermittent pattern of diseased and healthy tissue may have a "cobblestone"

Address for Correspondence: Prof. Miloslava Rabišková, Department of Pharmaceutics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, Brno 612 42, Czech Republic. Tel: 0042 54156 2860. E-mail: rabiskovam@vfu.cz

(Received 17 December 2010; revised 28 January 2011; accepted 01 February 2011)

Abbreviations:

AUC, area under the curve;
CD, Crohn's disease;
CMCNa, carmellose sodium;
IBD, inflammatory bowel disease;
MCC, microcrystalline cellulose;
NO, nitric oxide;
PPARγ, peroxisome proliferator-activated receptor gamma;
ROS, reactive oxygen species;
TNBS, 2,4,6-trinitrobenzenesulfonic acid;
TNF, tumor necrosis factor;
UC, ulcerative colitis;
5-ASA, 5-aminosalicylic acid

appearance[10]. Main features of UC and CD are compared in Table 1 [11,12].

The degree of disease activity of UC and CD can vary from mild to highly severe[13] and acute flare-ups are followed by the periods of remission[14]. In the acute phase, patients often receive intense therapy, that is, the dosage schedule with multiple tablets several times a day. These complicated dosing regimens are inconvenient for patients and negatively affect their compliance[15,16]. On the other hand, patients in the quiescence state of disease with the absence of symptoms are non-adherent too[17]. Precisely, lack of patients' compliance in frequent lifelong treatment greatly increases the risk of clinical relapse and disease activity, and in addition represents an important barrier to the successful management of the patients[18,19]. Thus, new once or twice-daily dosing formulations could improve patient compliance. They are at least as effective as more frequent dosing tablets[6,15,20,21].

As the etiology of IBD is still not well-known, therapy is symptomatic and targets general inflammatory mechanisms[22]. IBD is considered a chronic inflammatory disorder characterized by the development of intestinal inflammation resulting from the transmural infiltration of neutrophils, macrophages, lymphocytes, and mast cells, ultimately giving rise to mucosal disruption and ulceration[23]. Activated macrophages produce a host of proinflammatory cytokines, including IL-1, tumor necrosis factor-α (TNF-α, i.e. a cytokine involved in systemic inflammation, the member of a group of cytokines that stimulate the acute phase reaction), and the chemokine IL-8[24]. Increased levels of inflammatory cytokines are secreted in the colonic mucosa of IBD patients, leading to the production of other inflammatory mediators such as nitric oxide (NO), reactive oxygen species (ROS), eicosanoids, and so on. These mediators contribute to tissue necrosis and mucosal dysfunction[25]. Recently, the peroxisome proliferator-activated receptor (PPARγ) has been described as a regulator of cellular proliferation, apoptosis, and anti-inflammatory response possibly through several signaling pathways[26].

Table 1. Comparison of ulcerative colitis and Crohn's disease (adapted from refs. 11 and 12).

Feature	Ulcerative colitis	Crohn's disease
Distribution	Diffuse, distal predominance	Segmental or diffuse, often proximal predominance
Rectum	Always involved	Often spared
Microscopic distribution	Diffuse	Often focal
Depth of inflammation	Mucosal	Transmural
Sinus tracts and fistulae	Absent	Often present
Strictures	Absent	Often present
Fissures	Absent	Often present
Granulomas	Absent	Often present

Pharmacotherapy of IBD

Since the precise etiology of IBD is still not clear, the mainstay of medical therapy depends on inhibition of the inflammatory mediators[27]. In past years, available treatments offered both imperfect control of symptoms and the possibility of serious side effects. Today, current treatments include anti-inflammatory drugs, antibiotics, and immunomodulators[28]. 5-Aminosalicylic acid (5-ASA)-related drugs are used under mild or moderate disease conditions, whereas steroids and immunomodulatory drugs are required for the treatment of more severe inflammation. However, the use of latter types of drugs is seriously restricted because of the more serious complications and toxic side effects, which are related to their systemic absorption[29,30]. As both CD and UC remain medically incurable and associated with potentially significant morbidity, the focus of a treatment must be to reduce or eliminate symptoms, optimize nutritional status, prevent complications, and minimize the potential psychological effects of these chronic illnesses[31].

5-Aminosalicylic acid: mesalazine (5-ASA), sulfasalazine, olsalazine, and balsalazide

Formulations containing 5-ASA have become the gold standard of treatment for mild-to-moderate active UC, based on an extensive and long history of their efficacy and safety. They are effective in the induction and maintenance of IBD remission[32,33]. Furthermore, 5-ASA may provide protection against the development of colorectal cancer in patients suffering from IBD[34]. Free 5-ASA is a zwitter ion and when administered orally, it is rapidly and nearly completely systematically absorbed from the proximal small intestine and then extensively metabolized to *N*-acetyl-5-ASA by *N*-acetyltransferase-1 in intestinal epithelial cells and the liver[35,36]. This metabolite is therapeutically inactive[35]. Excretion is primarily

through the urine as a mixture of free 5-ASA and *N*-acetyl 5-ASA[37]. From large intestine, 5-ASA and its metabolite are excreted in feces[5,38].

Strategies for colon delivery

Optimal delivery of 5-ASA in the treatment of UC demands release in the colon, where 5-ASA acts topically at the inflammatory lesions and can exert anti-inflammatory effects. Therefore, strategies to protect orally administered 5-ASA from its release, absorption, and metabolism until it reaches the colon have been developed to provide effective therapy with minimal side effects[36,39]. In general, four primary approaches have been proposed, namely prodrugs, pH- and time-dependent formulations, and colonic microflora-activated systems[40]. Sulfasalazine, olsalazine, and balsalazide are prodrugs (Table 2). Sulfasalazine consists of 5-ASA linked to sulfapyridine by an azo-bond. Olsalazine comprises two molecules of 5-ASA linked by an azo-bond as a dimer, and balsalazide consists of 5-ASA linked by an azo-bond to inert carrier 4-aminobenzoyl-β-alanine[36,38,41]. Due to the azo linkage, the compounds pass unchanged through the small intestine and undergo their metabolism by the bacterial azoreductase enzymes when they reach the colon releasing 5-ASA[42,43]. Prodrugs are available either in a form of tablet or gelatine capsule and are listed in Table 2. Sulfapyridine, the therapeutically inactive moiety of sulfasalazine, is absorbed systemically from the colon and is responsible for most of the hypersensitivity and intolerant side effects[44,45]. Olsalazine is associated particularly with an increased incidence of dose-dependent diarrhea. The azo-bond prodrugs represent dose limitations in the treatment of IBD, rely on their ileic secretory effects, which can lead to diarrhea[43]. Hence, various new concepts for the safer treatment of IBD without these side effects are desirable. The majority of commercial available colon-targeted products containing 5-ASA is based on pH-dependent concept (Table 3). Most commonly used polymers for controlled release preparations are methacrylic acid copolymers—Eudragit® L 100-55, Eudragit® L 100, Eudragit® S 100, which dissolve at pH 5.5, 6.0, and 7.0, respectively[46]. Inter- and intra-individual variability, the similarity in pH between the small intestine and the colon, and very different data of the colonic pH in patients with IBD in literature make pH-dependent systems less reliable and site-specificity of drug release in colon more unpredictable[47,48]. However, in CD with the inflammation throughout the intestinal tract these forms can be valuable. Controlled release mechanism of mesalazine through semipermeable ethyl cellulose membrane (Pentasa) tends a time-dependent release of the drug, starting in the duodenum and proceeding till rectum. Pentasa is pH-independent formulation containing mesalazine pellets with moisture-sensitive membrane and is available as a tablet, a capsule, or a sachet[3]. Therefore, Pentasa preparation is as well more useful for CD patients who often have inflammation of the small intestine[49]. On the other side, time-dependent systems confront the complication in predicting the accurate location of drug release due to inherent variability in GI transit times. The onset of initial drug release occurred in the small intestine in some subjects, whereas in others the formulations passed ascending colon intact. Moreover, accelerated GI transit in patients with UC has been observed[47,48,50]. Hence, these factors limit time-dependent systems for colon delivery especially in IBD. On the other hand, microflora-activated systems seem to be very promising for specific colon-targeting, because they are independent of pH and transit time variations along the GI tract and the drug release starts upon activation after arrival into the colon[51]. Bacteria population, which is essentially absent from the stomach or the small intestine, increases sharply in the colon[52]. These anaerobic bacteria produce a wide range of degrading enzymes. A number of naturally occurring polysaccharides are stable in the upper GI tract, but they are degraded by the colonic bacteria[51]. Hence, diverse polysaccharides, such as pectin, guar gum, amylose, chitosan, and so on, have been designed and applied for colonic delivery systems[53–57]. Nevertheless, the colonic microflora varies substantially between individuals, reflecting diet, age, and disease and thus microbially triggered drug delivery could be altered[58]. It may be concluded that each of these approaches represents a unique system in terms of design with some advantages, but has certain shortcomings, and

Table 2. Oral 5-ASA prodrugs and their dosage forms (adapted from refs. 35, 36, 38, and 41).

Product	Drug	Dosage form	Formulation	Site of delivery	Drug content
Azulfidine Salazopyrine	Sulfasalazine	Tablet	5-ASA linked to sulfapyridine by azo-bond	Colon	500 mg (200 mg 5-ASA)
Azulfidine EN-tabs Salazopyrine EN-tabs	Sulfasalazine	Tablet coated with cellulose acetate phthalate	5-ASA linked to sulfapyridine by azo-bond	Colon	500 mg (200 mg 5-ASA)
Colo-Pleon	Sulfasalazine	Tablet coated with Eudragit® L 100-55	5-ASA linked to sulfapyridine by azo-bond	Colon	500 mg
Dipentum	Olsalazine	Capsule	5-ASA dimer linked by azo-bond	Colon	250 mg (225 mg 5-ASA)
Colazide	Balsalazide	Capsule	5-ASA linked to 4-amino benzoyl-β-alanine (inert carrier) by azo-bond	Colon	750 mg (262 mg 5-ASA)
Colazal		Tablet (clinical testing)			1100 mg (400 mg 5-ASA)

Table 3. Mesalazine preparations used in oral application (adapted from refs. 14, 21, 36, 38, 41, 47, and 76).

Product	Formulation	Release pH	Site of delivery	Drug content
Asacol Asacolitin Ipocol Mesren Pentacol	Eudragit® S-coated tablets (delayed release)	pH > 7	Terminal ileum Colon	400 mg 800 mg
Salofalk Mesasal Claversal Calitofalk	Eudragit® L-coated tablets (delayed release)	pH > 6	Jejunum Terminal ileum Colon	250 mg 500 mg
Pentasa	Ethyl cellulose-coated pellets available as capsule, tablet, or sachet (slow continuous controlled release, time-dependent release)	Release via diffusion	Duodenum Jejunum Ileum Colon Rectum	250 mg 500 mg tbl, cps 1000 mg 2000 mg sachet
Apriso	Eudragit® L-coated matrix granules with polyacrylic polymer in the core available in gelatin capsule (delayed, extended, and continuous release)	pH > 6	Small bowel Colon	375 mg
Salofalk Granu-Stix	Eudragit® L-coated matrix granules with Eudragit® NE 40D in the core, available in a sachet (delayed and sustained release)	pH > 6	Distal small bowel Colon	500 mg 1000 mg 1500 mg
Lialda (USA) Mezavant (EU)	Eudragit® L- and Eudragit® S-coated lipophilic and hydrophilic matrix tablets (delayed release)	pH > 7	Terminal ileum Colon	1200 mg

at present no ideal system for colon-specific drug delivery exists for the treatment of IBD.

Single unit forms versus multiparticulate systems

Pellets as a multiparticulate drug delivery system offer the benefits as compared with conventional preparations, such as tablets. The size of pellets (~1 mm) guarantees their continuous and unhindered transit through the GI tract and thus they are less influenced by gastric emptying and intestinal transit[42,59]. Hence, the pellets can be taken independently from meals[39]. In addition, in the colon, pellets are slowly diffused over a wide area and are retained longer in the ascending colon than a tablet (28 h compared with 15 h, respectively) that could be favorable in the treatment of IBD, where local concentrations at the sites of inflammation are required[60–62]. Furthermore, pellets that are easier to swallow and as effective as tablets seem to enable a larger dose to be taken comfortably and conveniently, thereby potentially improve patients' compliance, treatment response, and quality of life[63,64]. Moreover, pellets could be widely and uniformly dispersed in the GI tract surfaces, which on the one hand increase the drug–tract contact surface and thus improve drug bioavailability and on the other hand reduce local irritation[47,59,65]. Finally, the release failure of the individual unit hardly affects the total release behavior due to the multiple units, thus risk of systemic toxicity is reduced[47,59].

Pellet characteristics and preparation

Pellets are small, free-flowing, spherical particles of drugs and excipients; they possess narrow size distribution and for pharmaceutical purposes they have a diameter of 0.5–2.0 mm. Pellets are prepared using several pelletization methods such as solution, suspension, or dry powder layering on inactive starters, extrusion/spheronization, rotoagglomeration, spray drying, or spray congealing[66].

Solution or suspension layering is the oldest pelletization technique in which drug solution or its suspension is sprayed onto inactive spherical particles of sucrose or microcrystalline cellulose (MCC) in fluid bed equipment or coaters. These pellets are uniform in size distribution and exhibit very good surface morphology. In dry powder layering process, inactive starters are wetted with an adhesive solution in a rotating pan, rotoprocessor, or rotogranulator, and drug powder is added simultaneously. Lower drug loading up to 50% is main disadvantage of the layering process. Homogeneous particles of matrix structure also cannot be prepared by this method. Mechanical properties of layered pellets, that is, their hardness and friability depend to certain degree on the properties of starter used[67].

Extrusion/spheronization, which is widely used in pharmaceutical industry, was developed in Japan in 1964. It comprises four main steps: wetting of homogenized dry powder mixture of a drug and excipients; extrusion when the plastic mass is passed through the extruder to form rods of spaghetti-like structure; spheronization including breaking of extrudate to short cylinders, converting them to spheres and smoothing their surface; and drying step using various drying procedures[68,69]. Particles of high drug loading up to 90%, homogeneous matrix structure, uniform size, and good mechanical properties could be achieved. Main disadvantage of this method is a several steps process.

Rotoagglomeration in rotogranulators or rotoprocessors is a newer pelletization method starting with

homogenized dry powder mixture that is wetted and turned into the spheres by the action of three forces: centrifugal, fluidization, and gravitational. Pellets are dried and can be coated in the same equipment, and this is main advantage of this method. Particles of homogeneous matrix structure and drug content of 65% can be obtained. Their particle size distribution is however broader than that resulting from the above mentioned two methods[70,71].

Spray drying is a method of converting solution or suspension droplets into porous spherical particles by hot air in a production chamber. It is used generally to form fast-dissolving particles[72]. Spray congealing involves cold air to solidify droplets of a melted liquid or dispersion. Particles of prolonged drug release can be obtained[73].

Spherical pellets have ideal shape for coating application. Coated or uncoated particles can easily modify the release profile of drugs and therefore are widely used in controlled drug release. In general, they are semi-products and turn into the final medicament when filled into hard capsules or carefully compressed into disintegrating tablets.

From promising candidates to commercialized products

The main objective in the treatment of IBD is to deliver drugs to the sites of inflammation to achieve maximal drug concentration and to reduce side effects due to the abrupt release of drug in the upper GI tract. In this context, many research groups have attempted to develop various multiparticulate systems for successful colon-specific drug delivery. First, novel 5-ASA formulations, that is, promising candidates for the treatment of IBD evaluated *in vitro* are reported; second, systems containing 5-ASA tested *in vivo* in trinitrobenzene sulfonic acid (TNBS)-induced rat colitis model are mentioned; and finally, multiparticulate-commercialized 5-ASA formulations administered to healthy volunteers or IBD patients in a clinical trials are presented.

In vitro evaluation

Gupta et al.[74] prepared layered 5-ASA pellets and studied the influence of multiple coatings from aqueous dispersions of polymethacrylates on drug release. Inner coating layer consisted of Eudragit® RL and RS (2:8), and outer layer of Eudragit® FS of different coating levels (15–30%). Eudragit® FS 30D is a new polymer resin composed of methacrylic acid, methacrylate, and methylmethacrylate, which dissolves rapidly at pH ≥ 7.5. Dissolution testing involved media of two different pH values in each set, that is, first the samples were exposed to 0.1 N hydrochloric acid for 2 h and then they were transferred into the buffers of pH 6.5 or 7.0 or 7.5, respectively, for 12 h. No drug release was observed at pH 6.5. At pH 7.0, prolonged 5-ASA release after a lag time of 15–60 min depending on the coating level was determined. Similarly, at pH 7.5 again prolonged drug release was measured, but without lag time at any of the coating levels. With respect to the obtained results, this delivery system might prove successful for the drug transport to the ileo-colonic part of GI tract in a sustained-release fashion.

Zambito and Di Colo[75] studied *in vitro* the release of 5-ASA from chitosan matrices of 50 mg weight and 6 mm diameter obtained by the compression of 5-ASA with chitosan or chitosan hydrochloride microspheres. Drug release study was carried out in phosphate-buffered saline (PBS) pH 7.4 after 4 h of incubation in rat cecal contents or in bicarbonate buffer pH 7.0. All (100%) or almost all (90%) the drug amount was released from the chitosan hydrochloride-based matrices after 2.5 h at pH 7.4 depending on the incubation in rat cecal contents or a bicarbonate, respectively. The drug release from chitosan-based matrices was lower (70%) and independent of the incubation buffer used at the same time and conditions. For *in vivo* application, the matrices are supposed to be filled into enteric-coated capsules to prevent the drug release in upper GI tract.

In another study, Rudolph et al.[14] compared the dissolution profiles of 5-ASA pellets prepared by extrusion/spheronization and coated either with Eudragit® FS 30D dispersion or with Eudragit® S organic solution or aqueous dispersion. Dissolution testing was performed for 6 h in different media of pH 1.2, 6.0, 6.5, 6.8, 7.2, or 7.5, respectively. No drug release was observed at pH 1.2, 6.0, and 6.5 and its release was slow at pH 6.8. The release of 5-ASA from Eudragit® FS-coated pellets was in the average about 20% an hour at pH 7.2 and was completed within 2 h at pH 7.5. For pellets coated with Eudragit® S, fast release was observed at pH 7.2 and 7.5. The multiunit dosage form coated with Eudragit® FS 30D exhibited release pattern more appropriate to the pH profile of the ileum and the colon observed in UC patients and reported in some articles[76,77].

Milojevic et al.[78] presented 5-ASA pellets prepared using extrusion/spheronization and subsequently coated by amylose–Ethocel® coating (1:4). For drug release studies, dissolution medium of 0.1 M HCl with pepsin was used for the first 3 h and the test continued in PBS of pH 7.2 with pancreatin for an additional 21 h. No drug release was observed in pH 1.2, and only 10% of 5-ASA was released after next 21 h in pH 7.2. *In vitro* fermentation studies were performed in batch culture fermenter with mixed fecal bacteria. Gut microflora was capable to degrade amylose–Ethocel® coating and the drug release was almost completed after 8 h. These results indicate that this coating could be used for a range of drugs requiring their delivery into the colon.

Calcium alginate beads as core carriers of 5-ASA coated with inner Aquacoat® film and outer layer of Eudragit® L 30D have also been studied for drug delivery into the lower intestinal tract[79]. Dissolution started in acidic medium of pH 1.4 for 2 h, and afterward the medium was changed with simulated intestinal fluid of pH 7.4 for 22 h. Outer coating prevented 5-ASA dissolution in acidic medium and then the drug release rate was controlled by the inner film and alginate in the core. This dosage form

is reported to deliver a drug to the ileo-colonic part in a sustained released fashion with minimal early release in the upper GI tract.

Cheng et al.[59] reported 5-ASA pellets produced by extrusion/spheronization and coated with Eudragit® L 100 and Eudragit® S 100 (1:4). For drug release, three dissolution media of different pH values 1.2, 6.0, and 7.2 were sequentially used. Drug was released <1.0% in pH 1.2 until 2 h, and <3.0% in pH 6.0 PBS at 1 h after 2 h. When the buffer of pH 7.2 was employed, >80% of 5-ASA was released after 1.5 h. Thus this formulation could achieve pH-specific drug delivery under pH 7.2.

Different coating composed of nutriose:ethyl cellulose (1:3 to 1:5) was studied on pellets with 5-ASA prepared using extrusion/spheronization[80]. Nutriose is a starch derivative, which is preferentially degraded by microflora enzymes in the colon of IBD patients compared with healthy people[81]. Dissolution studies were performed in three media of pH 1.2 (2 h), 6.8 (9 h), and 7.0 containing fresh fecal samples from IBD patients (10 h). The release of 5-ASA was suppressed in pH 1.2 and 6.8 corresponding to the upper GI tract conditions, but the release rate significantly increased in the last medium. Proposed formulation provides an interesting approach of a colon-targeted system.

From the above mentioned experiments, it can be concluded that the appropriate dissolution method using buffers simulating pH values in different GI tract parts of IBD patients as accurately as possible for time intervals corresponding to the residence times in stomach and intestine, and including either fecal bacterial enzymes or β-glucosidase in the medium mimicking colonic compartment[82], would be crucial for the *in vitro* evaluation of 5-ASA colon-targeted systems.

Nevertheless, the promising candidates for novel developed preparations selected in *in vitro* testing should be considered carefully and necessitate always *in vivo* investigations to verify their efficacy, because *in vitro* data cannot reflect inflammatory state in IBD.

Preclinical studies in animals

Several models of experimental colitis have been developed to investigate the molecular and cellular mechanisms of inflammation and immunological disorders. Currently, hapten-induced colitis, in which TNBS shares important similarities with human CD such as transmural inflammation, lymphocyte infiltration, Th1-dominated cytokine profile, and stricture formation. This model is suitable to study anti-inflammatory agents and/or their delivery systems during the course of developing and resolving inflammation[83,84].

Wei et al.[85] reported bacterially triggered film-coated pellets efficient for colonic targeting and effective in the treatment of IBD in rats. 5-ASA pellets were prepared by extrusion/spheronization (the formulation comprised 5-ASA, lactose, and MMC) and coated with chitosan/Kollicoat SR 30D film. The coated pellets were tested for drug release for 2 h in 0.1 M HCl, the study was continued for 3 h at pH 6.8 and finished in a PBS pH 6.8 with cecal content or β-glucosidase for 10 h. Only a small amount of 5-ASA was released from the coated pellets in the upper GI tract conditions and its release increased in the presence of rat cecal bacterial enzymes or β-glucosidase enzyme. For *in vivo* evaluation, pellets were administered to TNBS-induced colitis in rats. A significant decrease of colonic damage score was observed after oral administration of pellets compared with the untreated colitis control group. Colon/body ratio was found to be lower and level of myeloperoxidase activity in the colon was markedly decreased in comparison with the colitis control. The coated pellets provided an effective and prolonged local therapeutic concentration for the treatment of IBD with a potential of reducing the adverse effects.

Another approach for colon-specific delivery was presented in the study of Mladenovska et al.[86] 5-ASA-loaded alginate microparticles were prepared by spraying of aqueous dispersion of alginate and 5-ASA into the solution of chitosan and $CaCl_2$ in acetic acid placed in the apparatus collector. Drug release studies were performed in different dissolution media and correspondent residence times were included: stomach—120 min. (pH 1.2, 0.1 M HCl), duodenum—10 min (pH 6.0, PBS), jejunum—120 min (pH 6.8, PBS), and ileum–colon (a suspension of fresh rat cecal content in bicarbonate buffer pH 7.0) until 24 h. In conditions simulating gastric content, the 5-ASA release was about 27%, with increasing pH, slower release was observed (2 h after the pH change to 6.8 only 34% of drug was released). The release was completed in simulated colonic conditions in 24 h. The particles were administered orally to rats with TNBS-induced colitis. The sixth day maximal activity of inflammation was observed. From eighth to the 21st day, the decrease in colonic inflammation was proved (total damage score, colon/body weight ratio, myeloperoxidase activity decrease). For biodistribution studies, radiolabeled [^{131}I] 5-ASA microparticles and [^{131}I] 5-ASA as an aqueous suspension were compared. Dominant localization of 5-ASA in the colon and smaller amount of systemically absorbed 5-ASA were observed when microparticles were administered. Thus this microparticulate system may be promising for clinical treatment of colonic IBD.

Eudragit® FS 30D coating (15% w/w) was applied on 5-ASA pellets prepared using extrusion/spheronization and consisting either of the drug (30%), MCC (25%), and chitosan (45%) or the drug (30%) and MCC (70%)[87]. Three continual dissolution tests using the media of different pH values, that is, 1.2, 4.0, 6.8, and 7.5 were used. First 5 h, the dissolution was performed in the same dissolution media, that is, pH 1.2 for 2 h and pH 6.8 for 3 h, mimicking thus the upper part of GI tract. Then either pH of 7.5 for the remaining 15 h or for 0.5 h following pH 4.0 for the next 14.5 h was used, respectively. Third testing was done in pH 4.0 from fifth to 15th hour simulating the lowest pH value described in the inflamed colon. Very slow drug release has been observed when maximal pH value of dissolution medium reached only

6.8, that is, 31.47% or 9.38% of 5-ASA was released from chitosan containing coated pellets and pellets without chitosan, respectively, at the end of the dissolution test. On the other hand, complete drug release was determined 1 h after the pH change to 7.5 (lag time 5 h) in pellets containing chitosan, and 4–15 h after this pH change in pellets without chitosan depending on the medium used. Pellets were tested *in vivo* on rats with TNBS-induced colitis. Clinical activity score, colon/body weight ratio, and myeloperoxidase activity were determined to quantify the severity of the colitis. In all treated groups, all the observed parameters decreased and reduced significantly the inflammation in colon. Slight anti-inflammatory effect of chitosan was also observed in accordance with recently published data[88,89]. Based on the results obtained *in vitro* and confirmed *in vivo* on rats, this novel Eudragit® FS coating used in a combination of either chitosan/MCC or MCC in the pellet core can control very well 5-ASA colonic release within extended time period from 1 to 15 h.

The results of *in vivo* studies for promising multiparticulate systems developed for the treatment of IBD have to provide the information of therapeutic effects (determination of colon/body weight ratio, assessment of the damage score, measurement of myeloperoxidase activity, TNF-α, etc.), biodistribution data (confirmation of the dominant localization of 5-ASA in the colon), and pharmacokinetic data (demonstration of low systemic bioavailability).

Clinical studies

Three hundred sixty-two patients with mild-to-moderate UC were enrolled in the randomized–controlled trial to compare the efficacy and the tolerability of 5-ASA pellets with a tablet formulation[63]. Pellets were administered orally as one sachet of 1.5 g, taken twice daily, tablets as two 500 mg dose, taken three times daily, both in the period of 8 weeks. Pellets were as effective as tablets in UC patients, allowed less frequent dosing in an easier to swallow formulation that could improve patient compliance, treatment response, and quality of life.

In another study by Farup et al.[15], 227 patients with mild-to-moderate UC were randomized to the treatment either with prolonged-release pellets or prolonged-release tablets for 8 weeks. 5-ASA (4 g daily) given as pellets twice and four times daily, supplied in packets of 1 g, was found at least as effective as tablets containing 500 mg of the drug four times daily. In addition, the twice-daily dosing regimen was preferred by the patients.

Thirteen patients with UC and CD were enrolled in the pharmacokinetics study of mesalazine pellets coated with Eudragit® L (Salofalk®) in children[64]. Plasma and urinary concentrations of 5-ASA were determined. It has been demonstrated that about 80% of the parent drug was delivered to the colon that provide a higher local activity in colonic mucosa and lower systemic absorption of 5-ASA as compared with Salofalk® tablets. Salofalk® tablets were examined in earlier multiple dosing study in children by Klotz[90]. In comparison with previous experience in adults[35], pharmacokinetics of mesalazine administered as pellets appear to be similar in both populations[64].

A study of GI spread of oral prolonged-release mesalazine pellets (Pentasa) dosed either as tablets (2 × 500 mg) or sachets (1 g) was undertaken in eight healthy volunteers[62]. *In vitro* dissolution profile at pH 7.5 and *in vivo* disposition of the pellets in terms of gastric emptying, small intestinal transit, and colon arrival was comparable. Sachet with 1 g dose offers the advantage of fewer oral doses and easier swallowing.

Brunner et al.[39] investigated GI transit and release of 5-ASA from pellets versus tablets administered as single dose of 500 mg of 5-ASA in 14 healthy male volunteers. The GI transit of ^{153}Sm, incorporated into the formulations, was followed by gamma-scintigraphy. The dissolution tests were carried out in buffer at pH 1.2 for 2 h, and then at pH 6.8 for 1 h (tablets) or 5 h (pellets). The lower dissolution rate was measured for pellets (>85% of the drug released after 5 h in pH 6.8) than for tablets (>85% of the drug released after 30 min in pH 6.8). Drug release was verified by assessing 5-ASA plasma pharmacokinetics. The both formulations released active 5-ASA in the same target region and passed through the GI tract under fasting conditions in comparable times (3.3 ± 1 and 3.8 ± 1 h for pellets and tablets, respectively). However, area under the curve (AUC) values of plasma were significantly lower for pellets when compared with tablets (968 ± 629 ng h/mL and 2206 ± 1767 ng h/mL, respectively). This finding was explained by the more prolonged drug release from the pellets found *in vitro* assuming that this release will remain constant also *in vivo*. Slower and more prolonged release of 5-ASA passage through the stomach independent of concomitant food intake and better palatability gets more advantages for pellets compared with tablets.

Roda et al.[76] conducted a randomized study in 23 healthy volunteers comparing pharmacokinetics of new enteric-coated pellet formulation administered in 1.2 g sachets in one dose with an equimolar dose of three separated enteric-coated commercially available tablets (Pentacol, 400 mg). New formulation is composed from 5-ASA pellets (5-ASA, MCC, carmellose sodium [CMCNa]) coated with Eudragit® S 100 and Eudragit® L, which ensure a complete release of the active ingredient at pH 7.5 after 45 min. The C_{max} and AUC values were similar for both formulations (1554 ± 612 ng/mL, 28120 ± 7820 ng h/mL for pellets and 1471 ± 585 ng/mL, 28770 ± 9770 ng h/mL for the tablets, respectively). The use of pellet formulation allows to reduce the daily dosages and to improve the patients' compliance.

Frequently, clinical studies are conducted in healthy volunteers and do not take in consideration pathophysiological conditions associated with IBD as accelerated transit time, alternation colonic microflora, and so on. Clinical studies carried out in IBD patients showed equal efficacy of multiparticulate formulations and tablets.

Simplified dosage regimen and easier administration make multiparticulate systems preferable in patients and resulting in presumably improved long-term compliance.

Novel 5-ASA preparations on the market

New mesalazine formulations include coated granules (Apriso and Salofalk Granu-Stix) and multimatrix tablets (Lialda, Mezavant)[5,36,38].

In Apriso (Table 3), 5-ASA is incorporated in retarding polyacrylate multiparticulate matrix core that allows drug release after a 6- to 7-h period throughout the terminal ileum and colon. The core is protected with Eudragit® L coating that dissolves at pH ≥ 6, which means a benefit for the patients with bowel pH < 7. These coated granules are filled into gelatin capsule that dissolves quickly in the stomach to disperse particles into the digestive tract. Capsules combine delayed, extended, and continuous-release mechanisms and could be administered as a single dose, with or without a food. A once-a-day dosing encourages compliance in patients[5,32,91].

Salofalk Granu-Stix® represents mesalazine multiparticulate formulation in a sachet (Table 3). Matrix cores containing 5-ASA and retarding polymer Eudragit® NE 40D are coated with Eudragit® L. Eudragit® NE 40D is aqueous dispersion of a neutral copolymer based on ethyl acrylate and methyl methacrylate. It is insoluble but swellable in water polymer with low permeability independent on pH. Due to its high flexibility, it requires no plasticizer and is suitable for matrix structures[91]. Coated matrix granules enable delayed and prolonged release of the drug from small bowel until the colon or rectum[92].

MMX mesalazine (Multi Matrix System; Lialda or Mezavant) is based on tablet coated with a gastro-resistant pH-dependent polymer film of Eudragit® L and Eudragit® S that provides drug release at a pH above 7.0, initiating in the terminal ileum and continuing throughout the colon (Table 3). Mesalazine in the amount of 1.2 g is embedded in lipophilic and hydrophilic matrices, which disintegrate slowly over 24 h. This MMX release system permits less frequent dosing (once or twice daily) with fewer tablets and is more appropriate for patients experiencing difficulty with a high pill burden[5,36,38,80,93].

Conclusion

It can be concluded that many improvements in drug delivery into the inflamed areas of the gut in IBD patients have been achieved in recent years. Multiparticulate dosage forms such as pellets showed their benefits, that is, prolonged presence close the site of action, improved drug bioavailability, and easier administration of large drug doses, compared with tablets. Furthermore, multiparticulate systems allow once-daily dosing regimen leading to the better patient compliance, therapeutic efficacy, and diminution in risk of disease relapse. Several new multiparticulates were introduced to the market; others products are undergoing clinical studies; and many of these preparations are investigated. Thus microparticulates proved their great significance in the pharmacotherapy of IBD.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Hanauer SB. (2006). Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis, 12 (Suppl 1):S3–S9.
2. Baumgart DC, Sandborn WJ. (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet, 369:1641–1657.
3. Fiocchi C. (2002). Inflammatory bowel disease. Current concepts of pathogenesis and implications for therapy. Minerva Gastroenterol Dietol, 48:215–226.
4. Elson CO, Sartor RB, Targan SR, Sandborn WJ. (2003). Challenges in IBD Research: updating the scientific agendas. Inflamm Bowel Dis, 9:137–153.
5. Lichtenstein GR. (2009). Mesalamine in the treatment of ulcerative colitis: novel therapeutic options. Gastroenterol Hepatol, 5:65–73.
6. D'Haens G, Hommes D, Engels L, Baert F, van der Waaij L, Connor P et al. (2006). Once daily MMX mesalazine for the treatment of mild-to-moderate ulcerative colitis: a phase II, dose-ranging study. Aliment Pharmacol Ther, 24:1087–1097.
7. Ando T, Watanabe O, Nobata K, Ishiguro K, Maeda O, Ishikawa D et al. (2008). Immunological status of the stomach in inflammatory bowel disease—comparison between ulcerative colitis and Crohn's disease. Digestion, 77:145–149.
8. Gariballa SE, Gunasekera NP. (1994). Bilateral foot drop, weight loss and rectal bleeding as an acute presentation of Crohn's disease. Postgrad Med J, 70:762–763.
9. Tresca AJ. (2009). The differences between ulcerative colitis and Crohn's disease. About.com Guide [Online]. Available at: http://ibdcrohns.about.com/od/ulcerativecolitis/a/diffuccd.htm. Updated on May 2, 2009.
10. Low RN, Sebrechts CP, Politoske DA, Bennett MT, Flores S, Snyder RJ et al. (2002). Crohn disease with endoscopic correlation: single-shot fast spin-echo and gadolinium-enhanced fat-suppressed spoiled gradient-echo MR imaging. Radiology, 222:652–660.
11. Guthery SL. (2007). Inflammatory bowel disease as a complex genetic disease [Online]. Available at: http://jorde-lab.genetics.utah.edu/medstudents_2007/Guthery_IBD_lecture.pdf
12. Wijmenga C. (2005). Expressing the differences between Crohn disease and ulcerative colitis. Plos Med, 2:e230; quiz e304.
13. Frieri G, Giacomelli R, Pimpo M, Palumbo G, Passacantando A, Pantaleoni G et al. (2000). Mucosal 5-aminosalicylic acid concentration inversely correlates with severity of colonic inflammation in patients with ulcerative colitis. Gut, 47:410–414.
14. Rudolph MW, Klein S, Beckert TE, Petereit H, Dressman JB. (2001). A new 5-aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis. Eur J Pharm Biopharm, 51:183–190.
15. Farup PG, Hinterleitner TA, Lukás M, Hébuterne X, Rachmilewitz D, Campieri M et al. (2001). Mesalazine 4 g daily given as prolonged-release granules twice daily and four times daily is at least as effective as prolonged-release tablets four times daily in patients with ulcerative colitis. Inflamm Bowel Dis, 7:237–242.

16. Hawthorne AB, Rubin G, Ghosh S. (2008). Review article: medication non-adherence in ulcerative colitis—strategies to improve adherence with mesalazine and other maintenance therapies. Aliment Pharmacol Ther, 27:1157–1166.
17. Shale MJ, Riley SA. (2003). Studies of compliance with delayed-release mesalazine therapy in patients with inflammatory bowel disease. Aliment Pharmacol Ther, 18:191–198.
18. Han SW, McColl E, Barton JR, James P, Steen IN, Welfare MR. (2005). Predictors of quality of life in ulcerative colitis: the importance of symptoms and illness representations. Inflamm Bowel Dis, 11:24–34.
19. Lakatos PL, Lakatos L. (2008). Once daily 5-aminosalicylic acid for the treatment of ulcerative colitis; are we there yet? Pharmacol Res, 58:190–195.
20. Prantera C, Rizzi M. (2009). 5-ASA in ulcerative colitis: improving treatment compliance. World J Gastroenterol, 15:4353–4355.
21. Sandborn WJ. (2008). Oral 5-ASA therapy in ulcerative colitis: what are the implications of the new formulations? J Clin Gastroenterol, 42:338–344.
22. Egan LJ, Sandborn WJ. (1998). Drug therapy of inflammatory bowel disease. Drugs Today, 34:431–446.
23. Talero E, Sánchez-Fidalgo S, Calvo JR, Motilva V. (2007). Chronic administration of galanin attenuates the TNBS-induced colitis in rats. Regul Pept, 141:96–104.
24. Sands BE. (2000). Therapy of inflammatory bowel disease. Gastroenterology, 118:S68–S82.
25. Reinecker HC, Steffen M, Witthoeft T, Pflueger I, Schreiber S, MacDermott RP et al. (1993). Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clin Exp Immunol, 94:174–181.
26. Cabrero A, Laguna JC, Vázquez M. (2002). Peroxisome proliferator-activated receptors and the control of inflammation. Curr Drug Targets Inflamm Allergy, 1:243–248.
27. Hanauer SB. (2004). Medical therapy for ulcerative colitis 2004. Gastroenterology, 126:1582–1592.
28. Kornbluth A, Sachar DB; Practice Parameters Committee of the American College of Gastroenterology. (2004). Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol, 99:1371–1385.
29. Campieri M, Ferguson A, Doe W, Persson T, Nilsson LG. (1997). Oral budesonide is as effective as oral prednisolone in active Crohn's disease. The Global Budesonide Study Group. Gut, 41:209–214.
30. van Dieren JM, Kuipers EJ, Samsom JN, Nieuwenhuis EE, van der Woude CJ. (2006). Revisiting the immunomodulators tacrolimus, methotrexate, and mycophenolate mofetil: their mechanisms of action and role in the treatment of IBD. Inflamm Bowel Dis, 12:311–327.
31. Markowitz J. (2008). Current treatment of inflammatory bowel disease in children. Dig Liver Dis, 40:16–21.
32. Kruis W, Bar-Meir S, Feher J, Mickisch O, Mlitz H, Faszczyk M et al. (2003). The optimal dose of 5-aminosalicylic acid in active ulcerative colitis: a dose-finding study with newly developed mesalamine. Clin Gastroenterol Hepatol, 1:36–43.
33. Sutherland L, Macdonald JK. (2006). Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database Syst Rev, 4:CD000543.
34. Bernstein CN, Eaden J, Steinhart AH, Munkholm P, Gordon PH. (2002). Cancer prevention in inflammatory bowel disease and the chemoprophylactic potential of 5-aminosalicylic acid. Inflamm Bowel Dis, 8:356–361.
35. Sandborn WJ, Hanauer SB. (2003). Systematic review: the pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. Aliment Pharmacol Ther, 17:29–42.
36. Sandborn WJ. (2007). New advances in 5-ASA therapy for ulcerative colitis. Medscape gastroenterology [Online]. Available at: http://cme.medscape.com/viewarticle/553568. Accessed on March 3, 2007.
37. Pierce D, Martin P, Kern M, Homolka R. (2007). MMX™ Mesalazine: a pharmacokinetic evaluation of dose proportionality and the effect of food. Gut, 56 (Suppl 3):A158.
38. Ng SC, Kamm MA. (2008). Review article: new drug formulations, chemical entities and therapeutic approaches for the management of ulcerative colitis. Aliment Pharmacol Ther, 28:815–829.
39. Brunner M, Greinwald R, Kletter K, Kvaternik H, Corrado ME, Eichler HG et al. (2003). Gastrointestinal transit and release of 5-aminosalicylic acid from ^{153}Sm-labelled mesalazine pellets vs. tablets in male healthy volunteers. Aliment Pharmacol Ther, 17:1163–1169.
40. Chourasia MK, Jain SK. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci, 6:33–66.
41. Leopold CS. (1999). Coated dosage forms for colon-specific drug delivery. Pharm Sci Technol Today, 2:197–204.
42. Marakhouski Y, Fixa B, Holomán J, Hulek P, Lukas M, Bátovský M et al.; International Salofalk Study Group. (2005). A double-blind dose-escalating trial comparing novel mesalazine pellets with mesalazine tablets in active ulcerative colitis. Aliment Pharmacol Ther, 21:133–140.
43. Kles KA, Vavricka SR, Turner JR, Musch MW, Hanauer SB, Chang EB. (2005). Comparative analysis of the *in vitro* prosecretory effects of balsalazide, sulfasalazine, olsalazine, and mesalamine in rabbit distal ileum. Inflamm Bowel Dis, 11:253–257.
44. Loftus EV Jr, Kane SV, Bjorkman D. (2004). Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. Aliment Pharmacol Ther, 19:179–189.
45. Harrell LE, Hanauer SB. (2004). Mesalamine derivatives in the treatment of Crohn's disease. Gastroenterol Clin North Am, 33:303–17, ix.
46. Khan MZ, Prebeg Z, Kurjakovic N. (1999). A pH-dependent colon targeted oral drug delivery system using methacrylic acid copolymers. I. Manipulation of drug release using Eudragit L100-55 and Eudragit S100 combinations. J Control Release, 58:215–222.
47. Asghar LF, Chandran S. (2006). Multiparticulate formulation approach to colon specific drug delivery: current perspectives. J Pharm Pharm Sci, 9:327–338.
48. Yang L, Chu JS, Fix JA. (2002). Colon-specific drug delivery: new approaches and *in vitro*/*in vivo* evaluation. Int J Pharm, 235:1–15.
49. Burakoff R. (2000). Crohn's disease of the small intestine. Curr Treat Options Gastroenterol, 3:59–68.
50. Klotz U. (2005). Colonic targeting of aminosalicylates for the treatment of ulcerative colitis. Dig Liver Dis, 37:381–388.
51. Sinha VR, Kumria R. (2004). Polysaccharide matrices for microbially triggered drug delivery to the colon. Drug Dev Ind Pharm, 30:143–150.
52. Linskens RK, Huijsdens XW, Savelkoul PH, Vandenbroucke-Grauls CM, Meuwissen SG. (2001). The bacterial flora in inflammatory bowel disease: current insights in pathogenesis and the influence of antibiotics and probiotics. Scand J Gastroenterol, 36(Suppl): 29–40.
53. Lee CM, Kim DW, Lee HC, Lee KY. (2004). Pectin microspheres for oral colon delivery: preparation using spray drying method and *in vitro* release of indomethacin. Biotechnol Bioprocess Eng, 9:191–195.
54. Ji CM, Xu HN, Wu W. (2009). Guar gum as potential film coating material for colon-specific delivery of fluorouracil. J Biomater Appl, 23:311–329.
55. Siew LF, Man SM, Newton JM, Basit AW. (2004). Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance *in vitro*. Int J Pharm, 273:129–134.
56. Ferrari PC, Oliveira GF, Chibebe FCS, Evangelista RC. (2009). *In vitro* characterization of coevaporates containing chitosan for colonic drug delivery. Carbohydr Polym, 78:557–563.
57. Zhao W, Song L, Deng H, Yao H. (2009). Hydration, erosion, and release behavior of guar-based hydrophilic matrix tablets

containing total alkaloids of *Sophora alopecuroides*. Drug Dev Ind Pharm, 35:594–602.
58. Edwards C. (1993). Interactions between nutrition and the intestinal microflora. Proc Nutr Soc, 52:375–382.
59. Cheng G, An F, Zou MJ, Sun J, Hao XH, He YX. (2004). Time- and pH-dependent colon-specific drug delivery for orally administered diclofenac sodium and 5-aminosalicylic acid. World J Gastroenterol, 10:1769–1774.
60. Abrahamsson B, Alpsten M, Jonsson UE, Lundberg PJ, Sandberg A, Sundgren M et al. (1996). Gastro-intestinal transit of a multiple-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with the emphasis on colon. Int J Pharm, 140: 229–235.
61. Zhang H, Alsarra IA, Neau SH. (2002). An *in vitro* evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon. Int J Pharm, 239:197–205.
62. Wilding IR, Kenyon CJ, Hooper G. (2000). Gastrointestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. Aliment Pharmacol Ther, 14:163–169.
63. Raedler A, Behrens C, Bias P. (2004). Mesalazine (5-aminosalicylic acid) micropellets show similar efficacy and tolerability to mesalazine tablets in patients with ulcerative colitis—results from a randomized–controlled trial. Aliment Pharmacol Ther, 20:1353–1363.
64. Wiersma H, Escher JC, Dilger K, Trenk D, Benninga MA, van Boxtel CJ et al. (2004). Pharmacokinetics of mesalazine pellets in children with inflammatory bowel disease. Inflamm Bowel Dis, 10:626–631.
65. Kramar A, Turk S, Vrecer F. (2003). Statistical optimisation of diclofenac sustained release pellets coated with polymethacrylic films. Int J Pharm, 256:43–52.
66. Ghebre-Sellassie I. (1989). Pellets: a general overview. In: Ghebre-Sellassie I, ed. Pharmaceutical Pelletization Technology. New York: Marcel Dekker, pp. 1–13.
67. Gryczová E, Dvořáčková K, Rabišková M. (2009). Evaluation of pellet friability. Čes Slov Farm, 58:9–13.
68. Pérez J, Rabisková M. (2002). Influence of the drying technique on theophylline pellets prepared by extrusion–spheronization. Int J Pharm, 242:349–351.
69. Häring A, Vetchý D, Janovská L, Krejcová K, Rabisková M. (2008). Differences in characteristics of pellets prepared by different pelletization methods. Drug Dev Ind Pharm, 34:289–296.
70. Krejcová K, Rabisková M, Vetchý D, Polásek E, Tomásek V, Prokopová A. (2006). The influence of drug solubility and particle size on the pellet formulation in a rotoprocessor. Drug Dev Ind Pharm, 32:585–593.
71. Gu L, Liew CV, Heng PW. (2004). Wet spheronization by rotary processing—a multistage single-pot process for producing spheroids. Drug Dev Ind Pharm, 30:111–123.
72. Vehring R. (2008). Pharmaceutical particle engineering via spray drying. Pharm Res, 25:999–1022.
73. Emås M, Nyqvist H. (2000). Methods of studying aging and stabilization of spray-congealed solid dispersions with carnauba wax. 1. Microcalorimetric investigation. Int J Pharm, 197: 117–127.
74. Gupta VK, Beckert TE, Price JC. (2001). A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. Int J Pharm, 213:83–91.
75. Zambito Y, Di Colo G. (2003). Preparation and *in vitro* evaluation of chitosan matrices for colonic controlled drug delivery. J Pharm Pharm Sci, 6:274–281.
76. Roda A, Simoni P, Roda G, Caponi A, Pastorini E, Locatelli M et al. (2009). Pharmacokinetics and safety of a new 1200 mg single-dose delayed release mesalazine microgranule formulation. J Bioequiv Bioavail, 1:44–51.
77. Press AG, Hauptmann IA, Hauptmann L, Fuchs B, Fuchs M, Ewe K et al. (1998). Gastrointestinal pH profiles in patients with inflammatory bowel disease. Aliment Pharmacol Ther, 12:673–678.
78. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG et al. (1996). Amylose as a coating for drug delivery to the colon: preparation and *in vitro* evaluation using 5-aminosalicylic acid pellets. J Control Release, 38:75–84.
79. Lin SY, Ayres JW. (1992). Calcium alginate beads as core carriers of 5-aminosalicylic acid. Pharm Res, 9:1128–1131.
80. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Flament MP et al. (2009). Novel polymeric film coatings for colon targeting: drug release from coated pellets. Eur J Pharm Sci, 37:427–433.
81. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Dubreuil L et al. (2009). Colon targeting with bacteria-sensitive films adapted to the disease state. Eur J Pharm Biopharm, 73:74–81.
82. Dvořáčková K, Bautzová T, Rabišková M. (2011). Dissolution study in the evaluation of oral preparations with controlled drug release. Chem Listy, 105:50–54.
83. Strober W, Lúdvíksson BR, Fuss IJ. (1998). The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn disease. Ann Intern Med, 128:848–856.
84. Lamprecht A, Ubrich N, Yamamoto H, Schäfer U, Takeuchi H, Maincent P et al. (2001). Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. J Pharmacol Exp Ther, 299:775–781.
85. Wei H, Li-Fang F, Min B, Yong-Zhen C, Bai X, Qing D et al. (2010). Chitosan/Kollicoat SR 30D film-coated pellets of aminosalicylates for colonic drug delivery. J Pharm Sci, 99:186–195.
86. Mladenovska K, Raicki RS, Janevik EI, Ristoski T, Pavlova MJ, Kavrakovski Z et al. (2007). Colon-specific delivery of 5-aminosalicylic acid from chitosan–Ca-alginate microparticles. Int J Pharm, 342:124–136.
87. Bautzová T, Rabišková M, Lamprecht A. (2011). Mucoadhesive pellets for the treatment of inflammatory bowel disease. Int J Pharm, in press.
88. Seo SB, Jeong HJ, Chung HS, Lee JD, You YO, Kajiuchi T et al. (2003). Inhibitory effect of high molecular weight water-soluble chitosan on hypoxia-induced inflammatory cytokine production. Biol Pharm Bull, 26:717–721.
89. Kim KW, Thomas RL. (2007). Antioxidative activity of chitosans with varying molecular weights. Food Chem, 101:308–313.
90. Klotz U. (1995). [5-Aminosalicylic acid and chronic inflammatory bowel diseases in children]. Klin Pediatr, 207:285–287.
91. Evonik Industries. (2009). Eudragit® Application Guidelines, 11th Ed. Darmstadt: Evonik.
92. Cohen RD, Lichtenstein GR, Safdi AV. (2008). 5-ASA treatment for ulcerative colitis: what's on the horizon? Gastroenterol Hepatol, 11 (Suppl 24):5–14.
93. Grosso A. (2009). A review of mesalazine MR formulations in ulcerative colitis. Br J Clin Pharm, 1:333–336.