Expert Opinion

Azo compounds in colon-specific drug delivery

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Azo compounds have the potential to act as drug carriers that facilitate the selective release of therapeutic agents to the colon, and also to effect the oral administration of those macromolecular drugs that require colon-specific drug delivery. With some further research-driven refinements, these materials may lead to more efficient treatments for local conditions, such as colonic cancer or inflammatory bowel disease. This article provides an overview of the azo-based systems developed to date, identifies the requirements for an ideal carrier, and highlights the directions for further developments in the field of azo group-facilitated colonic delivery.

Keywords: azo polymers, azo reduction, biodegradation, colonic targeting, drug delivery

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1. Introduction

1.1 The colon – a site for drug delivery

Prompted by the acknowledgements that the absorption of active agents via the colonic mucosa is favoured by long transit times, low proteolytic activity and high efficacy of absorption enhancers, the last two decades have witnessed many researchers becoming focused on local treatment via orally administered colon-specific drug delivery [1-16].

The selective delivery of drugs into the colon through the oral route may be particularly effective for the therapy of local conditions such as inflammatory bowel disease (IBD; including ulcerative colitis and Crohn's disease) and colonic cancer. This selectivity may also improve the systemic bioavailability of drugs that are unstable at the low pH of the stomach, or those that interact with bile salts and hydrolytic enzymes. Targeting a drug to the colon has also the advantage of preventing the nausea and vomiting that are commonly associated with irritation of the gastric mucosa [17]. Once-a-day dosage forms may also benefit because, for some substances, the colon behaves like a homogeneous reservoir that releases the active agent into the bloodstream at a constant rate and in a regulated mode [18].

1.2 Physiology of the colon

It is not only the physiological characteristics of the colon that dictate the design requirements for the development of vehicles for successful drug delivery to this part of the gastrointestinal tube, but also those of the other parts of the alimentary canal [19].

A successful colon-specific drug delivery system needs to be insensitive to the variations in gastric emptying time, which in turn are determined by a multitude of factors, including size, density, dietary intake, posture, gender, age, exercise, emotional state, stress and disease [20-25]. By contrast, transit time along the small intestine is notably constant (3 ± 1 h) and independent of dosage form or dietary intake [26]. The size of the dosage form is known to affect emptying times: single units are

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retained at the ileocaecal junction for a long and variable time [27], whereas particulate systems tend to regroup at the ileocaecal junction before moving into the large intestine [28]. A long residence time at the ileocaecal junction can lead to premature release of the drug, especially as the slower flow rate encourages the accumulation of large numbers of gastrointestinal bacteria in this region [13].

An added complication in the design of colon-specific delivery vehicles is presented by the retrograde contractions of the proximal colon, which prolong the retention of the luminal content in the caecum and the ascending colon [11]; colonic motility is reported to be proportional to the caloric content of ingested food (fat and high quantities of carbohydrates stimulate colonic motility [18]).

The pH gradient along the gastrointestinal tract is another factor of significance in the development of orally administered colonic dosage forms. The terminal ileum is reported to present the most alkaline environment (pH 7.5 ± 0.5) [29]. At the upper end of the colon, polysaccharide fermentation leads to the formation of short chain fatty acids, which effect a drop in pH (6.4 ± 0.6). This change in pH provides the means for the inhibition of proteolytic activity in this part of the intestine. The pH rises again in the transverse (6.6 ± 0.8) and further in the descending colon (7.0 ± 0.7) [11]. Disease can affect the pH of the colon: in a study involving 7 patients affected by ulcerative colitis, it was found that untreated subjects presented lower luminal pH (4.7 ± 0.7), compared with treated patients (pH = 5.5 ± 0.4); no such variation has been evidenced in patients affected by Crohn's disease [30].

The vast flux of water from the colonic lumen to the systemic circulation may be responsible for the colonic absorption of water-soluble drugs. This passive diffusion mechanism is controlled by the physicochemical properties of the drug molecule, and appears to be particularly well suited for the delivery of hydrophilic molecules in the 250 – 400 Da molecular weight range [31,32]. Carrier-mediated uptake is limited to B and K vitamins that are produced by the local flora. In general, the absorption of drugs in the colon appears to be more dependent on processes such as receptor-mediated endocytosis, fluid phase pinocytosis and, above all, paracellular transport (upper limit of 350 Da) [33]. Several absorption enhancers have been investigated for possible use in colon-specific formulations. Substances such as Ca^{2+} chelators or lipid/surfactant formulations have been shown to perform better in the colon than in the upper intestine [34].

The colonic environment presents some important barriers to drug delivery. The drug could be subject to physical entrapment into the faecal matter or to non-specific interactions with undigested foodstuffs. Furthermore, it could be degradable by the enzymes that are produced by the local microflora [35]. Attractive or repulsive interactions are also possible between the drug and the negatively charged mucus. The pH gradient at the layer of unstirred water between the mucus and the epithelium may represent a further barrier to the absorption of drugs in the colon [36-38]. The capacity for drug absorption may be altered drastically during the diseased state: increased intestinal permeability is typical of IBD [32,39]. Also, there is evidence to suggest that the function of the tight junctions is altered in IBD patients [40].

The gastrointestinal tract harbours a complex ecosystem, which accommodates 400 – 500 distinct species of aerobic and anaerobic bacteria. The total living population of the gastrointestinal tract (GIT) amounts to 10^{14} units (the entire human body is comprised of 10^{13} eukariotic cells [41]). The composition and concentration of microflora along the digestive tube is highly dependent upon the nature of each segment and upon its physiological condition. In general, an increase in the anaerobic/aerobic bacteria ratio is observed along the aboral direction. As the passage of the stool along the colon is slower than bacterial doubling time, the proliferation of the microbes in this part of the gastrointestinal tract is favoured. The predominant species in this region are anaerobic bacteria [42]. Colonic bacteria live in a symbiotic relationship, affording many benefits for the host. Water and salt homeostasis depend on the absorption of short chain fatty acids produced by the bacterial metabolism of carbohydrates and proteins in the colon. The resident flora is also involved in the synthesis of vitamin B and K and is responsible for the stimulation of the gut immune system. From a pharmaceutical point of view, colonic bacteria play another important role in that they facilitate the metabolism and absorption of many drugs, such as sulfasalazine, isonicotinuric and salicyluric acids, L-DOPA, lactulose, digoxin and cyclamates [43].

The composition of the bacterial population of the GIT is known to be affected by certain diseased states. For example, in acute diarrhoea the local microflora can be outnumbered by the pathogen. Keighley *et al.* [44] investigated the influence of IBD on the composition of the intestinal microflora and found that although Crohn's disease patients present extreme changes, in ulcerative colitis the microflora is not affected.

In the colon, the energetic requirements of the vast residing microflora are satisfied by the fermentation of a great variety of substrates that cannot be digested in the small intestine. As only the indigestible part of the food reaches the colon, bacteria that populate this part of the GIT produce specific reductive enzymes that allow them to use substrates such as disaccharides, trisaccharides and mucopolysaccharides as carbon sources [45]. The reductive enzymes that are produced by colonic bacteria include β-glucuronidase, β-xylosidase, α-arabinosidase, β-galactosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase [10,46].

The microflora of the colon is also responsible for its redox potential, which is in turn related to total metabolic activity. The redox potential of the proximal small intestine is reported to be -67 ± 90 mV, that of the distal small intestine -196 ± 97 mV, and in the colon it is reportedly -415 ± 72 mV [47]. As the

redox potential changes along the GIT, it may be used as a trigger for specific drug delivery; the very low redox potential of the colon is particularly useful for the reduction of azo or disulfide bonds [48].

2. Targeting strategies

The most promising colon-specific drug delivery systems considered so far have been categorised into four broad classes [2,9,49,201]:

- Time-controlled systems are designed to delay the release of a drug until the delivery system reaches the colon; such systems are not designed to respond to inter- or intra-individual variations in gastrointestinal transit time.
- Pressure-controlled systems use the transient increases in luminal pressure that are associated with the strong peristaltic waves of the colon; the efficacy of such systems has not as yet been proven.
- pH-controlled systems respond to changes in the local pH environment of the gastrointestinal tract, especially to the small drop in pH (~ 0.5) between the distal intestine and the colon, and to the more pronounced drop (to ~ pH 6.5) at the proximal colon; the performance of these systems is often sensitive to food- or disease-induced variations in pH.
- Enzyme-controlled systems exploit the hydrolytic capacity of the enzymes present in the large intestine to release active agents from prodrugs, coated systems or hydrogel networks.

Due to their site-specificity, enzyme-controlled systems seem to hold the most promise as potential vehicles for the delivery of active agents to the colon: the reductive degradation of azo compounds – one class of such systems – was first observed in studies of the metabolism and absorption of food dyes [50].

3. Site specificity through azo reduction

Aromatic azo compounds can be reduced to hydrazo derivatives and, further, to primary amines. The reduction of such azo compounds by colonic microflora – although not yet fully understood – is one of the most extensively studied bacterial metabolic processes; hypotheses have attempted to implicate azo-reducing enzymes and non-specific electron shuttles, but considerable work is needed before the mechanism of azo reduction is fully deconvoluted [51,52].

Work so far has established the presence of reducing agents both inside and outside the walls of bacterial cells [12,53,54]. Based on the observation that some azo compounds that cannot penetrate the bacterial wall are susceptible to colonic azoreduction, it has been claimed that the reduction process may be facilitated by extracellular or membrane-bound enzymes [55]. Consistent with this suggestion, some aerobic azo reductases have been isolated [56], but as no evidence for the existence of anaerobic enzymes has been found [55], it is possible that anaerobic azo reduction occurs via a co-metabolic reaction involving soluble flavins that act as electron shuttles between the reduced form of nicotinamide adenine dinucleotide phosphate-linked flavoproteins and other electron acceptors [53]. The feasibility of azo reduction by non-specific enzymes has been demonstrated in experiments using a series of single-species cultures of intestinal micro-organisms (*Clostridium, Salmonella, Bacillus, Eubacterium* and *Escherichia coli*), all of which were seen to facilitate the protonation of the azobond [57].

Brøndsted and Kopeček have suggested that azo reduction may proceed via either one of two possible pathways: the transfer of two electrons via a hydrazo intermediate (**Figure 1**) or, alternatively, a one-electron-transfer mechanism [58]. Consistent with the former pathway, researchers have reported evidence for the formation of a hydrazo compound [59,60], which is also supported by the suggestion that the number of electrons involved in the degradation of an azo bond must be two or four [61]. The existence of the more difficult to detect, radical intermediate, has not, as yet, been witnessed.

Interestingly, a study by Nam and Renganathan [62] has shown that azo dyes can be reduced non-enzymatically, by NADH, in mildly acidic environments (pH 3.5 to 6.0). The same workers have also suggested that enteric bacteria may not be able to reduce azo bonds, for the simple reason that such bonds do not occur in nature.

The molecular design considerations for pharmaceutically acceptable azo compounds are imposed not only by the need to achieve good reduction rates, but also by the necessity to avoid potential toxicity: some aromatic amines that are derived from the reduction of azo compounds can be metabolised to electrophilic derivatives capable of binding covalently to DNA [63].

4. Azo compounds as prodrugs

Azo prodrugs of 5-aminosalicylic acid (5ASA) – an anti-inflammatory agent used primarily for the treatment of IBD – bestow an important advantage to the therapeutic moiety: the premature absorption of the active agent in the small intestine is minimised as the molecular size and hydrophilicity of the prodrug molecule are increased. Sulfasalazine – one of the marketed 5ASA prodrugs – is made by attaching 5ASA to sulfapyridine via an azo bond (**Figure 2**). Consequent to this modification, the upper-gut absorption of the active agent has been shown to be limited to ~ 12% of the administered dose [64,65]. Olsalazine, a prodrug molecule with a symmetrical structure (**Figure 2**), has been designed for the purpose of reducing the side effects of sulfapyridine [66]. Olsalazine is not as effective a treatment for ulcerative colitis as sulfasalazine – probably due to the slower degradation of the azo bond, which promotes the acetylation and excretion of the active agent [67,68]. In an attempt to improve the efficacy of 5ASA, the drug was conjugated to 4-aminophenylacetic

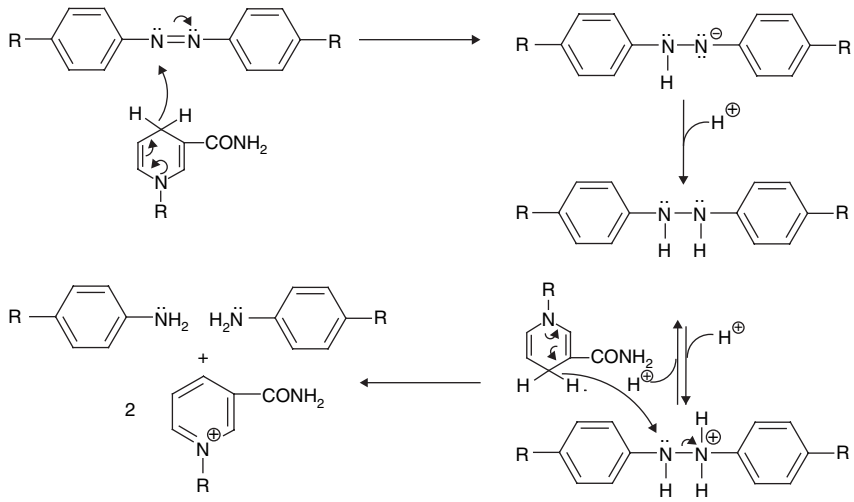

Figure 1. The mechanism of reduction of the azo group by hydrogen nicotinamide adenine dinucleotide, as proposed by Nam and Reganathan [62].

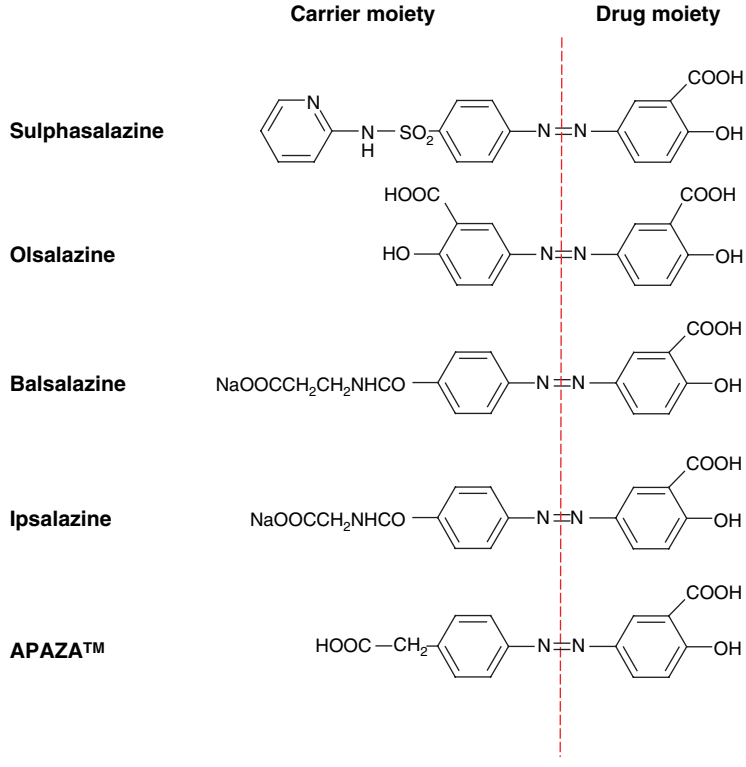

Figure 2. Chemical structures of various 5-aminosalicylic acid prodrugs.

acid (4-APAA; an anti-inflammatory drug that acts by a different mechanism). Using a rat model of experimental colitis, the resulting prodrug, APAZA™ (Biocon Ltd) [202] (**Figure 2**), has been shown to be 10- to 20-times more potent than sulfasalazine [69].

Based on the hypothesis that the steric hindrance associated with macromolecular chains would prevent absorption prior to the cleavage of the azo bond, the use of polymeric azo prodrugs has been considered as a means of inhibiting the absorption of the active agent in the upper part of the intestine. As the polymeric carrier is generally not absorbed, due to its high molecular weight, the same systems offer the possibility of reduced risk to adverse reactions. Additionally, such systems allow the use of biodegradable spacers, which in

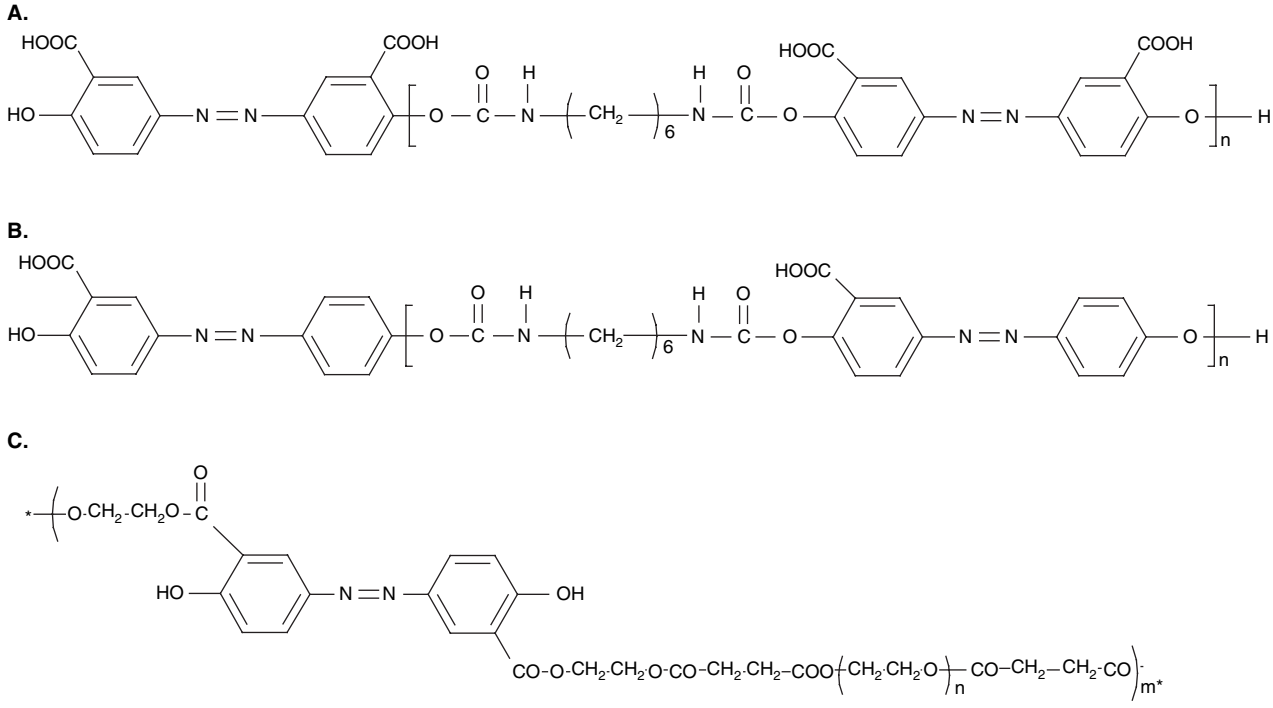

Figure 3. Examples of linear polymeric azo prodrugs.

turn have been shown to provide a means of controlling the rate of release of the active agent [70]. A further advantage of polymeric carriers is that they are often amenable to chemical functionalisation, which in turn permits the regulation of their physicochemical properties [71]. However, a fundamental drawback of such systems is that they tend to release the active agent very slowly, often resulting in subtherapeutic dosing profiles; the use of bioadhesive polymers [71,72], and the modification of the hydrophilicity of the polymeric chain (**Figures 3A** and **3B**) may provide the means for addressing this issue [73].

Wang and coworkers [74] prepared a linear azo polymer by the conjugation of poly(ethylene oxide) chains to the carboxylic groups of olsalazine (**Figure 3C**). The workers showed that varying the length of the poly(ethylene oxide) chain impacted upon the degree of hydration and, in turn, affected the rate of release of olsalazine. A further advancement in the field of linear polymeric prodrugs saw the development of polymeric chains that exhibit pH-dependent degradation, namely copolymers of azo derivatives of 5ASA with hexamethylene diisocyanate [75]: the hydrolysis of the urethane bond at neutral to basic pH was shown to favour polymeric degradation and drug release, but the release of 5ASA in rat caecal extract was too slow (extending to ~ 80 h from the onset of the experiment) for therapeutic application [76]. This finding points to the need to examine the implications of employing such systems under regimes that are imposed by conditions that reduce intestinal transit time, such as diarrhoea, or under those that demand the administration of co-therapeutants that cause a reduction in the viable colonic bacteria count (e.g., antibiotics [77]).

5. Bioadhesive azo polymers for prolonged drug release

The use of bioadhesive drug carriers is commonly assumed to increase the bioavailability of oral therapeutics by extending the contact time with the gastrointestinal mucosa, by maintaining an elevated concentration of the drug at the site of action, and by inhibiting the contact of the drug with the potentially degrading enzymes of the intestinal lumen. On the basis of these assumptions, azo prodrugs of 5ASA, ciclosporin A, or 9-aminocamptothecin (9-AC) have been combined with bioadhesive polymers [52,78-80].

In an effort to design a drug carrier that responds to the distribution profiles of gastrointestinal enzymes, and rationalised in terms of the adhesion specificity of some bacteria (e.g., *Shigella flexneri*) for colonic epithelial cells [81] – through binding via glucose- and fucose-specific lectins at the surface of colonic enterocytes [82] – a copolymer of *N*-(2-hydroxypropyl)methacrylamide and *N*-methacryloylglycylglycine was partially functionalised with an azo derivative of the drug and partially with a saccharide (fucosylamine, glucosamine or mannosamine), **Figure 4A** [79]. It has been claimed that in the upper intestine, the peptidic bond of this system would be protected by steric effects and that backbone degradation and release of the prodrug (aminoacid derivative) will be subsequent to azo reduction

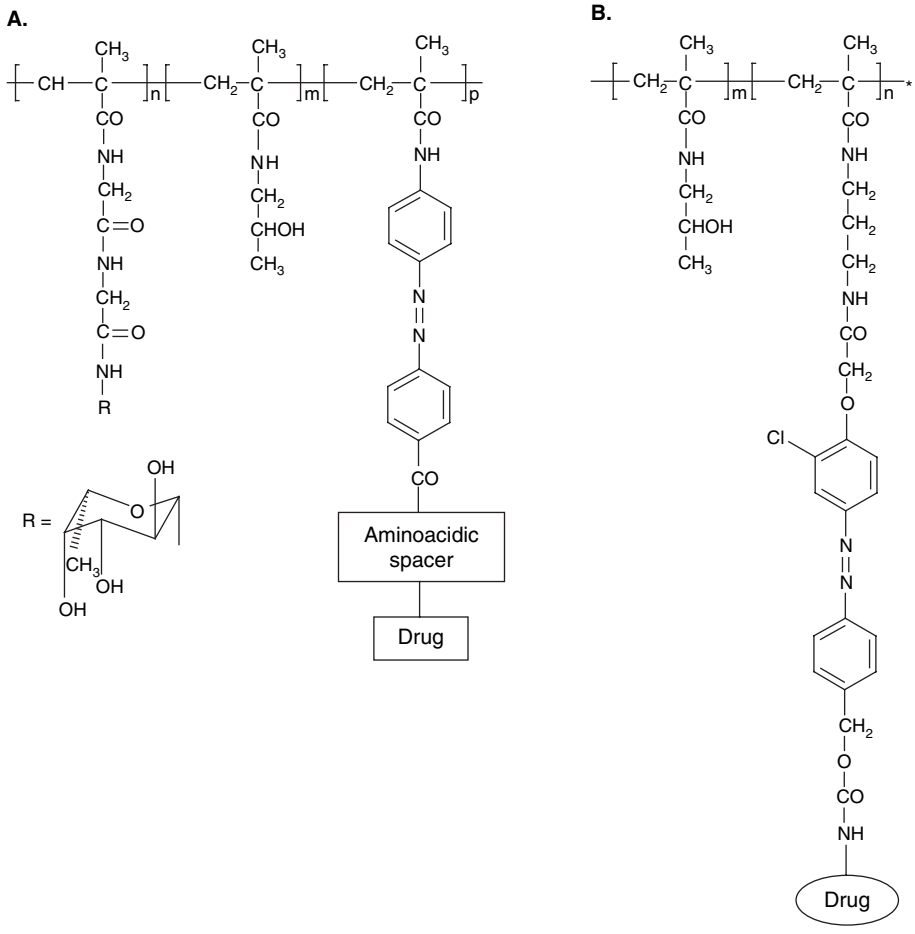

Figure 4. Structures of bioadhesive, colon-specific hydroxypropyl methacrylamide copolymers containing: A. an aminoacid spacer [79], and B. a self-eliminating group [84,85].

in the colon, which will precede the liberation of the active agent from the prodrug through the action of aminopeptidases. Complementary work has concluded that conjugates containing a leucine–alanine spacer release the drug more readily than structures containing alanine alone [79,80]. In all systems considered so far, the drug release rate from the peptide-drug system has been found to be too low to achieve therapeutic concentrations of 9-AC in the colon [83].

It has been suggested that drugs containing amino groups may be attached to certain polymeric carriers via a carbamic link with a bifunctional *p*-amino-benzyl alcohol (**Figure 4B**), which acts as a self-eliminating group [84,85]: enzymatic cleavage of the azo moiety results in the formation of an electron-donating amine, which in turn promotes the decomposition of the unstable carbamic acid derivative, releasing carbon dioxide and freeing the parent drug [84,85]. Results from an *in vitro* release study have suggested efficacious drug release [86]. This finding is consistent with data from an *in vivo* study, which showed that polymeric conjugates, utilising the 'self-eliminating group' principle, effect high local concentrations of 9-AC and result in improved antitumour efficacy and reduced systemic side effects [87].

The authors of the present review have developed azo-crosslinked poly(acrylic acid) derivatives that exhibit pH-dependent swelling and show specific *in vitro* degradation in the presence of colonic extracts [88,89]. The carriers have also been shown to develop bioadhesive capabilities following the cleavage of the azo crosslinker. *In vivo* distribution studies (rat model) using ^{14}C-radiolabelled congeners of the polymeric carriers have shown that progressive swelling and muco-adhesion prolong the residence time in the lower bowel to > 48 h [90]. Parallel *in vivo* studies in a rodent model of reactivated hapten-induced colitis considered the effect of the drug-free polymers on the expression of the inflammation marker myeloperoxidase [91]: rats treated with the polymer on alternate days, before and after colitis reactivation (for a total of 14 days), presented myeloperoxidase activity values comparable with those of healthy animals, and with those of animals that had not received a reactivation dose of the hapten (**Figure 5**). These data provided strong evidence that azo crosslinked poly(acrylic acid) derivatives exhibit

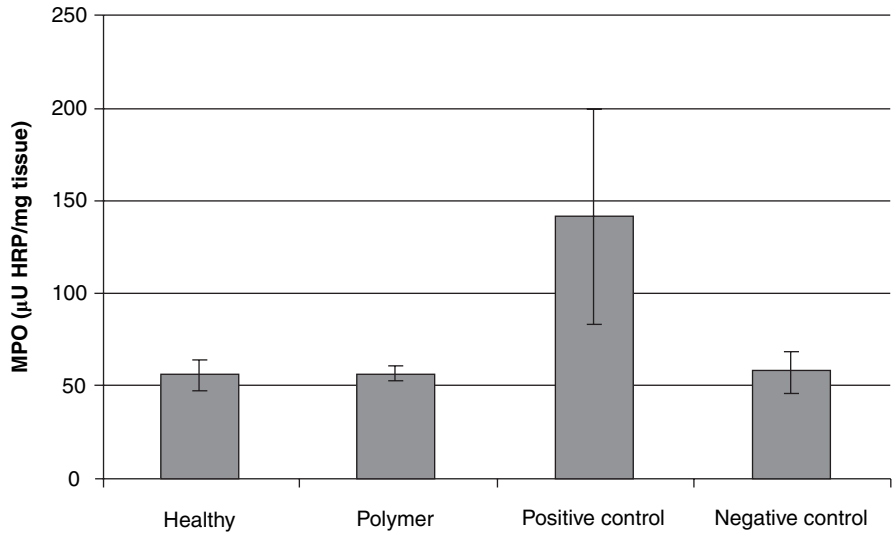

Figure 5. Potential mucosal-coating properties of azo-crosslinked poly(acrylic acid), expressed as capacity of reducing the activity of the inflammation marker myeloperoxidase (MPO) in a rat colitis model. Myeloperoxidase activity is measured as corresponding horseradish peroxidase (HRP) capacity for reducing hydrogen peroxide. The data are presented as mean ± SEM (n = 6); in all cases $p > 0.05$ (one-way ANOVA followed by Turkey's post hoc test) [90].
ANOVA: Analysis of variance; SEM: Standard error of the mean.

mucoadhesion specificity in the colon, and indicated their potential to act as a protective mucosal coating in the treatment of IBD [90]. Scientific developments have now reached a stage where the effectiveness of these mucoadhesive formulations needs to be tested in human volunteers [92]. Additionally, molecular-level investigations are needed to establish the precise nature of the interaction between colonic mucous and the bioadhesive polymer [93].

6. Use of azo hydrogels in stimulus-responsive systems

The highly hydrophilic nature of biocompatible hydrogels [94,95], which offer a desirable drug diffusion pathway [96], combined with their capability to exhibit responsiveness to external stimuli makes these systems good candidate vehicles for drug delivery.

One of the primary concerns in the design of any hydrogel intended for drug delivery is the control of its degree of swelling – a factor which governs the loss of drug prior to arrival of the carrier at the site of action. Kopeček's group incorporated azo crosslinked *N*-substituted acrylamides (**Figure 6**) into the structure of a series of hydrogels [58,97-99]. The pH sensitivity of the dynamic swelling behaviour was seen to increase with increasing proportions of ionisable functionalities in the polymer structure, whereas the equilibrium degree of swelling is reported to be dependent on the hydrophobic/hydrophilic ratio of the monomeric constituents (both features of some significance, as drug release involves diffusion through the matrix, which in turn is highly sensitive to the swelling behaviour and to the degradation characteristics of the polymeric network). The biodegradability of these hydrogels is reported to depend not only upon their swelling behaviour but also upon the crosslinking density and the size of the crosslinker. A limited degree of swelling allows the enzymes and the electron mediators to interact with the azo bonds of the polymer without inducing the premature release of the drug. As a rule, the higher the number of crosslinker units in the structure the lower the mobility of the polymeric chains, and the lower the overall swelling and rate of degradation. This structural feature is interlinked with the size and chemical nature of the crosslinker: a long spacer between the azo bond and the polymeric backbone may facilitate the access of reduction mediators, even in highly crosslinked systems.

Shantha *et al.* [100] demonstrated the selective *in vitro* release of 5-fluorouracil from copolymer hydrogels of methacryloyloxy azobenzene and hydroxyethyl methacrylate (HEMA) in the presence of colonic microflora, but this system was not proposed as a suitable candidate for clinical use because the reduction of the azo bond is accompanied by the release of aniline.

Based on the premise that the reduction of the azo group in the colon would facilitate the swelling of the gel, and the subsequent microbial degradation of the polysaccharides would trigger the release of the drug, van den Mooter's group attempted to enhance colon specificity by combining azo bonds with polysaccharides [101]. Copolymers of 4,4′-di(methacryloylamino)azobenzene with methacrylated inulin or dextran exhibited swelling behaviour that was inversely proportional to the concentration of the azo compound, due to higher crosslinking density and

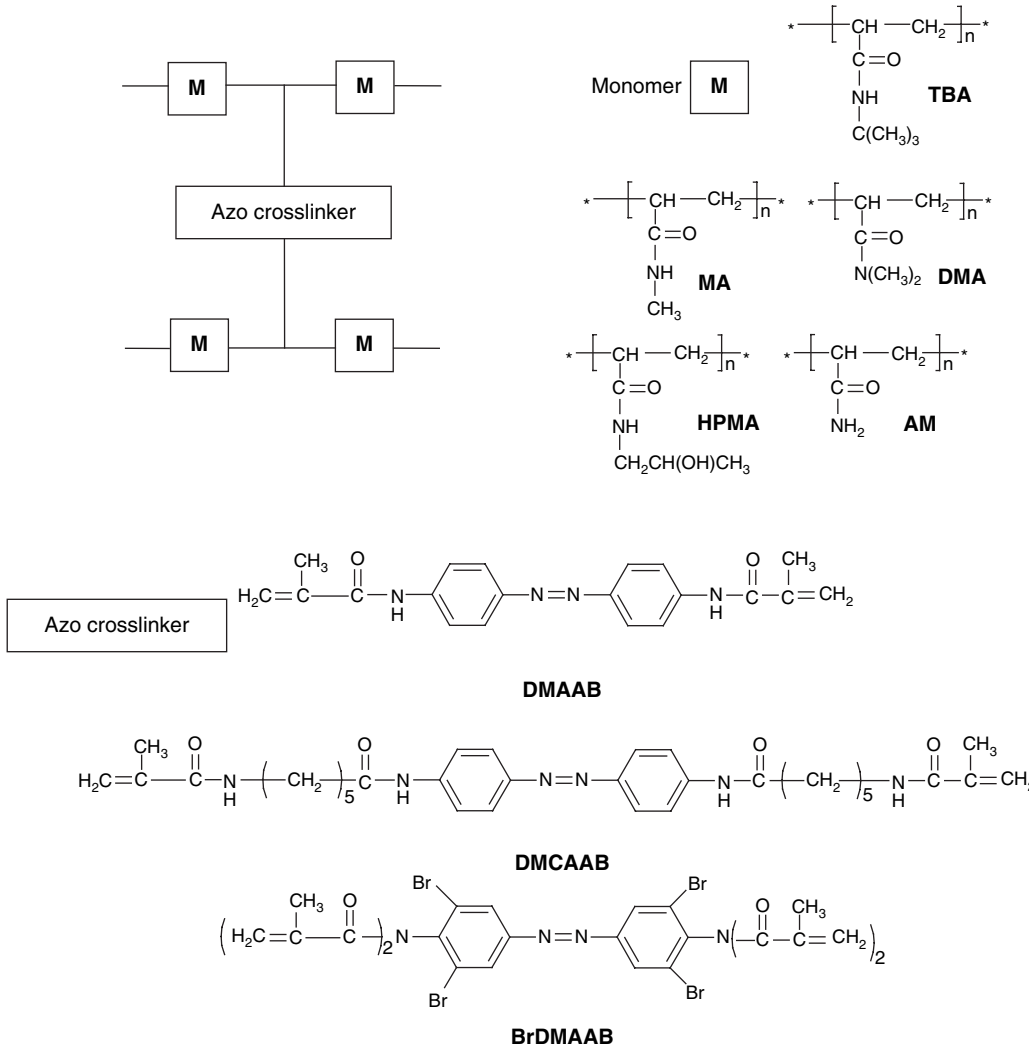

Figure 6. Chemical structures of monomers used for the preparation of azo-crosslinked hydrogels.
AM: Acrylamide; BrDMAAB: 3,3′,5,5′-Tetrabromo-4,4,4′,4′-tetra(methacryloylamino)azobenzene; DMA: *N,N*-dimethylacrylamide; DMAAB: 4-4′-Di(methacryloylamino)azobenzene; DMCAAB: 4-4′-Di(*N*-methacryloyl-6-aminohexanoylamino)azobenzene; HPMA: *N*-(2-hydroxypropyl)methacrylamide; MA: *N*-methylacrylamide; TBA: *t*-Butyl acrylamide.

increased hydrophobicity [102,103]; the use of dextran-based analogues is reported to afford a better control of the cross-linking density. Parallel *in vitro* studies suggested that the colonic degradation of these hydrogels would be very limited, probably due to the relative chemical stabilities of the inulin and dextran analogues.

A similar approach was adopted by Liu *et al.* [104], who synthesised konjac glucomannan-grafted azo-crosslinked hydrogels: acrylic acid moieties were incorporated to confer pH sensitivity and hydrophilicity, and swelling and rate of drug release were controlled by varying the degree of cross-linking. *In vitro* investigations confirmed pH sensitivity, and studies in the presence of endo-1,4-β-mannonase showed the slow release of a model drug (bovine serum albumin). However, these materials were characterised by rather long degradation times.

Yin *et al.* [105] studied aromatic azo-crosslinked copolymer systems containing pH-sensitive carboxylic acids and *n*-alkyl methacrylate ester moieties. These hydrogels are reported to change their swelling behaviour within a narrow pH window and to exert control over the premature loss of incorporated drug by exhibiting limited, but progressive swelling in the small intestine.

7. Azo polymeric coatings for monolithic dosage forms

The development of azo polymers as coatings for solid dosage forms offers an attractive alternative to the use of azo hydrogels in that the physicochemical properties of the drug do not impose any restrictions upon the drug-loading process.

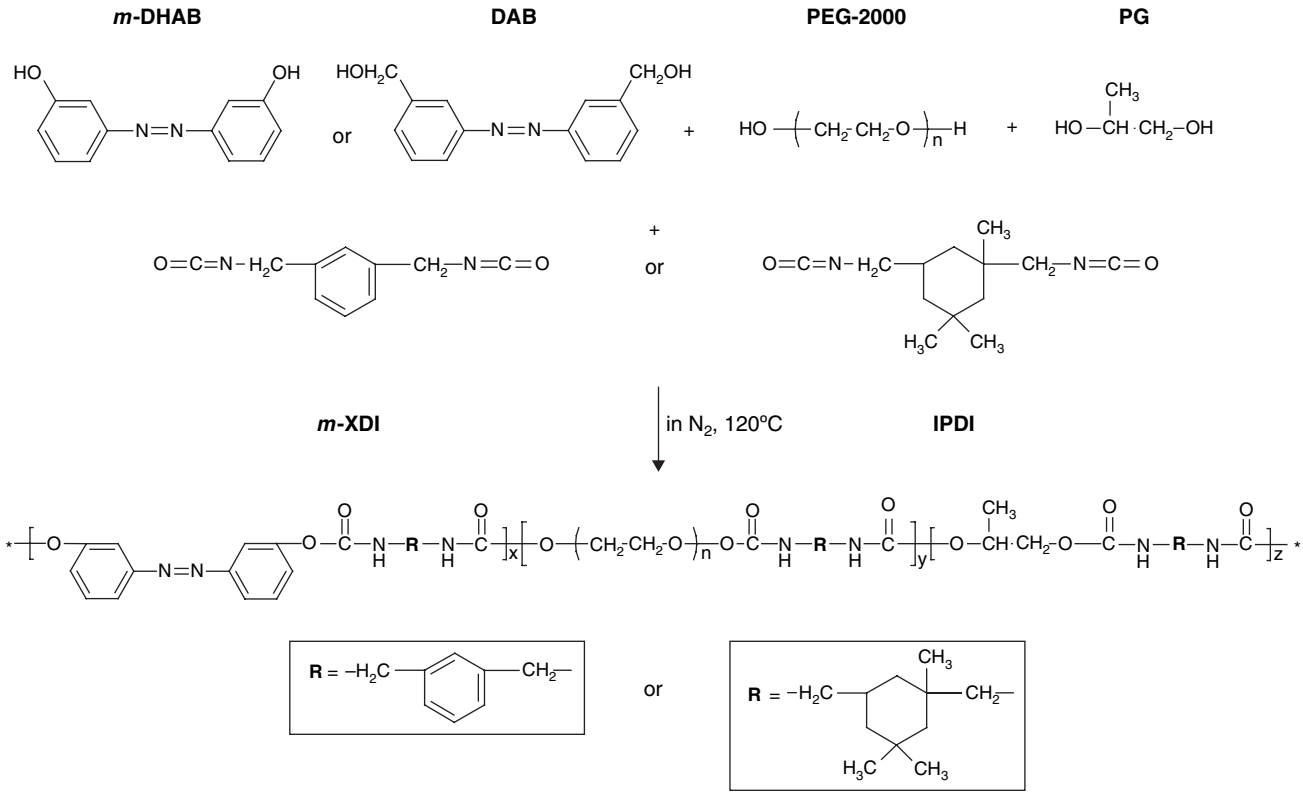

Figure 7. Chemical structure and synthetic procedure for the preparation of linear azo-containing polyurethanes.
DAB: *m,m′*-Di(hydroxymethyl)azobenzene; IPDI: Isophorone diisocyanate; *m*-DHAB: *m,m′*-Dihydroxyazobenzene; *m*-XDI: *m*-Xylylene diisocyanate; PEG-2000: Poly(ethylene glycol), PG: Propylene glycol.
Mw = 2000.

Azo polymers were first investigated as coating materials for colon-specific dosage forms as early as 1986, by Saffran *et al.* [106], who aimed to deliver vasopressin and insulin to the colon via the oral route. The work used copolymers of styrene and HEMA that were crosslinked with divinylazobenzene or with substituted divinylazobenzenes. These early materials, which were not solvent processable due to their high degree of crosslinking, exhibited poorly defined degradation profiles.

Kimura and coworkers [59,60], in an attempt to improve the solubility and film-forming properties of such azo polymers, synthesised linear polyurethanes in which the azo moiety was incorporated into the main chain. The copolymers were comprised of three segments: i) *m,m′*-di(hydroxymethyl)azobe nzene; ii) hydrophilic poly(ethylene glycol) (PEG); and iii) hydrophobic *m*-xylylene diisocyanate (**Figure** 7). *In vitro* degradation studies showed that the azo bonds of these systems could only be reduced partially and, further, that the reduction process was reversible on exposure to air. Drug release studies were also conducted following incorporation of a hydrophobic drug into polymer-coated pellets: the release rate was seen to be highly sensitive to the relative amounts of azo moieties and PEG units. Faster release was obtained either by increasing the azo content of the copolymer or by increasing the amount of PEG. The researchers claimed that effective targeting may be achieved by considering the interplay between hydrophilicity, degradability and crystallinity in the copolymers. However, *in vivo* studies using pellets coated with a soluble inner layer of (carboxymethyl)ethyl cellulose and an outer azo-containing polyurethane layer showed that the activity onset of incorporated peptides (insulin and calcitonin) occurred well before transit through the ileo-caecal valve [107].

In a further development, van den Mooter's laboratory prepared polymeric coatings comprising of a mixture of HEMA and methyl methacrylate (MMA) that was polymerised in the presence of the bifunctional azo compound 4,4′-di(methacryloylamino)azobenzene [108]. *In vitro* characterisation work indicated that high hydrophilicity is required if these coatings are to be efficiently degraded in the colon. Again, the control of pH-dependent swelling provided the means of improving performance [109]: the incorporation of methacrylic acid into the structure suppressed precolonic swelling and enhanced specificity. Parallel *in vitro* release studies using ibuprofen showed that methacrylic acid-containing polymers are capable of releasing the drug within a therapeutically relevant timescale. The same workers observed that these pH-independent systems require ~ 24 h of incubation in the bacterial medium before they release their

ibuprofen content. However, considering that capsules and tablets generally pass through the colon in ~ 20 – 30 h, it would be reasonable to conclude that neither of these systems is suitable for the systemic delivery of peptides, hormones and other drugs with narrow therapeutic windows [110].

The performance of capsules coated with an azo-crosslinked copolymer consisting of HEMA/MMA in a 5:1 ratio has been evaluated in studies involving a single human volunteer, and the data have been compared with those obtained using the commercially available pH-sensitive polymer Eudragit® S (Röhm Pharma). For this work, the gastrointestinal transit of barium sulfate-loaded capsules was followed by X-ray imaging. The data revealed that intact HEMA/MMA-coated capsules reached the colon at ~ 10 h from administration, and those coated with Eudragit S disintegrated in the pH environment of the small intestine [111]. The results suggested that the combination of pH sensitivity and enzymatic degradation could prove a useful molecular design strategy for the development of successful colonic delivery systems.

8. Conclusions

The use of azo compounds as a means of achieving colon-specific drug delivery has evolved significantly since the initial observation that azo dyes degrade in the colon. In terms of molecular design, several of the azo compounds that have been synthesised are now approaching a level of refinement that will soon allow the selection of candidate materials for clinical evaluation. Owing to the high site specificity that appears to be achievable with azo-polymeric carriers, these systems have gained considerable ground over the azo-prodrug alternative. Although some work is still needed to optimise the capability of polymeric azo compounds to act as carriers of therapeutic agents to the colon, recent animal experiments have shown that azo polymers offer significant promise in the therapy of colon-related conditions by acting as a protective barrier for the colonic mucosa; essentially, a 'plaster for the colon' that can be administered orally.

9. Expert opinion

The degradation of azo-polymeric carriers represents a most promising strategy for the delivery of active agents to the colon. Chemical synthesis work has generated several materials that exhibit the fundamental features needed to achieve colon specificity, and, with a few additional refinements, the present limitations of azo polymers – in terms of their loading capacity for the transport of active agents – are certain to be overcome. In parallel, molecular manipulation techniques are at a level of advancement that allows the fine tuning of the polymer structure through the incorporation of features that permit the colon-delivery vehicle to develop selective mucoadhesive properties and to respond to changes in pH as it travels along the gastrointestinal tract.

Primary among the molecular design considerations for new azo materials is the refinement of their solubility characteristics. For example, an azo polymer that can be processed in a pharmaceutically acceptable solvent but which is insoluble in gastrointestinal fluids would provide the ideal coating material for the delivery of active agents in monolithic form. Another molecular design challenge is presented by the need to exert fine control over the colonic degradability of the material: as azo degradation is primarily determined by the degree of crosslinking in the polymer structure, the chemical design stage needs to be informed by *in vivo* studies that address the relationship between particle size, degree of crosslinking and colonic residence time. The hydrophilic–lipophilic balance of the azo polymer is also important: high hydrophilicity impacts directly upon the equilibrium degree of swelling, which in turn affects the azo-degradation process. However, as the same parameter also influences the rate of drug release, the adjustment of the hydrophilic–lipophilic balance can only be made following consideration of the demands imposed by each active agent.

At present, the employment of azo-based carriers in clinical trials appears to be inhibited by two interlinked barriers: firstly, limited data regarding inter- and intra-subject variability of the azo-reduction process impede the design of molecular systems that can be confidently proposed as reliable candidates for such studies, and, secondly, the selected materials need to be subjected to toxicological studies. The importance of toxicological evaluation is appreciated when the carcinogenicity and mutagenicity of some of the azo dyes evaluated by the food industry are considered. Nevertheless, azo polymers for colon-specific drug delivery offer an advantage over their low molecular weight analogues in that they can be designed to degrade to fragments that cannot be absorbed systemically because of their high molecular weight.

The combination of molecular features that promote mucoadhesive behaviour (e.g., agglutinins) with functionalities that act as penetration enhancers (e.g., poly(acrylic acid) or EDTA) represents a further research area that may lead to improvements in the efficiency of drug absorption through the colonic mucosa. It is also possible that the mucoadhesive selectivity of azo polymers will be improved to the extent that specific sites within the colon can be targeted. As our understanding of the physiological and pathological characteristics of the colon advances new options for achieving highly specific targeting may become available, as is exemplified by the discovery that in both colonic cancer and Crohn's disease there is an overexpression of folic acid receptors in the diseased tissue [112,113], it is possible that, in future generations of azo polymers, folic acid receptors will provide the entities for highly specific targeting.

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